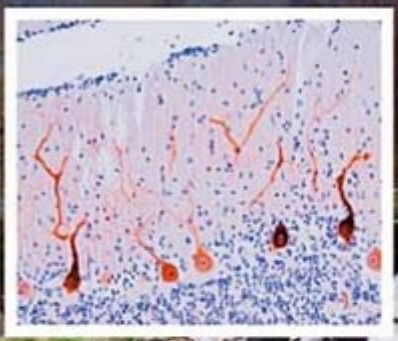

DISEASES OF SHEEP

FOURTH EDITION

Edited by I.D. Aitken

Blackwell Publishing

Moredun

Diseases of Sheep

Fourth Edition

Edited by

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Former Director, Moredun Research Institute, Edinburgh

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Foreword to the Fourth Edition

One of the challenges we face at Moredun is ensuring that the high-quality scientific research into diseases of sheep, and the improvements in animal health that are the outcome of that research, are made widely available to producers, advisors, veterinarians and the allied scientific community. Like its predecessors, this new edition of *Diseases of Sheep* is a major contributor to ensuring that that happens by enlarging on earlier work and incorporating new information gained over recent years.

The UK and international contributors to this excellent publication are experts in their respective areas of work and all who are involved with sheep will benefit from the wide range of knowledge and experience that the authors bring to their contributions.

Livestock production carried out with high standards of health and welfare is demanded by our consumers. This publication is an important link in the chain of achieving this for the sheep sector.

The Moredun Foundation is indebted to Professor Ian Aitken for once again editing this revised and updated edition of an established authoritative book.

John Ross CBE, FRAgS
Chairman
Moredun Foundation for
Animal Health
and Welfare

Preface

The first edition of this book in 1983 was spearheaded by Dr W.B. Martin, the then Director of the Moredun Research Institute. It had two main purposes – first to remedy the current dearth of readily accessible information on sheep diseases and second, through royalty income, to assist the Institute's parent body (the Animal Diseases Research Association) in its bid to purchase additional grazing land at Gilmerton, the Institute's original site. Both objectives were met.

At Bill's invitation I joined him as co-editor for the second and third editions, both of which incorporated new knowledge on the causes and control of diseases and widened international coverage to major sheep-producing areas of the world. In that, we were fortunate in enlisting the support of colleagues both at home and abroad, many of whom had links with Moredun as scientific collaborators or visitors. When a fourth edition was suggested we were encouraged by initial soundings of former contributors but, sadly, Bill died before the work was formally commissioned. As a personal tribute to a friend and professional colleague I accepted editorship of this new edition.

The aim has been to build on what has gone before by updating information on specific diseases, by providing accounts of current knowledge in other areas related to the production, health and welfare of sheep and by adding to the international spectrum. As with other production animal species, generic issues such as welfare, judicious use of therapeutic resources, international movement of disease, emerging disease threats and zoonotic hazards, among others, have been addressed.

Compilation of a multi-authored work is primarily dependent on the inputs of individual contributors, specialists in their own fields, and I am indebted to them for the quality of their contributions and for their forbearance in dealing with editorial queries and occasional harassments. In particular, I acknowledge new authors who took on responsibility for chapters previously written by others and who, like their predecessors, have been generous with their time and expertise. From the outset, the Institute and the Moredun Foundation (ADRA's successor) have been supportive, particularly in making available the secretarial support of Mrs Christine Curran who has been the skilful hub of the vast electronic traffic involved in bringing the task to completion. To her and to all present and former colleagues at Moredun who have assisted my editorial efforts I owe sincere thanks. As with the second and third editions the Institute library will be the beneficiary of accruing royalties.

While none involved would claim that the coverage is comprehensive in every particular, together, the 75 chapters of this edition provide an authoritative source of information for veterinarians, sheep specialists and others for whom sheep health and welfare has importance. The last two decades have seen a burgeoning of printed and electronic output in the animal health field generally and it is gratifying that *Diseases of Sheep* continues to be well regarded by a demanding readership. Bill Martin would have appreciated that and I trust that this new edition upholds the reputation of its forerunners.

Ian Aitken

Part I
Introduction

1

Sheep: a UK perspective on a world resource

D.A.R. Davies

Sheep and goats are classified as belonging to the order Artiodactyla, sub-order Ruminantia family Bovidae, sub-family Caprinae and to the genus *Ovis* and *Capra*, respectively. Generally, they can be distinguished by a number of appearance traits. The chromosome number of the domesticated sheep (*Ovis aries*) is 54 and that of domesticated goat (*Capra hircus*) is 60, so successful interbreeding is ruled out. Both depend on forage as their major food source. In the wild, they occupied somewhat different ecological niches; goats were found in the more inhospitable mountain areas browsing on a mixed flora of bushes and shrubs and poor grass, while sheep mainly grazed the lower pastures but also browsed on the young shoots of bushes and trees.

HISTORY

Sheep were relatively easy to tame and consequently were one of the first species to be domesticated and used as a source of food and fibre [1, 2]. It is probable that domestication came partly via imprinting of any captured very young lambs on humans, but a number of behavioural and other factors also came into play and made domestication of adults relatively easy. The wild sheep were significantly smaller than today's domesticated types, were relatively docile and showed a submissive behaviour when captured. They would have been easy to catch and handle, and even the males would not have posed a major threat to their captors. Like humans, the natural social structure was based on a hierarchy with a dominant leader. In time, this would often have resulted in the shepherd assuming the role of the latter, especially if the person was associated with an agreeable food source. In addition, control of the domesticated population was relatively easy because sheep at pasture generally grazed as a flock and confined their grazing activity to a specific home range area or areas and so did not stray widely, and could be confined with primitive fences.

The earliest records of domestication have been found in areas of south-western Asia. Much has been written about their dispersion with their keepers throughout Europe, Asia and Africa and, in more recent centuries, the introduction of domesticated types into North and South America and to Australia and New Zealand.

GEOGRAPHY

Today, sheep are widespread throughout the world with the total number, although declining, estimated to be almost 1000 million [2]. Countries with the largest populations are shown in Table 1.1. Within the world population there is a considerable amount of genetic diversity. This is mainly attributable to the effects that natural and human-controlled selection have had in the establishment of populations that are adapted to a variety of different climatic conditions but also reflects the relative importance to the sheep economy of the three main products: meat, milk and wool.

On a global scale the effects of the above can be summarized in the following way. The sheep of tropical regions of Africa have adapted to the hot climate and the problem of heat dissipation by having hair, not wool, and a stored energy source in the tail rather than in the subcutaneous fat layer.

Large numbers of fat-tailed sheep are also found in most Asian countries. Often, the sheep are owned by poor people and live in difficult environmental

Table 1.1: World sheep populations 2002–3

Country	Millions
China	139.2
Australia	98.0
Iran	53.0
Former Soviet Union	51.9
India	42.0
New Zealand	39.0
Turkey	29.4
United Kingdom	24.9
Spain	23.0
Argentina	12.5
Uruguay	10.7
World total (including other countries)	992.7

Source: www.britishwool.org.uk/a-factsheet4.asp

conditions where winters are very cold and winter food is scarce. They are able to survive such conditions by having a thick coarse wool cover and by being able to draw on the large stores of energy in the form of fat around the internal organs and in the tail that are built up during the summer when food is more plentiful. The animals are multipurpose, being slaughtered for food and, in some cases, being a source of milk and milk products, but the main product of the industry is coarse wool that is used for carpet making.

In Europe, sheep developed to live in cold and wet inhospitable regions do not have fat tails but have a thick subcutaneous fat cover as well as large deposits of internal fat. Again, the adults have carpet-quality wool and the young are born with a thick felt-like birth coat that enables them to survive wet and cold conditions.

The best quality fine wool production is mainly centred on the Merino and Romney breeds. Dry arid subtropical areas are particularly well suited to this type of production because, although the poor quality of the diet reduces yield, it also results in an increase in the fineness of the staple. Large populations exist in Australia and South Africa and in Argentina and Uruguay. The decline in wool values in the past few decades has seen a substantial reduction in numbers and a move to meat production in many of the better land areas of these countries.

It is in the temperate grassland zones of both hemispheres that there is the greatest emphasis on meat production associated with the most intensive systems, highest stocking rates and the most productive breeds and crossbreeds.

Ewes are a local source of milk for human consumption in many poor communities, but it is mainly in the flocks of European countries that border the Mediterranean and some areas of south-west Asia that milk and milk products are the major output of the sheep flock.

BREEDS

Undoubtedly, in almost every country a number of distinctive populations would have evolved over the ages, but breeds as they are now known are a relatively recent development. In the UK, developments in agricultural practice in the eighteenth century made it possible to keep livestock over winter. This allowed stock keepers like Robert Bakewell (1725–95) and his several disciples to develop and use techniques to standardize local populations and create breeds. Initially, they were developed from the undefined local stocks but subsequently new breeds usually resulted from blending together, via crossbreeding and selection, individuals from a number of the breeds already in existence.

Breeds continue to be developed, usually in response to changing demands of the sheep industry. The number in the world has been estimated to be near 300. In the UK, including the imports in recent decades, there are now more than 70 [3]. In contrast, almost all sheep in Australia are Merino or part Merino crossbreds. Some breeds have a global distribution and large breed populations, while at the other extreme there are some that are confined to their area of origin and have very small numbers. Quite often there are a number of breeds that fulfil the same role, but a few have an almost unique combination of characteristics. It is now accepted that the conservation of all breeds, whatever their present status, is an effective means of preserving genetic variation that may be useful in future times. In the UK, the activities of the Rare Breeds Survival Trust in the past four decades has revitalized interest in several breeds that were at risk of, and in some cases near, extinction, because of their small numbers [4].

It has also to be recognized that, having created breeds, many flock-masters carry out crossbreeding to utilize the complementarity that exists between two or more breeds and/or the improved performance due to the hybrid vigour of the crossbred offspring.

The development of breeds resulted in the establishment of populations in which a desirable performance trait was well expressed and appeared to be loosely linked to the animals' appearance. Selection within a population resulted in a reduction in genetic variability, an increase in homozygosity and animals that had a more uniform phenotypic appearance and produced offspring that resembled the parents. At the same time, the difference between groups became more distinct and breeds became distinguishable and identifiable on the basis of a small number of the easily recognized traits listed below:

- **Face colour**. Colours may be black, various shades of brown, white, distinctly patterned or variously speckled and are entirely genetically controlled.
- **Fleece colour**. Again, this is genetically determined. The predominant colour is white, although some breeds have black and some a brown fleece and a fourth group have distinctive patterns of wool colours.
- **Face wool cover**. This can vary from none (clean face) to substantial. Many breeds have a clean face apart from a wool top knot.
- **Horned/polled**. In primitive breeds both sexes are horned, in others only the male has horns and in many of the commercially important breeds both sexes are polled. Differences are genetically controlled.
- **Tail length**. Many northern hemisphere breeds like the Finn, North Ronaldsay and others have short tails. In Asia and Africa fat-tailed sheep predominate.
- **Ears**. These may be prick or lop.

All of the above traits, but particularly face colour, can be considered to be simple phenotypic markers of an animal's genotype and production performance. Animals/breeds of a certain appearance may be expected to produce within a specified range of performance, but because there is no genetic correlation between the appearance and performance traits a crossbreeding programme could remove the link. Also, within breeds, variation in appearance is not likely to be associated with differences in production performance.

Mature size/weight and conformation

This is an appearance trait that is genetically linked to some performance traits. Genotype has an important influence but environmental effects can modify both the size and weight of an animal. Variation in skeletal size (or bone) is considerable; added to this are differences in the amount of flesh (lean meat muscle and fat) covering the skeleton. The latter is usually described in terms of condition score on a 1 (thin) to 5 (fat) scale and is often more influenced by previous nutrition and production than genotype [5]. Gender heterogeneity also contributes to size differences, with ewes usually being 65–70 per cent of the weight of rams of the same breed. All of the above contribute to the wide difference in weight between a 25 kg female Soay, considered to be Britain's smallest and most primitive breed, and a very large 150 kg Oxford Down breed ram [3].

The ideal size to maximize biological and financial efficiency will depend on a number of factors. The small size of hill breeds is considered to be a survival and economic advantage when animals have to perform in harsh environments. On the other hand, in more favourable conditions larger well-fleshed and more productive animals are likely to be preferred.

Performance traits

These include all the important traits directly influencing fibre and meat production as well as those involved in reproduction, behaviour and disease resistance. In many cases performance differences within breeds are just as great as those between breeds. This can be attributed in part to environmental influences, but for most production traits there is also some genetic variation. Generally, genetic variation is multigenic, but an increasing number of genes that have a major effect on performance have been identified. Breeders therefore need to select their animals knowledgeably and to realize the opportunities that exist to improve the genetic worth of their breed or flock by selective breeding.

FIBRE PRODUCTION

In primitive types, coat cover includes hair and a soft downy undercoat. Breeds of sheep that have evolved in tropical areas have hair and not wool, and moult annually. Wool is the inherent fibre coat of the sheep of temperate and cold climates. It has excellent insulation and water-repellent qualities, and provides the necessary protection from excessive cold and wet winter conditions. Apart from exceptions like the Wiltshire Horn, sheep do not moult and the fleece has to be removed annually by shearing [3]. The contribution of wool to the income of a sheep farm has diminished considerably since the development of synthetic fibres. Presently, the cost of this operation relative to the value of the fleece often leaves little or no profit margin and makes the Wiltshire Horn and its crossbred derivatives attractive propositions. In Australia, much of the breeding effort is directed towards the development of sheep that require less care by having short tails and the natural ability to moult breech wool. The recent identification of a Merino line that has a bare crutch would seem to be the ideal sheep for the system. Most of the traits associated with wool production have a medium or high heritability and selection programmes to improve quantity and quality continue [6].

Nutrition has important influences on wool growth and high nutritional demands in pregnancy result in reduced fleece weights. On the other hand, undernutrition will give rise to finer fibres. In practical circumstances a balance has to be achieved between the effect of the above on incomes via meat and via wool production.

The value of the fleece can vary considerably depending its the weight and quality. The former is determined by staple length and density of the fibres, and the quality by the fineness and crimp of the fibres. Some of the UK Longwool breeds claim yields up to 10 kg compared with an average of approximately 2.5 kg for lowland types and less than 2 kg from some hill breeds. Hill breeds tend to produce coarse wools having a staple diameter of 35–50 μm that is used mainly for carpet and rug making. Most lowland breeds produce a better-quality finer material with a diameter of 24–33 μm used for hosiery and knitwear [3]. The Merino produces a heavy fleece of 5–12 kg of very fine wool with a diameter less than 24 μm that is used for the manufacture of high-quality garments [6].

Table 1.2: World production of raw wool (thousands of tonnes)

Country	Production
Australia	544
China	302
New Zealand	235
Former Soviet Union	133
Argentina	72
Turkey	70
United Kingdom	50
South Africa	45
Uruguay	43
Pakistan	40
World total (including other countries)	2194

Source: www.britishwool.org.uk/a-factsheet4.asp

Wool has been harvested and used for garment and carpet making for centuries. Despite the advent of synthetic materials it remains a commodity that is traded internationally. The countries that are the main producers are listed in Table 1.2. China, with its rapidly expanding textile industry, has become the major importer of top-quality material from most of the main producing countries. This includes 45 per cent of the total and almost 70 per cent of the fine and super fine Australian clip, 50 per cent of the medium quality export from Uruguay and the greatest part of the slightly coarser New Zealand export.

MEAT PRODUCTION

The main meat product is lamb. It is defined as the meat from animals slaughtered up to 1 year of age. Mutton is the product from older animals including both male and female culls from the breeding flock. In most countries it makes a small contribution to the total production and is generally used for manufacturing purposes.

Consumption of lamb is influenced by tradition and is related to the size of the sheep population of the country. In the European Union (EU), it varies from nearly 14 kg per person per annum in Greece to 6.3 kg in the UK and only 1.2 kg in Germany [7]. There is a considerable amount of trade in meat products within Europe and between it and New Zealand and

Australia. The latter are major exporters to a number of South-east Asia and Middle Eastern countries, but more than 50 per cent of both lamb and mutton from the main exporter, New Zealand, comes to Europe.

Currently, New Zealand has a tariff-free entitlement to export the carcass weight equivalent of 227 000 tonnes of sheep meat to the EU. Approximately 17 per cent of this is to Germany, 14 per cent to France and 42 per cent to the UK [8]. Actual imports have been slightly below entitlement with 67 000 tonnes of lamb and 16 000 tonnes of mutton estimated to have been imported into the UK in 2004 [9]. Historically, all the lamb was frozen product and filled a deficit in supply from home production during the early months of the year. The UK is now almost self-sufficient, exporting approximately 77 000 tonnes and importing 8000 tonnes of lamb to and from other EU countries [9]. The New Zealand export also includes an increasing amount of chilled product that is more comparable to that which is home-produced. The above means that there is now more competition in the market and the price obtained by the farmer in both the UK and New Zealand is influenced significantly by the volume and value of imports and exports.

The efficient production of quality lamb meat for an affluent society is the main focus for producers. Lambs from larger breeds grow significantly faster than those of small breeds provided they are supplied with sufficient nutrients by their dams and/or by grazing good-quality pastures and/or by consuming a high concentrate diet. In ideal conditions growth rates to slaughter weight of large breeds like the Suffolk will reach 500 g/day and those of hill breeds like the Welsh Mountain with a mature weight less than half that of the Suffolk will be no more than 250 g/day. Efficiency and hence profitability of lamb meat production systems is increased by using a fast-growing large breed sire and a medium or small breed or crossbreed dam that has a reduced maintenance cost.

Generally, but not always, lambs will be slaughtered when they reach 50 per cent of their potential mature weight. This is considered to be the optimum point because it usually coincides with the skeleton being well covered with flesh, the muscle-to-fat ratio being high and the later maturing expensive lumbar and hind leg joints being well developed. The EU carcass classification grades reflect the variation in the latter and provide a clear guide to the producer of the quality of carcass required [10].

In recent years, consumer demand for lean meat of low fat content has resulted in much of the breeding effort being focused on improving the quantity and quality of meat production. Sire Reference Schemes and Best Linear Unbiased Prediction (BLUP) statistical techniques have been used to extend the use and accuracy of breeding values and hence the response to selection [11]. The use of ultrasound sonography and, more recently, X-ray computed tomography (CT) have allowed more accurate assessments of body lean and fat content to be made. Ultrasound has been used to measure the depth of the eye muscle (*m. longissimus dorsi*) and that of subcutaneous fat cover in the region of the third lumbar vertebra [10]. A whole body CT scan provides a more accurate estimate of fat and muscle content of the whole body and constituent parts [12]. It also gives an accurate estimate of the hind leg musculature that previously has been determined by eye. Considerable emphasis is placed on the trait and the double muscling seen in some breeds like the Texel and more noticeably in the Beltex. The Callipyge gene has also been identified as having a major positive effect on lumbar and hind leg muscle development but, unfortunately, it also has a deleterious effect on meat tenderness [13].

The development of lumbar and hind limb musculature is economically beneficial and does not result in reproduction problems, but selection for large heads and wide shoulders is misguided because of the low value of these parts of the carcass and the compromise of welfare that results from an increase in the incidence of dystocia and difficult lambings.

REPRODUCTION

The profitability of a sheep enterprise is linked to output per hectare. The latter is influenced by stocking rate and output per ewe. The annual output of lamb per ewe is thus a major influence on the efficiency and profitability of lamb meat production and in turn is determined by the number of parities per year and the number of surviving offspring per parity.

Parities per year

The gestation period generally varies between 144 and 150 days but in some breeds individual animals

will have normal pregnancies outside this range. Most sheep developed in temperate zones have a seasonal breeding pattern and come into oestrus in the autumn in response to shortening day length. In the northern hemisphere all breeds will show oestrus between September and January but some will start at least a month earlier and some will continue to show oestrus into March. Ewes of the Dorset Horn breed will mate in almost any month of the year. Breeds developed and used in tropical countries often have two crops of lambs per year. In temperate regions this level of production is not possible and even obtaining three crops in 2 years is demanding so is practised only occasionally.

Producers, however, utilize the variability in length and timing of the breeding period to vary the date of their lambing to suit particular environmental and economic conditions.

Prolificacy: lambs produced and sold per ewe

The published information available on the mean litter size of UK breeds indicates figures for mature ewes ranging from 1.2 to 2.7, thus giving a wide choice from which to select a breed that is appropriate for the location and management system [3]. In difficult hill conditions dealing with significant numbers of twins is problematic and the target output will be a litter size of 1.1. In low input lowland conditions the ideal flock will have a litter size of 1.7 and a lambing percentage near 150 with the majority of ewes producing only singles or twins. In commercial high output intensive systems producers aim to achieve a litter size between 2 and 2.2 giving, after losses, a lambing percentage of 180–200 per cent.

In all conditions lambs that are surplus to the ewe's rearing ability create difficulties and need to be either cross-fostered on to another ewe or else artificially reared. The latter technique is now well developed and results in lambs being reared successfully, but is expensive of both time and money. The most efficient way of dealing with greater lamb numbers in commercial production is to ensure that the ewes are managed correctly and have the genetic ability not only to bear but also to rear up to three lambs.

Selection to increase the prolificacy of breeds has usually resulted in a poor, slow response but, overall in the UK, this is not a major constraint because there are breeds that theoretically have more than enough genetic capacity to meet the required prolificacy needs of sheep producers in all conditions. The performance of hill breeds is usually limited by nutrition, and in lowland conditions a lambing percentage figure of 200 can be reached by breeds like the increasingly popular Lleyn and with the help of a little hybrid vigour by various F1-crossbred combinations sired by Bluefaced Leicester and Border Leicester breed rams.

Even higher prolificacy breeds are available such as the Finn, Romanov, Cambridge and British Milk Sheep [3, 14]. Mature ewes of all of the latter breeds are capable of an average litter size figure in excess of 2.5. At this level more than half of the flock will be producing triplets and quadruplets. In smaller flocks it is possible to devise a high input management system that will cope with this performance level and ensure survival of at least 90 per cent of lambs born alive. Commercial flock-masters are unlikely to be interested in large numbers of such sheep because of the problems outlined above but several are known to keep small flocks to source home-produced crossbreds.

In the UK, the payment of subsidies to the sheep industry has reduced the importance of prolificacy to profitable farming. The recent change to a single farm payment system designed to maintain a suitable environment may or may not change flock-masters' views on targets [15]. Whatever the general view, high prolificacy sheep are likely to continue to have a role in lamb production. Ultrasound scanning of ewes in mid-pregnancy can be used to determine accurately the number of lambs. This allows feeding to be adjusted to minimize problems in late pregnancy and also indicates the housing and care that will be needed at lambing. Problems of dealing with numbers of triplets and quadruplets and a very variable colostrum yield up to 12 hours post-partum are being addressed, but breeding strategies must be focused on contributing a product that is more uniform in its reproductive performance and also one that has good conformation and carcass qualities. An important reason for the interest in super-prolific breeds like the Booroola Merino and Inverdale is that, unlike the breeds mentioned above, their ovulation rate is controlled at a single fecundity gene Fec.B and Fec.X, respectively [14]. This makes it much easier to introgress the high fecundity/prolificacy allele into other populations of meat breeds with the aim of producing sheep that are both highly prolific and have excellent carcass quality.

Another attraction of a single gene effect is that the progeny of a homozygous ram will carry a copy of the fecundity allele and thus be uniformly productive. There are usually major differences in performance between animals that carry none, one or two copies of the fecundity allele. The downside is the possibility that the effect of a single copy of the gene is too large to be manageable and cannot be reduced by further crossbreeding.

Milk production

One of the difficulties of ewes rearing triplets is the competition for the available milk supply that can result in uneven growth of lambs and an increase in teat damage. Theoretically at least, this could be overcome by having ewes with four functional teats. It is recorded that Alexander Graham Bell had a population of sheep with up to eight nipples. It is known that ewes and rams in many breeds have more than two nipples and that some ewes produce significant amounts of milk from the anterior extra teats. Using animals from a number of sources a population of four-teat sheep that produce 20–30 per cent of their total yield via the front teats has been developed in the UK [16]. The benefit of having such sheep is a more even distribution of the milk supply when triplets are being reared; twins may also benefit by being able to suck both front and rear teats.

The milk production *per se* of most breeds is not well documented and can only be deduced from early lamb growth up to 6 weeks of age when it is the main source of nutrients for the offspring and when yields are maximum. Daily yields of 3–4 kg are normal but if ewes are well fed these can increase to 5 or more litres [17]. Ewes' milk contains almost 200 g/kg of total solids, fat levels average 67 g/kg and protein 56 g/kg [18]. At such performance levels the demands of lactation are considerable and equivalent to that experienced by a dairy cow yielding 35–40 litres/day. The nutrients in the milk are digested very efficiently and yields of 5 litres will support twin lambs growing at 450 g/day and triplets growing at 300 g/day.

The above figures are seldom replicated in hand-milking systems partly because without the stimulus of suckling the let-down is incomplete and partly because of the reduced number of occasions that milk is withdrawn from the udder; lambs will suck up to 10 times per day. Some breeds like the Friesland that have been developed specifically for milk production have an efficient let-down and also have high yields with reported figures for a 7-month lactation varying from 250 to 600 litres [3]. Consequently, this breed, and some more modern derivatives like the British Milk Sheep, feature in almost all of the few flocks that produce milk in the UK.

Table 1.3: Ewes and lambs put to ram in main sheep-producing countries of the EU in 2004 (1000 head)

Country	Total ewes and ewe lambs to ram	Milk ewes and ewe lambs to ram
Spain	17 352	3 475
United Kingdom	16 266	
Italy	7 255	5 804
Greece	6 972	6 300
France	6 749	1 618
Ireland	3 469	
Portugal	2 312	587
Germany	1 595	

Source: www.epp.eurostat.cec.eu.int/

Most of the milk produced is manufactured into cheese or yoghurt, so compositional quality is important.

Large numbers of ewes are kept for milk production in southern Europe (Table 1.3). In the south of France the local Lacaune breed provides the milk for the production of the famous Roquefort cheese. Flocks are large and are part of a sophisticated breeding programme that includes a well-organized recording scheme, scientific evaluation of sires and the widespread use of top rams by artificial insemination [19].

Milk production can give very good financial returns, but labour costs are high and provision has to be made for the rearing of lambs. This is achieved in small-scale low-cost systems by separating ewes and lambs for part of the day and in larger scale operations by completely removing the lambs from their dams either soon after birth or at 6 weeks of age and then feeding them an appropriate diet.

BEHAVIOUR AND HEALTH

The improvement of production traits referred to above has been the main focus of breeders and

scientists in the past 50 years. The extent of the effect on natural behaviours and resistance to disease of this selective breeding, coupled with improved management, nutrition, vaccination and drug use, has not been fully assessed and there is limited information about current between- and within-breed variation for most behaviour and health traits.

It is known that the home range grazing behaviour referred to above remains important and very much in evidence in the hefting behaviour demonstrated by UK hill breeds and the flocking instinct is seen in most breeds. There is evidence that in other important traits such as mothering ability and lamb teat-seeking and sucking behaviour, both of which affect lamb survival, there has been some loss of these instinctive behaviours [20, 21].

Behavioural difficulties are of course only part of the many factors that influence lamb survival at birth. In the immediate post-partum period others include birth coat type and dystocia problems, referred to above, that are influenced by both the size and shape of the ewe pelvis and the size of the lamb. Many of these factors are not considered to be vitally important in intensive systems with ewes housed for lambing and 24-hour care provided, but if labour and other input costs have to be reduced to achieve economic sustainability then consideration will have to given to the breed and type of sheep that has the least number of problems at lambing. In New Zealand, selection for easy care sheep involving minimal human interference at lambing time has been practised for some time. The extent to which practices employed in New Zealand can be imitated elsewhere without compromising acceptable welfare standards, not just at lambing but throughout the production year, will have to be addressed by producers contemplating an easy care system.

Flock-keepers have relied for decades on a mix of husbandry, vaccination and a variety of drugs to control disease. All of these are valuable and necessary for the well-being of the sheep but they tend to be short-term solutions and their blanket use may be concealing natural variation in susceptibility to disease.

At the present time there is a huge increase of interest in selection for disease resistance. Two very different examples of genetic variability linked to disease are cited below. Scrapie is a transmissible spongiform encephalopathy (TSE) that has been known to be a fatal condition for more than a century. Polymorphisms at codons 136, 154 and 171 of the prion protein have been found to be linked to susceptibility and it is now possible to select animals that, when challenged, do not show clinical signs of the disease. In the UK there is a National Scrapie Plan that includes an EU directive for the compulsory testing and restricted use of rams [22].

Intensive production, high stocking rates and fertilizer use have required the regular use of anthelmintics to control nematodes but worm resistance to the available drugs has invoked a rethink. There is now evidence from a number of breeds that 20–40 per cent of the variation in faecal egg counts of weaned lambs and periparturient ewes is the result of genotype differences. In addition, a strong favourable genetic correlation has been found between faecal egg counts and growth rates of lambs [23, 24]. Identification and use of resistant animals has consequently become a major objective in several breeding programmes throughout the world.

The success of the above programmes should be a spur for the setting up of programmes to identify variation in and then selection for resistance to a number of other conditions that create difficulties for the sheep industry. Lameness is probably the major health and welfare problem for sheep and shepherds, and some sheep are more susceptible than others. It would be a huge benefit if either within or across breeds resistant populations could be established to reduce the number of painful cases and the time that has to be spent preventing infection and curing affected animals.

The time seems opportune for breeders, geneticists, veterinary surgeons and scientists to talk, exchange ideas and challenge present concepts about the most efficient measures of disease control in the short and long term.

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Part II
Welfare

2

Sheep welfare: standards and practices

A.C. Winter and J.L. Fitzpatrick

Sheep are kept under a wide variety of extensive and intensive conditions, which bring with them a range of associated welfare problems. From the time sheep were first domesticated, their appearance and breeding have been manipulated according to the particular demands of society; this is particularly true of wool type and yield, carcass shape, litter size and breeding season. These factors can have many welfare implications. In the UK, sheep production has retained a largely natural or 'green' image, in contrast to intensive farming of pigs and poultry, which must not be lost if the confidence of the consumer is to be maintained.

LEGISLATION

The welfare of farm animals in the UK is currently regulated by a number of Acts of Parliament. New legislation is being discussed in a draft Animal Welfare Bill that brings together and modernizes welfare legislation for farmed and non-farmed animals. This was published in July 2004 and awaits time for discussion in Parliament. Once enacted, owners and keepers will have a duty to promote the welfare of their animals and a new offence of failing to take reasonable steps to ensure an animal's welfare will be introduced.

Until 1968 the main legislation lay in the Protection of Animals Act 1911, which aimed to prevent cruelty to animals, in particular 'unnecessary suffering by anything which is done or omitted to be done'. Following the Report of the Brambell Committee, which considered intensive methods of farming, statutory provisions for the welfare of livestock were introduced in the Agriculture (Miscellaneous Provisions) Act 1968. Legislation under this Act includes the prohibition of certain operations on animals with or without the use of anaesthetics (for sheep this specifies freeze dagging, short-tail docking and tooth grinding), and provides for the production of welfare codes. It also includes the Welfare of Farmed Animals Regulations 2000 under which owners and keepers have to take reasonable steps to ensure the welfare of animals under their care and not cause any unnecessary pain, suffering or injury. A code of recommendations for the welfare of sheep was first published in 1977, with a new version in 2000, revised in 2003 [1]. Welfare codes do not have the force of law, but failure to abide by their recommendations can be used as evidence of poor welfare standards. Law relating to transport is described in Chapter 5.

The Council of Europe produced recommendations concerning sheep in the Standing Committee of the European Convention for the Protection of Animals kept for Farming Purposes in 1992 [2]. A European Commission Council Directive (98/58/EC) on the protection of animals for farming purposes that sets standards relating to the welfare of all farm animals in the EU was implemented in the UK by means of the Welfare of Farmed Animals Regulations 2000, mentioned above. The EU has recently published an animal welfare action plan for 2006–10 [3]. On a much wider scale, the Office International des Epizooties (OIE) has set up an animal welfare working group to provide guidance on global welfare standards.

FRAMEWORK FOR ASSESSING WELFARE STANDARDS

In the UK, an independent body, the Farm Animal Welfare Council (FAWC), set up in response to the Brambell Report, keeps under review the welfare of farm animals at all stages from farm to slaughter, and advises the agriculture ministers on any matters of

concern, changes required to welfare codes, legislation, etc. The welfare of farm livestock is generally considered under the so-called 'Five Freedoms' developed by FAWC, which, although defining ideal states, form a logical and comprehensive structure for the analysis of standards within any system. These promote 'positive' welfare as opposed merely to absence of cruelty.

The Five Freedoms are:

1. **Freedom from hunger and thirst** by ready access to fresh water and a diet to maintain full health and vigour.
2. **Freedom from discomfort** by providing an appropriate environment, including shelter and a comfortable resting area.
3. **Freedom from pain, injury or disease** by prevention or rapid diagnosis and treatment.
4. **Freedom to express normal behaviour** by providing sufficient space, proper facilities and company of the animal's own kind.
5. **Freedom from fear and distress** by ensuring conditions and treatment to avoid mental suffering.

These freedoms require that people who are caring for livestock should provide:

- caring and responsible planning and management
- skilled, knowledgeable and conscientious stockmanship
- an appropriate environment
- considerate handling and transport
- humane slaughter.

INDICATORS OF WELFARE

Assessment of welfare may be qualitative or quantitative. Qualitative indicators include fear, timidity, agitation, pain, calmness, curiosity and play [4]. Behavioural indicators of stress may include vocalization, panting and markedly increased locomotory activity [5]. Quantitative indicators include physiological changes, e.g. heart rate, plasma cortisol and catecholamine concentrations, altered reproductive parameters, increased disease incidence, and reduced growth rate. Responses of sheep to chronic stress are diffuse and subtle [6].

Sheep show particularly large physiological responses to the approach of a dog (especially an unfamiliar one) and introduction into a new flock, and find isolation, capture and inversion more aversive than physical restraint. They also find shearing stressful [7]. A system of harvesting wool, without the need to shear, by injecting a protein which causes cessation of wool production in the follicle for a few days, is being developed in Australia.

Among farm animals sheep are particular stoics and exhibit few obvious signs of pain or distress. As a species subject to predation, masking visible indicators from predators may have given sheep an evolutionary survival advantage. As human observers generally lack the ability or skills to discern their outward signs, methods for objective assessment of distress or pain in livestock, including sheep, are being used to provide a scientific understanding that, in turn, can help to improve welfare.

Many studies have measured cortisol as an indicator of stress, pain and poor welfare. The acute phase proteins (APP) in serum are also useful in categorizing disease severity that may then be correlated with pain and welfare status. APPs are serum proteins that increase in concentration during infection or inflammation [8]. They are relatively stable and persist for a number of days or even weeks after the original insult or stimulus. The APPs, haptoglobin and serum amyloid A, correlate with clinical mastitis in cattle [9]. They or other APPs may be useful biological correlates of inflammatory disease in sheep.

PAIN IN SHEEP

Sheep are susceptible to many diseases in which inflammation is a key component of the pathophysiological process. Inflammatory diseases include foot and limb lesions such as foot-rot, interdigital dermatitis and, increasingly, contagious ovine digital dermatitis (CODD), pneumonias, mastitis, and dermatitis commonly associated with sheep scab. In these conditions, infections with pathogens including bacteria, viruses or parasites induce the inflammatory cascade with the release of mediators such as histamine, complement, cytokines and prostaglandins. In addition to their effect on blood flow and the immune response, some mediators are known to alter pain processing at local tissue levels where pain receptors (nociceptors) may be affected [10]. Mediators may also affect the central nervous system by altering pain processing at the spinal cord level. When these changes occur, animals are said to be 'hyperalgesic', i.e. they respond to an unpleasant stimulus at a level which would not affect

normal individuals [11, 12]. Thus, hyperalgesia can be an indirect indicator of the presence of pain and has been described in a number of species, including humans, cattle and sheep.

In farm animals hyperalgesia is measured by application of a mechanical stimulus, usually to a limb, using a gas-driven device, which increases gradually in pressure until the animal responds by moving (e.g. kicking or shifting weight) [13]. This allows measurement of pressures over time to be correlated with different indicators of disease. To date, studies have focused on diseases with recognized indicators of severity such as mastitis (clinical signs in the mammary glands, visible alteration in milk, and/or the presence of somatic cells in milk) and lameness where locomotion, lameness and lesion scoring can be employed. Hyperalgesia has been demonstrated in lame sheep and cattle [14, 15]. Sheep with severe lameness had a significantly lower threshold to a mechanical stimulus than matched 'sound' controls, indicating that hyperalgesia was present and suggesting that the sheep were affected by a painful condition. The duration of hyperalgesia varied in different studies, with some suggesting that lameness may induce pain for up to a week while others showed that the effect lasted for 3 months, even though the sheep were no longer lame [16]. It is likely that the duration of pain will vary with the aetiology of the lameness due to the wide variety of causes of this condition in sheep (see Chapter 39). Hyperalgesia has also been described in naturally occurring mastitis, including acute mastitis in dairy cows and chronic mastitis in sheep.

In the UK, non-steroidal anti-inflammatory drugs (NSAIDs) are used to treat farm animals affected by inflammatory diseases (see Chapters 73 and 74). Their use tends to be reserved for cases demonstrating obvious clinical signs and where the signs, such as anorexia or dullness, indicate a relatively severe form of disease often with systemic involvement. The use of NSAIDs in sheep tends to be more limited than in cattle and pigs. Few studies on the effect of NSAIDs in diseases of sheep have been reported, but flunixin meglumine did reduce hyperalgesia in cases of foot-rot with the responses to a mechanical threshold returning to normal levels [17]. That NSAIDs are used only rarely to treat sheep may result from a lack of perception of need for anti-inflammatory or analgesic therapy, from cost considerations or from lack of products specifically licensed for use in sheep. However, in the EU, products authorized for other food-producing animals can be used under the 'Cascade' system. As more knowledge is gained on the pain associated with disease in sheep, it is to be hoped that drugs which alleviate pain will be used more frequently and strategically.

Many facets of disease influence the overall contribution to pain in individual sheep, and on a flock basis. These include the intensity and duration of the disease in individual sheep, and also the prevalence of the disease when pain is assessed at the flock level. Clearly, measurement of disease intensity is not sufficient, since pain of low intensity but long duration may be of different significance to an individual animal within a flock than a high intensity pain for a short duration, although in terms of flock welfare, both may have comparable impact. Thus, the need to assess the severity of disease and associated pain as a function of duration, intensity and prevalence is increasingly recognized. The difficulty in achieving this ambitious goal is compounded by the fact that on many farms estimation of disease parameters is hampered by poor disease recording systems and reduced availability of labour for regular inspection of flocks.

SETTING STANDARDS

FAWC's report on the welfare of sheep in the UK [18] made many recommendations to improve standards of sheep husbandry. The latest welfare code for sheep takes into account many of the points made.

Several other Member States of the EU have organizations that perform similar roles to FAWC, but not Spain, which is the only other state with a sheep population approaching that of the UK. Of other countries with a large sheep population, New Zealand is notable in having a similar organization, the National Animal Welfare Advisory Committee (NAWAC), which has produced a Code of Recommendations and Minimum Standards for the Welfare of Sheep. Australia also has a Model Code of Practice for the Welfare of Sheep.

Various farm assurance schemes, which aim to ensure that a minimum standard of welfare is maintained on approved farms, have developed in the UK [19] in an attempt to reassure consumers that satisfactory standards are being adhered to on farms, during transport and at slaughter. All assurance schemes are accredited by the UK Accreditation Service and, for those covering sheep, standards are based on the

sheep welfare code. Farm inspections are necessary before acceptance on the schemes. Of all current schemes, the RSPCA's Freedom Food scheme focuses most explicitly on welfare.

An Animal Health and Welfare Strategy (AHWS) [20] has recently been developed for Britain, setting out the position it is hoped to achieve by 2014. Within the overall aims there are some differences in implementation by devolved authorities; for example, in Scotland, funding provision encourages farmers to discuss issues relevant to their farm with their veterinary surgeon and to plan improvements.

EFFECT OF FINANCIAL SUPPORT ON WELFARE

Sheep farming within the EU comes within the remit of the Common Agriculture Policy (CAP) and up to 2004 was supported by payment of a subsidy, the Sheep Annual Premium (SAP) with farms having individual sheep quotas. Under reform of the CAP, Single Farm Payments (SFP) have been introduced from 1 January 2005, whereby subsidies have been decoupled from production, allowing farmers greater freedom to farm to the demands of the market and without minimum or maximum limits on numbers of sheep they may keep. To receive the payment, farmers must keep their land in good agricultural condition and comply with the standards on public health, animal and plant health, the environment *and animal welfare* (so called cross-compliance). Price support through SFPs is likely to fall progressively over the next 10 years.

Prior to reform of the CAP, price support formed a significant part of the income of sheep farmers in the UK [21], particularly in hill and upland areas, where up to £30 per eligible ewe was paid in some years; this support could form up to 70 per cent of the gross margin of the enterprise. With the introduction of SFPs it remains to be seen what the impact will be on sheep numbers. Sheep farmers may be facing income reductions of 20–50 per cent, leading to the possibility of farm aggregation with larger flocks with lower inputs, changes that could have very profound effects on sheep welfare, since any fall in income reduces investments in labour, feedstuffs and veterinary care. Producers may aim to increase income from the market by improving quality of slaughter lambs, or may seek to minimize costs by reducing inputs or sheep numbers [22]. If inputs are reduced, emphasis will need to be placed on the ability of sheep to lamb unassisted without housing and to thrive on a grass-only diet [23]. Other strategies such as reducing stocking density for environmental reasons could, however, be advantageous for the sheep. New Zealand is one country where all price support has been removed. After some upheavals, the industry has adapted and survived.

EFFECT OF FARMING SYSTEM ON WELFARE

The husbandry system under which sheep are kept has a great influence on their welfare. In some countries, for example Greece, where the farming system has changed from traditional extensive to intensive, the disease incidence has been so great that some farmers have reverted to extensive systems. Extensively farmed sheep, which are perceived as being in the most natural system, are at risk of undernutrition and extreme weather conditions, particularly in winter, but are at low risk from infectious diseases. They may be inspected only at irregular intervals because of the impossibility of covering the large areas of remote or difficult terrain over which they graze at very low stocking densities [24, 25]. Large numbers of sheep kept in the mountainous areas of UK and in New Zealand, for example, fall into this category.

Sheep on enclosed land are usually kept at much higher stocking densities and are particularly at risk of diseases caused by internal parasites such as roundworms and coccidia, and infectious diseases spread by close contact such as foot-rot; however, regular inspection is much easier. In the UK, many sheep are housed for at least part of the production cycle, particularly for protection against severe weather during lambing time or for fattening lambs in the winter. Housing can have a major effect on welfare (good or bad), depending on the quality of management.

GENERAL MANAGEMENT

Shepherding

The skill and experience of the shepherd, the person actually caring for the sheep, is probably the most

important factor influencing their welfare. The country and type of system determines how much contact the shepherd has with the sheep. With flocks on enclosed ground in the UK, daily inspection is possible, so any welfare problems should be detected and dealt with quickly. In extensive systems in remote or mountainous areas, sheep may be seen only at gathering times, perhaps three times a year; they live, therefore, more or less as feral animals, so it is important that hardy breeds, adapted to the conditions, are not replaced by less hardy breeds in the pursuit of increased output. Access to remote areas can be improved by constructing hill roads and the use of all-terrain vehicles. In many parts of mainland Europe, sheep grazing remote areas are still managed by traditional methods, with a shepherd constantly herding them and maintaining flock structure. The number of sheep that can be cared for by one shepherd is also an issue. In the UK, an upper limit of about 1000, with extra help at busy periods such as lambing, was suggested by the FAWC in 1994 but the number per shepherd has probably risen since then; 30 years ago it was common for one person to be responsible for no more than 350–400 ewes. In countries such as Australia and New Zealand, the number of sheep kept per shepherd is generally much larger.

In the UK, there is a potentially serious problem with an ageing population of hill shepherds in particular, with few young shepherds coming forward to replace them, because of the arduous nature of the job. Availability of training is also an issue, as the widespread network of training groups run by the Agricultural Training Board in the 1970s and 1980s no longer exists, although in some areas newer regional organizations, colleges and some veterinary practices do provide training opportunities. Training of stock keepers is crucial and local training groups perform an extremely valuable function if they can deliver practical 'hands-on' tuition given by highly experienced instructors, including veterinarians and farmers.

Food and water

Sheep require an adequate diet in order to breed and grow to their full potential (Chapter 8). In periods of drought or severe winter weather, supplementary feeding will be necessary if sheep are not to suffer from malnutrition. With extensively managed sheep, it is good practice to provide easily accessible stores of feed in case of sudden onset of severe weather. The widespread uptake of scanning for fetal numbers has improved the welfare of hill sheep in particular, since twin-bearing ewes can be removed for better treatment in less harsh conditions. This has helped to overcome the problem of chronic undernutrition caused by overgrazing land in summer, with ewes entering the winter period in poor condition.

It is essential that all sheep have access to food at the same time when limited amounts are being fed, i.e. adequate trough space, otherwise the weaker animals will be at a disadvantage. Floor feeding is an alternative. Composition of the food is also important; a tendency to buy concentrates on price (as low as possible!) rather than quality, can lead to problems such as pregnancy toxaemia. When sheep are housed, they are entirely dependent on the shepherd for their food and water supply.

Condition scoring is the most usual means of assessing body state, although regular weighing is used for sheep of a uniform type. In the UK, a condition score of less than 2 for lowland sheep and 1.5 for those on the hill in a significant number of the flock can indicate inadequate care and requires positive steps to rectify the situation.

Sheep also need a regular supply of water, although intake may be low if they are on a diet with a high moisture content, for example root crops. Lactating ewes can have a high water requirement. Some hill ewes are reluctant to drink from troughs and need a supply of running water.

Housing and handling

Winter housing can improve the welfare of sheep and shepherd; it can also reduce lamb losses and improve profit margins, but has to be managed well otherwise severe disease problems can arise. Adequate amounts of dry bedding are essential. Dirty, wet conditions underfoot will predispose to infections such as foot-rot, pneumonia, and scouring and polyarthritis in young lambs. Unacceptably wet bedding is a common consequence of feeding low-dry-matter silage.

A well-designed and maintained handling system is crucial to good flock management. This should include facilities such as a dip or shower to apply preventive treatments for external parasites where necessary; a footbath and a device to turn over sheep so that the shepherd can work in a normal standing position when foot trimming, can help to raise standards of foot care.

Health and disease

The animal health and welfare strategy (AHWS) for Great Britain [20] brings together current thinking and policies and lays out aims for the future. It emphasizes that prevention is better than cure and advocates health planning and other proactive services to improve health and welfare of all animals.

Any disease has an adverse effect on welfare, in particular those such as lameness or parasites (external or internal) that affect large numbers of the flock. Individual sick sheep should also be dealt with by receiving appropriate treatment or, if untreatable, should be culled (see 'Casualties' below). Fitness of the flock should be maintained by an annual inspection of breeding animals, with particular attention being paid to soundness of teeth, including molars. Animals that are unfit through problems such as tooth disease, chronic lameness, mastitis or breeding difficulties should be culled. The development of written health and welfare programmes covering the yearly production cycle for all flocks, with appropriate expert advice, is a major recommendation of the Welfare Code and its importance is emphasized in the AHWS. Some diseases such as foot-rot and sheep scab must be tackled on a flock or even regional basis for control to be successful. Scab has been controlled successfully in some areas of Scotland by co-operation of all farmers in a defined area. Foot-rot has been almost eliminated from New South Wales, Australia, as the result of an eradication programme. Other infectious diseases which lead to chronic weight loss for which there are control programmes in some countries include Johnes disease and maedi-visna.

Breeding and lambing time

The period around lambing is when most deaths occur (75 per cent of ewe deaths and an average UK lamb mortality rate of up to 20 per cent). Lamb survival is an important indicator of welfare standards within a flock, although allowances have to be made for the effect of exceptional weather conditions. Even so, good shepherding and a range of management factors including pregnancy diagnosis, better ewe feeding, housing, good preparation and planning can keep lamb mortality under 10 per cent even in harsh conditions [26]. Training of staff in lambing and lamb survival techniques is of paramount importance.

Lamb survival is enhanced when strong bonding occurs between ewe and lamb(s). There are behavioural and physiological adaptation differences between hill and lowland breeds that influence lamb survival [27]. For ewes lambing outside, bonding occurs most readily where the animals are not disturbed from the lambing site. The welfare of mother and offspring is therefore best met by regular inspection from a distance, with intervention only when lamb or ewe survival is threatened [28]. In extensive conditions, the best use of labour may be to separate and supervise twin-bearing ewes and to leave single-bearing older ewes on the open hill to lamb with little or no supervision. In indoor lambing flocks, provision of well-bedded individual pens for ewe and lambs for 24 hours or so after lambing allows bonding and reduces the risk of mis-mothering.

Manipulation of breeding by techniques such as intravaginal progestogen sponges, artificial insemination (AI) and embryo transfer can have adverse effects on welfare if operators are not properly trained and facilities (e.g. number of lambing pens) are not adequate. In the UK, performance of embryo transfer and laparoscopic AI is restricted to veterinarians. Genetic selection for increased yield of meat and carcass shape, etc. can have adverse effects on ease of lambing (Chapter 11).

Casualties

Chronically sick animals, which have no carcass value, and injured animals, even if they have a value, may not be legally transported in the UK (other than for veterinary attention) and are a particular welfare concern. They should be killed humanely on the farm as soon as possible. Guidance has been given on transport of casualties [29] and on dealing with casualties in general [30].

Mutilations

The benefits of carrying out any procedure that is classed as a mutilation must outweigh the disadvantages, such as the acute and chronic pain resulting from the procedure, which should itself be minimized. The main concerns lie with tailing and castration, which are dealt with in Chapter 4.

Human selection for maximum fine wool production, exemplified by the Merino breed which has many folds that increase skin area, has produced an animal

that is very susceptible to fly strike. This has resulted in two types of mutilation which reduce soiling of skin and therefore attractiveness to blowflies. The first, the 'mulesing' operation (cutting away the woolled skin immediately adjacent to the hairless area around the anus), reduces the risk of fly strike in the folds of wool-covered skin in the perineal area. The disadvantages are the pain caused at the time of the operation and during healing, and the risk of infection and fly strike during healing. The advantage is that the resultant scars avoid the contamination of wool with faeces and urine, so flies are less likely to be attracted to the site. Research into alternatives to mulesing has been carried out over many years and has been given additional impetus by increasing public questioning of the procedure because of the adverse welfare impact referred to above. The most promising alternative involves injection of collaginase intradermally around the breech. This causes local bruising and scabbing of the skin with loss of wool from the treated area. The procedure appears to be painless [31]. Preventive chemical treatments are an alternative, but mean frequent, often protracted and stressful musterings.

The other operation is 'pizzle-dropping', that is, cutting the skin of the prepuce so that it hangs free of the abdomen, facilitating urine drainage; this prevents 'pizzle rot' (balanoposthitis). This procedure and mulesing are both banned in the UK.

Identification by tagging or tattooing may also be viewed as a mutilation, although some form of permanent marking is usually necessary at least for management reasons. In countries of the EU double tagging of all sheep born after 9 July 2005 is mandatory for identification and tracing purposes, although the UK has a temporary derogation which allows single tagging. Electronic identification of sheep in all EU countries will be mandatory from 1 January 2008.

CONCLUSION

Flock welfare is best safeguarded by good advance planning by well-trained staff equipped with modern aids to assist in handling sheep. A health and welfare programme incorporating correct nutrition, appropriate vaccines, parasite control measures, etc. will ensure optimum flock health. Treatment of sick sheep and culling of those unfit to breed before the next breeding cycle will help to maintain a good welfare standard throughout the flock. The UK Sheep Veterinary Society has produced a policy statement [32] encompassing many of the issues addressed in this chapter.

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3

Welfare of fetal and newborn lambs

D.J. Mellor

During the past 50 years, curiosity driven research and the use of the fetal sheep as a model for human clinical application vastly improved knowledge of fetal physiology in general, and of fetal neurological development and functionality in particular [1, 2]. Over the same period, applied research, driven primarily by economics, led to major advances in knowledge of the causes and prevention of neonatal lamb mortality

and provided practical means for minimizing such losses with intensive [3–5] and extensive [6] lambing management systems (see Chapter 10).

Although virtually none of this research was conducted with the welfare of the fetus or newborn explicitly in mind, recent re-evaluations of the literature have provided some novel welfare insights [1, 2, 7, 8]. They are supported by sound scientific evidence that the fetal lamb is actively maintained in a continuously unconscious state throughout pregnancy and that consciousness appears for the first time only after birth. The evidence is summarized and some implications for the welfare of fetal and newborn lambs are outlined here.

PREREQUISITES OF SUFFERING

Significant suffering, in whatever form, is the antithesis of good welfare [9, 10]. To suffer, or indeed experience good welfare, internal and external stimuli must be able to elicit impulse transmission along nerves from peripheral sensory receptors to the animal's brain, and its brain structures must be operationally sophisticated enough to transduce those nerve impulses into perceived sensations. Moreover, the functional state of the brain must be able to support consciousness, as unconsciousness nullifies perception. Finally, for welfare to be compromised, the intensity, duration and/or character of the sensations the animal perceives must result in noxious or aversive experiences.

Thus, the prerequisites of suffering are the presence of a nervous system of sufficient sophistication to perceive sensations, a functional brain state that supports consciousness and sensory input that is experienced as noxious or aversive.

PHYSIOLOGICAL BACKGROUND

Prenatal development of the neural apparatus

Neurological development in the embryo and early fetus is not apparently sufficient to allow impulse transmission in sensory nerves to be perceived as sensations. Initially this is because nerve tracts and brain structures are too rudimentary and sparsely connected. Although developmental sophistication progressively increases, it is only after critical connections have been made between the cerebral cortex, the part of the brain responsible for consciousness, and subcortical brain structures, principally the thalamus, that sensory perception would become possible [1, 2]. This occurs about two-thirds of the way through pregnancy in fetal lambs.

The sensory environment *in utero* is significant and varied, and by late pregnancy the fetal lamb is responsive to stimulation in most of the sensory modalities evident after birth [8]. The development of these mechanisms prenatally allows the newborn to use sight, hearing, smell, taste, touch, proprioception (awareness of body orientation and posture) and thermal sensitivity to bond successfully with its mother and secure its survival during the critical first few minutes and hours after birth [1, 8]. It is evident, therefore, that fetal sense organs do operate *in utero*, but this does not mean that the fetus perceives or experiences the associated inputs as sensations. For that to occur the fetus would need to be conscious.

The absence of fetal consciousness

As just noted, the nervous system is too immature to support any activity resembling consciousness during the embryonic and early fetal stages of development. Thus, even if the physiological environment of the brain permitted it, neural development could not support fetal consciousness until after establishment of the critical corticothalamic connections. Moreover, the electrical activity of the fetal cerebral cortex (ECoG) provides apparently definitive evidence that fetal consciousness is absent before and throughout the last half of pregnancy in sheep [1, 2]. Fetal ECoG activity is initially non-existent. It then evolves through rudimentary and discontinuous patterns into undifferentiated continuous activity, and, finally, during the final few weeks before birth, it matures into two discrete states that resemble the rapid eye movement sleep and non-rapid eye movement sleep patterns seen normally in postnatal lambs. These are all states of unconsciousness. Thus, although the fetal neural apparatus may be able to support consciousness during late pregnancy, the evidence is that the fetal lamb remains unconscious throughout [1, 2].

Maintenance of fetal unconsciousness

This conclusion is supported by evidence that a number of suppressors act *in utero* to inhibit fetal neural activity. Thus, fetal, placental and uterine tissues play a key role in providing chemical and physical factors that together actively maintain the fetus in a continuously unconscious state [1, 2]. These factors include the following: (1) a powerful ECoG suppressor and unconsciousness-inducing agent (adenosine), produced by fetal tissues and the placenta, the concentrations of which markedly increase during hypoxaemia and decrease during experimentally induced fetal oxygen abundance; (2) two neuroactive steroids (allopregnanolone, pregnanolone) which have well-established anaesthetic, sedative/hypnotic and analgesic actions and are synthesized by the fetal brain and placenta; (3) a potent sleep-inducing hormone (prostaglandin D_2); (4) other ECoG inhibitors produced by the placenta; and (5) warmth, together with (6) buoyancy and cushioned tactile stimulation within the uterine environment.

Changes in neural state at birth: the onset of consciousness

Fetal unconsciousness persists throughout labour until after birth [8]. Indeed, it often becomes deeper, because transient hypoxaemia-induced increases in adenosine concentrations during uterine contractions, when sufficiently strong and protracted, can cause almost complete suppression of the ECoG (indicated by a near isoelectric trace). This marked inhibition and lesser such effects are usually rapidly reversed when fetal normoxaemia is restored between labour contractions. Likewise, when placental oxygen supply is lost with severance of the umbilical cord immediately after birth, the ECoG of the newborn would progress towards an isoelectric state, reached after 60–90 seconds. Such cortical inhibition can only be reversed with the successful onset of breathing.

Breathing begins despite cortical inhibition because brain stem activities supporting the initiation of breathing, and other functions, are safeguarded, even during protracted hypoxaemia [2, 8]. Umbilical cord severance, hypoxaemia, hypercapnia (elevated carbon dioxide tensions) and other factors in the newborn lamb elicit gasping that leads to successful inflation of the lungs (if mature enough), then to the onset of breathing and a rapid elevation in oxygen tensions, which eventually rise to well above maximum fetal levels. It is this oxygenation of the newborn which, together with loss of the placenta as a source of adenosine, would result in a rapid decrease in blood and cerebral adenosine concentrations and a decrease in adenosine inhibition of the cerebral cortical functions required for consciousness. Consciousness therefore appears for the first time only after birth.

Loss of the placental source of other cortical inhibitors, including allopregnanolone and pregnanolone, would also contribute to the onset of consciousness in the newborn, but the decline in their inhibitory effects may take longer than for adenosine. However, once the putatively dominant inhibition of adenosine has declined, any residual inhibition by other such factors is likely to be offset by ECoG activators that begin to operate just before and during labour, especially during the final delivery stage and immediately after birth [8]. These neuroactivators include 17β-oestradiol, which is a potent neuroactive steroid with widespread excitatory effects in the brain, noradrenaline released from excitatory locus coeruleus nerves that extend throughout the brain and promote arousal and alert vigilance, and a barrage of novel sensory inputs associated with the newborn's first exposure to air, gravity, hard surfaces, unlimited space and, usually, to cold ambient conditions. Sight, hearing and taste may also contribute novel stimulatory impulse barrages.

WELFARE OF THE FETUS

Concerns about fetal welfare arise when movements *in utero* are observed after maternal slaughter at commercial abattoirs and elsewhere, or during disease control programmes, and when exposed fetuses flinch and/or kick in response to needle insertion or other such invasive procedures. These movements are sometimes taken to indicate that the fetus is conscious, can suffer and does so under those circumstances. However, there is now a sound scientific basis (summarized above) for concluding that the fetal lamb is never conscious *in utero*, so that its welfare cannot be compromised.

Slaughter of pregnant sheep

Except when religious slaughter is practised, commercial slaughter of sheep, whether pregnant or not, is preceded by a stun to first make the animal unconscious, and is accomplished by a neck cut that severs the major vessels carrying blood to and from the brain. The purpose is to cause a catastrophic and irreversible drop in blood supply to the brain that leads to disordered brain function, to the impossibility of regaining consciousness and eventually to brain death, and, over the same period, to cessation of the heart beat [1].

Blood loss is very rapid, so that blood flow through the brain and other tissues of the body ceases quickly (i.e. usually within 10–20 seconds) [1]. In pregnant animals, blood flow and the accompanying oxygen supply to the uterus would cease over the same period. This means that oxygen supply to the fetus, via the placenta, would stop within 10–20 seconds. In fetuses within a few weeks of birth this leads very rapidly, i.e. within 60–90 seconds of the cessation of blood flow, to the disappearance of most detectable electrical activity in the cerebral cortex [1, 2].

This means that well within 60 seconds of effective slaughter of the pregnant ewe by a neck cut, the already unconscious fetus progresses to a state of profoundly deep unconsciousness and to an inevitable death provided that oxygen supply is not reinstated [1]. Likewise, although progress to fetal death may be somewhat more protracted after killing the ewe instantaneously with a penetrating captive bolt or by shooting, but without a neck cut, the implications for the continuously unconscious fetus are the same. Thus, fetuses that remain in the uterus until they are dead cannot suffer at any stage of the slaughter process. Their welfare is safeguarded. The movements often observed before fetal death are subcortical reflex responses to the increasing hypoxaemia and hypercapnia, and are not a cause for welfare concern [1, 8].

Collection of fetal tissues at slaughter

If fetuses are kept in the uterus until they are dead no welfare compromise can occur. Likewise, suffering cannot occur if, immediately on exposure, living fetuses are killed by a neck cut, captive bolt, or a blow to the head with a suitable blunt instrument. In contrast, welfare compromise can occur if living fetuses with mature lungs (i.e. within a week of birth) are removed from the uterus in circumstances where they could begin to breathe and successfully oxygenate their brain tissues to levels compatible with consciousness [1, 11]. Precautions to prevent this include not removing living fetuses from the uterus until at least 5 minutes after the maternal neck cut (by which time the ECoG would be isoelectric and the brain would be anoxic) and by preventing them from successfully breathing air (e.g. by keeping the head in the uterus, clamping the trachea). Such precautions should be applied to all near-term fetuses that might be able to breathe successfully, however immature they appear to be. Provided that these precautions are taken, flinching or kicking by living fetuses in response to invasive procedures (e.g. needle insertion, cutting) are subcortical reflexes, unrelated to consciousness, and are not a cause for welfare concern. Safeguards for fetuses during slaughter of pregnant animals, based on these observations, have been incorporated into Office International des Epizooties draft global guidelines for the slaughter of animals for human consumption [12].

Fetotomy (embryotomy)

Fetotomy, often incorrectly called embryotomy, raises welfare questions on those occasions when living fetuses need to be dismembered *in utero* in order to manage intractable dystocia. The above analysis and the further observation that the fetus cannot be aroused from its normal unconscious state to consciousness by noxious stimulation [2], show that, despite withdrawal and other reflex responses to cutting, it cannot experience pain or suffer. Nevertheless, killing such fetuses first [1] may be preferred in light of the emotional challenge of dismembering a physically responsive fetus, notwithstanding its unconscious state. On those occasions when a jammed lamb is partially delivered and obviously conscious, it must be killed before fetotomy is undertaken (see Chapter 11).

WELFARE OF THE NEWBORN

Consciousness appears after birth only when breathing oxygenates the lamb sufficiently to remove the dominant neuroinhibitory effects of adenosine on its brain function. The lamb that never breathes will never become conscious and will die without suffering.

The lamb with compromised lung function that does breathe, but not successfully enough to achieve an oxygenation-induced reduction in adenosine to levels compatible with consciousness, will also die without suffering. In contrast, most newborn lambs become conscious within minutes of birth [1, 7] and, once conscious, they have the potential to experience noxious and other sensations and to suffer if the intensity, duration and/or character of those sensations are sufficiently noxious or aversive.

The causes of neonatal lamb mortality and morbidity are well understood [3–6] but, until recently [7], their welfare implications had not been considered. There was a general presumption, however, that neonatal problems that result in death or severe debility must involve severe suffering, but this may not always be the case [7].

The major subjective experiences of welfare concern in the newborn lamb are apparently breathlessness, hypothermia, hunger, sickness and pain, and reference to documented responses of lambs, and to appropriate human experience, suggests that breathlessness and hypothermia usually represent less severe welfare insults than do hunger, sickness and pain [7].

Breathlessness

Breathlessness due to compromised lung function is linked to both hypoxaemia and hypercapnia, but hypercapnia is its primary cause. When the hypoxaemia is severe the newborn would be unconscious, as already noted, or its consciousness would be significantly dulled. Furthermore, hypoxaemia impedes heat production so that such lambs would be likely to become hypothermic in their usual birth environment, and hypothermia itself dulls consciousness (see below). Accordingly, both moderate hypoxaemia and hypothermia may reduce the noxious experience of breathlessness by dulling consciousness. On the other hand, as breathlessness is primarily due to hypercapnia, conscious lambs that are only mildly hypoxaemic and hypothermic may experience breathlessness at noxious levels.

Hypothermia

Hypothermia precedes death in most lambs that die in the perinatal period, and the majority of surviving lambs exhibit transient hypothermia during the first few hours after birth. Human experience shows that hypothermia can occur without the knowledge of the person and that as it becomes more severe, within the range commonly exhibited by newborn lambs before death, it dulls cognitive ability and consciousness. This is likely to reduce noxiousness of experiences associated with hypothermia. On the other hand, excessive cold can cause distress because of the marked physiological demands of producing heat at maximum rates for long periods and, possibly, because of cutaneous cold pain.

Hunger

Hunger becomes a welfare concern when its intensity rises significantly above usual daily levels. Starvation is relatively common in newborn lambs. Starved lambs experience severe hunger, the noxiousness of which may be reduced when hypoglycaemia causes sufficient hypothermia to depress consciousness, or when the starved newborn sleeps or becomes comatose. The duration of starvation-induced hunger is shorter in cold than in warm environmental conditions.

Sickness

Sickness, which is usually associated with infections in newborn lambs, can apparently be a relatively benign or noxious experience, depending on its cause. Thus, sleep, drowsiness, coma and hypothermia, which may be major features of a sickness episode, tend to be more benign in their effects and, if present, may reduce the noxiousness of distress and pain associated with some infections. Alternatively, distress and pain associated with infections may be very severe and not accompanied by these alleviating factors.

Pain

Pain arising from injuries or infections can be accompanied by alleviating factors such as sleep, immobility and treatment, and, alternatively, may be exacerbated by the nature and severity of the injury or tissue damage, and be of such an intensity or character that natural means of reduction, such as sleep and immobility, are not effective.

Concluding comments

Clearly, two or more of these experiences can overlap, sometimes where one reduces the effects of another

(e.g. where hypothermia dulls consciousness in hungry or sick newborns), and sometimes with greater negative welfare consequences (e.g. sickness plus pain). Although in the former cases the extent of welfare compromise may not be as bad as originally thought, all significant welfare compromise is of concern. In the case of newborn lambs, minimizing such compromise can be achieved by the application of successful, science-based lambing management strategies that have been developed over the past 50 years [3–7].

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4

Sheep welfare: castration and tail docking

V. Molony and J.E. Kent

Since sheep were first domesticated castration has been carried out to eliminate indiscriminate breeding, and tail docking to reduce the damaging effects of fly strike. Both practices are still considered by sheep farmers, in many parts of the world, to make management easier and more productive, sufficiently to outweigh the costs. Under the changing circumstances of sheep husbandry, management and marketing,

however, new costs must be taken into consideration, including the perception of consumers of suffering by the animals. It has been made clear by independent organizations such as the Farm Animal Welfare Council in the UK that castration and tail docking of lambs are of public concern.

JUSTIFICATION FOR CASTRATION AND TAIL DOCKING

Most sheep farmers accept that castration and tail docking of sheep should be carried out only after careful consideration of the benefits and costs to the animals, to their owners and consumers. Others carry out these procedures as part of an established tradition and may not recognize the need to justify them.

COSTS AND BENEFITS OF CASTRATION

In addition to preventing indiscriminate breeding, castration reduces unwanted sexual and aggressive behaviour, and makes male lambs easier to handle and to keep penned.

It is claimed that castrated lambs show more weight gain and have a better eating quality than uncastrated lambs: the meat from these lambs is more tender, easier to cook and has less smell (taint). However, the evidence for these claims is not accepted as conclusive by others who claim that uncastrated lambs grow faster, have more lean meat, are just as tender, are no more difficult to cook, and that the meat does not smell any stronger than that from castrated lambs.

Meat processors also claim some benefits of castration, the skin being easier to remove if there is more subcutaneous fat and no scrotum is present.

The main costs of castration are to the lambs, which suffer both from the procedure and the loss of their sexual potential. Various studies provide substantial experimental evidence that they suffer considerable pain and/or distress from the commonly used methods [1–7] and there is little experimental evidence that they do not. The feelings of those who are obliged to carry out these procedures also need to be considered. The costs to the owner are in time, instruments, and any losses in productivity such as increased perinatal mortality and increased disease susceptibility due to reduced intake of colostrum, immunological deficiency induced by the pain/distress or by increased handling stress to very young lambs. Costs to the consumer include more expensive lamb when castration decreases productivity and unwanted perceptions of animal suffering for which they accept some responsibility.

COSTS AND BENEFITS OF TAIL DOCKING

Fly strike is a major disease and welfare problem in many parts of the world and tail docking is accepted as the most effective and most economical way of reducing the severity of this problem [8–10]. If fly strike does not occur or can be easily avoided, e.g. by preventing faecal soiling, there appear to be few other substantial reasons to tail dock. However, the appearance of lambs of some breeds with long tails is considered, by some, to detract from their value, and failure to comply with such aesthetic considerations can have significant economic costs.

CONTROL OF CASTRATION AND TAIL DOCKING

The '*three R's*' used as the basis for control of scientific procedures on animals can be applied to castration and tail docking of lambs: use of the procedure should be reduced (fewer lambs should be treated), replaced (alternatives should be found) and refined (better and more humane methods should be sought).

Reduction and replacement can be achieved if lambs from rapidly growing breeds/strains are slaughtered before sexual behaviour and fertility become a problem for management and before the quality of the meat is reduced [10]. If the profit from individual lambs is high enough, the management costs for raising fast-growing lambs and the alternative of separating ram and ewe lambs could be accepted, especially if it added marketing appeal as a welfare-friendly production system. The further development of such systems may require government control of lamb production and, although this has been part of agricultural policy in

Europe for many years, only recently has government control been influenced by animal welfare considerations. However, high-cost production systems with good welfare standards will be undermined and may be abandoned if they are in direct competition with lower cost systems with poorer welfare standards. Thus, the introduction of welfare considerations into the sheep industry is heavily dependent on its consumers valuing lamb highly and paying accordingly.

Refinement requires clear understanding and awareness of the welfare costs and of practical ways to minimize them, involving research to establish and upgrade 'best practice'. Research has been carried out both under experimentally controlled conditions and by sheep farmers 'in the field' [12] and has led to adoption of different approaches to castration and tail docking in different parts of the world. In the UK, the rubber ring method has evolved as the method of choice for castration and tail docking of young lambs but its use is restricted by law to lambs of under 1 week old. In 2002 in New Zealand [10] about 20 per cent of lambs were castrated; 40 per cent were made bilateral cryptorchids by applying rubber rings and the remaining 40 per cent were left intact.

Several research groups have assessed the pain/distress suffered as a result of castration and tail docking of lambs by measurement of the effects of various methods on the behaviour and physiology of lambs [1–6, 12, 13]. There appears to be agreement that, without effective analgesia, lambs suffer considerable pain/distress from all the commonly used methods [7, 14–19], and that less painful methods should be used. Video and graphical evidence can be seen at www.vet.ed.ac.uk/animalpain/. Economic pressures require that any such methods should be quick, easy, effective and reliable.

METHODS OF CASTRATION AND TAIL DOCKING

Societies whose way of life depends on animals generally show them great respect, regret the need to carry out procedures such as castration or tail docking and show distaste for the process. There are also instances where societies appear to have 'ritualized' these procedures to help those carrying them out to cope with the pain/distress caused to the animals.

What method is 'best' for castration and for tail docking?

There is no simple answer to this question as the method that may be best for very young lambs is not necessarily optimal for older lambs, and methods best in some countries may not be appropriate in others. After deciding that lambs need to be castrated it is also necessary to decide how important it is that they are made completely infertile and that none show sexually associated, deleterious behaviour. This will assist in making a choice between surgical removal of the testes, induction of ischaemic testicular necrosis or some other approach.

A brief description of the advantages and disadvantages of generally practised methods is included. Details of how they are applied should be sought elsewhere (e.g. [2–5, 16, 19]).

Surgical castration and tail docking

The main advantages of this method are that it is certain, and that pain can arise only from the remaining tissues. The disadvantages are those of any surgical interference, including: pain, stress, inflammation, infection, haemorrhage and the time needed for healing, all of which can reduce productivity and most of which can be minimized by established surgical skills, the use of local anaesthesia and treatment with non-steroidal anti-inflammatory drugs. The disadvantages are considered to outweigh the advantages in some countries.

Bloodless castration

The main advantages are that, because the skin remains intact, there is much less chance of infection, it is quicker and is considered by some to be easier to carry out than the surgical method. Successful crushing with a bloodless castrator occludes the blood supply to the testes and destroys the nerves to and from the tissues beyond the crush, making the tissues insensitive immediately and causing atrophy of the testes. The main disadvantages are rupture of crushed blood vessels can lead to haematomas and failure to apply the instrument correctly may not subject the spermatic cord to the necessary destructive forces to ensure atrophy of the testicular tissue. The bloodless castrator can produce a burst of intense pain as it is

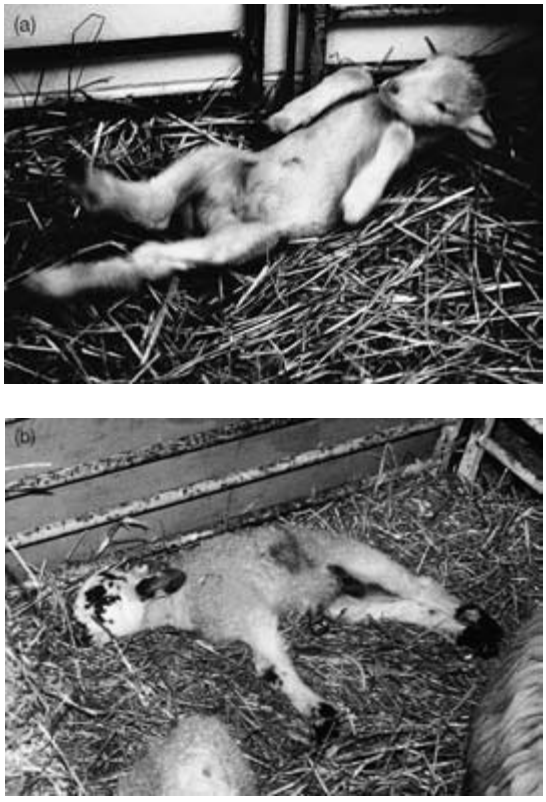

Figure 4.1: Behavioural changes in lambs following application of rubber rings for castration: (a) lamb rolling and kicking soon after being castrated and tail-docked; (b) after a period of restlessness, they become exhausted (about 60 min after ring application) and assume lateral recumbency.

applied which should be short lived but can be followed by considerable pain from damaged tissues proximal to the line of the crush [16, 19]. Bloodless castrators are not often used for tail docking, due to the operators' perceptions of the severity of this method and its unreliability if the unwanted tail is not cut off at the same time.

Ischaemic castration and tail docking with rubber rings

The advantages are that rubber rings are quicker and easier to use, as well as being more reliable than bloodless castrators. They can be used both for castration and tail docking. and the Elastrator instrument used to apply them is easy to use, cheap, robust, reliable and durable.

The disadvantages are that lambs experience acute pain for up to 2 hours (Figure 4.1) followed by chronic inflammation, sepsis and further pain until the affected parts fall off and healing occurs [20, 21]. This can take more than 6 weeks for lambs with large scrotums [22].

All of these methods produce behavioural and physiological changes that have been interpreted as indicative of considerable acute pain and which require methods for its reduction or alleviation to improve the welfare of the lambs.

METHODS FOR REDUCING PAIN FROM CASTRATION AND TAIL DOCKING

The easiest, quickest and cheapest approach to this problem could be to adopt production aims and husbandry methods that do not require these procedures. If these approaches cannot be used, the least painful practical alternatives should be adopted.

Local anaesthesia

Since the development of potent local anaesthetics it has been possible to eliminate or greatly reduce the acute pain from castration and tail docking provided adequate amounts of anaesthetic are injected close to the nerves involved and sufficient time is allowed for the anaesthetic to block conduction [13]. Two, more humane, methods for field use have been described [12, 17]. In each, rubber rings are applied with either needleless injection of local anaesthetic or application of a bloodless castrator to make the tissues at and beyond the rubber ring insensitive. These pain reduction methods can be applied at the same time as the rubber ring and the lambs need not be handled twice. They take approximately twice as long to carry out as the usual rubber ring method, suitable needleless injectors are costly and there can be increased handling stress to the lambs [12].

The skill needed to apply a bloodless castrator effectively, quickly and safely after a rubber ring is much less than when it is applied separately to each spermatic cord in the conventional way, i.e. without a

rubber ring [1, 5]. Pain suffered by lambs when a bloodless castrator is applied can be eliminated or reduced by including injection of local anaesthetic as part of the method [17]. Some operators will need to be convinced that the overall suffering of lambs is significantly reduced by this combined method compared to other methods [1, 5, 23, 24] before their dislike of crushing can be overcome.

LEGISLATION TO CONTROL CASTRATION AND TAIL DOCKING

Although it may be claimed that castration and tail docking are being carried out using humane methods without the need for any legislation, many societies consider it necessary to help to ensure the welfare of the lambs by setting minimum standards for application of particular methods. Local anaesthesia for castration and tail docking is now compulsory in Switzerland and Austria, and other countries may adopt this approach in future.

Setting standards for the different methods requires not only assessment of the costs and benefits, but also compliance with similar legislation for the production of all lambs for sale in a particular market. This is necessary to avoid putting those producers who comply at a competitive disadvantage to those who do not.

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5

Sheep welfare: transport of sheep

J.A. Earl

Concern regarding the welfare of animals during transport has led to the funding by the European Union (EU) of research on the welfare of sheep during transport. Attention has been focused on the stressful effects of transport, including food and/or water deprivation, the effects of loading and unloading, differing space allowances, differing journey times, and surveys of deaths and injuries.

RELEVANT UK LEGISLATION

The present relevant UK law is The Welfare of Animals During Transport Order 1997, in response to the EEC Directive 91/628 as amended by 95/29, and was made under the Animal Health Act 1981. The Order imposes a 14-hour journey limit by road for weaned sheep if vehicles are of an approved standard, which includes the presence of adjustable ventilation, carriage of appropriate food and equipment to allow the connection of a drinking-water supply. A 1-hour rest for food and water can be followed by a further 14-hour journey before a 24-hour rest period is required. For unweaned lambs, a 9-hour journey limit, followed by a 1-hour break before a second 9-hour journey is allowed. An outline of European regulations on transport of animals is given at the end of the chapter.

THE SCIENCE OF WELFARE DURING TRANSPORT

Sheep have probably evolved not to show many outward signs of distress because of the way this would

attract predators in the wild; as a result they show a passive response to pain and stress. Consequently, biochemical, physiological and behavioural parameters are involved in assessing the effects of transport and can be related to the 'Five Freedoms' of animal welfare that are outlined below:

1. Freedom from hunger and thirst

Water and food deprivation

Total freedom from hunger is not possible when transporting a grazing animal, but data indicate that sheep tolerate hunger and water deprivation extremely well [1], confirmed by the absence of a stress response after 48-hour of food and/or water deprivation (in non-transported sheep) at normal (20°C), high (35°C) or low (7°C) temperatures [2]. Dehydration has been assessed by measuring blood osmolality, albumin levels, haematocrit, skin thickness and body-weight changes, but no evidence has been found that dehydration occurs on road journeys lasting 9–31 hours [3, 4].

Although loading can lead to an increase in haematocrit, probably through splenic contraction, a progressive decrease follows, together with a fall in osmolality (possibly due to cortisol protecting the plasma volume), with a consequential absence of thirst. Rising concentrations of blood urea, β-hydroxybutyrate and free fatty acid relate to metabolic stress caused by lack of food. Free fatty acid levels rise for the first 24 hours of food deprivation, whereas β-hydroxybutyrate is a better indicator of longer term food deprivation. Neither food deprivation nor dehydration affects prolactin release, although growth hormone values tend to rise with food deprivation [2].

The conclusions are that sheep tolerate food and water deprivation extremely well, even in adverse environmental conditions, and that food and water deprivation are not the main source of stress during transport.

Weight loss

Weight loss during transport has been attributed to food and water deprivation, metabolism and excretion, and typically falls in the range 6–10 per cent of body weight during road transport of up to 24 hours [3, 4]. This period of food deprivation in untransported sheep usually causes a similar, but smaller degree of weight loss. Up to 4 days might be required for lost body weight to be restored if only hay and water are offered, but weight restoration and recovery of metabolic parameters to normal is more rapid if concentrates are fed [4].

Eating and drinking behaviour

Sheep eat before drinking during the post-transport rest, even when dehydrated [4, 5], which is probably related to sodium and fatty acid absorption from the digestive tract. Therefore, if access to water is limited during lairage, restricting dry feed also would be advisable, otherwise eating without the opportunity to drink could allow an unrelieved thirst to develop.

Although this might not be stressful to sheep, it does suggest that a motivation is being frustrated, which in welfare terms should be avoided.

Different feeds are eaten at different rates, and this is seen in sheep after a period of deprivation.

Concentrates are consumed much more rapidly than hay, especially if they are a familiar source of food. Hence, for sheep to recover quickly after transport, they require concentrates, but need to be familiar with them in order to eat them in a short space of time.

Cockram [6] found no differences in lying behaviour or eating patterns in sheep given 0, 3 or 12 hours of rest in the middle of a 24-hour period of transport, suggesting that a continuous journey for a period of 24 hours is at least tolerable if other conditions are satisfactory.

2. Freedom from discomfort

Fatigue

Creatine kinase and either lactate dehydrogenase or blood lactate have been used as indicators of muscular exertion. Sheep transported in conditions that allowed them to lie down showed little evidence of fatigue, even after journeys of 24 hours [4]. Similarly, only minimal tissue damage due to transport was found [3]. However, studies involving the transport of slaughter-weight lambs (37–39.5 kg) at a variety of stocking densities for a period of 24 hours during winter indicated that fatigue, as evidenced by elevated creatine kinase levels, can occur at a stocking density of

approximately 0.23 m^2 per unshorn lamb. Stocking densities ranging from 0.26 to 0.34 m^2 per unshorn lamb, and from 0.18 to 0.3 m^2 per shorn lamb were not associated with elevated creatine kinase levels [4]. Under current EU regulations, unshorn lambs between 26 and 55 kg should receive 0.2–0.3 m^2 space allowance.

The ability of older sheep, such as cull ewes, to withstand long journeys without fatigue is likely to be less than that of young lambs, but might depend on the state of health or debility, rather than age itself.

Environmental stress

Sheep are unable to show behavioural adaptation to a rising temperature on a lorry, and a high stocking density could lead to heat stress through the increased degree of physical contact between sheep. Similarly, a high stocking density might limit the cooling effects of transport, particularly for shorn sheep; sheep morbidity and mortality are far more likely to be associated with hyperthermia than hypothermia.

3. Freedom from pain, injury and disease

Stocking density can influence the incidence of bruising through the effects of falling and riding. Low density contributes to falling, and a high density to riding and trampling. The position of a lamb or sheep on a lorry also influences the incidence of bruising, with those at the front of a lorry or on the bottom deck being more susceptible to injury [7]. Sheep can be more aggressive with each other when on rough journeys, which might contribute to increased bruising.

Deaths in transit

In one survey the number of deaths in sheep during transit was 1 in 5500, less than one-tenth of the percentage of deaths in poultry, and approximately one-quarter of that in pigs. Deaths can be related to pre-existing disease and the percentage can vary with the time of year. Death rates might also be higher when lambs have been through an auction, as opposed to having travelled direct from farm to slaughterhouse, but the distance travelled in these two situations could vary.

4. Freedom to express normal behaviour

The freedom to behave normally requires sheep to be able to lie down, which tends to occur after 3–4 hours of transport provided space allows [6]. With insufficient space allowance, fatigue could occur on journeys of over 3 hours' duration. Other normal behaviours, e.g. grazing or flocking, clearly cannot occur, although sheep have been observed to ruminate during transport. A comprehensive study of the effects of driving events such as acceleration, braking, turning and gear changes, etc. on the behaviour of 1-year-old sheep in transit [8] demonstrated clear benefits in terms of increased lying-down (and therefore reduced falling and loss of balance) and increased rumination when motorways were used for the transport, as compared to minor roads. This study further suggested that driving style and therefore driver education could have an influence on the welfare of the transported sheep. Mixing batches of lambs might not lead to significant antagonistic behaviour, fighting being more common between lambs that are familiar with each other.

5. Freedom from fear and distress

Cortisol, glucose, adrenaline and prolactin have been used as indicators of short-term stress, whether psychological or physical in origin. Elevated cortisol and prolactin concentrations and a fall in osmolality and haematocrit for 3 hours after loading sheep into vehicles have been reported [3], but little evidence was found that transport caused much response after that time. Blood glucose concentrations can fluctuate rapidly in response to environmental stimuli and tend to be raised at the start of a journey, probably in response to loading. Heart rates increase when sheep are disturbed by loading and unloading, when a vehicle stops and starts, or during the first few hours of transport [2, 3, 9]. It is likely that this is indicative of both fear of handling and of a strange environment. No evidence for motion sickness has been found in sheep, unlike pigs, but, because ruminants do not vomit readily, this comparison is hard to make. High noise levels in a vehicle are not associated with any alterations in stress indicators, which suggests that high noise levels are not stressful [10].

Miscellaneous effects

Shearing

An increased likelihood of bruising could result from impacts in the absence of wool, and there is a reduced likelihood of 'wool pull' during handling, but shorn lambs have been found to have few hormonal responses differing from those of unshorn lambs. The behaviour of sheep in a lairage has been found to differ; shorn lambs eating more rapidly and for longer periods than woolled sheep. Shorn and unshorn sheep do have differing critical temperatures, however.

Meat quality

Dark cutting is a potential problem in sheep, but there is little evidence about this.

Breed differences

Upland sheep can show a greater cortisol response to transport than lowland sheep, although whether this is due to differences in genotype or experience is not possible to say.

A comprehensive literature review regarding the effects of road transport on slaughtered sheep is available [11].

Long-distance transport by sea

A stress syndrome that includes reduced appetite and water intake, digestive disorders, diarrhoea, posterior paralysis and rapid death has been described in sheep transported by ship for several weeks from New Zealand and Australia to the Middle East [12]. The death rate for sheep on such journeys is usually about 2.5 per cent, although it can reach 8 per cent and on occasion even 100 per cent if a catastrophe occurs. On such long journeys, the recommendation is that sheep should be fed and watered twice daily. Most deaths on these voyages from New Zealand and Australia are due to inanition, but pneumonia also is a major cause of loss in sheep from the former country, and salmonellosis and trauma important causes of death in sheep from the latter. Paradoxically, the sheep succumbing to inanition are those that are well nourished after leaving good pasture in August but are less attuned to fat mobilization than sheep from dry pasture in May [13].

CONCLUSIONS

Scientific evidence suggests that optimum road transport conditions for sheep include:

- Gathering by a known person, and known dog if necessary.
- Careful loading on and unloading from a vehicle with an in-built forced ventilation system.
- A space allowance to permit lying down, at least for journeys over 3 hours.
- Steady driving over smooth roads.

Journeys of up to 24 hours ought to be continuous with as few stops for loading/unloading as possible. For journeys over 24 hours, a 24-hour travel time before a rest period might be advisable as a compromise between further loading/unloading and freedom from hunger. This only applies if the other criteria are met. Rest periods of sufficient duration should be given to allow adequate recovery before further transport. This could take up to 6 days, depending on the foodstuff offered.

EUROPEAN UNION REGULATIONS

The present European legislation (EEC 91/628, as amended by 95/29) provides a regulatory framework whereby all Member States legislate to protect the welfare of animals during transport. This amended Directive sets agreed standards for the maximum journey times, feeding and watering intervals, resting periods, space allowances and means of transport. The intention has been to provide a common, satisfactory level of protection for animals transported within the EU without imposing unnecessarily stringent barriers to trade within the EU itself. Individual Member States are permitted to set stricter conditions for transport within their national borders and may opt for openly declared exemptions for areas of their country remote from the mainland part of the EU.

In addition, the EU has signed the Council of Europe *Convention for the Protection of Animals*

During International Transport, although this convention is not legally binding.

In November 2004, the European Council adopted a new Regulation on the protection of animals during transport, a radical overhaul of the previous Directive mentioned earlier, which will enter into force in 2007. The new Regulation introduces stricter rules for vehicle standards for journeys over 8 hours and introduces tools such as satellite navigation checks on vehicles [14].

Main elements of the new Regulation

The Regulation harmonizes national rules regarding transport, and defines more clearly the responsibilities at each stage of transport; training is given more importance, and markets and collection centres have additional rules imposed. In addition, enforcement is emphasized through increased monitoring and the more specific responsibilities.

For journeys over 8 hours, stricter standards are introduced: vehicles will have to provide drinking systems, and have alarmed-temperature monitoring and recording equipment.

The Regulation also introduces a ban on transporting heavily pregnant or postnatal females, and this specifies the final 10 per cent of the duration of a pregnancy and 1 week after having given birth, respectively. In addition, the very young, including lambs less than 1 week of age, should not be transported unless the journey is shorter than 100 km.

Although the maximum journey times and space allowances are unchanged from EEC 91/628 and 95/29, it is expected that the impact of the Regulation on animal welfare shall be the subject of a report within 4 years of the Regulation being brought into force, i.e. by 2011. This report may be accompanied by appropriate legislative proposals as regards journey times, resting periods and space allowances. Furthermore, deadlines have been given for establishing maximum and minimum temperatures for transport, and for applying navigation systems within transport vehicles.

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6

Slaughter of sheep

D.C. Henderson

In the UK and many other countries sheep are reared primarily for meat production and young sheep are slaughtered at the appropriate stage in the production cycle. Likewise, breeding animals that have reached the end of their productive lives are sent to the abattoir. This chapter considers welfare issues concerning slaughter of animals consigned in this way.

We have a responsibility to animals under our care to ensure that they are slaughtered or killed in a manner that does not compromise their welfare from the moment of leaving the farm until the moment of death. Ideally, slaughter should be as close to the point of production as possible but, unfortunately, in the UK (and some other European countries) many smaller slaughterhouses have closed in recent years and therefore some animals are subjected to longer journey times. This need not be detrimental to welfare if the standards of vehicles and driving are high, but must add to the stress of the procedure if journeys are excessively long or complex. This is of particular concern when transporting cast ewes, which are of low value and may be especially vulnerable to injury during transit.

LEGISLATION

On arrival at the slaughterhouse animals become the responsibility of the owners and UK legislation covering the running of premises is the Welfare of Animals (Slaughter or Killing) (WASK) Regulations, 1995. These state (Part 1, Introductory, 4. Humane treatment of animals): (1) 'No person engaged in the movement, lairaging, restraint, stunning, slaughter or killing of animals shall – (a) cause any avoidable excitement, pain or suffering to any animal; or (b) permit any animal to sustain any avoidable excitement, pain or suffering'. Additionally, any person involved in the process should have – 'the knowledge and skill necessary to perform those tasks humanely and efficiently in accordance with these regulations'.

In law, therefore, the welfare of animals should be safeguarded, and to a large extent is, but there remain some areas of concern that recently have been addressed by the Farm Animal Welfare Council (FAWC). This body was set up in 1979 to advise government of any legislative or other changes that may be necessary to safeguard the welfare of farm animals on agricultural land, at market, in transit and at the place of slaughter. In its 'Report on the Welfare of Animals at Slaughter or Killing – Part 1: Red Meat Animals' published in June 2003, FAWC considered five basic principles should be observed:

- pre-slaughter handling facilities that minimize stress
- use of competent well-trained, caring personnel
- appropriate equipment which is fit for purpose
- an effective process that induces immediate unconsciousness and insensibility or an induction to a period of unconsciousness without distress and
- guarantee of non-recovery from that process until death ensues.

The Report made a number of recommendations, some of which impact on the way sheep in particular are dealt with.

HANDLING OF ANIMALS

Animals arriving at the slaughterhouse should be unloaded as soon as possible and wherever possible on level ground. They should be inspected immediately by a member of staff trained in animal welfare so that any animal that has been injured in transit or is otherwise compromised can be moved to an isolation pen

designed specifically for that purpose. Any animal in pain or distress should be killed without delay by competent staff who should be available by day or night.

Apart from its use for ante-mortem inspection, the lairage is also a place where sheep can rest and recover after a journey, although it is unlikely that animals will be in lairage long enough for full physiological and behavioural recovery. Some premises provide field lairage contiguous to the slaughterhouse which can be beneficial to sheep especially to those unused to being confined indoors. However, it is important that these areas are managed appropriately to reduce the risk of disease build-up and to ensure reasonable conditions underfoot, and are not overstocked so as to provide at least some vestige of grazing.

Lairage design can do much to reduce the distress of animals in a strange environment; for example by taking account of the natural behaviour of the species involved so as to allow them to proceed without hindrance. Noise reduction measures should be in place and floors should be of non-slip materials and kept clean. The use of goads or sticks should never be necessary when moving sheep around the lairage.

The provision of food and water in lairage is important as some animals may undergo complex journeys without the opportunity to eat or drink; for example, via markets or collection centres. Legally, water must be available at all times but, in some instances, too few watering points are provided, they may be of the wrong type for sheep, troughs may be contaminated or pens overstocked. There is a need for guidance on optimum schedules for the feeding and watering of sheep (and other species) prior to slaughter.

In recent years it has become necessary to check the age of sheep by examining the teeth and their identity by reading ear tags. In some cases it is also necessary to deal with dirty animals to comply with the clean livestock policy. All these procedures require additional handling when, ideally, animals should be handled as little as possible. Producers can help by presenting clean animals for killing and the slaughterhouse can assist by providing suitable facilities and trained operators to shear sheep when necessary. Electronic identification of animals would assist in reducing stress.

RESTRAINT FOR SLAUGHTER

In small to medium slaughterhouses sheep may be penned together where they are to be stunned. Animals are walked from the lairage to the pens and allowed to settle while the slaughterer moves quietly around the pen stunning any animal when the opportunity arises. This may be by captive bolt or head-only electrical methods. There is a need for research into the design of stunning pens for sheep so as to reduce the risks of pre-stun shocks and to ensure that animals can be shackled quickly.

In plants with larger throughputs automated systems may be used to move and restrain animals for stunning. For example, V-shaped moving belts support the sheep so that their feet are off the ground to prevent struggling and possible injury. Some include fully automatic stunning systems, others are manual. It is important that the speed of the belts synchronizes with the speed of stunning so that sheep are not kept waiting in the conveyors and that emergency procedures are in place for breakdowns or other untoward events.

The UK Meat Hygiene Service (MHS) is responsible for the enforcement of welfare regulations at slaughter and Official Veterinary Surgeons (OVS) employed by the MHS oversee the treatment of animals from the moment they arrive at the slaughterhouse until the moment of death (among other duties). They therefore have a critical role in welfare supervision. It is their responsibility to monitor the efficiency of stunning and slaughter, but they cannot be everywhere at once and there is a need for more widespread use and development of devices that automatically monitor the efficiency of stunning equipment since this is an obvious area where animal welfare may be compromised.

Slaughter without pre-stunning

WASK exempts the slaughter – without the infliction of unnecessary suffering – of animals by the Jewish method (Shechita) and the Muslim method (Halal) with certain provisos. Sheep may be shorn around the neck, placed on their backs on a cradle and restrained by a handler while a transverse incision is made with a sharp knife that cuts through skin and muscle, severs all the major blood vessels, oesophagus, trachea, nerve trunks and other tissues in the neck, so that the animals bleed out until death ensues. In the case of Shechita, neither a pre-cut stun nor a post-cut stun is allowed by Jewish law. However, a proportion of Halal slaughter animals are pre-stunned. Sheep are not allowed to be moved until they have bled out when they are shackled and hoisted.

FAWC had major concerns regarding this method of slaughter particularly regarding pre-slaughter handling, the potential for pain and distress during exsanguinations, and the time to loss of brain responsiveness. Concerns on handling related to the skill, care and physical ability of the handlers restraining the sheep and, in some observed cases, the illegal practice of lifting young sheep by the fleece. It was considered more appropriate to use some form of crate – such as are used to hold animals for foot trimming – to restrain animals for slaughter.

FAWC's major concerns, however, were in regard to: (a) the pain inflicted by the drastic neck cut and (b) the time taken to loss of consciousness following the incision. Scientific evidence shows that sheep become insensible within 5–7 seconds of the throat being cut. (In adult cattle it may be between 22 and 40 seconds, and in calves up to 120 seconds.) FAWC therefore recommended that slaughter without pre-stunning was unacceptable and that the exemption under WASK should be repealed. Government did not accept this recommendation.

Other recommendations made by FAWC included the training and licensing of slaughterers, the formalizing of the role of Animal Welfare Officers (AWO), and research, development and technology transfer in a number of areas aimed at improving the welfare of sheep at slaughter.

Copies of the FAWC report referred to may be obtained from:

Farm Animal Welfare Council
1A Page Street
London
SW1P 4PQ
www.fawc.org.uk/reports/pb8347.pdf

Part III
Reproductive physiology

7

The reproductive cycle and its manipulation

D.C. Henderson and J.J. Robinson

THE REPRODUCTIVE CYCLE

The reproductive cycle of the ewe is controlled by the integrated activities of neuronal and endocrine pathways that link the brain, ovary and uterus. These time the occurrence of oestrus and ovulation, regulate the lifespan of the corpus luteum and thereby control the duration of the inter-oestrous interval.

The breeding season

Sheep are seasonally polyoestrus, short-day breeders. In temperate latitudes, their sexual activity begins with the declining day length of late summer and autumn and ends with the increasing day length of late winter, early spring. The blood metabolite that monitors information on the changes in day length is melatonin, a hormone secreted by the pineal gland in the brain during the hours of darkness. By responding to changes in day length, melatonin programmes the circannual reproductive rhythm to ensure that the breeding season occurs at the correct time of year. Melatonin can also be used to advance the breeding season by its oral administration or by its insertion as a subcutaneous implant.

Although behavioural oestrus and ovulation occur only during the breeding season, dynamic changes in ovarian follicular growth and regression continue to occur throughout the non-breeding season (anoestrus). These follicles can be further stimulated artificially, so allowing ewes to breed during anoestrus. Indeed, in equatorial regions, where day length varies very little, the dynamic state of the ovarian follicle population means that native ewes can breed at any time of the year. The availability of food is often the limiting factor, and this is governed largely by rainfall, altitude and temperature. In temperate regions, photoperiod is the principal controlling factor, with temperature and other climatic factors having only minor effects.

While most breeds of ram are able to mate at any time of year, the quantity and quality of the ejaculate deteriorates coincident with the ewe's non-breeding season. The libido of rams also declines during this period, and these factors have an important bearing on the success of lamb production systems that involve the induction of oestrus outwith the natural breeding season.

The length of the breeding season varies with breed. The Dorset Horn is capable of lambing at any time of the year, but this applies to individual ewes rather than to the flock in which an 8-month season of sexual activity could be expected. Mountain breeds, such as the Scottish Blackface, Swaledale, Welsh Mountain and Cheviot, have a much shorter breeding season of around 4 months. In between these extremes are the cross-breds, such as the Greyface and Mule. Despite this variation, most breeds are at peak fertility during the late autumn (October–November), and this is reflected in the highest lambing rates in late March and April. Recent studies of the Merinos D'Arles breed in France and the Small Tail Han breed in China have demonstrated a polymorphism of the Mel_{1A} receptor gene which is linked to an ability of individuals from these breeds to lamb out-of-season.

The transitional period

Enhanced pituitary activity, characterized by an increase in the frequency of luteinizing hormone (LH) pulses, occurs in the transition between anoestrus and oestrus. These endocrine changes stimulate an increase in the maximum size, and oestrogenic capacity

of ovarian follicles, culminating in a surge of LH and ovulation. In the absence of progesterone priming, which is normal at the beginning of the breeding season, the first ovulation is not accompanied by behavioural oestrus; rather, it is a 'silent' oestrus. Indeed, if the corpus luteum (CL), which is formed following the first ovulation of the breeding season, regresses prematurely, ovulation recurs, but it too may be 'silent' because of inadequate progesterone priming.

Ewes respond readily to stimulation during the transitional period, either to the introduction of a ram, which increases the frequency of LH pulses, or to an exogenous gonadotrophin source such as pregnant mare's serum, now known as equine serum gonadotrophin (ESG) or equine chorionic gonadotrophin (eCG). The latter is adopted in this chapter. Provided eCG is given following a period of progesterone priming, ovulation is accompanied by behavioural oestrus.

The oestrous cycle

Inter-oestrous intervals in the ewe vary from 14 to 18 days, with an average of 16–17 days. The first 13–14 days are the luteal phase of the cycle, characterized by progesterone secretion by the CL, and the remaining 3–4 days are the follicular phase, which is initiated by the decrease in luteal progesterone production. Falling progesterone levels increase the frequency with which LH is released from the pituitary gland, thereby stimulating oestradiol production and secretion by large non-atretic follicles. The rise in oestrogen causes behavioural oestrus, induces the pre-ovulatory LH surge and produces a concomitant increase in the release of pituitary follicle-stimulating hormone (FSH). These endocrine changes promote the final maturation of the follicle leading to its rupture and the release of the ovum.

Invariably, more than one follicle develops in the ovaries and therefore multiple ovulation is common. There is rarely more than a few hours' spread in the time of the rupture of follicles. The mechanisms controlling the number of follicles reaching maturity and ovulating are complex and not fully understood. Nonetheless, there is involvement of ovarian growth factors [e.g. insulin-like growth factor-1 (IGF-1) and the IGF-binding proteins (IGFBP); epidermal growth factor (EGF); transforming growth factors (TGF-α and -β) and ovarian peptide hormones (e.g. inhibin, activin and follistatin)], in that they interact to modulate the responsiveness of the follicular cells to gonadotrophins. Recent studies of genetic mutations have demonstrated the importance of two oocyte-secreted members (BMP15 and GDF9) of the TGF-β superfamily in the regulation of ovulation rate in ewes [1].

Oestrus and ovulation

The behavioural signs of oestrus last 1–2 days and average around 36 hours. As in other species, the signs are the result of high concentrations of circulating oestrogen, which, in the ewe, peak just before the onset of oestrus and immediately prior to the LH surge of early oestrus. This coincides with the lowest level of circulating progesterone, which has been falling rapidly from about day 15 of the cycle (day 0 = ovulation). Oestrus in the ewe is very subdued. If a ram is present, ewes in oestrus will seek him out, display a little tail wagging and may nuzzle his scrotum, but this is usually the limit of their activity. If a ram shows interest, they will stand to be mounted. If no ram is present, oestrus will go undetected, but will last several hours longer than when males are around. Ewe lambs and gimmers may show no outward signs of oestrus whatsoever, which can pose problems if inexperienced ram lambs are used. Therefore, it is essential to run mature rams of high libido with virgin females.

At the beginning of the breeding season, ovulation rates are lower and oestrus is generally shorter, less intense and less fertile. Ovulation is spontaneous and takes place towards the end of oestrus, some 18–24 hours after its onset. The ovulation rate depends on the number of follicles that mature and is influenced by a number of factors. These include nutrition during fetal life and again around 6 months before ovulation when the ovarian follicles leave their primordial pool. They also include the nutritional status and body condition of the ewe at the time of ovulation. Other contributing factors are breed, age, reproductive status (dry or lactating) and the season of the year. Optimizing as many of these factors as possible enhances ovulation rate. High ovulation rates do not necessarily translate into lambs born, however. This will depend on the activity and fertility of the rams and on the management of ewes during early pregnancy, with a view to minimizing early embryonic mortality.

Leuteinization and luteolysis

Following ovulation, the ruptured (Graafian) follicle becomes a corpus haemorragicum, which is transformed into a CL that immediately begins to secrete progesterone, reaching a peak around 6 days after ovulation. Progesterone inhibits the secretion of the pituitary gonadotrophins, so preventing the development of oestrogenic follicles, which would jeopardize embryo survival. Progesterone also primes the reproductive tract for the acceptance of a fertilized ovum.

If the ewe does not conceive, from around day 12 of the cycle the CL fades and circulating progesterone levels fall correspondingly. This process (luteolysis) is brought about by prostaglandin F_2 alpha ($PGF_{2\alpha}$), which is secreted in a pulsatile manner from the uterine endometrium in response to progesterone priming. $PGF_{2\alpha}$ is sustained in its action by oxytocin from the CL interacting with its endometrial receptors to ensure continued oxytocin production for the episodic secretion of $PGF_{2\alpha}$. However, if an early embryo signals its presence by producing ovine trophoblastic protein (oTP-1), which is an interferon (IFN_τ), the secretion of $PGF_{2\alpha}$ by the uterus is suppressed, the CL remains intact and the pregnancy is sustained.

Mating and fertilization

At ejaculation, a fit and fertile ram will deposit around 3000–4000 million sperm in the anterior vagina of the ewe. A ram may serve a ewe on more than one occasion and, in multi-sire groups, ewes tend to be served more often than in single-sire groups. The sperm have a long, tortuous and hazardous journey to reach the ampulla of the oviduct, where they meet the ova. Of the very large numbers of sperm that are deposited, only a few hundred complete the journey. Most never circumnavigate the convoluted cervix of the ewe and most that do are treated as 'foreign' material and are engulfed by white blood cells.

At fertilization, normally only one sperm fertilizes an ovum. Enzymes from the acrosome in the head of the sperm assist it to penetrate the outer membrane (zona pellucida) of the ovum. Once this has occurred, no further sperm may enter the zona unless the ovum is aged and degeneration has set in. Dual or multiple penetration is fatal to the ovum. If the sperm and/or ovum are aged by the time they meet – which would rarely occur following natural service, but could following the mistiming of artificial insemination – then fertilization may not occur, or if it does, embryo survival is likely to be compromised.

MANIPULATION OF BREEDING

Better understanding of how environmental and genetic factors influence the growth of ovarian follicles and ovulation rate encourages the application of new management strategies and techniques for enhancing overall reproductive performance. The ultimate aim in this application, however, should be to match the level of reproductive performance with the resources that are available.

Ovulation rate

While rams produce sperm 'on demand' from puberty until age or infirmity intervenes, the ewe is born with all the ova she will ever possess, most of which will either never develop or else perish when the follicle, in which each ovum is encapsulated, becomes atretic. The incidence of atresia increases as the follicles develop from the primorial quiescent state to ovulation – a process that takes about 6 months in the ewe. The increase in atresia reflects the increase in the sensitivity of the developing follicle to subtle shifts in the gonadotrophins so that, during the lifespan of the CL, numerous follicles emerge, grow and perish (atresia) before emergence of those that actually ovulate. The number of ova that develop to full maturity and ovulate can be altered in a number of ways.

Nutrition

Through its adverse effect on the development of the fetal ovary, undernutrition ($0.5 \times$ maintenance) imposed for the first 95 days of pregnancy can reduce permanently adult ovulation rate by 20 per cent [2]. Although not tested for their influence on ovulation rate, shortened periods of maternal undernutrition have also been shown to affect adversely the fetal ovary and are thus unlikely to be conducive to the full expression of ovulatory potential. These periods are the first 30 days of pregnancy, days 30–50 and days 50–65.

Undernutrition at around 6 months before ovulation is due to occur can also adversely affect ovulation rate. This is the time when ovarian follicles that are destined for further development are leaving their quiescent primordial pool. Improving the ewe's nutrition (flushing) in the 2 weeks before mating appears to alleviate the adverse effect of this earlier undernutrition.

Of course 'flushing' is a long established method of boosting ovulation rate. Ewes generally respond optimally to flushing when in medium body condition (2.5–3.5), rather than when excessively thin or fat. Thus, ewes should be condition-scored post-weaning, separated into groups and managed so that most are in appropriate body condition pre-mating.

Breeding and fecundity genes

Breeds differ considerably in ovulation rate, and cross-breeding is probably the simplest method of increasing (or decreasing) the fecundity of a flock. For example, in early or out-of-season breeding flocks, both fecundity and early breeding are essential characteristics of the ewe breed. By crossing the Dorset Horn ewe (a prolonged breeder) with the Finn ram (a prolific breed), the cross-bred female offspring posses intermediate fecundity, while retaining some ability to breed out of season. However, there are individual animals within a number of breeds throughout the world with abnormally high ovulation rates [3]. Such individuals include the Thoka (Iceland), Booroola Merino (Australia), Garole (India), Javanese (Indonesia), Inverdale Romney (New Zealand), Woodlands Coopworth (New Zealand), Belclare (Ireland), Cambridge (England), Lacaune (France), Hu and Han (China). Their prolificacy is due to a single gene or, in the case of the Belclare and Cambridge, two genes. The increase in ovulation rate for one copy of a prolificacy gene ranges from 0.4 for the Woodlands gene (maternally imprinted and only expressed when inherited from a sire) to 1.5 for the Booroola. The prolificacy gene in the Inverdale is located on the X chromosome. Ewes with a single copy of the gene (obtained by mating gene carrier rams to non-gene carrier ewes) have mean ovulation and litter size increases of around 1.0 and 0.6, respectively, but those with two copies (obtained by mating gene carrier rams to gene carrier ewes) are infertile. The development, in New Zealand, of a DNA test for the gene has led to a rapid increase in the inclusion of the gene into New Zealand flocks where all of the progeny (males and females) are slaughtered for meat production. The claimed benefits are avoidance of the reduction in litter size that often accompanies the use of superior meat-producing breeding ewes and elimination of the need for pre-mating flushing to boost lamb production. In view of the increasing number of prolificacy genes that are being identified and the variation in the inheritance patterns it is envisaged that marker-assisted genetic selection will be increasingly used to bring prolificacy more in line with environmental resources and market requirements.

Gonadotrophins

Ovulation rates can also be increased by using exogenous hormones or immunization techniques. Gonadotrophins such as eCG or ovine follicle-stimulating hormone (oFSH) can be used to superovulate ewes in embryo-transfer programmes. However, the response is variable and, while the ovulation rate of the flock or group overall can be increased substantially, some ewes apparently do not respond and others over-respond. Currently eCG is generally reserved for inducing oestrus and ovulation outwith the normal breeding season or ensuring good oestrous synchronization in a fixed-time insemination programme during the breeding season. In both situations it is given in a single injection following a period of progesterone priming. In contrast, oFSH is used for the induction of superovulation in embryo donor ewes to which, because of its short half life, it is given as a series of twice-daily injections before and immediately after the end of the period of progesterone priming.

Anti-steroid immunity

Among the many steroids secreted by the ovary is the androgen, androstenedione, which has a regulatory effect on ovulation rate through feedback on the hypothalamic–pituitary axis. Ewes immunized against androstenedione respond with significantly higher ovulation rates, but the response varies with breed and level of feeding and the bonus of additional ovulations may be nullified through high embryo mortality or excessive neonatal lamb losses. Products of this type, eCG given at progestogen withdrawal being another example, are vulnerable to misapplication, often through ignorance of the increased level of management necessary to cope with the larger litter

sizes induced by these drugs. The introduction of a very high prolificacy gene, such as the Booroola gene, presents similar problems. Mainly as a result of these problems, the androstenedione-protein immunogen product was withdrawn in the early 1990s. However, it is now available again. Despite research showing that immunization against the ovarian peptide hormone, inhibin, could increase ovulation rate there is no commercially available immunogen for inhibin. Nor is there a commercial product to exploit the experimentally proven enhancement of litter size that arises from short-term immunization against the oocyte peptide products of the genes (BMP15 and GDF9) responsible for the greater prolificacy of the Inverdale, Belclare and Cambridge breeds.

SYNCHRONIZATION OF OESTRUS AND OVULATION

A compact lambing, arising from a short mating period, can be achieved by judicious timing of ram introduction or by application of oestrous synchronization procedures that either mimic or control the function of the corpus luteum [4].

The ram or teaser effect

Suint, a mixture of secretions from the sebaceous and odoriferous skin glands in sheep, contains pheromones which, in the case of the ram, have a profound and immediate effect on anoestrus ewes that have been kept apart from males for several weeks. The effect is expressed as a rapid increase in the frequency of LH pulses, which increases the growth of ovarian follicles with a corresponding increase in their oestradiol production, which, in turn, triggers the release of the preovulatory LH surge. Therefore, in ewes approaching their natural breeding season or in those breeds and environments where anoestrus is shallow, the introduction of the ram increases the frequency of LH pulsing from the low incidence during anoestrus to the high incidence that marks the start of the breeding season and precedes the LH surge.

Ram-responsive ewes will ovulate within 2–3 days of ram introduction. However, ovulation is not accompanied by behavioural oestrus at this time ('silent heat'), since the ewes have not been subjected to a period of progesterone priming. This can be overcome by the use of exogenous progesterone and, providing their lifespan is of normal length (12–14 days), the following ovulation will be accompanied by behavioural oestrus. However, in some ewes, the CL has a short lifespan of around 4–5 days, with the result that the following ovulation also occurs in the absence of behavioural oestrus.

In practice, this means that in a group of ewes subjected to the ram effect, some will exhibit behavioural oestrus from around 18 days after joining, whereas the remainder will do so from around 24 days. Therefore, vasectomized rams introduced into a flock of ram-responsive ewes that are in anoestrus should be replaced by fertile rams 14 days after teaser introduction. This should result in most ewes being served in a 10-day period. Teaser rams need be with the flock for only a few days to produce the required effect. (The vasectomy operation is described in Chapter 74.)

Ram-induced ovulation is employed most successfully in the transitional period, when most ewes in the flock have not begun oestrous cycling but are almost ready to do so. While it can be used in deep anoestrus, the results are not usually as good and, unless the teasing is sustained by close ram-to-ewe contact (achieved by increasing stocking density), by joining with fresh teaser rams and/or by the introduction of an oestrous ewe to enhance the teasing stimulus, those ewes that fail to respond fairly quickly may remain in anoestrus for a protracted period. The major attribute of the ram effect, therefore, is that it encourages ewes to breed a few weeks earlier than they would do normally. However, the response varies from year to year, and there is a paucity of scientific information on why this should be so.

One important practical point is that if most ewes ovulate within a few days of teaser ram introduction, then they will exhibit behavioural oestrus together and, if normal ram-to-ewe ratios are used, rams will be overworked and either may not cope, or their semen reserves may deplete so quickly that conception rates and litter sizes to the first oestrus may be disappointing. To tease a flock satisfactorily one teaser per 100 ewes is adequate at high stocking densities but two or three are required for extensive rangeland systems where stocking density is very low. When the teasing process has been highly effective at least one fertile ram per 20–25 ewes will be required to cope

with the actual matings when the ewes exhibit behavioural oestrus.

In some countries, notably Australia, a favoured alternative to the vasectomy operation is wethers (castrated males) treated (androgenized) with proprietary testosterone preparations at weekly interval for 3 weeks. The response to this treatment should persist for a month or longer following the third injection, but it should be noted that, in Member States of the European Union, animals treated in this way are prohibited from entering the human food chain.

Progestogens and progesterone

Progestogen-impregnated intravaginal pessaries ('sponges') developed in Australia, or a controlled internal drug-releasing device (CIDR) developed in New Zealand, can be used in conjunction with eCG to induce oestrus and ovulation in anoestrous ewes or, in the absence of eCG, to synchronize these events in ewes during their natural breeding season. Sponges contain either of the two synthetic progestogens-medroxyprogesterone acetate (MAP) or fluorogesterone acetate (FGH) both of which are many times more potent than the natural progesterone contained in CIDRs. Blood concentrations of progestogens, in the case of sponges and progesterone in the case of CIDRs, are high during the insertion period (12–14 days), to mimic the lifespan of a normal CL. On removal, the precipitous fall in the concentrations of these hormones initiates the endocrine changes that lead to oestrus and ovulation, provided the ewes are already sexually active (oestrus cycling) at the time of sponge or CIDR insertion. If they are not sexually active, the progestogen or progesterone priming treatment must be supplemented with a gonadotrophin source, eCG, at the time of sponge or CIDR removal. The oestrus, which follows progestogen-eCG or progesterone-eCG treatment during the non-breeding season, is a fertile behavioural oestrus. However, the outcome of matings is dependent on a number of factors, in particular the ability and fertility of the rams during the non-breeding season. Generally speaking, the further in time from the normal breeding season of the ewe flock that induction of oestrus is attempted, the poorer will be the results in terms of conception and lambing rate. Other factors, such as the nutritional status of ewes and whether they are dry or suckling lambs will affect the results. It is important to recognize that when oestrus is induced during the non-breeding season, ewes that fail to conceive will return to anoestrus. Their failure to show a repeat oestrus cannot therefore be taken as a sign that they are pregnant. The exception to this would be in the transitional period, when ewes could be expected to continue oestrous cycling following induction.

Dose of eCG

The main factors affecting the dose of eCG are breed of ewe and time of year. Table 7.1 gives a guide to dosage, in international units (IU), for a small selection of breeds, which differ in the length of their breeding season. Immediately before it is used eCG

Table 7.1: Suggested doses (IU) of eCG in relation to ewe breed and month of mating at latitudes of 50–60°N

Month	Dorset Horn, Finn × Dorset	Suffolk and Suffolk Cross	Scottish half-breds, mules, greyfaces
July	600–500*	700–600	Poor results†
August	400–300	500–400	700–600
September	0	300–0	500–300
October	0	0	0

*The higher doses should be given at the beginning of the month and the lower doses towards the end.

†The poor results refer to natural matings, and are in contrast to the high conception and lambing rates that can be achieved following the intrauterine insemination of ewes of these breeds at an oestrus induced by an IM injection of 600–700 IU of eCG at sponge withdrawal.

must be reconstituted, from in-date freeze-dried powder that has been stored between +2°C and +8°C since, in solution, it degrades quickly.

For the induction and synchronization of oestrus, eCG may be given at the time of sponge removal, and at the amounts suggested in Table 7.1. For the superovulation of embryo donor ewes it may be given about 28 hours before sponge removal and at a much higher rate (1500 IU). For this purpose it may also be followed by an intramuscular injection of gonadotrophin-releasing hormone at the onset of oestrus. When ewes are being synchronized for fixed-time artificial insemination, eCG should always be used to reduce between-ewe variation in the timing of ovulation.

Damage at sponging

Although there are reports of ewes being damaged at sponge insertion, this should not happen. It is important that those not familiar with the procedure seek instruction from their veterinary surgeon or other experienced person. Not every ewe can be sponged. For some age categories, in particular ewe lambs and gimmers, the hymen may still be intact, and forcing the applicator through it may make sponge removal extremely difficult. It may even so damage the vaginal wall that the ewe is rendered permanently barren. Care should be taken also when removing sponges lest the string pulls out of the sponge. Often this can be avoided by inserting a clean, lubricated finger into the vagina, while at the same time applying steady traction on the string with the other hand. This approach ensures that the area around the sponge is lubricated and that the sponge is free of any adhesions that may have formed between it and the vaginal wall. If the string does become detached, a speculum and forceps are required to retrieve the sponge.

If sponging has not been done hygienically, there may be a degree of vaginitis, which may show as blood tinging on the sponge at withdrawal. This may interfere with the uptake of progestogen from the sponge and reduce the chance of the ewe conceiving. In early breeding flocks, sponging ewes during the summer may increase the risk of fly strike around the vulva, flies being attracted to the unpleasant smelling fluid that accumulates around the sponge and tracks back to the vulva. In addition to improving hygiene, sponges can be dusted with terramycin powder at the time of insertion, to minimize the incidence of these adverse effects.

Sponging young sheep

Ewe lambs and virgin gimmers may be sponged, although additional care must be taken and farmers must be reminded that the results are likely to be significantly poorer than in adult ewes, especially when ewe lambs are insufficiently grown. Ewe lambs should be given a small dose of eCG (e.g. 300 IU) at sponge removal, but high doses should be avoided.

Ram introduction

The timing of ram introduction following the removal of sponges is crucial. Ewes will begin to show oestrus from around 24 hours after sponge removal but most will not be in oestrus until 36–48 hours. Rams introduced immediately after sponge removal will repeatedly serve the first ewes to show oestrus and, in so doing, deplete their semen reserves, resulting in poor conception rates to the induced oestrus, a disappointing lamb crop and a more extended lambing. Therefore, rams should not be joined with ewes until 36–40 hours after sponge removal.

With this timing, most ewes will be served within 24 hours but there may be a few latecomers, so rams should be left with the ewes for at least 48 hours. They should then be removed, rested and fed until they are required to mate ewes that fail to conceive at the induced oestrus and return 15–20 days later. The rams should be joined with the ewes for the repeat matings 16 days after sponge removal and left with them for at least a week, since the synchrony is less precise at this second service.

Only mature rams should be used in synchronized matings as ram lambs are unsuitable through inexperience.

Ram-to-ewe ratios in synchronized flocks

In synchronized flocks, large numbers of ewes are mated over a very short period at the induced oestrus. During the breeding season, ram fertility should be satisfactory and one ram to 10 ewes (10 per cent) should be adequate. Outside the breeding season, ram libido and fertility are likely to be reduced and the need to resort to higher amounts of eCG to induce ovulation at this time makes sperm transport within the reproductive tract and fertilization more difficult. Therefore, the ratio should be increased to

around one ram to five ewes (20 per cent). At the repeat mating (first return) at least 3 per cent of rams should be used.

Ewes will be served more frequently if run with a group of rams rather than a single individual. Also, there is a risk in single-mating groups that the ram may be subfertile or infertile. Therefore, in pedigree flocks in which single-sire matings are essential, rams should be fertility tested close to the time of use. This may be justified in commercial flocks also, although the use of small groups, for example, 50 ewes with five rams, run in small paddocks, reduces the chance of ewes being served only by an infertile ram. Rams need not be raddled for the induced oestrus during the breeding season. Indeed, if used, harnesses may cause breast sores during this busy period if not fitted early (1 week before) and adjusted at least daily. However, rams must be raddled for the repeat services in order to identify early and later lambing groups.

Hand mating

In the absence of adequate ram numbers and if artificial insemination is not possible, hand mating can be employed. In this procedure, rams are lined-up in the shedding race and each in turn is exposed to a group of synchronized ewes. Following an observed mating, the ewe is drawn off from the main group and the ram joins the end of the ram queue, whereupon the next ram in the line-up is released to the group of unmated ewes. After mating, ewes remain separated from the main flock and are re-mated, using the same procedure, 8–12 hours later. This approach ensures a more uniform spread of the limited supply of semen across the flock.

Delaying mating

Under some circumstances conception and lambing rates may be improved if matings take place one cycle after the induced oestrus. This is because synchronization treatments can have an inhibitory effect on sperm transport. Delaying matings to the second cycle is possible, however, only in flocks that are synchronized either during their natural breeding season or close to it, so that there is no danger of them returning to anoestrus. Although the degree of synchronization is slightly reduced at the second cycle, the ram-to-ewe ratio should remain at around 10 per cent.

Artificial insemination

The high requirement for rams may pose problems in some synchronized flocks and in these circumstances the use of artificial insemination (AI), using either fresh or frozen semen, should be considered. Bearing in mind the generally lower conception rates obtained following oestrous synchronization, the sperm dose when using conventional cervical AI should be high (about 150×10^6 motile sperm, equivalent to 0.05 ml of dense semen at each of two inseminations carried out about 44 and 56 hours after sponge removal) in order to achieve lambing rates of around 70 per cent. Only fresh semen is recommended for cervical insemination. Lambing rates using frozen/thawed semen by this insemination route are seldom more than 50 per cent and often much lower, making it unacceptable.

Frozen/thawed semen can be used but it must be deposited directly into the uterus (a procedure currently carried out by laparoscopy) for acceptable results. As a result of the success of this insemination method most of the major pedigree sheep flocks in the UK are now involved in sire reference schemes for genetic improvement. Semen from selected rams is collected and frozen for subsequent distribution to flocks in widely different locations. Using the laparoscopic insemination technique lambing rates to fixed-time insemination with frozen/thawed semen in the UK sire reference schemes are around 70 per cent. The technique, however, requires special training and, in the UK, can be performed only by a veterinary surgeon. It involves a single insemination at about 56 hours after sponge withdrawal, i.e. a few hours later than for fresh semen inseminated by laparoscopy, to allow for the fact that freezing the semen induces its capacitation (change in the sperm membrane required for fertilization), thereby reducing sperm survival time in the reproduction tract.

Lambing time in synchronized flocks

Ewe conceiving at a synchronized oestrus will lamb over approximately 1 week. There should be no lambings over the following week and the repeats should lamb down in 8–10 days, the whole lambing taking around 3–4 weeks. Some of the benefits of a compact and predictable lambing are an improved ability to provide supplementary feed in relation to requirements

and a reduction in perinatal infections that tend to build up when the lambing period is protracted. On the other hand, additional skilled and semi-skilled labour, and, in the case of adverse weather conditions, housing for the flock over the lambing period, must be available to capitalize on the technique.

Alternative synchronization methods

Prostaglandin $F_{2\alpha}$ (or its analogues) can be used to synchronize groups of sexually active ewes, but has no place in out-of-season breeding programmes (other than to remove persistent CLs), because its mode of action is to cause regression of the CL.

The CL is responsive to the action of prostaglandin from around days 4–14 of the cycle (day 0 = oestrus) and progesterone levels fall to basal levels within hours of the administration of an adequate dose (12 mg of $PGF_{2\alpha}$ or, in the case of its analogues, 150 μg of cloprostenol or 6 mg of luprostiol). A suitable interval between the two injections required to synchronize all ewes in the flock is 9 days. Oestrus occurs about 35 hours after administration of prostaglandin, with ovulation about 24 hours later.

An abbreviated period (5 days) of progestogen priming followed by an injection of prostaglandin at sponge withdrawal has been shown to be highly effective for synchronizing oestrus during the breeding season. Another combination, albeit not quite as effective in that it achieves around 90 per cent as opposed to 100 per cent synchrony, is an intramuscular injection of 4 μg of buserelin, a gonadotrophin-releasing hormone (GnRH) analogue, followed 5 days later by prostaglandin. Again, this protocol is effective only in ewes that are sexually active.

Because of the additional costs incurred in using prostaglandin (which, in the UK, has to be administered by a veterinary surgeon) and the fact that the procedure can be applied to ewes only during their breeding season, the double prostaglandin technique is unlikely to be widely used. However, the treatments involving either a short period of progestogen priming (5 days) or an injection of GnRH followed, in each case 5 days later, by prostaglandin may be useful for the synchronization of additional recipient ewes at short notice in, for example, an embryo transfer programme.

Superovulation and embryo transfer

Successful embryo transfer requires close synchrony of oestrus between the donor ewe and ewes receiving her embryos, and is normally achieved by judicious timing of the withdrawal of the progestogen sponge following about 12 days of progestogen priming. For donor ewes of large body size, it may be beneficial to replace the sponge with a new one after 7 days. A suitable superovulatory protocol is twice-daily injections (about 12 hours apart) of FSH, starting on day 10 (day 0 = sponge insertion) and continuing until the day after sponge withdrawal. This represents a total of eight FSH injections over a period of 4 days, with the sixth injection coinciding with sponge withdrawal. Recipient ewes are synchronized by giving an injection of 400 IU of eCG at sponge withdrawal but, because ovulations tend to occur sooner in superovulated than non-superovulated ewes, it is normal to withdraw the sponges of the recipients about 4–6 hours before those of the donors.

Owing to the adverse effect of superovulation on sperm transport through the cervix, the preferred method of insemination in embryo donor ewes is directly into the uterus by laparoscope. Timing of insemination is critical and, for the FSH regimen just described, an appropriate timing is 46 hours after sponge withdrawal with about 50×10^6 motile sperm in each uterine horn. Embryos (blastocyst stage) for transfer to recipient ewes are collected 6 days later by methods that vary from laparotomy to laparoscopy or a modification of the laparoscopic technique. The latter involves exposure of the tip of the uterine horn through a small incision in the body wall. This facilitates the insertion of the embryo-flushing medium into the uterine horn and its collection from the horn, via a self-retaining catheter inserted by laparoscope some 2–3 cm posterior to the external bifurcation. Embryo transfer to recipient ewes is either by the laparoscopy or a modification of the laparoscopic technique, involving temporary exposure of the tip of the uterine horn through a small mid-ventral incision following its laparoscopically guided pick-up [5].

Ovum pick up and *in vitro* embryo production

The *in vitro* production of embryos from oocytes harvested by laparoscope is less traumatic than

conventional surgical embryo recovery. The technique, which involves aspiration of the ovarian follicles, can be carried out on lambs as young as 4 weeks of age. Despite their sexual immaturity ewe lambs respond well to gonadotrophin (FSH and eCG) stimulation and the numbers of *in vitro* produced blastocysts are as high as those of adult animals [6]. The technique can lead, however, to over-sized lambs and associated birth problems.

Melatonin

Melatonin treatment for out-of-season breeding was a logical progression from the use of lighting regimes, which involve the added costs of housing to induce oestrus. This hormone, which is secreted by the pineal gland, can be administered orally or by subcutaneous implant. Under UK conditions, the daily inclusion of 3 mg of melatonin in feed given to ewes in mid-afternoon (15.00 h) from late March onwards induces behavioural oestrus in mid-June. Daily timed administration is more effective than continuous release (intraruminal bolus or subcutaneous implantation) in that it closely mimics the artificial short-day treatments that are well known to advance the breeding season. On the other hand, the insertion of an implant, for example, has obvious practical advantages, but this method of administration appears to be effective under UK conditions only when initiated towards the end of June. With an approximate 60-day programming period before the reproductive axis is activated, this gives only a modest advance of the breeding season. However, when used in conjunction with the added stimulus provided by the judicious timing of ram introduction (5–6 weeks after insertion of the implant), an advanced and compact onset of the breeding season can be achieved. Under these conditions, melatonin has the added benefit of increasing ovulation rates, making them more like that of the peak breeding season.

Recent observations showing an association between alleles for the melatonin receptor 1a (Mel_{1A}) gene and aseasonality of breeding in the Merinos D'Arles in France and Small Tail Han Sheep in China imply that it may be possible, through the use of DNA markers, to incorporate out-of-season breeding attributes into breeds that currently are strictly seasonal [7].

Induction of lambing

This technique can be used where it is desired to lamb a group of ewes over a very short period. It is practical to do only when the group or flock has been synchronized in oestrus and mating dates are known, otherwise there is a distinct risk of reduced lamb survival due to excessive prematurity.

At normal parturition the birth process is initiated by the fetal lamb through a series of hormonal changes, which result in a surge of cortisol secretion, a decrease in progesterone and an increase in oestrogen. The latter triggers the release of $PGF_{2\alpha}$ from the uterus and the birth of the lamb.

Prostaglandin cannot be used in sheep for the induction of lambing because the production of progesterone from the placenta blocks its action. However, both oestrogens and corticosteroids have been used successfully, with the latter now the drugs of choice in practice.

Betamethazone and dexamethazone are the two most commonly used corticosteroids. Both are available in short-acting clear aqueous solutions and long-acting aqueous suspensions. There is variation in the response of ewes to these different preparations in terms of the time taken from treatment to lambing and in the spread of lambing. From the practical point of view, the latter is the more important, and the drug of choice would appear to be betamethazone suspension. Dose rates normally range from 8 to 16 mg, but the higher dose rate given by intramuscular injection results in a shorter time from treatment to lambing, usually between 26 and 62 hours.

This technique is used in frequent-lambing flocks, e.g. with three lambings in 2 years, where a high degree of synchrony at lambing is required both to allow for a high level of lambing supervision over a short period to ensure maximum lamb survival and also for ease of subsequent flock management. To avoid lamb deaths from prematurity, it is important not to use the technique too early in gestation. Assuming a mean gestation length of around 146/147 days for most sheep breeds, it is unwise to induce ewes on a flock basis before day 142 of gestation, although it is possible to induce individual ewes that are in difficulty with their pregnancy a few days earlier than this. Some breeds, such as the Finnish Landrace and its crosses, have shorter natural gestation lengths and have been induced successfully by injecting as early as day 138 of gestation. Depending on the chosen time for

induction, a small percentage of ewes may lamb before the treatment is applied, but this is acceptable when set against the benefits of better lamb survival. Ewes injected on the evening of day 142 could be expected to lamb from the morning of day 144 until the evening of day 145. Unlike in the cow, retained placentas and dystocia are not problems encountered following parturition induction in the ewe.

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8

Ewe management for reproduction

L.A. Stubbings

The ability of the ewe to breed successfully is the main determinant of economic efficiency in the majority of sheep systems throughout the world. While the variation in genetic potential between breeds is immense and significant advances have been made in the selection of superior genotypes, the final determinant of reproductive efficiency is most clearly linked to environmental restrictions and in particular those linked to nutritional sufficiency. The differences between biological and practical ceilings to productivity are often significant in sheep production. Genetic selection is largely based on ovulation rate. However, the application of Darwinian principles in the development of the sheep has resulted in an animal that is highly adapted to its environment and whose productivity is highly correlated to the resources on offer. Therefore, the genetic components of reproduction in the ewe are linked mainly to ovulation rate; the heritability of other traits being relatively low because of the influence of the environment.

The key to management for reproduction is twofold. First, to recognize the potential of the breed or cross and, secondly, to manage her in such as way as to optimize her output, relative to the economic returns and welfare considerations. Technological advances in recent years have served only to underline this relationship. The classic example is the immunization of ewes against androstenedione, an endogenous steroid that quantitatively inhibits ovarian follicle production [1]. A commercial preparation successfully removed the physiological restraint on

ovulation rate, but the product found little practical application because subsequent environmental limitations often resulted in no more lambs being reared and sometimes fewer lambs due to the increased demands of large litter sizes.

Shepherds have employed such strategies for many generations, basing management systems on the need to optimize lambing rate according to resources available. This chapter provides an overview of the principles behind the factors that affect reproductive performance and the practical implications this has on management of the ewe for optimal output.

NUTRITION

The impact of nutrition on the reproductive performance of the ewe was first demonstrated in the 1920s and subsequent research has elucidated some of the underlying mechanisms, while others still remain the subject of speculation. The effects of nutrition are largely mediated through the effects of energy intake and for practical purposes they can be broken down into short (current feeding levels in days or weeks), medium (weeks and months as it affects body condition) and long (months and years with effects also related to fetal and rearing phases) term effects.

Short-term nutrition

A plane of nutrition above that required for maintenance in the period just before mating (flushing) has long been recognized as having a profound influence on lambing rates. This influence is mediated through the effect of energy intake and is thought to influence all levels of the reproductive system including the hypothalamus, pituitary gland and ovary. However, recently published work [2] suggests that it is largely mediated via follicular development, with responses to and increases in the concentrations of glucose, insulin and leptin at the ovarian level in an acute (2–3 days in duration) response.

There is also a clear interrelationship between body condition and short-term nutrition (flushing), with the response to short-term energy intake being apparent only when ewes are in sub-optimal body condition in the 2–3 weeks pre-mating. This effect is mediated via an increased voluntary food intake of the leaner ewes over and above that demonstrated by fitter ewes [3]. This is shown in Table 8.1, where leaner ewes eat significantly more herbage when there is no restriction on availability. This has important implications for the management of ewes and allocation of resources. It clearly demonstrates that fit ewes do not need to be flushed, their condition alone ensuring good ovulation rates; in contrast, leaner ewes require a high herbage availability to optimize ovulation rate. It should be noted, however,

Table 8.1: The effect of pre-mating body condition on pasture intakes (dry matter/ewe/day) and lambing performance at two levels of pasture availability

Condition score					
5 weeks pre-mating		At mating	Pasture intakes (g dm/ewe/day)	Lambing rate*	Litter size†
>3.0	H	3.18	722	1.40	1.62
	L	2.96	728	1.10	1.50
2.5/2.75	H	3.05	746	1.53	1.70
	L	2.78	829	1.43	1.57
<2.25	H	2.86	1101	1.47	1.61
	L	2.46	778	0.93	1.33

H = high pasture availability, above maintenance; L = low pasture availability, maintenance only.
*Per ewe mated.
†Per ewe lambing.
Data from reference [3].

that ewes in poorer condition demonstrate lower embryonic success, so flushing only partially restores the full potential in these ewes.

Very high or very low levels of feeding in the periconceptual period are detrimental to reproductive performance. Severe undernutrition in the weeks before mating to 7 days after fertilization has been shown to have significant effects on subsequent placental and fetal growth patterns. [4, 5]. Maternal weight loss during this period affects feto-placental growth up to day 55 of pregnancy, particularly for multiple fetuses, with long-term developmental effects. Indeed, this has also been demonstrated in humans where specific long-term effects such as coronary heart disease in later life have been recorded. Overfeeding reduces embryo survival rates, possibly through increased hepatic blood flow, which increases the metabolic clearance of progesterone, high levels of which are required for early embryonic cell division.

Medium-term nutrition

Energy intake over a production cycle is reflected in the accumulation or loss of stored energy in the form of body fat. This is another demonstration of the adaptation of the ewe to her environment as she is programmed to superimpose information on the extent of her body reserves on her subsequent ability to rear a crop of lambs successfully.

Table 8.2 clearly demonstrates the positive correlation between the body condition score of a ewe at mating and reproductive performance [6]. The period between the end of one reproductive cycle (weaning) and the start of the next (mating) is therefore of vital importance since it is the time when body reserves can most easily and effectively be replenished.

Body condition can override any short-term effect of feeding level on ovulation rate, with ewes in intermediate (ideal) body condition at the time of mating unaffected by the level of feeding at the time of mating [7]. In effect, therefore, if the ewe is in optimal body condition at mating she does not need to be flushed and indeed will not exhibit the increased voluntary food intake of a lean ewe even if offered high herbage availability. This is in contrast to the lean ewe which, given the opportunity, will eat more and take advantage of the positive effect this has on follicular development.

Long-term nutrition

The onset of breeding activity and fertility at first oestrus in the ewe lamb are well known to be influenced by nutrition [2], body weight being the major determinant. To provide optimum conception rates, management must aim to attain at least 60 per cent of mature body weight at mating. A high plane of nutrition in adult life can only partially compensate for undernourishment in the rearing phase, due not only to the permanent effect on ovulation rate, but also on udder development and her overall capacity to survive and rear lambs.

Relatively recently, the effect of fetal nutrition on reproductive performance has been noted. Ewe lambs deprived of nourishment *in utero* produce more singletons in their first three pregnancies, and under severe restrictions of 50 per cent below maternal maintenance requirements the fetal ovary is impaired as early as day 47 of pregnancy [8].

Table 8.2: The effect of body condition score at mating on lambing percentage (lambs born per 100 ewes mated)

	Body condition score						
Breed of ewe	1.0	1.5	2.0	2.5	3.0	3.5	4.0
Welsh mountain	60	65	105	116	123		
Swaledale		78	133	140	156		
Mule			148	166	178	194	192
Scottish halfbred			148	170	183	217	202

Data from reference [6].

Minerals, trace elements and anti-nutritional factors

Deficiencies of a number of trace elements have been implicated in both reduced ovulation and embryonic survival rates. Copper, zinc, manganese, iron, cobalt, selenium and more recently iodine are all mentioned in the literature, but with tangible evidence only for selenium and iodine. Pre-mating supplementation of ewes grazing areas known to be deficient in selenium has been shown to reduce embryonic mortality in weeks 3–4 of pregnancy (see Chapter 54). Iodine has also been implicated in embryonic loss in New Zealand (see Chapter 67) and this may have increasing importance for flocks utilizing potentially goitrogenic forages in the peri-conceptual period. Kale, for example, contains the goitrogenic 5-methylcysteine sulfoxide which in addition to its effect on iodine requirements may also induce iron and copper deficiencies, the resultant anaemia increasing embryonic mortality.

Anti-nutritional factors to be avoided in this period include the phyto-oestrogen compounds found in the legumes, in particular red clover, the effects of which can also be long term. Coumesterol levels in lucerne, which are increased by fungal disease, and zearlenone, a mycotoxin produced by the fungus *Fusarium,* are known to reduce fertility in ewes grazing affected areas at the time of mating (see Chapter 67). Certain other plant poisons such as the steroidal alkaloid found in false hellebore cause embryonic death at about day 14 (see Chapter 56).

OTHER FACTORS

While the roles of infectious abortion agents and of other reproductive diseases are dealt with in subsequent chapters, the following section deals with factors related to management of the ewe.

Heat stress

There are many parts of the world where the effect of high temperatures on reproduction is of significant concern. Even in the UK, when mating takes place in July or August there can be periods when daytime temperatures are in excess of 30°C. Notwithstanding the effect this may have on ram fertility or mating behaviour, there may also be a reduction in embryo survival rates in such conditions. Experimentally, 80 per cent of fertilized ova can be rendered non-viable by placing ewes in rooms at 36°C immediately after mating [9], with the embryo most vulnerable in the first 3 days after fertilization. In the light of current concern regarding global warming this may become a more significant factor in some systems of production in the future. In the later stages of pregnancy, heat stress can also affect performance by reducing uterine blood flow. Those ewes most at main risk are unshorn, housed ewes and over-fat ewes at any stage of pregnancy.

Other stress factors

It has long been accepted that stress should be avoided in the pre-implantation stage of pregnancy (up to day 21). Factors to be avoided include gathering, handling, dipping and any change in diet that may interrupt nutrient supply. Scientific evidence for this is sparse and sometimes inconclusive with, for example, no significant effect of dipping in the peri-conceptual period [10]. However, others have reported detrimental effects following daily administration of adrenocorticotrophic hormone (ACTH) or repeated handling stress 4–6 hours/day in the period up to day 20, suggesting that it is the duration of the stress factor that is the determinant of any adverse effect [11].

Age

Age has a marked effect on lambing rates and, in particular, on embryo survival. Ewe lambs have a very high rate of embryonic wastage due to the inherently low potential for the fertilized ova to get to the implantation stage. Even when ova are harvested from ewe lambs and implanted into mature ewes, their survival rate is 40 per cent compared to 64 per cent for ova taken from adult ewes [12]. Shearing ewe lambs pre-mating has been shown to increase conception rates (73 vs 53 per cent to first service) and is probably linked to a short-term increase in nutrient intake, similar to that described for the adult ewe with respect to flushing.

Genetic potential

The wide variation in the genetic potential between breeds of sheep worldwide is a reflection of the diverse environmental conditions in which sheep have evolved. Most of the genetic variation is due to differences in ovulation rate rather than embryonic survival rates and is often associated with simple genes, for example the 'Boroola'. Of practical significance, however, is the fact that selection for increased ovulation rate also tends to result in an increased number of barren ewes in the population. Furthermore, embryonic wastage in the highly prolific breeds tends to occur at the implantation stage, which has important implications for subsequent lamb birth weights if it reduces the number of placentomes occupied by each surviving fetus.

Internal parasites

There is much concern internationally about the increasing prevalence of anthelmintic resistance (see Chapter 27). New UK guidelines [13] designed to reduce the selection pressure on helminths advise farmers not to drench ewes routinely pre-mating. This is based on the fact that fit, healthy adult ewes have a high degree of immunity to the majority of nematode species and therefore do not require treatment and will not respond in terms of their reproductive performance. However, immature ewes (ewe lambs and two-tooths) together with lean ewes, which are likely to have less than optimal levels of immunity, should be treated. It should also be noted that where *Haemonchus contortus* and/or liver fluke are known to be present, a strategy to control these parasites is essential for all adult sheep.

PRACTICAL IMPLICATIONS

The optimum lambing rate in a flock must be determined taking into account the resources available and potential of the ewes. The objective of the shepherd is to put these together into a management programme that will result in economic efficiency. As previously discussed, management of the ewe for reproduction involves both short- and long-term factors; in particular, body condition which requires good, long-term strategies to be in place.

Selection of breeding stock

Once the fundamental decision on breed type has been resolved, the role of management is to select animals which are 'fit to breed'. The removal from the flock of those ewes that are unlikely to conceive and/or rear lambs is essential. Culling policy is the cornerstone of the production cycle and remains the Achilles heel of many commercial flocks as they misguidedly try to reduce costs by minimizing the number of ewes they cull [14]. The number of ewes that are truly infertile (barren) is actually very small, yet the number that fail to produce live lambs is much higher at 5–7 per cent due to a failure to establish and maintain the pregnancy or to abortion (37.6 and 52.9 per cent, respectively) [15]. The number that subsequently fail to rear lambs is even higher, often in excess of 10 per cent. Ewes that are not fit to breed should be identified at all stages of the production cycle, for example at lambing if they prolapse, have little or no milk, etc. so that at weaning they can be removed. Further inspection 6–8 weeks pre-mating is also essential, with ewes that have failed to regain sufficient body condition also culled.

Pre-mating and mating management

The influence of body condition, both on mating performance and subsequent ability to sustain a viable pregnancy, is so profound that ensuring the majority of the ewes are in the correct condition at mating must be the overriding objective of management. While body condition cannot predict the performance of the individual ewe, it is an essential tool when managing groups of ewes, providing the shepherd with a relatively accurate assessment of potential and information on which to base the need for any supplementary feeding. Procedures for assessing and scoring body condition are given at the end of the chapter.

The ideal condition will vary according to breed and the lambing percentage required (Table 8.2). Lowland ewes should score 3.0–3.5 at mating; a pure hill breed would score 2.0–2.5 [6]. The time of weaning should take into account the amount of body condition to be regained by the next mating and grazing availability. Generally, a period of 10 weeks is recommended

between weaning and mating, during which time healthy ewes will regain 1.0–1.5 units of body condition (10–15 per cent of body weight) on unrestricted grazing. Condition scoring should be used at the start of this period to group according to the amount of condition to be gained, and then periodically to check that ewes are gaining as required and adjust groups and grazing availability.

Supplementation or the need for flushing will, as described earlier, depend on the body condition of the ewes and the amount of grazing available. Traditionally, many shepherds evoke the need for flushing by reducing body condition until 3 weeks before mating because they recognize that ewes in less than optimum condition at this stage respond to additional feeding in terms of their ovulation rate and subsequent litter size. However, it is important to note that this is mediated through the increased voluntary food intake of the leaner ewe. In practice, the response is reliant on the lean ewes not being subject to any restriction in forage intakes so they can exercise their increased appetite over fit ewes.

Sward height can be used as an accurate indicator of the need for supplementation to maintain nutrient (energy) intakes, because it has been correlated to herbage mass availability. Table 8.3 demonstrates the suggested levels of supplement for lowland ewes [16]. High levels of concentrate supplementation are to be avoided where possible due to the detrimental effect on embryo survival described previously.

Early pregnancy

In commercial sheep flocks, mating will normally take place over a period of about 6 weeks, which means that for any group of ewes there will be some just mated, others in the pre-implantation phase and some with established pregnancies. This has important practical implications and advice is based on the need to avoid stress, abrupt changes in diet or changes to the plane of nutrition for at least a 6-week period after mating commences. In practical terms, shepherds should aim to maintain a maintenance level of feeding through this period and be prepared to offer some supplement if grazing availability declines rapidly, which can be the case with late autumn or early winter matings in the UK. Note should also be taken of the effects of stress, in particular heat, so that ewes are given access to shade and not gathered in the heat of the day. Other routine tasks and disruptions should also be avoided in this period. Subsequent development of the placenta beyond implantation is discussed in Chapter 10.

Table 8.3: Rates of supplement for ewes pre-mating based on sward height

Grass height (cm)	Supplement required (g/ewe/day)
>4.0	No supplement
3.5	400 g
3.0	700 g
<2.5	700 g Additional forage (hay) required

Data from reference [16].

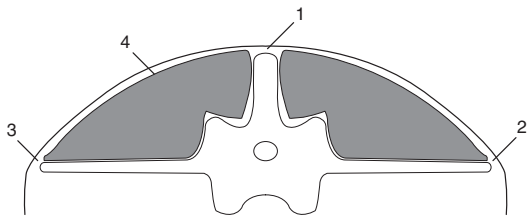
Rearing management

In addition to the management of replacements to ensure they reach optimum body weights at mating, nutrition in the rearing phase also has an important bearing on future reproductive performance. Ewe lambs that have been grown slowly, on a low level of nutrition, have lower ovulation rates than those that have grown more quickly. This is important in the UK since many replacement ewes are reared in the upland areas where there may be nutritional limitations. Purchasers of replacement sheep should therefore be wary of this when tempted to buy the cheap, smaller lambs at the end of the season. For those who breed their own replacements, the objective must be to provide for a good, planned level of growth up to mating and to remember that the potential of next generation females is also affected by events *in utero* in the peri-conceptual period of their dam.

BODY CONDITION SCORING

Assessing body condition is an important aspect of sheep management and also of considerable value in clinical appraisal. The following guide is drawn from published sources [17, 18].

To assess body condition, handle the ewes in the lumbar region, immediately behind the last rib. The prominence of the spinous (SP) and transverse (TP) vertebral processes are then felt and the

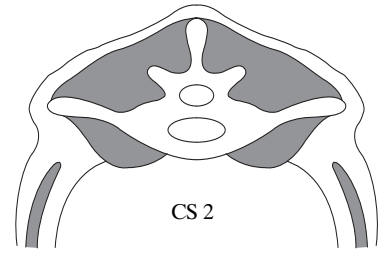
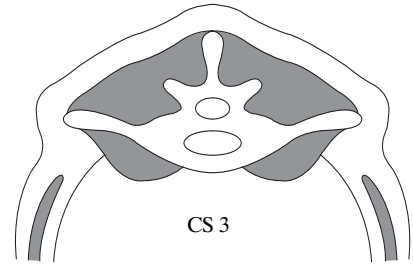
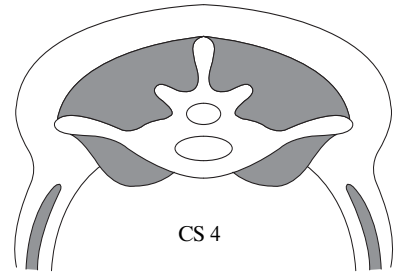


1 Prominence of spinous processes
2 Prominence of transverse processes
3 Cover over ends of transverse processes
4 Fullness of tissue between spinous and transverse processes

Figure 8.1: Anatomical features for condition scoring.

amount of eye (loin) muscle (longissimus dorsi) and degree of fat cover over both the SP and TP assessed (Figure 8.1).

From these features a scoring system from 0 to 5 has been developed that accurately reflects the body condition and hence body reserves of the ewe. In practice, half scores are also used because the difference between each whole score is relatively large. As a guide, each score equates to approximately 10 per cent of body weight, hence it takes 6–8 weeks on good grazing for a ewe to gain 1 condition score.

Score	Description	
0	Extreme emaciation. No fat or muscle detectable	
1	SPs and TPs are prominent and sharp, with gaps between the SPs palpable. No fat over the eye muscle	
2	SPs are prominent but smooth; each process can be felt only as corrugations. The TPs are smooth and rounded but it is still possible to press the fingers under them easily. Eye muscle is moderate with very little fat cover	 CS 2
3	The SPs are smooth and rounded and can only be felt using pressure. The TPs are smooth and well covered by fat, firm pressure being needed to feel over the ends. Eye muscle is full with moderate fat cover	 CS 3
4	SPs are detectable with pressure as a hard line between the fat-covered eye muscles. The ends of the TPs cannot be felt even with hard pressure	 CS 4
5	The SPs cannot be felt, only a depression between them. The TPs cannot be felt. The eye muscle is full with a thick covering of fat	

Target condition scores to be aimed for at different stages of growth and reproduction under UK sheep management systems are given in Table 8.4.

Table 8.4: Body condition targets

	Hill ewes	Upland ewes	Lowland ewes
At weaning	2.0	2.0	2.5
At tupping	2.5	3.0	3.5
Mid-pregnancy	2.0	2.5	3.0
At lambing	2.0	2.5	3.0

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9

Management and care of rams

J. Vipond and A. Greig

Rams are costly to buy yet receive relatively little extra attention on the farm. Their flock life should be three or four mating seasons, but an excessive number die or are culled while still relatively young. This may be due to insufficient management care and veterinary attention and over-reliance on concentrate feeding in early life. This chapter sets out the way to ensure rams have the right traits, mating capabilities required, and the management to ensure they work and justify their costs.

SELECTING A RAM

Replacement rams are usually purchased as ram lambs when 8 months old or as two-tooths (shearlings) at around 20 months of age. Alternatively, in pure-bred flocks, ram lambs with above average performance can be identified and retained. They will benefit from immunity to the farm disease problems, can be selected for 'get up and go' at birth and, as they can be reared less intensively than purchased replacements, have greater longevity and serving ability. Whether homebred or purchased, rams should be examined to check that they are well developed for their breed and age, without obvious physical defects and in good health. Sound feet and legs and a good mouth, with flat, square incisor teeth biting directly on to the dental pad, are required. Cheek teeth should be regular with no sharp protrusions at the outer edge as a result of uneven wear as these can damage the tissue on the inside of the cheek. The condition of these teeth can be checked from the outside by running the fingers along the jaws on each side of the face.

The ram's testicles should be palpated to confirm that they are well developed and normal. There should be two firm, evenly sized and well-formed testicles, which move freely within the scrotum; large testicles are a good feature (see Chapter 13 for recommended scrotal circumference). At the lowest part of each testicle is the epididymis which, in the breeding season, should be firm and about the size of table-tennis ball. Both the testes and epididymis should be free from obvious lumps. Ideally, rams should be bought 6–8 weeks before use, to acclimatize them to their new surroundings, and be kept separate on arrival from the established ram stud, to avoid fighting and injury. In the UK, purchased rams, as with any sheep, are potential sources of a number of infectious diseases and so should be subjected to the biosecurity measures. Under a purchaser's health plan, developed in consultation with a veterinary surgeon, quarantine for at least 4 weeks is typical. The specific diseases that need to be considered are maedi-visna (MV), sheep scab, caseous lymphadenitis (CLA), contagious ovine digital dermatitis (CODD) and resistance to one or more of the anthelmintic families. To prevent the introduction of MV, rams should be purchased from flocks which are MV-accredited, but where the MV status is unknown, the ram should be blood-tested on arrival or when aged over 12 months and again 6 months later. Such animals need to be kept in isolation until the results of the blood tests are known.

All introduced rams need to treated for both internal and external parasites. To prevent the introduction of worm resistance rams should be dosed sequentially with levamisole and one of the macrocyclic lactone (ML) products. When injected at the correct dose the latter products will also kill sheep scab mites – two doses of ivermectin brands are needed 7 days apart. Rams that come from a fluke area should be dosed with triclabendazole (see Chapter 28).

Purchased rams commonly spread CLA. Hitherto, careful examination of the area beneath the ear and around the jaw for evidence of the lumps caused by CLA has been the only 'test' available to flock-masters. However, in the UK a blood test has been developed and is used in a voluntary ram-monitoring scheme.

The full course of vaccines routinely used in the flock will ensure full protection before the breeding season commences. Vaccination against clostridial diseases is essential and pasteurella vaccination adopted where necessary. Where ticks are a recognized problem, replacement rams should be purchased from flocks within a tick-infested area. If this is not possible, or if the history is not known, ram lambs should be purchased and allowed to acclimatize for use the following year as shearlings. An unacclimatized ram that picks up ticks and develops tick-borne fever will become temporarily infertile, possibly for a period of several months. In addition to acclimatization, vaccination against louping-ill is advocated in areas where this disease occurs (Chapter 36).

RAM MANAGEMENT PRIOR TO MATING

As it takes 6–8 weeks to produce sperm, the following management timetable should be adopted.

Twelve weeks before mating

On farms where selenium deficiency is known or proven from blood test results, rams should be given an injection of a long-acting selenium preparation. Some of these products may cause a transient local reaction, so the preferred site is under the skin of the neck. Selenium is a critical component of the tail of the spermatozoa; larger numbers of ewes conceive and hold to service from selenium-supplemented rams. Blood-sampling ewes to assess flock selenium status is best done during August to November.

Six weeks before mating

Rams should be condition-scored and supplementary feeding of thinner ones started with a 16 per cent crude protein (CP) compound. Rams should be in above-average condition, condition score (CS) 3.0 but not over-fat (CS 3.5–4.0) at the start of mating. On good grazings concentrate feeding should start at around 250 g/day per head, gradually building up to around 500 g/day per head, continuing until desired condition is reached.

Concentrates and urinary calculi

Where rams are fed intensively with high levels of concentrate, urinary calculi (urolithiasis) can be a problem (see Chapter 55). The basic cause is precipitation in the urinary tract of an insoluble salt containing magnesium, ammonium and phosphate. Large stones may block the urethra with subsequent rupture of the bladder and death. Timely surgical intervention may prevent this (see Chapter 74).

The main reason for calculus formation is a high concentration of phosphate and magnesium salts in the urine brought about by excessive phosphorus (and magnesium) levels in a largely concentrate-based diet, compounded by inadequate water supply. Such calculi have never been found in grazing lambs.

Dietary factors that cause a high incidence of calculi in lambs are:

- High levels of concentrate feeding – availability of P is high from concentrates.
- Low forage intake – high forage diets reduce the availability of P.
- High P in the diet [over 4.6 g/kg dry matter (DM)].
- High Mg in the diet (over 2.3 g/kg DM).
- A low ratio of Ca:P in the diet (<1.5:1). (A high Ca:P reduces the absorption of P and so reduces urinary excretion.)
- Low water intake – increases the concentration of minerals in the urine.
- Genetics – Blackface and Texel breeds absorb more P from their diet than other breeds and are more at risk.

To prevent the formation of calculi:

- Feed diets low in P (<4.6 g/kg DM) and Mg (<2.3 g/kg DM) and maintain a high ratio of Ca:P. This ratio should be at least 2:1 but preferably nearer 3:1.
- Include 1.5 per cent salt in the diet to promote a higher water intake to dilute the urine. The total Na content should be about 6 g/kg DM.
- Ensure an adequate supply of clean water.

- Include ammonium chloride (0.5 per cent) in the diet to make the urine more acid so that crystals are less likely to start growing.
- Feed less concentrates and more forage.

Home-mixed concentrates should be supplemented with an appropriate mineral/vitamin mix, high in Ca (>25 per cent) and sodium (salt) and contain no P, Mg or copper. Other trace elements and vitamins should be included at the normal rate.

Dipping rams in organophosphate or high *cis*-cypermethrin dips will remove external parasites, but close to the breeding season rams are more susceptible to absorption of dip, thus avoid over-strength solutions, preferably dipping them after the ewes. Penning purchased rams after dipping with stock rams can reduce fighting. Examine the scrotum and sheath for any sign of thickening or abscesses, and seek veterinary advice if such abnormalities are found to be present; remove excess wool from the scrotum.

Check the brisket for sores which, if present, should be treated with an appropriate preparation. If there is no response to treatment, seek veterinary advice immediately. All feet should be examined for evidence of foot-rot, CODD or interdigital growths and appropriate treatment applied or veterinary assistance sought. Heavy breeds of ram are particularly prone to foot problems, and it is worth having them vaccinated against foot-rot 6 weeks before mating.

Rams release pheromones that help bring ewes into season. By keeping them completely separate from ewes until mating, a synchronization effect is produced, reducing the lambing period to around 3 weeks. In very hot weather, ensure rams have shade or house them in a large airy building, but do not run them on bedding that can generate heat, as this is detrimental to sperm production.

Two weeks before mating

Check condition score and adjust feeding if necessary. Re-examine the brisket and feet, and treat if necessary. If non-hardy breeds of ram are used on hill ewes, they should be trained to eat concentrates from a bucket tied in a high position to an all-terrain vehicle. They can then be fed easily among the ewes during the mating period.

Check that the testicles move freely in the scrotum, feel firm but not solid, are free from any hard lumps and have an epididymis the size of a table-tennis ball at the base. Examine the sheath and tip of the penis by putting the ram into the sitting position. The more upright the ram is sitting, the easier it is to protrude the penis, by gently pressing back the sheath with one hand while easing the penis upward with the other hand. A number of infections can affect the sheath or penis, which can prevent the penis being fully erected. Growths, pustules or ulcers at the opening of the sheath require veterinary attention.

Have any ram with doubtful reproductive capability examined by a veterinary surgeon who, in addition to a full physical examination, will probably take a semen sample to be evaluated with regard to sperm density, motility and abnormalities. A decision on whether or not to use the ram can then be taken.

MATING MANAGEMENT AND RAM TO EWE RATIOS

Rams (in particular ram lambs) should be monitored to confirm that they are able to mount and to serve properly. Traditional ram to ewe ratios in intensive flocks in the UK are one ram lamb per 30 ewes, and one experienced ram per 40 ewes (typically at least three rams in a group mating situation is preferred as single sire mating groups increase risk of reproductive failure and reduce lambing percentage). However, these ratios are not based on experimental trials. This contrasts with New Zealand studies with experienced rams and mature ewes (older than two-tooth) when no reduction in flock reproductive performance was evident at one ram to 210 ewes. Large-scale New Zealand farms use ratios of 1:100–150 regularly. The difference in ratios may be explained by differences in husbandry – in New Zealand, rams are not normally supplemented with concentrates or brought out by high feeding levels for sale or show as ram lambs. When carried to extremes these practices significantly depress mating ability. Several UK farmers now regularly use ram: ewe ratios of around 1:100 with the following provisos:

- Rams are not fed concentrates, are mature and in good condition score.
- Ewes have already had one crop of lambs previously and are in good condition and are on quality pasture (rule of thumb – graze half the sward height on offer then move on, e.g. start at 8 cm move at 4 cm, this gives high stocking rates and

avoids ewes grazing at low sward heights on poor quality material).
- Rams are run in groups of at least three rams (e.g. three rams + 300 ewes).

In hill flocks the normal ratio is three rams per 100 ewes as the rams may have much more ground to cover. Usually, two rams are turned out initially with the third being introduced after 17 days.

BATCHING EWES BY LAMBING DATE AND IDENTIFYING NON-WORKING RAMS

The use of a coloured crayon held in a harness (raddle) is a useful means of determining that lowland and upland rams are working satisfactorily. Rams with a raddle will leave clear marks on the ewes' rumps. These should only be used on all rams from the start of the tupping period where it is necessary to batch ewes by expected lambing date owing to shortage of lambing accommodation. Where all ewes can be housed at the same time, the use of raddles or marking fluid smeared on the brisket area will identify late lambers for delayed supplementary feeding. This is best achieved by raddling rams after the first 3 weeks of mating. In many situations, e.g. flat rate feeding or outside lambing on grass, the use of raddles is not justified. However, raddling with observation is useful to check that newly purchased rams are working. Where possible, rams should be inspected daily. Supplementary feeding of trained rams helps to maintain condition and allows raddle changes to be made without the stress of gathering the flock. Harnesses for raddles need adjustment as rams lose condition. Problem rams should be removed for treatment, and replaced if there is any doubt about their ability to work. In extensive hill conditions ewes can be observed from a distance to check that there is a ram with them but it is a mistake to gather ewes to the ram daily as the stress induced causes failure to conceive.

A ram in a single-sire mating group that is not serving ewes properly or is infertile will be seen surrounded by ewes 16–17 days after turnout. However, where several rams are run together at mating, ewes are likely to be served by each ram present, which results in a higher conception rate. Unless a specific pedigree mating programme is necessary single-sire mating groups should be avoided. Once rams are proven fertile, they generally do not need further testing other than the annual pre-tupping visual and handling check.

In purebred flocks the preferred ewes are those that hold to first service. Since the oestrus cycle lasts around 17 days, the preferred practice is to breed only pure replacements from ewes mated in the first 3 weeks of tupping. Purebred rams are then removed and a terminal sire used to sweep up. This saves valuable purebred rams from long tupping periods and excessive weight loss thereby increasing their useful life. When the rams have completed their work, they should be gathered together and managed apart from the main flock.

MANAGEMENT AFTER MATING

After mating, any rams considered unfit for a further year should be culled. Broken mouths, chronic lameness and 'old age' are the usual reasons for culling. Three-year old rams in hill flocks are often sold on to other farms to avoid father/daughter matings, which leads to inbreeding and reduced performance of ewes. These rams can work satisfactorily for a further year or two on lowland farms. Rams to be retained can be fed *ad libitum* hay plus 0.5 kg of concentrate (e.g. cereal, beet pulp or a 16 per cent CP compound ration) until CS 3.0 is reached. Ram lambs need extra supplementary feeding and should be fed separately from older rams; this also reduces the risk of spreading CLA. Cattle concentrates should not be used for rams as they may contain high levels of magnesium or copper.

Any open wounds around the head or brisket should be treated. Excess hoof growth should be trimmed and foot-rot treated using a footbath containing a 10 per cent solution of zinc sulfate. If there is any risk of ectoparasites, treatment by dipping or use of a pour-on should be carried out. In fluke areas, treatment for fluke is advisable. In the case of horned rams, check that horns are not touching the face: it should be possible to pass fingers between the horns and the head. In adult rams, up to 1–2 cm thickness of horn can be removed by cutting back parallel to the face.

WINTER FEEDING AND TREATMENT

Rams in reasonable body condition (CS 2.0–2.5) can be fed hay or silage to appetite with 0.2–0.3 kg of a 14–16 per cent CP sheep concentrate fed per head daily. Housed rams need 2 m^2 of space per head and trough space of 60 cm. Monitor health and body condition monthly. Free access to minerals is not recommended but if the ewes receive trace element supplementation, e.g. cobalt bullets, then treat the rams also. Copper supplementation should be discussed with your veterinary surgeon.

SUMMER AT PASTURE

Rams should be run on reasonable grass with shade available. A worming dose in May/June at shearing is recommended, particularly if on a pasture used year after year for the same purpose. In hill flocks, the rams can be run on to the open hill. They tend to stay together within a relatively well-defined area and usually do not mix with the ewes to any great extent. Rams may fight after shearing owing to their altered appearance, but are less able to do so if penned tightly together for a brief period.

Protect rams from pneumonia with a combined clostridial/pneumonia vaccine boost in April. To prevent head fly damage, routine summer dipping and/or pour-on treatment should be carried out. When treating with anthelmintics, ensure they receive a full weight-related dose, as rams are typically 30 per cent heavier than ewes of the same breed. Withhold food from rams for 12–24 hours before using the benzimadazole anthelmintic drenches, to increase the effectiveness of the treatment.

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10

The perinatal period

D.J. Mellor and J.C. Hodgson

The time just before, at and soon after birth (i.e. the perinatal period) arguably represents the most hazardous period in the life of a sheep. Annually, perinatal death averages 15 per cent or more of all lambs born in the UK, Australasia and elsewhere, and constitutes the greatest single source of loss to the sheep industry. In addition, impaired health and performance of newborn lambs (neonatal debility) is fairly common and represents a further loss that is less easy to quantify. However, nutrition of the fetus has a significant influence on its future productive performance [1].

Many causes of death and debility have been identified. Infections can be responsible for most problems in intensive indoor lambing systems, resulting

from animals in close contact and exposure of lambs to high levels of environmental infectious agents. However, taking all systems of production into account, available figures indicate that only 10–30 per cent of lamb deaths are attributable to infections, but the percentage may increase with increasing use of intensive rearing systems. Of the remaining 70–90 per cent, functional disorders (i.e. physiological impairments or pathophysiological changes) are the largest source of loss.

GENERAL PHYSIOLOGICAL CONSIDERATIONS

Development

The development of the embryo, fetus and newborn involves three interlinked processes: (1) *differentiation* or the formation of individual organs and tissues from the homogenous early daughter cells of the fertilized ovum; (2) *growth* or an increase in cell numbers (hyperplasia), cell sizes (hypertrophy) or both; and (3) *maturation* or the attainment of an adequate functional capacity of individual organs and tissues and their integrated operation in the whole animal. The progress of development is influenced by an inherent drive of organs and tissues to differentiate, grow and mature; processes that are presumably programmed genetically, and by environmental effects on these processes. In lambs, differentiation occurs mainly during the first 30–40 days after conception, growth is continuous although variable until adult size is attained, most maturation occurs continuously and at a variable rate until a few weeks after birth, and environmental influences operate throughout. The physiological state of a lamb at any particular time therefore results from all preceding interactions between the complex of factors that determine its stage of development and the effects of the environment. During the perinatal period particularly, marked changes occur.

Birth transitions in the lamb

Maturation of numerous fetal organs and tissues accelerates during the final 2 weeks of pregnancy in final preparation for the physiological adjustments which are necessary if the lamb is to survive its expulsion from the uterus at birth [2]. Birth usually occurs about 147 days after conception. At that time the fluid-breathing fetus must rapidly become air-breathing to survive the termination of placental gas exchange, a process that requires lung maturation, the establishment of pulmonary respiration and marked cardiovascular adjustments.

The fetal kidneys must mature sufficiently before birth to allow them to replace the placenta postnatally as the major organs of fluid and electrolyte balance and excretion. The loss of thermal insulation and placental nutrient supply necessitate a marked increase in heat production, which at first must be fuelled entirely from the lamb's body energy reserves; the mechanisms which achieve this must therefore be operational at birth.

The quiescence of lamb behaviour *in utero* must be replaced by the relatively vigorous activities of standing and teat-seeking. Subsequently, the onset of sucking introduces large volumes of nutrient-dense colostrum into the gut for the first time, and this both requires and induces gastrointestinal and metabolic adjustments.

Finally, the expulsion of the lamb from a sterile into an infective environment means that the lamb must acquire or develop the ability to resist pathogens soon after birth, achieved mainly by the passive acquisition of antibodies from colostrum and maturation of adaptive and innate aspects of immunodevelopment, such as gut closure.

Labour and birth

The maturational changes in the lamb during the final 2 weeks of pregnancy are accompanied by a complex sequence of hormonal interactions which remove a block to, and actively stimulate, contractions of the uterine muscle, cause relaxation of the cervix and delivery of the fetus(es). Important features of this sequence of events are reductions in placental progesterone and increases in placental oestrogen production, which cause parallel changes in the concentrations of these hormones in maternal plasma [2]. However, these hormone changes not only affect uterine activity, they also influence other functions in the ewe including the onset of milk production and ewe–lamb bonding [2].

Onset of milk production

Udder development during pregnancy consists of a massive increase in cell numbers associated with proliferation of the collecting ducts and terminal differentiation of milk-producing alveolar cells. Most visible udder growth occurs during the final 6 weeks of pregnancy. Just before parturition, however, there is a rapid transition from growth to secretion, manifest first when the alveolar cells begin to produce and secrete specific milk products, which accumulate in the gland as precolostrum and then colostrum.

The onset of copious milk secretion occurs soon after birth, when colostrum is sucked from the udder by the lamb, and thereafter milk production usually increases continuously for 1–3 weeks. The transition at birth from udder growth to secretion depends largely on the withdrawal of progesterone, which inhibits milk formation, and surges in prolactin, cortisol and oestrogen, which stimulate milk formation.

Withdrawal of progesterone also allows greater mammary blood flow, which increases the supply to the udder of nutrients, including precursors of milk constituents. Undernutrition during the last third of pregnancy raises plasma progesterone concentrations and depresses colostrum production [3].

Ewe–lamb bonding

Maternal behaviour patterns, leading to successful ewe–lamb bonding and ensuring that each ewe dries, protects and feeds her offspring, are under a large measure of physiological control. The odours of amniotic fluid, which at most times are repellent, usually become attractive to ewes at birth. The attraction is general initially, the fluid on virtually any lamb being acceptable, but rapidly becomes specific.

This period of general receptiveness to lambs is linked to the transient rise in maternal concentrations of oestrogen that occurs at birth, and is reinforced by expansion of the cervix and vagina during expulsion of each fetus. Thus, ewes are strongly motivated to lick newborn lambs, and this introduces them to the unique odours of each lamb, which they learn to discriminate as the period of general receptiveness fades during the first 3–5 hours after birth. In addition, licking helps to dry the lambs and may increase arousal and stimulate respiration; it also elicits from the lambs various behavioural and vocal responses, which further stimulate maternal interest and activity and lead to the lamb being accepted at the udder. Although the interacting mechanisms that induce maternal behaviour in ewes at birth have yet to be clarified fully, key features are the transient oestrogen rise and mechanical stimulation of the genital tract during fetal expulsion.

Integration of perinatal events

It is evident that a wide variety of activities in the fetus, placenta, uterus, udder and elsewhere in the mother need to be integrated if birth is to have a successful outcome [2]. The initiation and coordination of many of these diverse events is achieved by a single physiological signal originating in the fetus.

There is a marked rise in the activity of the fetal adrenal cortex, which progressively increases the secretion of cortisol during the final 2 weeks before birth. This cortisol surge has direct fetal effects, either by acting alone or synergistically with other hormones, or it acts indirectly in the fetus and mother by causing changes in the production of several fetal, placental and maternal hormones. By this means, fetal maturation, labour, delivery, milk production and ewe–lamb bonding are initiated and sequenced appropriately [2].

PATHOPHYSIOLOGICAL CHANGES

The border between normal and impaired function (i.e. between physiology and pathophysiology) is reached when an animal's well-being is threatened.

Placental insufficiency

Placental development begins about 30 days after conception. Placental weight increases until about 90 days gestation and thereafter remains fairly constant, but proliferation of blood vessels continues throughout and is associated with marked increases in blood flow to both the fetal and maternal sides of the placenta. However, placental size varies widely between fetuses at the same stage of gestation due to competition between litter mates for the fixed number

of implantation sites, variations in maternal nutrition or to other unknown factors. As a result, inadequate placental development accompanied by impaired transfer functions is usual in a small proportion of ewes in most flocks [2]. The most obvious consequence of placental insufficiency is fetal growth retardation.

During the final 30–40 days of pregnancy, low-weight fetuses of this type exhibit: (1) a long-term oxygen shortage (chronic hypoxaemia) with associated elevations in haematocrit and plasma concentrations of lactate and cortisol; (2) a chronic nutrient deficiency associated with low plasma glucose and fructose concentrations; and (3) other metabolic and hormonal disturbances. Fetal death is common when placental weight is near the bottom of the normal range and is probably due mainly to the restriction on oxygen and nutrient supply. Even when such fetuses survive to term, effects of the hypoxaemia persist, inhibit the lamb's heat production response to cold stress after birth and thereby increase its susceptibility to fatal hypothermia. However, in many cases these effects are transient, so that such lambs can be saved by keeping them in warm air (38–40°C) usually for no more than 6–10 hours, until the effects of the oxygen shortage have passed.

Intrapartum hypoxaemia

Even when placental weight is above average, fetal hypoxaemia can occur during birth as a result of umbilical cord compression and/or protracted labour [2]. Short-term or acute hypoxaemia, with its associated elevations in plasma lactate and cortisol concentrations, can also inhibit heat production sufficiently to jeopardize the survival of newborn lambs. In these cases, however, the inhibition lasts for only about 30 min, so that support for short periods in warm air (38–40°C) will usually overcome this problem.

Maternal underfeeding

Underfeeding ewes between about 30 and 90 days of gestation (the period of greatest placental growth) impedes placental development, so that proportionately more fetuses enter late pregnancy with small placentas and consequently must face the hazards described above. Moreover, if the underfeeding continues, such fetuses have the extra handicap that their nutrient supply will be reduced even further by the combined effects of placental insufficiency and maternal underfeeding. Underfeeding during early gestation may also retard fetal ovarian development, with the long-term potential to reduce adult ovulation rates [4]. This is an example of fetal programming of adult physiological capability, whereby events during fetal life having potential detrimental effects during adult life [1].

When ewes carrying fetuses with average or above average placental weights are underfed during late pregnancy, oxygen supply to their fetuses is not impeded and fetal death is rare. However, the inadequate nutrient supply does retard fetal growth, depletes fetal energy reserves, especially fat, and causes fetal hypoglycaemia with associated elevations in plasma cortisol and reductions in plasma fructose concentrations [2]. Postnatal heat production is not inhibited in lambs of this type unless they experience intrapartum hypoxaemia. However, their depleted energy reserves do reduce the period over which heat production can be sustained in the absence of feeding. This can be life-threatening, because undernourished ewes may exhibit poorer maternal care and abandon their lambs, and they do produce less colostrum and milk [2].

Moreover, colostrum composition may affect the absorption of macromolecules, as the absorption of marker immunoglobulin given by stomach tube 4 hours after birth is higher in lambs receiving colostrum from undernourished than from well-nourished ewes [5]. Recent work suggests that bioactive compounds present in colostrum, such as retinol and insulin-like growth factors and their binding proteins, in combination, may alter the absorption of macromolecules [6].

Specific effects of some dietary deficiencies have been defined. For example, lambs from ewes deficient in cobalt are apparently slower to start sucking and have lower plasma concentrations of immunoglobulin G than do lambs from cobalt-sufficient ewes [7]. Selenium deficiency may lower resistance to cold stress [8] and, conversely, chronic cold exposure of ewes induced by winter-shearing 4 weeks before lambing may have indirect beneficial effects on the newborn lamb by increasing the activity of the selenium-containing enzyme, type 1 iodothyronine 5′-deiodinase, in its tissues at birth, leading to increases in plasma tri-iodothyronine concentrations and in the thermogenic activity of brown adipose tissue [9].

Supplementing ewes during late gestation with vitamin E can also lower mortality amongst lambs born during the early part of the lambing season [10].

Premature birth

Restrictions on fetal oxygen and/or nutrient supply cause an early rise in the fetal plasma concentrations of cortisol which starts the birth process, so that it is not surprising that premature birth is a common consequence of placental insufficiency and severe maternal underfeeding during late pregnancy. Maternal stress can also cause premature birth, possibly because of placental transfer of maternal cortisol to the fetus. Many premature lambs are also immature and succumb quickly after birth, but some thrive, presumably because the fetal cortisol rise, although early, is protracted and large enough to allow adequate tissue maturation before birth.

Starvation of the newborn

A total or partial failure to ingest sufficient colostrum or milk occurs when lambs fail to suck adequately owing to weakness, competition with litter mates or inadequate mothering, or when colostrum/milk production is deficient. Such starvation has several pathophysiological consequences.

First, the lambs become hypoglycaemic as their body energy reserves are depleted and this leads to cerebral compromise in warm conditions and to hypothermia when cold. The hypoglycaemia can be exceptionally severe, with plasma glucose concentrations of about 0.5 mmol/1 compared to 4–8 mmol/1 in fed lambs. Starved hypothermic lambs with such low plasma glucose concentrations are protected from cerebral compromise only by their low body temperatures and the associated slow rates of brain metabolism. If they are rewarmed without providing additional glucose by intraperitoneal injection they will die in convulsions. The quantities of colostrum/ milk required to meet a lamb's energy needs and prevent hypoglycaemia during the first day after birth are surprisingly high; indoors (still, dry air at 2–10°C) a lamb needs about 210 ml/kg body weight, and outdoors (0–10°C, wind, rain) about 280 ml/kg [11]. Well-fed ewes produce more than enough colostrum to meet these needs, and hand milking ewes after injecting them with the hormone (oxytocin), which causes let-down, is a convenient and practical way of accumulating supplies of colostrum/ milk to feed surplus or orphaned lambs [11].

Second, starvation impedes normal gut maturation and growth, which could have long-term deleterious effects in those lambs exposed to short periods of starvation after birth [2].

Third, starvation deprives the newborn lamb of its only significant source of immunoglobulins which, in fed lambs, act within the gut or after absorption into the blood stream, or both, to help provide protection against infections that cause diarrhoea, watery mouth, pneumonia, septicaemia and other conditions. However, when lambs consume enough colostrum/ milk to meet their energy needs their intakes of protective antibodies are usually sufficient [2].

Use in diagnosis

The pathophysiological changes have been used to determine criteria which allow different forms of functional impairment to be identified in newborn lambs (Table 10.1). The necessary critical assessments are comparatively few and straightforward (weight, rectal temperature, haematocrit and the plasma concentrations of lactate and fructose at about 15 min after birth, and the age at death) and permit the identification of lambs that have experienced placental insufficiency, acute intrapartum hypoxaemia, inadequate thermogenesis and starvation (Table 10.2). Investigation of a commercial flock with an 18 per cent neonatal mortality rate showed that prenatal factors contributed to 71 per cent of the deaths, postnatal factors to 13 per cent and 16 per cent were undiagnosed [12]. That prenatal physiological impairments can be major sources of neonatal lamb loss needs to be borne in mind when strategies for improving neonatal health and vigour are being devised.

Table 10.1: Criteria for causes of death in lambs based on altered haematocrit, plasma composition, birth weight and rectal temperature measured soon after birth

Category	Diagnostic variable	Pathophysiological state indicated
Placental insufficiency	High haematocrit	Chronic fetal hypoxaemia
	High plasma lactate	
	Low plasma fructose	
	Low birth weight	Fetal undernutrition and growth retardation
	Low rectal temperature	Inhibited heat production
	Age at death <12 hours	
Acute intrapartum hypoxaemia	Haematocrit not high	Acute fetal hypoxaemia
	High plasma lactate	
	Low rectal temperature	Inhibited heat production
	Age at death <12 hours	
Inadequate thermogenesis	Haematocrit not high	Fetal normoxaemia
	Plasma lactate not high	
	Low rectal temperature	Excessive heat loss or inhibited heat production
Starvation	Normal haematocrit	No prenatal or intrapartum predisposing factors
	Normal plasma composition	
	Normal birth weight	
	Normal rectal temperature	
	Age at death >12 hours	

Table 10.2: A comparison of some features (mean ± standard deviation) of newborn lambs that survived and those that died, assigned to four pathophysiological categories [12]

		Lambs which died			
Diagnostic variable	Surviving lambs	Placental insufficiency	Acute intrapartum hypoxaemia	Inadequate thermogenesis	Starvation
Haematocrit (ml/dl)	46.0 ± 5.9	54.0 ± 6.4	42.0 ± 4.7	41.0 ± 5.2	45.0 ± 4.3
Plasma lactate (mmol/l)	8.7 ± 3.9	14.5 ± 5.1	14.5 ± 4.9	6.4 ± 2.43	6.5 ± 3.9
Plasma fructose(mmol/l)	1.8 ± 0.6	1.3 ± 0.4	1.8 ± 0.4	1.48 ± 0.48	2.0 ± 0.6
Birth weight (kg)	4.2 ± 1.0	2.6 ± 0.9	3.5 ± 0.7	3.60 ± 0.90	4.2 ± 1.2
Rectal temperature (°C)	37.9 ± 1.3	33.9 ± 2.7	35.8 ± 2.3	36.0 ± 3.7	39.1 ± 0.6
No. of lambs	600	20–24	31–34	11–12	11–13

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Part IV
Reproductive diseases

11

Genital abnormalities, obstetrical problems and birth injuries

J.C. Hindson and A.C. Winter

There are significant areas of reproductive loss in sheep even when nutritional inputs are optimal throughout the year and in the absence of reproductive infections. Such losses are made up of ewes that fail to breed because of abnormalities of the genital tract, prolapses and their complications (Chapter 14), and those associated with parturition. About 5 per cent of ewes in the UK die annually, three-quarters in the peri-parturient period. In addition, approximately 6 per cent of ewes fail to lamb each year. A survey of over 5000 ewes in the UK showed that 0.3 per cent were anoestrous, 0.3 per cent failed to become pregnant despite multiple matings, 2.4 per cent aborted and a further 3.4 per cent were barren, a total reproductive failure rate of 6.4 per cent [1]. In a slaughterhouse survey of over 33 000 sheep, the same author found that 6.6 per cent of ewes and 1.9 per cent of nulliparous sheep showed abnormalities of the reproductive tract [2].

FAILURE TO BREED

Developmental abnormalities

Smith *et al.* [2] described the range of congenital reproductive abnormalities that occur in sheep. These include cystic structures, hypoplasia or aplasia of part or the whole of the reproductive tract, uterus unicornis, fusion of the ovaries and intersex. Intersex sheep may show abnormalities of the vulva (underdevelopment or enlargement), clitoris (enlargement and protrusion from the clitoral fossa), shortened vagina or abnormally small teat size. Occasionally, gonads may be palpable in the inguinal region. Karyotyping may be used to identify animals with chromosomal abnormalities. Intersex animals have been reported in breeds producing large litters, such as the Cambridge.

Without normal ovaries, animals will not have an oestrous cycle and will not mate; therefore they can be identified and removed from the flock providing raddle markers are being used on the rams. Ewes with normal ovaries but defects of other parts of the tract are likely to show normal mating behaviour but fail to become pregnant. They can be identified if the raddle colour markers on the rams are being changed regularly, or are detected at scanning.

Acquired defects

Smith *et al.* [3] also reported on acquired reproductive abnormalities; 6.6 per cent of almost 10 000 cull ewes showed abnormalities such as bursitis, parametritis, abscesses and mascerated fetal remnants. Nulliparous sheep also showed acquired abnormalities such as hydrometra and follicular cysts. Animals with acquired abnormalities but functional ovaries may mate in the normal manner but repeatedly return to oestrus. In multiparous ewes, many of these abnormalities are presumably sequels to lambing difficulties. Laparoscopy to examine the ovarian areas may help in making a diagnosis.

OBSTETRICAL PROBLEMS AND DYSTOCIA

The normal delivery of a healthy full-term lamb and the rapid transfer of a 'parasitic' fetus living on a life-support mechanism in a fluid environment, through a rigid passageway of restricted size to an immediate

free-living state without physiological damage occurring is an event to wonder at. In a survey of 15 584 ewes, the incidence of dystocia was recorded as 3.1 per cent (3.5 per cent with single lambs and 1.3 per cent with twins) [4]. Manipulation of breeding, in the search for maximum output, increases the risk of dystocia. Changes in carcass conformation to increase yield of meat, removal of nutritional constraints to fetal growth, together with increasing tolerable litter size may all have an adverse influence. Work in New Zealand revealed a dystocia incidence of 20–31 per cent in a Romney stud flock [5], but this was reduced to 4 per cent or less within 4 years by culling ewes that required assistance at lambing and by selecting rams that sired lambs of lower birth weight. The application of severe culling, or even no intervention at all so that those ewes and lambs that get into difficulty do not survive to breed further, has been the basis of the development of 'easy care' sheep. In the Coopworth breed, mothering ability is central to selection of the next generation. Where this type of selection takes place, there is much higher maternal ability and better lamb survival compared with systems with no such selection. Scott [6] has drawn attention to the welfare aspects of common ovine obstetrical problems.

Dystocia

The most common types of dystocia in ewes are failure of the cervix to dilate (ring womb), malpresentation or posture of the fetus(es) and fetal oversize. In a survey of 328 dystocia cases submitted to a veterinary practice in west Wales, at least 50 per cent were due to presentation or postural abnormalities, and 26.8 per cent were the result of failure of the cervix to dilate [7]. Unless dealt with early and skilfully, cases of dystocia lead to losses of both ewes and lambs (see Figures 11.1 and 11.2 in the colour plate section). Losses in ewes can result from unskilled assistance causing damage or infection of the reproductive tract. Losses in lambs arise through factors such as hypoxia, subdural haemorrhages, fractured ribs, ruptured liver and as a result of poor mothering ability on the part of the dystocic ewe.

Failure of the cervix to dilate ('ring womb')

This is a complex problem due to many causes rather than one alone [8]. While the condition can appear to be idiopathic, it can be a complication of abortion or premature birth, since the absence of the normal hormonal cascade preceding parturition (decreasing progesterone, increasing oestradiol, prostaglandin and relaxin) will have left the cervix unprepared for the normal response to uterine contractions. The condition is also a common complication of prolapse of the cervix. The ingestion of foodstuffs contaminated with the fungus *Fusarium graminareum*, which has oestrogenic activity, also has been implicated. Ring womb can arise also where no fetal extremity is presented into the internal cervical os, with a lamb in transverse presentation for example, but in these cases manual dilation of the cervix is usually possible, since normal softening of the cervix will have occurred.

Malpresentation, position and posture

These are the commonest causes of dystocia. Some result from chance coincidence between random fetal movements and uterine or abdominal expulsive efforts, but many are associated with lack of space within the uterus. Large single lambs and large litters increase the risk, since there is insufficient space for the fetus(es) to adopt the extended posture for parturition to progress smoothly. Other cases can arise because of stress or disturbance of ewes, particularly immature ewe hoggs, through bad management practices interfering with the normal behaviour of the ewe during the early stages of parturition. There are many variations of abnormal presentation or posture. With anterior presentations, deviation of the head with retention of one or both forelegs, because of carpal or shoulder flexion, is common, as too is presentation of the head only. With posterior presentations, which form about 10 per cent of deliveries, hock flexion or breech presentation are the most common problems. Transverse presentations also may occur, probably the result of prolonged uterine contractions acting on a fetus already in an abnormal position. Most malpresentations are capable of correction by a competent person adopting a basic approach of correct diagnosis and use of adequate lubrication and other aids.

Oversize

This may be defined as relative or absolute. Relative oversize occurs when the ewe is unable to achieve

normal delivery, but the obstetrician can achieve it with full lubrication and careful traction. The aim is to deliver the lamb in such a position that the minimum diameter is presented at the pelvic inlet and minimum friction occurs during traction. Relative oversize often appears as a malpresentation, as expulsive efforts may force the head alone, or head and one limb, or limbs alone through the pelvic inlet. Absolute oversize is present when it is highly unlikely that the whole lamb will pass through the pelvis without trauma; in such a case surgery should be the method of choice, not a last resort.

Hindson [9] suggested a formula (slightly modified, below) to determine whether delivery is possible. This takes into account the most important factors governing ease of lambing, i.e. size of the ewe's pelvis, size of the lamb as estimated by limb diameter, parity of the ewe, whether the lamb is in anterior or posterior presentation and whether the ram used was of exaggerated muscular conformation. Ratio (R) of feto/maternal disproportion is given by

$$R = \frac{M}{F} \times \frac{P}{B} \times \frac{1}{E}$$

where M is the inter-ischial diameter of the ewe (cm); F is the digital diameter of the fetus measured at the fetlock (cm); P is the parity (primiparous = 0.95, multiparous = 1); B is the presentation (posterior presentation = 1.05, anterior presentation = 1); E is the conformation type of ram (exaggerated muscular type = 1.05, normal conformation ram = 1).

If $R = 2.3$ or less, surgery is indicated, if $R = 2.1$ or less, surgery is essential.

Oversize frequently is a flock problem. It is common in ewe hoggs, which are often mated when too immature; these must be at least two-thirds of mature body weight at the time of mating. Their feeding must be carefully controlled during the later stages of pregnancy to avoid overfatness and excessive growth of single lambs. Regular condition scoring and scanning for fetal numbers are good practices by which to avoid these problems. The search for extra carcass yield and 'improved' conformation has also increased the potential for oversize. Breeds with heavy musculature have wide thoracic and hindquarter diameters even as lambs, and this heavy muscle development requires thicker and stronger bone formation. Inevitably, one consequence is thicker bone in the maternal pelvis, potentially reducing the pelvic inlet area. This is shown in extreme form in the Beltex and Dutch Texel breeds, which have the added complication of 'double muscle' conformation.

Other causes of dystocia

Maternal factors include uterine inertia resulting from systemic illness such as hypocalcaemia, pregnancy toxaemia, septicaemia or premature onset of parturition; weak or overstretched abdominal muscles; constrictions of the birth canal, including pelvic deformity, torsion of the uterus or tightness of the vestibule or vulva.

Fetal factors include developmental defects such as duplication of the head or limbs, body fusion (Figure 11.3) ascites, anasarca, schistosome or hydrocephalus; prepartum fetal death can result in uterine inertia with putrefaction and emphysema grossly increasing the size of the fetus. Some of the most difficult cases are those in which the shepherd is in doubt as to the commencement of parturition. Full term has been reached with normal udder development and perhaps some vaginal discharge, but no evidence of fetal membranes or limb extremities. The ewe may or may not show maternal behaviour or signs of uterine or abdominal contractions. The usual underlying reason is that no part of the fetus is engaged in the pelvis and so maternal stimulation via the pelvic reflex has not occurred.

Figure 11.3: Conjoined twins – a difficult obstetrical problem to diagnose, often requiring a Caesarean operation to resolve.

The role of the veterinarian

As well as dealing with individual cases of dystocia, the veterinarian can have an overview of problems within a flock and should be able to identify areas for improvement in order to minimize losses. An extremely important role is educating the shepherd in obstetrical skills and in recognition and acceptance of the point at which a skilled professional should be called. The benefits from insistence on training are great and appreciated by all involved with lambing ewes. They also have very significant effects in improving welfare of ewes and lambs.

The veterinarian's approach to dystocia cases

A detailed study of obstetrical techniques is outside the scope of this book, see reference [10] for guidance; however, a basic approach should always be adopted and should incorporate the following guidelines.

If the starting point is a normal healthy lamb within a normal healthy ewe, the only criterion for success is a normal healthy lamb outside a normal healthy ewe. All else is failure. Unfortunately, farmers do not always present ewes in such an optimum state. It is then up to the skills of the clinician to make as good a job as possible in the circumstances (or to decide on euthanasia on welfare grounds), and to educate the farmer to present cases at an earlier stage in the future.

Examination should be carried out with absolute care, optimal hygiene and almost unlimited lubrication. As much time as is felt necessary must be taken in order to diagnose the problem. Administration of sacrococcygeal epidural anaesthesia may be helpful if the expulsive efforts of the ewe make diagnosis or manipulation difficult. Once diagnosis has been achieved, delivery should be straightforward. A combination of aids will be necessary for a successful outcome, such as continuous lubrication, positioning the ewe according to the particular circumstances of the presentation, lambing cords or snares, which can be applied to the head or limbs to identify those belonging to the same lamb, and traction coincident with the uterine and abdominal contractions of the ewe. The clinician must be absolutely satisfied that all lambs have been delivered before leaving the case.

Caesarean operation

This should not be a method of last resort, since a prompt decision to carry out surgery is vital in ensuring a successful outcome as far as both ewe and lambs are concerned. If the lambs have been dead for some time, the success rate is markedly reduced. In a series of 137 cases a ewe survival rate of 97.8 per cent was achieved [11] where live or freshly dead lambs were present, compared with 57.1 per cent where the lambs were autolysed and emphysematous.

Surgery should always be performed in cases of absolute oversize and should be considered for a large lamb in posterior presentation, where the risk of injury is high if traction is attempted. Surgery also will be necessary in many cases of ring womb and torsion of the uterus, and required for some cases of fetal developmental abnormality, even though the lamb will not be viable. For methods of anaesthesia and surgical procedures see Chapter 74.

Fetotomy

This must be carried out only on lambs that are already dead. Gross swelling in a 'head only' presentation is often dealt with by this method. In cases where autolysis of lambs is present, fetotomy, provided it is performed carefully, may result in a better chance of survival of the ewe than performing a Caesarean operation.

BIRTH INJURIES

The only involvement of the veterinarian in this area should be in correcting the mistakes of others, in the diagnosis of injury to either ewe or lamb that requires euthanasia to prevent further suffering, and on occasion to recognize a pattern that will lead to advice on changes in breeding policy (for example in the case of a high incidence of oversize) or further training for members of staff (evidence of uncontrolled and inadequately lubricated traction).

INJURIES TO THE EWE

Uterine rupture

Most cases result from mistakes by the shepherd, although there is also the possibility of idiopathic or spontaneous rupture, or of full uterine and maternal expulsive effort coinciding with a fetal movement

that results in damage by a projecting extremity. Diagnosis should be straightforward, but the decision as to what course of action to take following removal of the lamb is difficult. Suturing without carrying out a laparotomy is impossible. Surprisingly, some ewes will survive, providing the placenta is quickly expelled or removed, and antibiotics are administered both parenterally and into the abdominal cavity via the rupture. If the tear is severe, or the ewe is shocked, euthanasia is necessary on welfare grounds.

Cervical tearing

This is common following unskilled manual dilation of the cervix in cases of ring womb. Severe, sometimes fatal, haemorrhage may result. It may be possible to exteriorize the cervix by careful traction, sufficient to ligate the source of the bleeding. If the tear extends through to the abdominal cavity, the same comments apply as to uterine rupture. If the cervix is damaged, antibiotic cover is necessary to prevent chronic infection, which often results in straining and prolapse of the cervix days or even weeks after lambing.

Vaginal tearing and bruising

This again results from excessive interference or inadequate lubrication by inexperienced staff. Providing any tear has not extended to the abdominal cavity, recovery should result. Administration of antibiotics, non-steroidal anti-inflammatory drugs and epidural anaesthetic may all be necessary depending on the severity of the damage.

Vulvar injury

Occasionally this may result in scarring or deformity. Episiotomy is carried out occasionally to prevent uncontrolled tearing in extreme cases of constriction of the vulva.

When any of the above injuries is evident other than as isolated incidents, it is an indication that further training of staff is necessary.

INJURIES TO THE LAMB

These are almost always the result of oversize, prolonged delivery or excessive traction, and are an important cause of stillbirth or death during the neonatal period [12].

Subcranial haemorrhage

This is usually the result of prolonged second-stage labour and is common in extensive systems where lambing ewes are not frequently inspected for difficulties. In one survey of perinatal mortality in Australia [13], up to 86 per cent of lambs necropsied showed subdural, subarachnoid and extradural haemorrhages in and around the cranial and spinal meninges. If severe, the damage may prove fatal. If not, it is undoubtedly an important cause of reduced lamb viability because of reduced mobility, reduced sucking drive and poor bonding with the mother. With increased supervision, or in intensive units, a significant percentage can be saved by techniques such as feeding by stomach tube and general nursing in the first few days of life.

Oedema

This is most common in a 'head only' presentation. It may occur also during prolonged second-stage labour when some other part of the fetus becomes impacted within the pelvis. If the lamb is born alive, the oedema disappears within a few hours.

Ruptured liver

This condition is almost inevitably fatal. It usually results from forced delivery but can be caused by maternal trauma during the ewe's endeavours to stimulate mobility in the lamb. This problem has also been reported on a flock scale as a result of vitamin E deficiency [14].

Fractured ribs

Extreme traction of an oversized lamb, particularly if in posterior presentation, often causes fractures along the costochondral junctions of the rib cage. Diagnosis is straightforward, as inspiratory effort by the lamb produces an inward movement along this line with every breath. Treatment is not possible, but some lambs survive, providing underlying lung damage is not too severe.

Extensor paralysis of a forelimb

This is usually the result of delivery of a lamb with a forelimb retained and may be transitory or permanent.

Limb fractures and other trauma

Fractures may be due to trauma during delivery, but more commonly to the lamb being trodden on by the mother or other sheep in the immediate post-lambing period. Inadequate, particularly cramped, housing conditions often are implicated. Damage to the eyes, jaw or neck results from unskilled and incorrect techniques during delivery of lambs.

CONCLUSION

Since 90 per cent of veterinary visits to sheep units occur near to or at lambing time and 90 per cent of lamb deaths occur in the perinatal period, the clinician has a very significant role to play in both general sheep welfare and in reducing losses. Education of those directly responsible for day-to-day care of pregnant and lambing ewes is vitally important and can markedly reduce losses of both ewes and lambs.

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12

Neonatal conditions

D.C. Henderson

Most surveys of lamb losses in the UK and elsewhere usually quote figures for losses that include abortions, as well as deaths during the act of parturition and to disease and injury during the first week or two of life. Abortion is dealt with elsewhere in this book, as are other important conditions afflicting newborn lambs. This chapter will therefore deal with the general measures that can be used to reduce losses of lambs in the perinatal period, but will include a description of hypothermia and watery mouth, which are not dealt with elsewhere. (Table 12.1 lists the common conditions affecting newborn lambs in the UK for completeness.)

REDUCING LOSSES IN THE PERINATAL PERIOD

Many infectious agents encountered in a flock are as a direct result of purchased animals being introduced to the farm. Agents such as border disease virus (a cause of abortion) and the virus causing the skin disease known as orf (contagious pustular dermatitis) being examples. It is therefore imperative that newly purchased animals are isolated in a quarantine area, well away from the home flock, for a period of at least a month and preferably – in the case of breeding females – until well after lambing. During this time they should be carefully observed and any relevant treatments undertaken.

The flock should be fed so as to ensure that ewes are in appropriate body condition throughout pregnancy and in particular in relation to the number of fetuses present, as determined by ultrasound scanning. Mineral and trace element status of ewes should be monitored by blood sampling a representative sample of the flock as and when appropriate. These measures should ensure adequate placental development so that lambs are born within the normal weight range for the breed, that ewes produce an adequate quantity and quality of colostrum, and exhibit a strong bond with their offspring post-partum.

Preventive measures should be applied at appropriate times to reduce the risk of disease in ewes which

Table 12.1: Common conditions affecting newborn lambs

General conditions	Specific conditions
Birth trauma	Fetal anoxia; ruptured liver; fractured ribs; brain haemorrhage
Climatic/mis-mothering	Hypothermia (exposure/starvation)
Inherited conditions	Daft lamb disease
Congenital malformations	Hydronephrosis; cleft palate
Congenital non-infectious conditions	Swayback; white muscle disease
Congenital infections	Hairy shakers (border disease); campylobacteriosis
Enteric infections	Watery mouth; colibacillosis; salmonellosis; lamb dysentery; cryptosporidiosis; rotavirus
Respiratory infections	Pasteurellosis (acute septicaemia)
Navel infections	Joint-ill (polyarthritis); navel-ill; necrobacillosis; abscessation
Skin diseases	Contagious pustular dermatitis (orf)
Predation	Foxes, crows, etc.

may compromise their ability to care for their lambs. Vaccination of ewes against clostridial diseases (such as lamb dysentery, tetanus and pulpy kidney) is of particular importance in providing protection for lambs via colostrum.

Dividing the lambing flock into separate groups according to lambing date, fetal numbers or some other parameter is a wise precaution in the final trimester, especially in the case of housed animals or closely confined outdoor lambings. This should reduce the risk of spread of infectious diseases such as abortion and neonatal diarrhoea, but also assist in the correct feeding of ewes according to fetal numbers. Compromised animals, such as those suffering from lameness and which may have difficulty competing at the trough, should also be dealt with separately.

Shelter for both ewes and lambs is crucially important at lambing. Access to woodland or straw bale enclosures can mean the difference between life and death in inclement weather and especially in hill and upland conditions. This is particularly so where lambs may be compromised at birth (for example, through inadequate birth weight or because of infection) and thereby prone to hypothermia – a rapid and ruthless killer of the newborn (see below).

Ewes also require adequate space at lambing time with the provision of a sufficient number of lambing pens to assist with mothering-up. Contamination of the lambing area should be kept to a minimum by cleaning out frequently and providing copious amounts of clean fresh straw or other suitable bedding material. However, it should be remembered that even the cleanest area still presents a risk to the newborn and especially to compromised lambs – in particular to diseases that strike within hours of birth, such as watery mouth (see later).

The importance of a close ewe–lamb bond and the ingestion of adequate amounts of colostrum by the lamb cannot be overemphasized. Selection of breeding stock from ewes that give birth to viable lambs and rear them successfully year on year will assist in this regard. As ewes generally become better mothers with each successive lambing, increasing the average age of the flock should increase lamb survival rates, other things being equal.

Hygienic practices are essential when assisting ewes to lamb to prevent injury or infection and shepherds should therefore be encouraged to resist any unnecessary interference. Appropriate antibiotic therapy should be applied after manual interference.

In order to reduce the risk of infection gaining entry via the navel, it is essential that it is treated by soaking in tincture of iodine (e.g. 2.5 per cent sublimated iodine in absolute alcohol) immediately after birth to desiccate and disinfect the cord. This, together with adequate colostrum intake, will significantly reduce the occurrence of navel-ill, joint-ill and liver abscesses.

Ewes should be examined for the presence of adequate supplies of colostrum and lambs examined to ensure they have ingested sufficient. If there is any doubt the lamb should be assisted to partake from the ewe or be stomach-tubed with colostrum from its mother, another suitable ewe or from a colostrum bank. If the latter is not available then an alternative should be used, such as cow or goat colostrum (bearing in mind the risks from ovine anaemia factor or caprine arthritis encephalitis, respectively). Commercial colostrum substitutes are available.

Before moving ewes and lambs out of the lambing area to other quarters it is imperative to make a careful assessment of the ewe–lamb bonding, especially where the move is from housing to outdoors when weather condition may have a significant effect on vulnerable lambs. Any procedure that may hinder the lamb's ability to follow the ewe and take colostrum, such as castration and/or tailing, should be avoided and certainly during the first day of life, in bad weather or if lambs are in any way compromised, since it can be fatal under these circumstances. Indeed, careful thought should be given as to whether these mutilations are strictly necessary under individual flock circumstances.

The role of shepherds at lambing time is crucial. An adequate shepherd-to-sheep ratio to allow for close shepherding and a modern, informed approach to the vocation is essential. Farmers and others should appreciate the highly skilled nature of their shepherds' work and provide all necessary equipment, facilities and assistance to allow them to perform their duties at what is often a hectic and stressful time. Access to training in the modern methods used to ensure the survival and welfare of both ewes and lambs should be actively encouraged.

HYPOTHERMIA

A newborn lamb with a wet birth coat, recently ejected from the warmth and protection of the intrauterine environment is very vulnerable to heat loss, leading

Figure 12.1: A recently born wet lamb, at risk of hypothermia unless early attention is given.

to a lowering of body temperature or hypothermia. Chilling is a significant cause of death in the newborn, especially when lambs are born into a hostile environment, as may be found on hill and upland farms, especially in bad weather and particularly the combination of wind and rain. However, hypothermia may still occur under lowland conditions, even in the relative comfort of a well-bedded lambing shed. Multiple siblings are particularly at risk.

The lamb has a relatively large surface area in relation to body weight compared to an adult sheep so that heat is lost rapidly (through latent heat of evaporation) unless the dam licks the lamb dry immediately after birth, or it is towel-dried by the shepherd (Figure 12.1). Additionally, some breeds have a poorly developed fleece which may increase the risk.

If ewes have been appropriately fed during the second and third trimesters in particular, then lambs will be born within the normal weight range for the breed and number in the litter. They will also have accumulated sufficient depots of brown adipose tissue and carbohydrate in muscle and liver to help maintain a near-normal body temperature, of around 39–40°C, by metabolizing the fat for a period of hours after birth. This will also depend on the mothering ability of the ewe (licking the lamb dry and leading it to shelter), the prevailing weather conditions and a number of other factors.

Once the brown fat and carbohydrates reserves are exhausted the lamb will not survive unless it has access to adequate amounts of colostrum at regular and frequent intervals, as this is its only source of energy. Lambs may still succumb if the rate of heat loss exceeds the rate of heat production as may occur in an exposed site in severe weather. Once body temperature falls by only 2–3°C (to below 37°C or lower) the sucking reflex is lost and lambs will starve to death unless revived and fed by the shepherd.

Clinical signs

Mildly hypothermic lambs – between 39 and 37°C – can appear relatively normal in that they may still follow their dams, even though they may not attempt to suck. As body temperature dips below 37°C lambs become lethargic and disinclined to follow their mothers who may sometimes abandon them. A lamb whose temperature falls to 35°C may still attempt to stand but will quickly become laterally recumbent and eventually comatose at around 25°C. Death ensues when rectal temperatures fall below 20°C but they may die much earlier.

Pathology

Deaths due to hypothermia as a result of exposure usually occur within the first 6 hours of birth since the lamb's energy reserves will normally sustain it – even in the absence of colostrum – for around this length of time. At necropsy the birth coat may still be wet if

the lamb has been neglected by the ewe. Signs of prematurity may be noted in some cases as such lambs are particularly prone to chilling. Signs of trauma, such as fractured ribs or jaws, or congenital deformities such as cleft palate may be seen and in some cases may have contributed to the demise of the lamb. Brown adipose tissue will probably have been completely metabolized, the last vestiges being the deposits around the kidneys. Yellowish subcutaneous tissue may be present in the extremities (limbs, tail and ears) and haemorrhage may be seen in the meninges or in the subcutaneous or periosteal tissues. Lambs that have died within 6 hours of birth are likely to be hydrated, but older lambs that have succumbed to starvation–hypothermia (usually 12 hours plus) are generally dehydrated at necropsy. The gastrointestinal tract may be completely empty or may contain varying amounts of colostrum or milk in a clotted or undigested form. The latter may indicate that the lamb has been stomach-tubed shortly before death. Meconium may or may not be present in the gut and chyle may be present or absent in the lacteals. The absence of post-mortem findings associated with other neonatal disease will assist in diagnosis but hypothermia is common in the terminal stages of many disease conditions.

Treatment

Most hypothermia cases respond reasonably to treatment in the absence of other complicating disease. If it is to be successful, any decision regarding the treatment of hypothermia cases will depend on a number of factors. It is important to attempt to determine the age of the lamb in hours, since this will give an indication as to whether the lamb is likely to be hypoglycaemic or not. If the age is unknown then it is safest to assume that the lamb is over 5 hours of age for the reasons explained below. The rectal temperature should be taken and an assessment of its state of consciousness should be made.

A lamb with a temperature of 37–39°C (mildly hypothermic) should be brought indoors where possible or otherwise guided to shelter with the dam and any other siblings. An attempt should be made to determine why the lamb has become chilled as this will have a bearing on any action post-treatment. The ewe should be carefully examined for lack of milk or udder disease (mastitis, orf, etc.).

Figure 12.2: Hypothermic lamb being exposed to circulating warm air in a warming box.

If the lamb is wet it should be thoroughly towel-dried and checked for any abnormality that might compromise feeding or locomotion. The chilled lamb should be fed, either by putting it to the ewe's udder (which may assist in assessing the ewe–lamb bond) or by stomach-tubing, preferably with colostrum from the dam or from some other source, providing the lamb is less than 24 hours old. The group should be closely observed, the lamb fed at regular intervals and its rectal temperature monitored until it is back to normal (39°C). Fostering or artificial rearing should be considered early if there is any doubt about the ewe's ability or willingness to look after the lamb.

Lambs are at high risk if their rectal temperature is below 37°C. Lambs of under 5 hours of age will probably have some remaining metabolizable energy to draw on and can be safely warmed in a warming box without first being fed and provided they are dry (Figure 12.2). The lamb should be removed once body temperature has reached 37°C, fed frequently, put in shelter with the ewe and dealt with as above. Normal metabolism will raise the lamb's temperature to normal (39–40°C) but it must be monitored frequently.

Lambs below 37°C, but over 6 hours of age, will be hypoglycaemic to some degree, owing to the depletion of metabolizable energy reserves. It is imperative to supply these lambs with an energy source before they are rewarmed to prevent them succumbing to a hypoglycaemic fit. If the lamb is fully conscious it should be safe to administer colostrum or milk by stomach tube. If, however, the lamb is semi-conscious or unconscious (unable to hold its head up) then the swallow reflex will be absent and the lamb must not be stomach-tubed. The lamb will have to receive its

Figure 12.3: Intraperitoneal injection of warm glucose solution.

energy supply by injection, which is best achieved by injecting 10 ml/kg body weight of a 20 per cent solution of glucose at blood heat via the intraperitoneal route. The site for injection is 1 cm to the side and 2 cm below the umbilicus (with the lamb suspended between the knees). A 2.5 cm/19 gauge needle should be directed towards the tail head and the solution administered slowly (Figure 12.3). The lamb can then be safely warmed to 37°C as described above. Lambs with body temperatures as low as 20°C have been revived by this technique, but they require appropriate aftercare and close shepherding post-recovery if they are not to relapse.

Prevention and control

In order to avoid losses from hypothermia the management and feeding of the flock throughout pregnancy should be such as to ensure the birth of lambs of normal birth weight, an adequate supply of colostrum and strong mothering instincts in the ewe. There is no substitute for an adequate level of highly skilled shepherding at lambing time so as to detect lambs in difficulty quickly and deal with them promptly. (The reader is referred to the paragraph on husbandry measures at the beginning of this chapter.)

WATERY MOUTH

Synonyms: slavers, rattle belly

This rapidly fatal disease affects lambs within 72 hours of birth and is characterized by profuse salivation, gut stasis, collapse and death. It is particularly prevalent in intensively managed flocks, either indoors or at high stocking rates at pasture. Particularly prone are lambs out of primiparous, thin or otherwise compromised ewes. Twins and triplets are more at risk because of the reduced chance of ingesting adequate amounts of colostrum immediately after birth. Despite attempts at treatment the mortality rate is high and therefore the disease is best tackled in affected flocks by employing preventive measures. It is much less common in hill flocks due to the relative lack of contamination at the lambing site.

Cause

The disease is caused by inadequate intake of colostrum, together with the ingestion of Gram-negative bacteria (principally *Escherichia coli*) from the environment immediately after birth (bedding, fleece, udder, teats). In the absence of colostral immunoglobulins (which are particularly important in combating Gram-negative micro-organisms) and in the neutral pH of the abomasum and reduced motility of the gut of the newborn lamb, the Gram-negative bacteria multiply very rapidly. Upon the death of the bacteria, large quantities of endotoxin are released into the gut and it is these products that are largely responsible for the clinical signs of the disease and for the death of affected lambs.

Because of the mechanisms that allow large molecules to pass unaltered into the systemic circulation during the first day of life, a bacteraemia also occurs in lambs deprived of colostrum. Interestingly, the *E. coli* isolated from cases of this disease do not possess the adhesion (K99) antigen normally associated with Gram-negative infections.

Clinical signs

Lambs very often show the earliest signs of disease within the first day of life. Their movements become

Figure 12.4: Lamb affected with watery mouth. Note drooling saliva.

sluggish, they are disinclined to suck, hang their heads and appear severely depressed. Some develop a swollen, tense abdomen, which may cause respiratory distress. Gastrointestinal motility is reduced which makes lambs disinclined to suck or swallow. In consequence, copious amounts of saliva drool from the mouth, giving the disease its name (Figure 12.4). The term rattle belly arises from the sounds heard when some lambs are picked up, due to gas in the abomasum. Some lambs may be affected by diarrhoea, others have normal faeces, while others again produce no faeces owing to the sluggish movements of the gut failing to void meconium – the latter being a sign, rather than a cause, of the disease. Other signs include lacrimation and puffiness around the eyes. Lambs often become dehydrated and hypothermic, which hastens the onset of collapse, coma and death – often within 24 hours or less of the onset of clinical signs.

Pathology

Post-mortem signs are frequently non-specific and insufficient to declare a diagnosis. Externally, there may be signs of salivation, lacrimation, diarrhoea and dehydration. The abdomen may be distended and on internal examination the abomasum may be full of gas, saliva and perhaps traces of colostrum (especially if lambs have been stomach-tubed), but often the gastrointestinal tract may be completely empty. Meconium may be present in a proportion of cases.

Diagnosis

Diagnosis is based on the history of the flock, the clinical signs and the often non-specific post-mortem findings. A number of neonatal diseases of lambs may present with signs not dissimilar to watery mouth in their terminal states.

Treatment

In order to achieve any degree of success, cases must be detected early and treated promptly. Even then the success rate is likely to be disappointing, especially once symptoms such as salivation have begun. The first aim should be to prevent starvation and correct dehydration which is best done using electrolyte solutions, fortified with glucose to provide energy if necessary and administered by stomach tube provided the lamb is conscious. Such preparations should not be administered by intraperitoneal injection to watery mouth cases. Nor should such lambs be given colostrum or milk by stomach tube, as they will be unable to digest the food. The presence of fluids in the gut will tend to stimulate gut movement, as will a soapy water enema to remove meconium, which in turn will assist in evacuating bacteria and endotoxin.

The use of parenteral antibiotics is normally advocated, but it should be borne in mind that this is likely to increase circulating endotoxin initially. The simultaneous administration of a corticosteroid, such as dexamethazone, or, probably more appropriately, a non-steroidal anti-inflamatory, such as flunixin, should

help to reduce the risk of endotoxic shock, as well as to make the lamb feel more comfortable. Once fully recovered, the lamb will require frequent monitoring to see that it is mothered-up and sucking satisfactorily.

Prevention

As is the case with hypothermia and indeed for most neonatal diseases, it is crucial to see that all lambs obtain adequate amounts of colostrum as early as possible and certainly within 2 hours of birth. At-risk lambs, such as multiples or lambs out of primiparous ewes, should be stomach-tubed if necessary. Rubber ring application for castration or tailing (if indeed these measures are necessary) should be avoided during the first day of life, as this will deter the lambs from sucking colostrum. Lambing pens should be cleaned out frequently and well bedded with clean straw, and outside paddocks should not be too heavily stocked. If these preventive measures fail and an outbreak occurs, then the use of an oral antibiotic to all newborn lambs immediately after birth may be instigated, although this is considered bad practice in the light of the risk of antibiotic drug resistance. However, it should be borne in mind that this disease is painful and distressing, and therefore presents a serious welfare challenge in affected flocks.

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13

Ram infertility

A. Greig

To be considered fertile, a ram must have sufficient libido to be interested in mating, and be fit to seek out and serve ewes properly and to inseminate them with fertile semen. At an average ram-to-ewe ratio of 1:50 anything that detracts from any of these parameters will produce a degree of infertility that may be of sufficient magnitude to cause a flock problem, depending on the mating situation in the flock. In the UK, the level of ewe infertility in the national flock can be obtained from the Meat and Livestock Commission data. However, there are no accurate figures for the incidence of infertile rams, although an estimate of 3.6 per cent has been made. A limited field study [1] found that about 30 per cent of rams were less than fully satisfactory in getting ewes in lamb, and 10 per cent were infertile. A similar proportion, i.e. 33 per cent of rams, suspected of being infertile were found to have recognizable abnormalities of the reproductive tract, other body systems or both [2], thus highlighting the merit of annual examination of the ram stud and subsequent observation of the animals at work.

RAM EXAMINATION

Flock-masters should subject their ram stud to annual physical examination at least 6 weeks, preferably 10 weeks, before the breeding season. In this regard

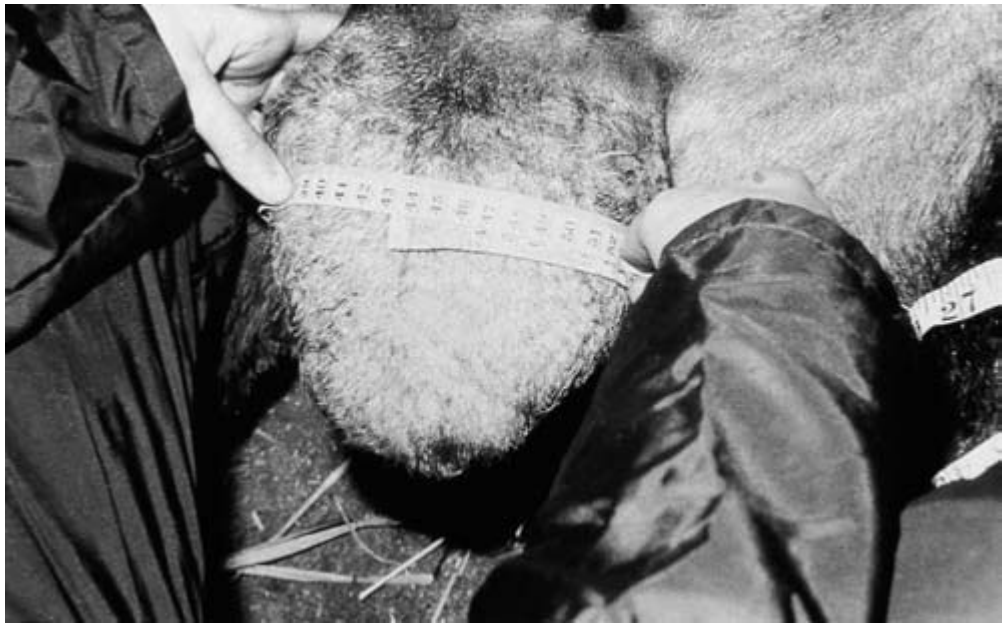

Figure 13.1: Measuring scrotal circumference.

the English Beef and Lamb Executive (eBLeX) have produced very useful literature entitled 'MOT Your Ram – For Better Returns'. This examination should also comprise the preliminary to an investigation into the reproductive capability of a ram with suspect fertility. The animals should be observed and their general condition, alertness and free locomotory movement assessed. Rams should be at a condition score of between 3.5 and 4.0 at the time of joining the ewes. Overfat rams are liable to be lazy, while rams in poor condition may well lose interest before the breeding season is completed. The poor state of the latter may well reflect an underlying medical condition, e.g. chronic pneumonia, parasitism or paratuberculosis. The scrotum and scrotal contents should first be examined with the animal in a standing position so that the normal hang of the testes in the scrotum can be assessed. The scrotum should preferably be clipped free of wool to reduce any chance of testicular overheating in hot weather and the scrotal skin should be thin and freely moveable over the testes with no evidence of mange, abscesses or other signs of previous injury. The scrotal circumference should be measured at its widest point using a tape measure (Figure 13.1); reference sizes are shown in Table 13.1.

Table 13.1: Normal scrotal circumference (cm) of rams within 1 month of the normal breeding period

	Breed type	
Age (months)	Down and Longwool	Hill
6–8	26–34	24–32
8–12	28–38	26–36
12–18	30–40	28–38
>18	30–44	30–40

Scrotal circumference has been correlated positively with sperm output and ovulation rate, number of multiple births produced and age of puberty of female offspring [3]. The testes should be gently handled and compressed to assess their resilience and tone. They should be of a similar size, move freely within the scrotum and give only a little under compression. A hard painful testicle is likely to reflect orchitis, whereas a soft one has probably undergone degeneration leading to atrophy. Likewise, the epididymes should be examined along their length paying particular attention to the head (Figure 13.2), which is a common site of epididymitis or spermato-

Figure 13.2: Handling the heads of the epididymes.

coele. In a ram ready to work, the tails of the epididymes should be firm and be between 2 and 3 cm in diameter. The ram should then be restrained on its rump while the scrotum is further visually examined, before the sheath, preputial orifice and brisket are similarly assessed.

- The inside of the thighs should be purple with waxing evident.
- The penis should be extruded to ensure that there are no adhesions that would prevent it being erected through the preputial orifice, and the glans, vermiform appendix and shaft should be examined.
- The brisket should be examined for evidence of brisket sores, which could reduce the ram's willingness to serve and certainly preclude the fitting of a crayon harness.
- The conformation of the ram's limbs is important, since defects such as straight hocks with sloping pasterns have been associated with reduced working life. All feet should be examined for evidence of foot-rot, scald, interdigital fibromas or other causes of lameness.
- Finally, the teeth, bite, eyes and nostrils should be inspected.

Ultrasonographic examination of the scrotum undertaken in the standing sheep using a 5 MHz linear scanner connected to a real-time, B-mode ultrasound machine or 6.0 MHz frequency sector transducer [4] provides invaluable information regarding location

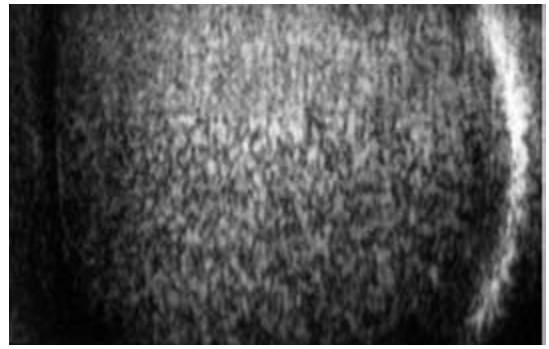

Figure 13.3: Ultrasonograph of normal testicle.

of any lesions. If necessary, ultrasonographic examination of normal rams can be undertaken first to establish normal measurements and sonographic appearance before progressing to those rams with scrotal swelling(s) or other palpable abnormalities.

Sequential examination of the pampiniform plexus, head of the epididymis, testicle and tail of the epididymis is undertaken as the transducer head is moved distally over the lateral aspect of each spermatic cord and testicle. The pampiniform plexus reveals a matrix of hyperechoic (bright white) lines throughout the conical anechoic (black) area. The normal testicle appears as a uniform hypoechoic area (Figure 13.3) with a hyperechoic mediastinum clearly visible. The tail of the epididymis is distinct from the testicle and considerably smaller in diameter (2 cm compared to 7 cm) with a distinct capsule. It may prove difficult to obtain good contact between the linear probe head and the smaller diameter tail of the epididymis.

It is generally accepted in the UK that the routine pre-mating evaluation of semen from all rams in a stud is not justified and should be restricted to those that are either suspected of being infertile on past history or in which an abnormality in the testes/epididymes has been palpated. Collection of semen into an artificial vagina produces the best samples, but with untrained rams in the field electro-ejaculation using either a transistorized rectal probe or variable output electro-ejaculator is generally adopted. Usually the animal is restrained in a standing position, the lubricated probe is inserted into the rectum by the operator, the electrodes are held over the area of the seminal vesicles and current applied in 4-second bursts. Simultaneously, a second person collects the ejaculate in a polythene bag applied over the preputial

orifice. However, when bacteriological examination of semen is required, e.g. for *Brucella ovis*, the animal should be restrained on its side, the penis extruded and secured with cotton gauze and semen collected in a sterile container. Semen density and motility are each scored 1 (poor) to 5 (high) and should be assessed immediately before preparing smears stained with nigrosin eosin. Semen from normal rams is milky white, density 4 or 5 and exhibits strong wave motion (5), and, on microscopy, has a low percentage of dead and abnormal sperm. Figures in excess of 20 per cent for both dead and abnormal sperm are not normal. Only two parameters need be used in assessing semen: motility and percentage of abnormal sperm [5]. Any sample with motile sperm and less than 30 per cent abnormal would be considered satisfactory. Other work has shown that semen samples had to contain as low as 40 per cent normal live sperm before fertility was adversely affected.

If the first sample is poor, a further sample should be obtained for examination, particularly if the ram has no history of infertility and appears clinically normal. If doubt remains about fertility, the animal should be run with a small number of ewes, noting his activities and the return rate of the ewes to service.

The British Veterinary Association has produced Certificates of Veterinary Examination for rams intended for breeding. In addition, the certificates provide a checklist for ram examination, guidelines for veterinary examination of a ram intended for breeding and procedures for electro-ejaculation.

CAUSES OF INFERTILITY DETECTABLE ON CLINICAL EXAMINATION

Conditions of scrotal skin

Scrotal mange, dermatitis, skin abscesses and excess scrotal wool are conditions that, by increasing the temperature of the scrotal contents, can cause testicular tubular degeneration, resulting in semen of reduced density and increased numbers of morphologically abnormal and immature sperm. Semen changes can occur as early as 3 days after an increase in scrotal temperature.

Conditions of the testes

Cryptorchidism

Cryptorchidism is due to a hereditary factor transmitted by the ram. The testes may be palpated in the inguinal canal or lie within the abdomen and thus be unpalpable. In a recent study 13 of 70 cases of cryptorchidism were bilateral and 57 were unilateral (K.C. Smith, personal communication, 2005). With the exception of one individual the testes were both in either the inguinal position or in an abdominal position. With the latter the undescended testes were found in the same position within the abdomen.

Hyperthermia of the testes in these abnormal positions causes degenerative changes so that spermatogenesis does not occur. Thus, if bilateral, the ram will be sterile while, if unilateral, normal semen will be produced from the normal descended testicle, but such a ram would be expected to impregnate fewer ewes successfully. However, because of the hereditary nature of this condition, cryptorchid rams should not be used in flocks breeding stock rams. The incidence of cryptorchidism is reported to be around 0.6 per cent in rams in the UK.

Testicular hypoplasia

Like cryptorchidism, this is usually an inherited trait with either or both testicles affected to varying degrees and a reduction in scrotal circumference from normal being manifest. Histologically, the spermatogenic epithelium fails to develop, but the interstitial tissue and cells of Leydig are normal. If hypoplasia is severe and bilateral, the ram will be sterile whereas, if unilateral and slight, the semen will be of reduced density and contain an increased percentage of abnormal sperm. This inherited trait has to be differentiated from the underdeveloped testes that arise from the long-term administration of anabolic steroids.

Testicular atrophy

This refers to a marked reduction in size and increased firmness of a previously normal testicle in an animal with an earlier history of satisfactory fertility, and represents the end-point of extensive tubular degeneration. It is usually bilateral, but can be unilateral when the neighbouring testicle has suffered acute orchitis. Semen samples are of reduced density with

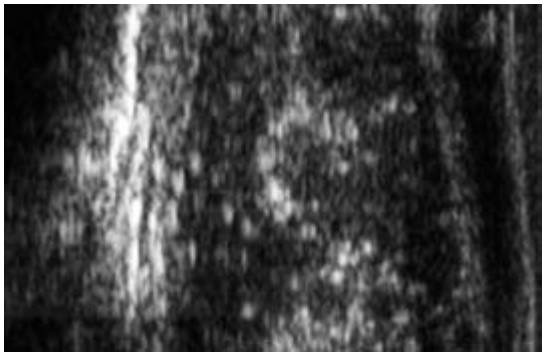

Figure 13.4: Ultrasonograph of atrophic testicle.

increased numbers of abnormal spermatozoa, particularly detached heads and tail deformities. On ultrasound examination an atrophic testicle appears more hypoechoic than a normal testicle with many hyperechoic (white) dots (Figure 13.4).

Orchitis

Inflammation of the testicle, whether caused by an infectious agent, e.g. *Arcanobacterium pyogenes*, *B. ovis*, *Corynebacterium ovis*, or trauma, is rapidly reflected in subnormal fertility. In the acute stage, heat, pain and swelling are evident, while chronic orchitis is characterized by reduced testicular mobility and induration of testicular tissue. When orchitis affects both testicles, infertility is permanent while, if unilateral, the contralateral testicle undergoes degenerative changes during the acute phase but regeneration may follow, so that a degree of fertility will return. Semen changes occur early in the process, so that ejaculates contain inflammatory exudate and only a few normal sperm.

Recent studies provide clear evidence that *A. pyogenes* is pathogenic for the ovine genitalia; however, methods of transition of the organism from commensal to pathogenic state are not clear. It was also noted that some degree of fertility was restored in the late stages of the disorder. Ultrasonography proved very useful both in the acute and chronic stages of the orchitis [6].

Treatment with systemic antibiotics can give variable results. When unilateral, removal of the affected organ early in the disease may prove the best course of action.

Neoplasia

Testicular neoplasms are rare, but Sertoli cell tumours and seminomas have been described.

Conditions of the epididymes

Epididymal hypoplasia or aplasia

The epididymis is the site where sperm mature and are stored, and therefore any reduction in the size of this organ will adversely affect its function and, hence, semen quality. Epididymal hypoplasia and aplasia are considered to be congenital abnormalities.

Spermatocoele

This condition arises from an obstruction of the epididymis and can occur at puberty from imperfect formation of epididymal tubules or as a sequel to an inflammatory condition. Stasis of sperm ensues and the increased accumulation of spermatozoa results in duct dilation, destruction of duct epithelium and release of sperm into the interstitial tissue, which evokes a granulomatous reaction (Figure 13.5). Part or whole of the epididymis can be involved, but the tail is most commonly affected.

On palpation, the affected portion of the epididymis will feel enlarged and nodular. Gross pathological examination shows the epididymis to consist of green–yellow milky or caseated material surrounded by fibrous tissue. The testicle is generally unaffected. Cultural examination frequently is unrewarding. Spermatocoele is generally progressive and, if bilateral, will result in infertility. Before gross changes are palpable, semen quality deteriorates with reduction in sperm numbers and an increase in the percentage of abnormal forms. If unilateral, the affected testicle can be removed.

Epididymitis

Worldwide, this is the most common cause of infectious infertility in rams, with *B. ovis* most frequently incriminated, particularly in mature animals [7]. Various non-enteric Gram-negative, pleomorphic organisms identified as *Actinobacillus*, *Haemophilus* or *Histophilus* spp. have been isolated from cases of epididymitis particularly in younger animals [8, 9].

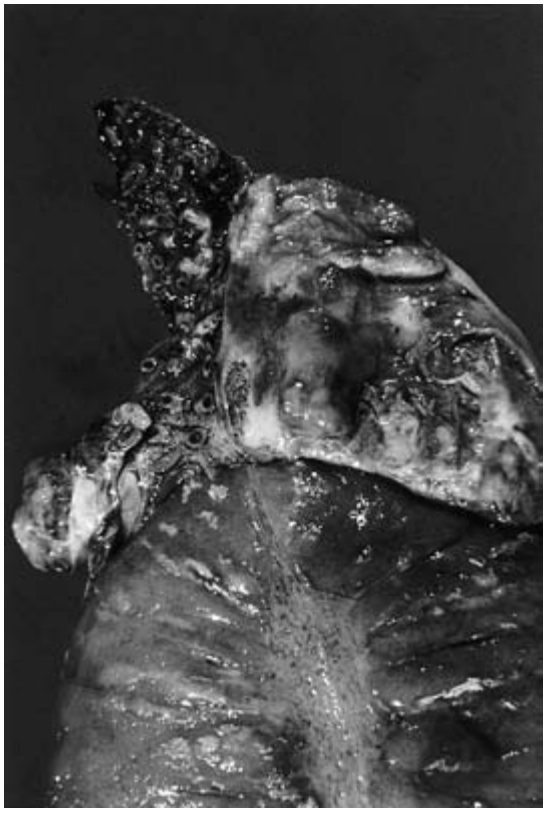

Figure 13.5: Spermatocoele affecting head of epididymis.

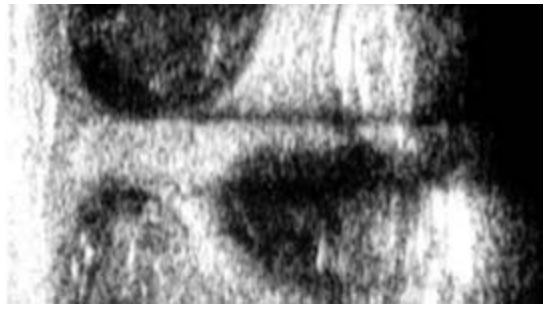

Figure 13.6: Epididymitis: ultrasonograph of head of epididymis.

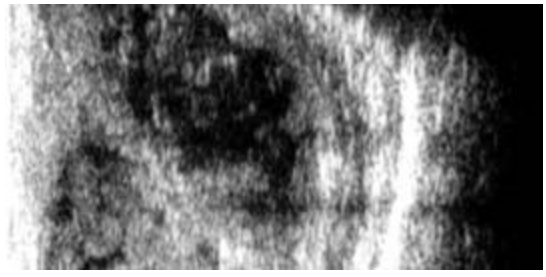

Figure 13.7: Epididymitis: ultrasonograph of tail of epididymis.

These organisms are commensals of the preputial cavity and probably migrate to the deeper organs of the reproductive tract under the influence of systemic hormonal stimulation [10]. Recent studies [11] showed that *A. seminis* resulted in a mild to severe epididymitis after inoculation by a number of routes. *B. ovis* has also been shown experimentally to cause epididymitis, and infection across mucous membranes is probably the natural route of transmission. Sporadic cases of epididymitis can be caused by *Actinomyces*, *Pasteurella*, *Staphylococci* and *Streptococci*, while concurrent orchitis and epididymitis may suggest a traumatic origin.

In the acute stage, pain and swelling will be evident, while induration will be a feature of the chronic disease. Rams with palpable epididymitis usually produce a semen of inferior quality, containing leucocytes. These cells also have been noted in subclinical cases of *B. ovis* infection and may thus be an aid in making a diagnosis. In countries where *B. ovis* infection is recognized, serological screening of ram studs is frequent.

Ultrasonographic examination of rams with epididymitis reveals a normal pampiniform plexus. Typically, the swollen epididymis appears as multiple 1–5 cm diameter anechoic areas containing many bright spots (Figures 13.6 and 13.7) surrounded by broad hyperechoic lines (fibrous capsule) extending up to 1–2 cm in thickness typical of thick-walled abscesses. The abscesses generally involve the tail of the epididymis (Figures 13.7 and 13.9) but may extend proximally to involve the body and head of the epididymis (Figures 13.6 and 13.8). (Figures 13.8 and 13.9 are in the colour plate section.) The testicle is embedded within this fibrous tissue reaction, is much reduced in size, and appears more hypoechoic than normal and contains numerous hyperechoic spots consistent with testicular atrophy. In sheep with unilateral epididymitis the contralateral testicle is much smaller than normal and appears more hypoechoic than normal.

Treatment and prognosis are the same as for orchitis, except that, with *B. ovis* infection, culling is recommended.

Conditions of penis and prepuce

Inability to erect fully or extrude the penis, arising from a developmental or congenital defect or adhesions, result in a ram being unable to serve properly. Balanitis (Chapter 21) can be a problem in certain flocks. Preputial prolapse leading to ventral deflection of the erect penis has been recorded occasionally.

Conditions outwith the reproductive tract

Brisket sores

These are not uncommon and, if present, preclude the use of a crayon harness. They arise possibly from chafing by a loose-fitting harness, aggravated when the animal lies around in areas devoid of grass. They are easier to prevent than treat and may require topical, and possibly systemic, antibiotic therapy and even minor surgery.

Foot conditions

In one study [2], these comprised a major finding, whether as overgrown horn, foot-rot, scald or interdigital fibromas. As well as making the animal unwilling to walk and therefore follow ewes, infectious conditions of the feet will cause testicular degeneration and thus reduce fertility.

Arthritis

The elbow, carpus and tarsus in that order of prevalence can be affected with arthritis. The stage and degree of inflammation is reflected in the ability or otherwise of the ram to work.

PHYSIOLOGICAL/PSYCHOLOGICAL FACTORS AFFECTING FERTILITY

Season

Sperm production occurs throughout the year but volume and density reach low levels in the spring. This seasonal effect is more marked in the hill breeds, but should always be taken into account when assessing semen quality outwith the normal breeding season.

Sexual inhibition

The common practice of running ram lambs and shearlings in bachelor groups, possibly compounded by heavy feeding, has been postulated as a cause of failure to work when first introduced to ewes in oestrus. Most animals will overcome this inhibition within a few days.

Social dominance

While the competitive drive amidst groups of rams will generally increase mounting activity, an individual animal, particularly if older, may dominate the others. If the dominant one is infertile or subfertile, the effect is compounded and obviously his covering ability will be stretched to the maximum and probably exceeded.

Immaturity

This term has been applied to rams that produce 'normal' semen and mate properly and yet have high return rates. The sperm transit time in the relatively short epididymis may have an adverse effect on sperm fertility.

Body condition

A greater problem, particularly with pedigree Down breeds in the UK, is overfatness as a possible cause of infertility. Overfat animals will be less willing to move and may be too heavy for some ewes.

ACKNOWLEDGEMENT

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14

Prolapse and hernia

B.D. Hosie

Prolapse is the posterior displacement, eversion and external protrusion of one or more of the vagina, uterus and rectum. The condition occurs most commonly in advanced pregnancy and less frequently after lambing. Prolapse of the rectum alone can occur post-weaning in lambs. Hernia is the term applied to protrusion of portions of intestine through a natural or accidental opening of the musculature, but not the skin, of the abdominal wall of lambs to form a visible localized swelling. Herniation of the uterus through the abdominal wall can occur in late pregnancy.

The welfare of sheep affected by prolapse and hernia is of considerable concern. Some presentations of prolapse and hernia appear as flock problems, which require an effective strategy for their prevention and treatment.

PROLAPSE

Vaginal prolapse antepartum

Synonyms: pre-partum cervical vaginal prolapse; vaginal eversion; vaginal prolapse

Cases of vaginal prolapse can be expected among pregnant ewes in all but the most extensively managed,

least productive flocks. The condition is of considerable welfare concern as well as causing a significant economic impact on flocks that experience a high prevalence of the condition. Veterinary surgeons should advise their clients on the correct treatment and care for affected ewes. While understanding of the causes remains poor, predisposing factors have been identified and prevention strategies described. These can be included in the health plan for flocks that experience outbreaks of vaginal prolapse.

Cause

Vaginal prolapse ante-partum in ewes was the subject of considerable research in the 1950s and early 1960s and many factors, some contradictory, were associated with the problem. For the vaginal wall to prolapse, the vaginal and vestibular tissues must be relaxed and readily distensible. Both features are influenced by the compliance of the vaginal wall, which is affected by the amount and distribution of collagen and smooth muscle, and the action of hormones. Vaginal compliance, capacity and stretchability were examined in normal sheep during the oestrus cycle and in pregnancy [1], and the influence of steroid hormones considered [2]. While both oestradiol and progesterone had a modest effect in increasing 'vaginal capacity', both hormones also reduced the compliance of the vaginal wall.

Clinical signs

Vaginal prolapse antepartum may precede lambing by up to 55 days, but most cases occur within the final 21 days of pregnancy. Acute cases with rupture of the vaginal wall are observed [3] but, typically, the prolapse develops over a few days, often returning spontaneously when the animals rise. Later, the vagina fails to return to its normal position, and the prolapse progresses until the vagina is completely everted and the cervix is visible (see Figure 14.1 in the colour plate section). Initially pink, moist and smooth, the vagina becomes swollen, oedematous and congested if not replaced and is very susceptible to injury. After prolonged exposure, the dried vaginal mucosa becomes rough and haemorrhagic and gangrene may develop. Straining becomes a feature of the condition when the mucosa is irritated or obstruction of the urethra leads to severe distension of the urinary bladder.

Vaginal rupture results in herniation of the caecum, ileum and colon, and, occasionally, the uterus [3]. Death from haemorrhage and shock is rapid. A Norwegian report of 17 cases of ovine vaginal rupture suggests the condition results from excessive tenesmus associated with uterine torsion following previous scarring of the vaginal wall [4]. Several authors [2, 3, 5] consider vaginal rupture results from a preceding vaginal prolapse, combined with protracted tenesmus.

Epidemiology

While an understanding of the pathogenesis of vaginal prolapse antepartum is poor, appreciation of predisposing factors has advanced.

Breed

The overall prevalence found in a survey conducted in the Scottish Borders was 1 per cent, but there were marked differences between breeds and their crosses [6]. Pure-bred hill flocks had few cases of vaginal prolapse (0.2 per cent) and most cases occurred in the highly prolific greyface (Border Leicester cross Scottish Blackface) or mule (Blueface Leicester cross Scottish Blackface) ewes mated to Suffolk rams (1.8 per cent).

Litter size

The risk of a ewe developing vaginal prolapse rises with increasing litter size. Independent studies in New Zealand [7] and Scotland [5] found that the risk of vaginal prolapse was five times greater in ewes bearing twins and 11–12 times greater in those bearing triplets than in ewes with single lambs. These marked differences probably explain, in large part, the different breed susceptibilities, as hill sheep generally carry only one lamb, while cross-bred ewes carry twins or triplets.

Age

The risk of vaginal prolapse increases with age, possibly because the proportion of ewes carrying twins and triplets increases amongst the older age groups. In addition, there is an accumulation of risk as 35–40

per cent of ewes that survive vaginal prolapse are affected in future pregnancies.

Nutritional factors

A study of cross-bred ewe flocks did not associate a high prevalence (>3 per cent) of vaginal prolapse with any particular feedstuffs or the presence of oestrogenic mycotoxins [8]. Mild hypocalcaemia found in some ewes recently affected by vaginal prolapse was considered to be a consequence of the stress and trauma of the prolapse rather than the cause. However, vaginal prolapse was more prevalent in flocks managed as a single group or small number of groups fed on an unrestricted basis. In those flocks, some ewes were overfed, particularly where there was a considerable spread of lambing dates. An epidemiological study in New Zealand identified access to salt and feeding swedes in the latter part of pregnancy and weight gain between the start of mating and scanning (mean 95 days) as risk factors [7]. The former may contribute through their effect on water intake leading to distention of the bladder.

Treatment

In one study [5], in which none of affected ewes was treated by a veterinary surgeon, 20.7 per cent died. The veterinary surgeon has a clear role in setting criteria by which the shepherd can determine which cases of vaginal prolapse to treat himself and which require veterinary intervention or euthanasia. Cases that fail to respond to treatment within 24 hours or that relapse require veterinary attention. Treatment and management of vaginal prolapse has been described [9, 10], and reference to those papers for detailed information is recommended, particularly the benefits of caudal epidural anaesthesia and the role of real-time B-mode ultrasonography [10]. Shepherds should be urged to seek assistance and training from their veterinary surgeon if the case mortality rate exceeds 10 per cent.

Assessment

Evidence of exhaustion or shock indicates a poor prognosis. Hypocalcaemia should be treated. The prognosis is good when the vagina is clean, moist and warm, but deteriorates as time progresses and the vaginal wall becomes dry, purple, bruised, dirty and cold. Evidence of the fetal membranes indicates that the cervix is open and fetal death, metritis and the death of the ewe are all possible sequelae. Euthanasia may be necessary for some cases.

Anaesthesia

Caudal epidural anaesthesia is effective at controlling straining. Local anaesthetic such as 2 per cent lignocaine gives control for a few hours, while much longer anaesthesia and analgesia (up to 36 hours) can be obtained by using a combination of lignocaine and xylazine.

Replacement

Traditional advice was to raise the hindquarters during this procedure, to reduce pressure from the stomach contents and unborn lambs on the vagina. Cradles can be constructed to help or an assistant may raise the hindquarters of the ewe from the floor. With epidural anaesthesia, the vaginal prolapse can be replaced in the standing ewe as the tenesmus stops. The vaginal mucosa should be carefully cleaned with warm, mild disinfectant and the prolapse gently reduced by pushing with bent fingers or the palm of the hand. A very distended urinary bladder should be allowed to empty with gentle pressure. Occasionally, a veterinary surgeon may need to empty the bladder by insertion of a hypodermic needle.

Retention

The bruised tissues cause persistent straining and, once replaced, the vagina must be physically restrained to prevent a recurrence of the prolapse. Various methods are available, such as tying wool across the vulva, stitching or the use of a truss or retainer device. The least invasive method that will retain the prolapse should be chosen. Webbing trusses are available commercially or can be made from baler twine and are effective for ewes in the early stages when there is little straining. The truss requires daily inspection and adjustment to ensure it does not chafe or cut or become displaced. Trusses should be removed at lambing and cleaned, disinfected and dried before storage. Many shepherds favour plastic T-shaped retainers that are inserted into the vagina but many veterinary surgeons believe they cause a greater risk of metritis and persistent straining. The retainer can

be held by tie strings fastened to the wool of the ewe or by creating a harness from binder twine. These retainers must be checked daily.

Most veterinary surgeons prevent recurrence by suturing the lips of the vulva after epidural anaesthesia. The most popular suture patterns are horizontal mattress or the method of placing a subcutaneous purse string suture around the vulva. The stitches must be into the perineal skin and not vaginal mucous membrane, as the latter results in urine scalding with secondary bacterial infection. Stitches are cut when lambing commences. The Buhner suture tied with a double bow is recommended as it can be slackened as the prolapse resolves and/or the signs of first stage labour are noted.

Supportive treatment

Affected ewes should receive a course of antibiotic injections and be clearly identified. More severe cases benefit from administration of a non-steroidal anti-inflammatory analgesic such as flunixin to reduce the discomfort to the ewe and reduce the chances of a relapse. The health of the ewe should be monitored for evidence of hypocalcaemia or pregnancy toxaemia, when appropriate therapy is required.

Complications

Fetal death may occur as a consequence of the prolapse and metritis may follow if the dead fetus is not removed promptly. Persistent tenesmus after replacement may cause rectal prolapse or recurrence of vaginal prolapse. Tenesmus might be prevented by correcting the cause or through epidural anaesthesia with lignocaine and xylazine. Rectal prolapse can be retained by a Buhner suture or, if severe, amputated under anaesthesia (see Chapter 74).

Incomplete cervical dilation may occur at parturition. While patient and careful digital pressure to the cervix may cause a dilation, often a Caesarean section is preferable, with an improved prognosis for the ewe and the lambs.

Prevention and control

In outbreaks of vaginal prolapse, veterinary surgeons can have a valuable role in overseeing the management of the flock to prevent further cases and to alert the shepherd to other health and welfare problems such as pregnancy toxaemia, that may develop. They can include a programme to control vaginal prolapse in the flock health programme for flocks in which the condition is a perennial problem. Culling of affected ewes is generally advised because almost half will prolapse in a subsequent pregnancy. In addition, the trauma to the reproductive tract may render such animals liable to infertility, dystocia or metritis.

In flocks that experience an outbreak of vaginal prolapse, a reduction in the intake of roughages and bulky feeds usually alleviates the immediate problem, but the adequacy of the flock's nutrition must be maintained. This should be assessed by body condition scoring about 10 per cent of the flock and measurement of serum betahydroxybutyrate (BOHB) on about 20 ewes in the group. If there is a great variation in the ewes' body condition scores, the flock should be split into more manageable groups by condition score and, if known, according to litter size and lambing dates. The feeding should be in accord with their requirements, taking into account body condition and BOHB levels.

In flocks where vaginal prolapse antepartum is a perennial problem with more than 3 per cent of the flock affected, greater control should be exerted over the flock's nutrition in the last 8 weeks of pregnancy. The flock should be divided in mid-pregnancy into groups with similar nutritional requirements on the basis of litter size, as determined by real-time ultrasonic scanning and expected lambing dates. The hay or silage should be analysed and rations formulated that will allow the pregnant ewes to lose body condition from a score of 3.0–3.5 at mating to 2.0–2.5 at lambing.

Eight weeks before lambing, the lean ewes (body condition score 2.0 or less) are identified and removed for additional feeding. About 3 weeks before lambing, the body condition of the ewes should be reassessed and blood taken from 10 to 20 ewes in each group for BOHB estimation. The target BOHB concentration of 0.8 mmol/l is suitable for flock groups with a wide range of expected lambing dates or litter size. Where the flock is divided into groups according to lambing date and litter size, a mean BOHB concentration of 1.1 mmol/l is an acceptable target [11]. The ration can be modified in the light of these findings. Where this approach is combined with the culling of ewes that are affected by vaginal prolapse, the reduction in the prevalence of vaginal prolapse antepartum is dramatic and of benefit to both sheep and shepherd.

Vaginal prolapse postpartum

This condition may occur several days or some weeks after lambing. The author investigated an outbreak where the condition affected ewes suckling their first lambs. The affected ewes tended to be smaller than average. Trauma to the cervix and vagina at lambing was suspected as a predisposing factor. As vaginal prolapse postpartum is seldom found sufficiently early to institute treatment most cases are humanely destroyed.

Prolapse of the uterus postpartum

This condition occurs sporadically after apparently normal lambings and is unrelated to vaginal prolapse antepartum. It is usually associated with younger ewes and may be a consequence of a hypocalcaemia. Provided the prolapse is treated promptly, utilizing the techniques described for treating vaginal prolapse antepartum, recovery is usually uneventful.

Rectal prolapse

In addition to occurring in adult ewes as a complication of vaginal prolapse, rectal prolapse in fattening lambs is due to tenesmus caused by irritation to the mucosa from coccidiosis, parasitic gastroenteritis and fly strike. Ultra-short docking of lambs in the USA, so that virtually no tail remains, results in a significant increase in the incidence of rectal prolapse in fattening lambs [12]. Treatment of the affected animal is as described for vaginal prolapse antepartum, and specific treatment of the group for the predisposing causes is described. The practice of ultra-short docking should stop.

HERNIA

Umbilical hernia

Open umbilical hernias occasionally occur in newborn lambs when loops of intestine pass through a patent umbilical ring. The prognosis is good, provided the shepherd can immediately return the loops of intestine to the abdomen and close the cord with a clamp or suture. Otherwise the prognosis is grave and euthanasia should follow.

Surgery can be considered for closed umbilical hernias in older lambs, although most resolve without intervention. The decision rests on the size of the hernia, the likelihood that loops of intestine will become strangulated and the value of the lamb.

Inguinal and scrotal hernias

These hernias are found in lambs rather than older sheep, although scrotal hernias can cause infertility in rams owing to raised intrascrotal temperature. Inguinal hernias are usually a consequence of raised intra-abdominal pressure, as might occur among lambs on artificial or highly fermentable diets. The pressure is believed to force intestinal loops though the inguinal ring to become visible as a swelling at the groin and inner thigh. Death follows strangulation or rupture of the herniated intestine. To avoid further cases, the diet should be changed to include more roughage.

Scrotal hernias were widely recognized when surgical castration was commonly practised. They vary in size from small and difficult to palpate at the scrotal neck to a gross enlargement of the scrotum due to herniation of intestine. Affected males should not be used for breeding, as the condition is inherited.

Ventral hernia

Ventral or abdominal hernias usually occur in the latter stages of pregnancy, when increased intra-abdominal pressure and possibly other non-specific factors such as previous dystocias and undernutrition weaken the muscle and tendinous support of the abdominal wall. Rupture of either the pre-pubic tendon or the fibrous aponeurosis of the abdominal muscles allows the uterus and abdominal viscera to fall into the sac formed by the skin and subcutaneous muscles. The affected ewe will have difficulty in rising and walking and, if it survives to parturition, the lack of abdominal muscular contractions means the fetuses do not engage or enter the pelvic canal. Ewes with ruptured pre-pubic tendons should be destroyed. Some cases of flank hernia can be assisted to lambing when surgical repair will permit the ewe to nurse its lambs. Thereafter, such ewes should be culled.

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15

Mastitis and contagious agalactia

G.H. Watkins and J.E.T. Jones

MASTITIS

Mastitis in ewes occurs in all countries of the world where sheep are kept, but most attention has been paid to the disease in those countries in which there are substantial milking flocks, e.g. France, Italy, Spain and Greece. In the UK, ovine mastitis was first investigated systematically some 75 years ago by Leyshon [1] in the eastern counties of England. The bacteria that he identified as the principal causes of mastitis are those we still regard as the most important.

Sheep farmers and veterinarians are increasingly concerned about the economic implications of mastitis. Adverse effects include acute disease leading to death of the ewe, acute disease followed by systemic recovery and the necessity to cull the ewe because of a permanently damaged udder, replacement costs and veterinary costs. Severe depletion of milk production may lead to starvation and death of lambs. Clinical and subclinical mastitis may lead to reduced milk yield and suboptimal growth of lambs. In both acute and chronic mastitis, affected ewes may suffer considerable discomfort, and the alleviation of pain is an important consideration.

There is little information on the economic effects of mastitis in relation to culling. In one investigation in which 1650 ewes were examined at the time of

slaughter, 10 per cent had evidence of mastitis [2]. Available surveys on diseases of sheep show that mastitis is one of the most important causes of mortality and of the enforced culling of ewes. A survey between 1985 and 1987 of some 70 lowland flocks with a collective population of about 30 000 ewes showed the incidence of mastitis varied considerably among flocks and ranged between 1 and 15 per cent of the ewes, with 10 per cent of the flocks having an incidence greater than 10 per cent [3]. The average incidence in lowland flocks is probably 4–5 per cent. Most of the recorded cases of acute mastitis in lowland flocks occurred in the first week and between the third and fourth weeks of lactation. However, since many less severe cases were not detected until the time of weaning, the period of peak incidence of this form of mastitis is not known.

Cause

In the UK, *Staphylococcus aureus* and *Mannheimia* spp (especially *M. haemolytica* A2 and A6, *M. glucosida* A11 and untypable strains) are the two most important causes, accounting for 80 per cent of cases of severe mastitis [3]. *Escherichia coli*, *Streptococcus* spp and coagulase-negative staphylococci cause most of the remaining 20 per cent and most occur in the first week of lactation. Other bacteria incriminated sporadically include *Arcanobacterium pyogenes*, *Bacillus cereus*, *Clostridium perfringens*, *Listeria monocytogenes*, *Actinobacillus* spp, *Pseudomonas aeruginosa* and the fungus *Aspergillus fumigatus*. *Leptospira hardjo* has been reported to cause agalactia but not mastitis. Mycoplasmal mastitis, discussed below, is not found in the UK.

Chlamydophila abortus mastitis has been produced experimentally in sheep, but there is no evidence that it causes mastitis in field circumstances or that it is present in the milk of ewes suffering from enzootic abortion. However, ewes that have aborted and some clinically healthy ewes in affected flocks excrete *Chlamydophila* in the birth fluids, which may soil the udder and the teats and thus easily contaminate milk. Is it therefore possible that ewe's milk for human consumption could become contaminated with *Chlamydophila* [4].

One non-bacterial cause of mastitis is the maedi-visna virus, which can cause indurative lymphocytic mastitis in ewes and may lead to reduction of growth rate in lambs. Maedi-visna is described in Chapter 31.

Clinical signs

Acute mastitis

Some cases are so severe and the course of the disease so rapid that no signs are observed and the ewe is found dead; fortunately, such cases are uncommon. In less severe forms of the acute disease, affected ewes have a swollen, painful udder, generally involving only one 'half'. The skin over the affected half may become red or purple, and this colour change may spread along the abdomen. At first the udder feels hot but, as the disease progresses, there may be extensive necrosis of the glandular tissue and, at this stage, the udder becomes cold and clammy. The secretion from the gland is usually sanguineous. The ewe is obviously ill, limb movement is stiff, body temperature is high and the ewe may become isolated from the flock; her lambs may show signs of starvation. If the ewe survives, these general signs of illness disappear but part, or the whole, of the affected half may slough. Healing of the skin takes several weeks.

Chronic mastitis

This develops from the acute form of the disease. It is manifested by hardness of the udder and by the presence of single or multiple abscesses within the gland and often the subcutis. The teat is sometimes swollen, and the teat canal may contain a hard core of inspissated pus. These changes are easily detected when the ewe is examined prior to mating when the gland has involuted. Single or multiple milk cysts may be mistaken for abscesses.

It had become common to refer to mastitis observed at the pre-mating examination of ewes as post-weaning mastitis. The great majority of cases of so-called 'post-weaning mastitis' are examples of pre-weaning mastitis detected post-weaning. They represent the sequelae of acute mastitis that developed during lactation [5].

Subclinical mastitis

Subclinical mastitis has been reported frequently in many countries where sheep are kept for milk

production, but to only a very limited extent where flocks are reared predominantly for meat and wool production as, for example, in the UK, New Zealand and Australia. The principal bacteria associated with subclinical disease are coagulase-negative staphylococci, various streptococci and, occasionally, *S. aureus* and *Mannheimia* spp. In affected ewes, the somatic cell count in milk exceeds 10^6/ml.

The Whiteside and California mastitis tests are suitable screening tests, but bacteriological examination and milk somatic cell counts (SCC) are necessary for confirmation. These tests have been comprehensively evaluated for the diagnosis of subclinical mastitis in meat-producing flocks [6]. In Mediterranean countries, SCC and bacterial culture are the main determinants for classifying lactating dairy ewes as 'healthy', 'infected' or 'doubtful' [7].

Investigations of subclinical mastitis in lowland ewes in several flocks in southern England showed that the predominant bacterial isolates from the milk of affected glands were streptococci, coagulase-negative staphylococci, *Mannheimia* spp and *S. aureus*, There was a significant association between the development of clinical mastitis and antecedent subclinical mastitis caused by the same organisms [8]. The possible effect of subclinical mastitis on milk production in flocks in the UK is not known, but it has been shown experimentally that milk yield and growth rate of lambs are significantly reduced [9].

Pathology

In well-developed lesions of the udder of ewes in which clinical signs have been present for 2 or 3 days, the affected half is much larger than the unaffected half, and there is often subcutaneous oedema, especially in the area surrounding the base of the teat. The mammary parenchyma is intensely and diffusely reddened, although, in the early stages, the reddening is confined to individual lobules, giving the cut surface of the gland a mottled appearance. As the inflammatory reaction progresses, tissue degeneration and necrosis result in the separation of the parenchyma from the skin and from the unaffected half. The lactiferous ducts are plugged with clots of milk or exudate, and there may be evidence of venous thrombosis.

Histologically, those areas of the gland that macroscopically are intensely reddened are hyperaemic and haemorrhagic. There is massive infiltration of leucocytes into the alveoli and degeneration, necrosis and desquamation of alveolar epithelium.

Diagnosis

It is not usually possible to associate the clinical and pathological features of acute mastitis with a particular causal organism. The changes brought about by *S. aureus* and *Mannheimia* spp. are essentially similar. Provisional identification of the bacterium may be made from microscopic examination of mammary secretion. The majority of the bacteria causing mastitis will grow on blood agar incubated aerobically for 24 hours. The routine use of other media and of anaerobic incubation is usually unrewarding. About 10 per cent of milk samples from mastitic ewes do not yield any bacteria when cultured.

Epidemiology and transmission

S. aureus inhabits the skin, mouth, nasal passages and vagina in a proportion of healthy ewes, but the carriage rate varies between flocks. It is isolated sometimes from normal teat skin and, rarely, from the teat canal in the absence of mastitis. *S. aureus* can cause dermatitis of the teat and udder skin and these lesions predispose ewes to mastitis. Severe outbreaks of staphylococcal dermatitis associated with mastitis have been described [10].

Mannheimia spp colonize the nares and tonsils of sheep during the first few days of life. They are also present in the mouths of ewes and lambs and, soon after lambing, on the teat skin of ewes. By contrast, *Mannheimia* spp are not isolated from the teat skin of ewes before lambing or after weaning, thus demonstrating that they are present on the teat skin only when lambs have been sucking [11]. As the teat skin is constantly being exposed during the sucking period, even small numbers of *Mannheimia* spp on the skin of the teat may gain access to the mammary gland through the teat canal. Because fewer than 10 colony-forming units of a virulent *M. haemolytica* are required to produce mastitis experimentally [12], the potential for disease is evident. Experimentally, some isolates of *M. haemolytica* multiply in the gland after a short lag phase of 3 hours and elicit a neutrophil response within 12 hours [12]. *In vitro*, *Mannheimia* spp adhere to and are internalized by mammary epithelial cells

within 2 hours, suggesting that these are important early stages in the development of mastitis caused by these bacteria [14].

Factors predisposing to entry of bacteria into the teat canal and thence to the gland have not been investigated in the ewe to the extent they have in the cow. Suggested factors include anatomical defects of the teat and indiscriminate sucking, which is common when ewes and lambs are housed. Lesions on the skin of the teat (e.g. orf pustules, bites and abrasions from vigorous sucking) predispose particularly to staphylococcal mastitis. All may facilitate spread of mastitis pathogens from affected to non-affected ewes. In dairy sheep flocks, the incidence of *Mannheimia* spp mastitis declines rapidly when lambs are removed, while the incidence of staphylococcal mastitis is unaffected, indicating that the main source of infection of *Mannheimia* spp for the teats of the ewe is the mouth of the lamb.

Possible predispositions to mastitis are not fully understood. In housed sheep, high stocking density (less than $2\,m^2$ per ewe) has been shown to lead to increased somatic cell counts and increased incidence of subclinical mastitis, as well as lower milk yield and protein and fat content [14].

Treatment, prevention and control

Most of the bacteria causing mastitis are sensitive to several antibiotics. Parenteral administration of an appropriate antibiotic, e.g. one of the penicillins or tetracyclines, will usually lead to recovery of the ewe but almost always the affected half of the udder is permanently damaged and lost to milk production. Even at an early stage of the disease, treatment is often disappointing and may not result in recovery of the affected gland.

There is no treatment for chronic mastitis; most affected ewes are culled, although genetically valuable ewes with no functional gland are often retained in a flock.

Currently, there are no effective vaccines nor are there any proven means of prevention. Greater understanding of the predisposing causes of mastitis may lead to appropriate preventive husbandry and managemental procedures. Empirical measures that may help are the segregation and culling of affected ewes so as to minimize spread of infection. In flocks in which there is a high incidence of mastitis, the teats should be examined regularly and any lesions present treated immediately and not allowed to become foci of infection. If there is coliform mastitis, faecal contamination of the udder, prevalent in indoor lambing systems, should be reduced by regular provision of clean bedding or eliminated by putting ewes and lambs outdoors as soon as possible.

Administration of intra-mammary antibiotic to dairy ewes in the dry period has been shown to reduce the incidence of subclinical mastitis in the next lactation [15]. The efficacy and cost-effectiveness of this measure for the control of mastitis in meat-producing flocks require investigation under carefully controlled conditions.

Recent work on dairy sheep in France has shown that high SCC is heritable (0.15), as well as being an accurate indicator of subclinical mastitis. It may, therefore, be possible to breed ewes selectively with a lower risk of development of subclinical mastitis [16]. However, at present, no evidence is available of genetic influences on clinical ovine mastitis in meat-producing sheep.

CONTAGIOUS AGALACTIA

Contagious agalactia affects sheep, goats and, possibly, South American camelids. It has been recognized in southern Europe and the Middle East for almost 200 years and has since spread to most other parts of the world, but does not occur in the UK, where it is a notifiable disease.

Cause

Traditionally, contagious agalactia has been ascribed to *Mycoplasma agalactiae.* Disease caused by this species has been comprehensively reviewed [17] and remains the most important cause of contagious agalactia in sheep. Three other mycoplasma species have been shown to cause similar disease and they are now included as possible causes of contagious agalactia. One, *M. putrefaciens,* has not been isolated from sheep and will not be considered further. Both *M. capricolum* subsp. *capricolum* (Mcc) and the large colony variant of *M. mycoides mycoides* (MmmLC) infect goats more readily than sheep. MmmLC is

distributed widely, occurring in areas free of *M. agalactiae*, but has been isolated only rarely from diseased sheep. Mcc is thought to be able to infect sheep in the absence of goats and has a greater predilection for joints than the other pathogenic mycoplasmas [18]. It has been isolated only once, from genital lesions, in sheep in the UK.

Clinical signs

In sheep, disease is usually chronic. Infection is followed by a period of malaise, anorexia and pyrexia that coincides with bacteraemia. Pregnant animals may abort and occasionally sheep die at this stage. Severe mastitis follows; both glands are often swollen, hot and tender, and milk is yellow and granular, with many clots. Later, as the mammary gland becomes atrophied and fibrosed, milk production may cease and lambs sucking affected ewes often die at this stage. Acute mastitis is followed by arthritis characterized by accumulation of synovial fluids in one or more, usually carpal or tarsal, joints. Keratoconjunctivitis often occurs concurrently with arthritis. Pneumonia is not a consistent finding in adult sheep, but is more common in younger sheep, in which it may lead to rapid death without development of other signs.

Diagnosis

Clinical diagnosis is based on a high prevalence of mastitis, arthritis and keratoconjunctivitis in the flock, but laboratory confirmation should be sought. *M. agalactiae* is present in large numbers in mammary secretions and joint fluid, which are the samples of choice in the acute stage of the disease. *M. agalactiae* is not fastidious, may be cultured in modern broth media, and may be distinguished from other mycoplasmas by its inability to ferment glucose, by growth inhibition and by fluorescent antibody tests using specific hyperimmune rabbit sera. Recently, polymerase chain reaction (PCR) tests to identify specific sections of mycoplasmal DNA have been used to identify cultured isolates and these can be applied to clinical samples to accelerate laboratory diagnosis. Failure to detect the causative mycoplasma is not uncommon in chronically infected animals.

Serology is important for screening sheep before export or import and for investigating long-standing disease, where culture is unlikely to be rewarding. The complement fixation test is still used for international certification, but it suffers from lack of sensitivity. Enzyme-linked immunosorbent assays have been developed, but many of these suffer from a lack of specificity. A review of laboratory diagnosis of contagious agalactia is available [19].

Epidemiology and transmission

If ewes are infected when they are not lactating, latency may ensue, resulting in clinical signs of contagious agalactia developing at the onset of lactation. Excretion often precedes clinical signs – after experimental inoculation by the nasal or conjunctival route, clinical signs occurred after 20 days but excretion commenced after 1 day from the nose and 9 days in the milk [20]. After recovering from contagious agalactia, sheep may continue to excrete *M. agalactiae*, from the nasal cavities, conjunctivae, ear canals, vagina and mammary glands, without showing clinical signs of disease. Asymptomatic carriage in the middle ear occurs, and mites resident in this site may act as both reservoir hosts and vectors of mycoplasma infection. Asymptomatic carriers play an important role in the maintenance and spread of infection.

Lambs are usually infected by sucking infected milk, which can contain large numbers of mycoplasmas. Adult sheep may be directly infected from respiratory secretions of sheep or goats, or from contaminated fomites such as milkers' hands and bedding. Infection is possible by the intra-mammary, respiratory or oral routes, and mycoplasmas can survive in a dormant state in soil and bedding for several weeks.

Treatment, prevention and control

Appropriate antibacterials, given early in the disease, can bring about some clinical improvement but antibacterial therapy is thought to promote carriage of mycoplasmas in joints and other tissues where penetration of the antibacterial is poor, with subsequent recurrence of disease and shedding of bacteria.

In endemic areas, it may be possible for a flock to remain free of contagious agalactia through strict biosecurity and dairy hygiene measures and serological testing of replacement animals. Infected animals should be isolated and culled. Occurrence of

contagious agalactia in countries free of the disease would usually result in culling of the affected flock.

Both inactivated, adjuvanted and live attenuated vaccines for *M. agalactiae* infection have been used in countries bordering the Mediterranean. Both reduce the severity of clinical signs but neither prevents excretion of mycoplasmas, and vaccinated animals may become carriers of *M. agalactiae*. Vaccination, therefore, may help to reduce the incidence and severity of contagious agalactia in endemic areas but is not compatible with attempts to eradicate the disease.

ZOONOTIC IMPLICATIONS

In dairy sheep it is important to note that some strains of *S. aureus* causing mastitis produce enterotoxin and therefore the ingestion of milk may result in food poisoning. *B. cereus*, which causes sporadic cases of mastitis, may also cause food poisoning. Both *L. monocytogenes* and *Streptococcus equi* subsp. *zooepidemicus* are rare causes of mastitis in ewes, but their presence in sheep milk could result in infection in humans.

In Britain, there is currently no statutory requirement for heat treatment or pasteurization of sheep milk for liquid consumption or for manufacture but, in Scotland, untreated milk may only be sold directly from the farm of production to the final consumer. These regulations are periodically reviewed, and pasteurization of sheep milk may be obligatory in future.

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16

Chlamydial abortion

I.D. Aitken and D. Longbottom

Synonyms: enzootic abortion of ewes (EAE), ovine enzootic abortion (OEA)

Chlamydial abortion causes serious reproductive wastage in many sheep-producing regions of the world, with the notable exceptions of Australia and New Zealand, and occurs most commonly in flocks that are intensively managed over the parturient period. Despite the availability of well-publicized voluntary control measures, chlamydial abortion is still the most commonly diagnosed cause of infectious abortion in the UK and other countries of northern Europe. In southern Europe, *Brucella melitensis* infection is of greater prevalence. Chlamydial abortion also affects goats and, less commonly, cattle and deer.

CAUSE

The causative organism belongs to the highly specialized bacterial family *Chlamydiaceae*, which has a biphasic developmental cycle that alternates between an extracellular infectious phase and an obligatory intracellular replicative phase that is not infectious [1, 2]. The extracellular round (300 nm diameter) infective form, designated the elementary body (EB), has a thick wall around a dense internal core and stains Gram-negative. In contrast, the larger (0.5–1.6 μm diameter), thin-walled intracellular reticulate body (RB), has more homogeneous granular contents and does not survive outside the host cell. Adhesion of the EB to the outer membrane of a susceptible cell leads to its engulfment and occupancy of a membrane-lined vacuole formed from the cell wall, known as the chlamydial inclusion (Figure 16.1), to which cellular lysosomes are not attracted, thus allowing the EB to evade the defensive reaction of the host cell. Structural modification of the EB wall prepares the emergent thin-walled RB for its metabolic parasitism of the host cell and successive rounds of binary fission. After a 24–36-hour period, the accumulated progeny RBs revert to the EB form and are released by rupture of the cell or by exocytosis to renew the cycle of infection.

In the laboratory, chlamydiae can be grown readily in the yolk sac of the developing chick embryo and in

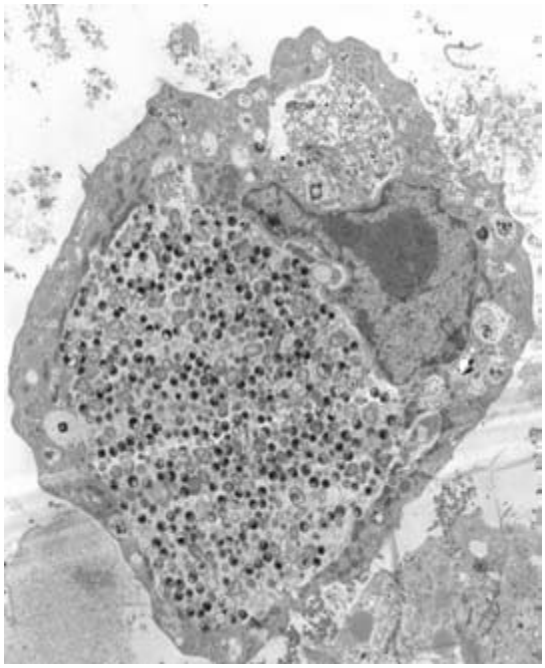

Figure 16.1: Chlamydial inclusion within an infected cell; both pale reticulate bodies (RB) and smaller, dark elementary bodies (EB) are visible.

cell cultures. Infectivity is destroyed by heat (10 min at 60°C) and by treatment with formalin or ether. However, infective particles can survive for weeks at low environmental temperatures and for years at −70°C.

The *Chlamydiaceae* underwent significant taxonomic reclassification in 1999 [3] based on ribosomal RNA phylogenetic analysis, and both morphological and phenotypic information. The family, which previously comprised the single genus *Chlamydia* and four species (*C. trachomatis*, *C. pneumoniae*, *C. psittaci* and *C. pecorum*), now also includes a new genus, *Chlamydophila*, and five new species (Table 16.1). Two of the new species, *C. muridarum* and *C. suis*, are present with *C. trachomatis* in the emended genus *Chlamydia*. The other three new species, *C. abortus*, *C. felis* and *C. caviae*, join *C. pneumoniae*, *C. psittaci* and *C. pecorum* in the new genus *Chlamydophila*. *C. trachomatis* and *C. pneumoniae* are principally pathogens of humans, while the other seven species infect a wide range of avian and non-human mammalian hosts. At least two species, *C. psittaci* and *C. abortus*, are recognized as causing zoonotic infections in humans [2]. Of the animal pathogens, three have been associated with chlamydial infections in livestock: *C. pecorum* infects ruminant and porcine species, and is associated with a number of conditions such as conjunctivitis, pneumonia, arthritis and apparently innocuous enteric infection, especially in sheep; *C. suis* appears restricted to swine, causing reproductive, respiratory and enteric infections; and *C. abortus* (formerly immunotype 1 *Chlamydia psittaci*) causes abortion in ruminants, particularly sheep and goats and swine.

Table 16.1: Family *Chlamydiaceae*: genera, species and principal hosts

Genus	Species	Principal host(s)
Chlamydia	*C. trachomatis*	Man
	C. muridarum	Mouse
	C. suis	Pig
Chlamydophila	*C. abortus**	Ruminants, pigs
	C. felis	Cat
	C. caviae	Guinea-pig
	C. pneumoniae	Man, koala, horse
	*C. psittaci**	Birds, including poultry
	C. pecorum	Ruminants, pigs

* Species with proven zoonotic potential.

All chlamydial species share a heat-stable lipopolysaccharide antigen that is the basis of the widely used *Chlamydiaceae*-specific complement-fixation (CF) test. However, interpretation of CF test results in sheep can be confounded by false positives, perhaps attributable to concurrent infection with *C. abortus* and *C. pecorum*, requiring recourse to species-level serological discrimination. Western blotting, immunofluorescence tests and a number of enzyme-linked immunosorbent assay (ELISA) procedures, based on native chlamydial antigen preparations, developed principally for research can be used selectively for this purpose but are not appropriate for large-scale routine application [4]. More recently, a competitive ELISA based on the *C. abortus* major outer membrane protein (MOMP) [5] and an indirect ELISA based on polymorphic outer membrane protein POMP90 [6] have been developed and shown to be both sensitive and specific in differentiating animals infected with *C. abortus* from those infected with various *C. pecorum* subtypes. The completion of the

sequencing of the *C. abortus* genome [7] and the future genomic sequencing of representative strains of the different subtypes of *C. pecorum* will enable more specific diagnostic tools to be developed in the future.

CLINICAL SIGNS

Chlamydial abortion is without specific premonitory signs, although closely observed individual ewes may display some malaise and exhibit slight vaginal discharge for a few days before expelling dead, moribund or weak lambs. It is the latter event, generally occurring some 2–3 weeks before term, that is the first indication of a problem. Although the fetal membranes present a variable extent of necrotic change, most lambs aborted at this late stage in pregnancy are well developed and quite fresh (Figure 16.2a in the colour plate section), the absence of autolytic change indicating that death *in utero* has been fairly recent. Some aborted lambs may appear 'pot bellied' owing to accumulation of blood-tinged fluid in serous cavities, and the fleece may be partially covered with, or discoloured by, flecks of pink–brown material originating from placental exudate. However, true degenerative changes such as corneal opacity and easily detachable wool, indicative of death some days or weeks before abortion, are seen in only a few cases. Generally, premature liveborn lambs are weak and, under farm conditions, rarely survive, even with nursing. Their deaths contribute to the sum of reproductive wastage caused by chlamydiae. In contrast, some ewes with placental infection give birth to live lambs, which they rear successfully and, in multiple births, the delivery of one dead lamb and one or more live weak or healthy lambs is not uncommon. For several days after abortion, ewes pass varying amounts of a discoloured uterine discharge but otherwise are clinically normal. The discharges eventually dry up, future breeding potential is not impaired and ewes are clinically immune, although some may excrete chlamydiae at their next oestrus and at subsequent lambing [8]. Occasionally, the placenta is retained and an associated metritis develops, leading to loss of condition and death as a result of secondary bacterial infection. Although yet to be confirmed under natural conditions, experimental studies suggest that inapparent fetal death and resorption in mid-gestation may be a previously unrecognized consequence of chlamydial infection in pregnancy and account for any above-average number of barren ewes in a flock affected by chlamydial abortion [9].

PATHOLOGY

Placentitis, the major and typical gross pathological component of chlamydial abortion, is a direct consequence of chlamydial colonization that starts about 8 weeks before the normal time for parturition. Experimental studies have established that, even if ewes are infected at an earlier stage of pregnancy, florid chlamydial growth in the placenta and ensuing pathology do not begin until after 90 days of gestation when rapid fetal growth commences [10], although chlamydial antigen can be detected in placental tissue some 3–4 weeks earlier. The reasons for the temporal restriction of placental pathogenesis have not been established. Possibly, contemporary physiological changes in hormonal activities serve to relax the suppression of chlamydial growth known to be exerted by interferon-gamma (IFN-γ) and other cytokines [11]. The crucial lesion occurs in the vascular placentome, the intimate apposition of the uterine endometrial caruncle and the fetal chorionic cotyledon that is the anatomical and physiological unit for maternal–fetal transfer of oxygen and nutrients (see Figure 4 in reference [2]). Chlamydiae infect and replicate within the trophoblastic epithelial cells of the chorionic villi in the hilus of the placentome, in which they produce visible cytoplasmic inclusions [12]. The placentomal infection and accompanying cellular disruption provoke an infiltration of inflammatory cells, consisting mainly of monocytes/macrophages. Loss of epithelial cells and progressive necrosis of the underlying villi and tips of caruncular septa are accompanied by accumulation of necrotic cellular debris and organisms in the placentomal haematomas. With time, infection spreads to the peri-placentome and then to intercotyledonary regions of the chorion, where damage of chorionic epithelial cells, oedema and cellular infiltration of the underlying stroma give rise to the reddened and thickened placental membranes that are characteristic of this disease, as well as the accumulation of a cream or straw-coloured exudate adherent to the surface of the placenta.

In contrast, endometrium opposed to infected and diseased chorion shows only limited infection and loss of epithelial cells. The associated cellular reaction is generally mild but, in severe cases, necrosis and sloughing of endometrial epithelium is accompanied by a more vigorous inflammatory response. As infection progresses in the placental membranes, vasculitis and thrombosis occur, most likely triggered by the release of chlamydial lipoolysaccharide (LPS), which has been shown experimentally to induce the pro-inflammatory cytokine, tumour necrosis factor-alpha (TNF-α) [12]. TNF-α mRNA is also readily expressed in the mononuclear cells of infected placentas, and of the inflammatory exudates, as well as in the cells of inflamed arterioles and arteries suggesting that production of TNF-α damages the placenta and thereby contributes to abortion or premature birth [12]. Not all placentomes of a placenta become infected and, in those that do, the degree and extent of inflammatory and destructive change is variable. However, impairment of the functional integrity of even a proportion of placentomes may compromise maternal–fetal exchange and contribute to fetal debility. In the later stages of gestation, chorionic epithelial cells constitute the major source of progesterone, the hormone responsible for maintaining pregnancy. Progesterone also interacts with locally synthesized oestradiol and prostaglandin in regulating parturition. Chlamydial infection alters the pattern of secretion of these three hormones, an alteration which may precipitate premature labour [13]. Thus, abortion is likely to result from a combination of several pathological, immunological and physiological events, including destruction of tissues by the pathogen and the concomitant release of LPS, the disruption of the normal hormonal control of pregnancy, vascular thrombosis and a pro-inflammatory immune response (IFN-γ and TNF-α) by the fetus. Interestingly, re-challenge of previously infected ewes also results in placentitis, although this may be dose-related and appears slower to develop when compared to naïve-challenged ewes, but these animals do not abort [14]. It is unclear why this is the case, but it is likely to be a consequence of the maternal acquired immune response. In the fetuses of naïvely challenged ewes, changes are confined to development of inflammatory or necrotic foci in the liver, lymphatic organs, lungs, skin and brain, although seldom are the changes so severe or extensive as to be grossly visible. Congestion and pinpoint white foci have been reported, but are not consistent features. Similar changes are observed in the fetuses of rechallenged ewes, although necrotic foci appear to be less frequent [14].

DIAGNOSIS

Abortion of well-preserved lambs in the final 2–3 weeks of pregnancy and an associated necrotic placentitis provide reasonable grounds for a provisional diagnosis (Figure 16.2a, b in the colour plate section) but, superficially, some cases of toxoplasmosis may present similarly and more than one micro-organism may be involved in an outbreak of abortion.

Necrotic placentitis is not a feature of the less common or sporadic causes of infectious abortion, e.g. *Listeria* and *Campylobacter*. Rarely, abortion occurs as a consequence of infection of pregnant ewes with the rickettsia, *Coxiella burnetii*, which is infectious for humans, causing an influenza-like illness known as Q-fever. Vast numbers of the organism are shed in infected placentas and uterine discharges. In stained smears, *C. burnetii* resembles *C. abortus*, but the two are antigenically unrelated and may be distinguished serologically.

Diagnosis of chlamydial abortion is readily confirmed by the demonstration of large numbers of EBs in smears made from diseased placental tissue and stained by a modified Ziehl–Neelsen procedure to reveal red bodies, singly and in clusters, against a blue background (Figure 16.2c in the colour plate section). Under dark-ground illumination, the stained bodies stand out clearly as bright green coccoid structures. If placental material is lacking, vaginal swabs from ewes that have aborted or the still-wet fleece of a recently aborted or stillborn lamb are useful alternatives for making smears. However, chlamydiae are likely to be less abundant in these sources, and they do not occur consistently in fetal stomach contents. For submission to a diagnostic laboratory, a small piece of affected placenta, free from gross contamination, should be placed in a suitable transport medium, such as SPG buffer (sucrose [74.6 g/l], KH_2PO_4 [0.512 g/l], K_2HPO_4 [1.237 g/l], L-glutamic acid [0.721 g/l]), supplemented with 10 per cent fetal bovine serum and non-penicillin antibiotics such as streptomycin and gentamycin. Under these conditions, chlamydiae survive for several days and will be evident in tissue smears. If necessary, isolation of chlamydiae may be attempted by

inoculation of tissue extracts into chick embryo yolk sacs or on to cell culture monolayers. Commercial kits are available for detecting chlamydial antigen in placental material [15]. If smears fail to detect any organisms and culture is not an option, then amplification of chlamydial DNA by the polymerase chain reaction (PCR) is a highly sensitive alternative approach [16]. There is a wide range of family- and species-specific chlamydial PCR protocols described in the literature, and the technique is beginning to be introduced into diagnostic laboratories throughout Europe.

In tissues, intracellular chlamydial inclusions can be demonstrated by Giemsa-staining of suitably fixed thin ($<4\,\mu m$) sections. However, immunological labelling methods, such as the direct immunoperoxidase technique [17], are simple, specific and rapid. Serology or PCR can be used to confirm or refute diagnosis. Infected or vaccinated ewes generally have low or moderate titres of CF antibodies but aborting ewes experience an episode of chlamydaemia, which often stimulates a post-abortion rise in antibody titre. Paired blood samples, one collected at or soon after abortion and the second about 2–4 weeks later, should reveal this rise. A less satisfactory alternative is to compare single samples from representative aborting and healthy ewes. Dubious outcomes, which may be due to concurrent infection with *C. pecorum*, may be resolved by Western blotting or by ELISA, as discussed earlier. The development of a DNA microarray hybridization assay using the ArrayTube™ platform that specifically differentiates chlamydial species holds much promise for the direct detection and identification of organisms from clinical samples [18]. Specific diagnosis early in the course of an outbreak allows the right control measures to be put in place but diagnostic monitoring should be maintained for the duration of abortions so that any other causes are not missed.

EPIDEMIOLOGY AND TRANSMISSION

Characteristically, chlamydial abortion occurs in flocks that practise intensive management over the lambing period, but is uncommon under extensive management such as occurs in UK hill flocks. In intensive systems, the practice of successive use of the same lambing field or sets of lambing pens, outdoor or indoor, can result in considerable environmental contamination by abortifacient chlamydiae. Exposure of susceptible females to such heavy infection is a major component in transmission of the disease. Ewes which abort or drop stillborn or weak lambs as a result of placental infection shed vast numbers of chlamydiae in the diseased placentas and uterine discharges. Under average UK spring weather conditions, chlamydiae remain viable for several days and afford opportunity for spread of infection. Survival is likely to be longer at temperatures near or below freezing. Susceptible ewes probably become infected by the oral or oro-pharyngeal route, and the tonsils may be involved as a primary focus. Generally, the infection remains inapparent, and the current pregnancy is not threatened unless it has more than 5–6 weeks to go to term. In flocks that have experienced chlamydial abortion or undetected placental infection, a proportion of animals will become intestinal carriers of *C. abortus* for an undefined period and intermittently shed chlamydiae in the faeces. The significance of this carriage to the epidemiology of abortifacient disease has not been established, nor has its relationship to enteric infection with *C. pecorum*. Intestinal infection with either or both species of *Chlamydophila* does not induce immunity against placental infection, but may complicate serology, as may *C. pecorum* infection at other sites.

In rams, *C. abortus* infection can spread to genital tissue, inducing orchitis, and the organism can be detected in semen during the acute phase of infection. Chronic testicular infection can result in palpable abnormalities. However, venereal transmission generally has been held to be uncommon or to have only a minor role in the epidemiology of chlamydial abortion. More recent experimental findings raise doubts about those assumptions. Following abortion induced by subcutaneous inoculation of *C. abortus*, ewes developed persistent but non-pathological infection of the reproductive tract, which was reactivated at subsequent breeding seasons, with transient excretion of chlamydial antigen detected at each oestrus. Further, careful deposition of *C. abortus* into the vagina of susceptible virgin yearlings at oestrus 5 weeks before natural mating resulted in delivery of underweight lambs of low viability but no abortions [19]. There was some placentitis, and chlamydial antigen was demonstrated in vaginal swabs and in the lambs. The extent to which cyclical vaginal excretion

of chlamydiae occurs in the field remains to be investigated together with any propensity for mechanical ewe-to-ewe venereal transmission by rams, which ultimately might cause or contribute to reproductive loss.

Live lambs born to dams with active placental infection and lambs fostered by ewes that have aborted or produced dead lambs are very likely to be infected as a result of close contact with their mothers, although no clinical evidence of infection may be apparent. Neither colostrum nor milk is a direct vehicle for transmission of chlamydiae from ewe to lamb, although infected utero-vaginal excretions contaminating the udder and teats could contaminate milk also. Experimental and field evidence suggests that, if bred in their first year, up to one-third of neonatally infected ewe lambs may develop placental infection, and some may abort in that first pregnancy. Clean flocks usually become infected by introduction of infected replacement females, which disseminate infection when they abort or lamb down. In-contact ewes of any age are likely to become infected, and the following year can bring a serious outbreak of abortion involving up to 30 per cent of the ewes. Thereafter, only the younger females are likely to be affected and an annual incidence of 5–10 per cent can be expected unless control measures are introduced. Clean replacements joining a flock that is already infected run a high risk of picking up chlamydiae at their first lambing, and some are likely to develop placental infection and may abort in the following year.

CONTROL AND PREVENTION

In an outbreak, the primary aim is to limit spread of infection. Ewes that abort or deliver dead or weak lambs should be clearly marked and isolated from other sheep until their uterine discharges have dried up (about 7–10 days). Aborted fetuses, dead lambs, placentas and contaminated bedding must be removed and destroyed. Lambing pens in which abortions have occurred should be cleaned and disinfected and, if possible, not re-used. Thorough hand-washing immediately after dealing with abortion material and before tending to other animals should be practised. As lambing progresses, continued vigilance is needed to ensure detection and swift isolation of all affected ewes particularly those that prematurely deliver live rather than stillborn lambs.

Strict segregation of aborting ewes is contrary to control procedures in other infectious abortion diseases such as border disease and campylobacteriosis, in which spread of infection to non-pregnant females is encouraged as a means of establishing immunity. If more than one agent is involved in an abortion problem, control of chlamydial infection should be the priority.

Treatment with long-acting oxytetracycline (20 mg/kg intramuscularly) may be used to moderate the severity of incipient chlamydial abortion; suppression of chlamydial multiplication extends the duration of threatened pregnancies. If necessary, antibiotic cover can be repeated at 10–14-day intervals, until lambing is completed. This therapy will reduce the number of organisms shed, but it will not eliminate infection nor can it reverse the pathological damage already done to a heavily infected placenta. Thus, some abortions and stillbirths will occur despite treatment. The use of oxytetracycline should be restricted to emergency situations and never advocated as a routine seasonal procedure.

Ewes that have experienced chlamydial abortion or placentitis are able to breed again normally in following seasons and maintain successful pregnancies. However, the possibility that a proportion may carry persistent infection of the reproductive tract and excrete organisms during oestrus should be borne in mind in devising a future control strategy. If they are to be retained, ewes with a history of abortion should be bred separately, using rams exclusive to the group or introduced only after 'clean' ewes have been mated. Separation for lambing would be advisable also.

The most effective way to avoid introducing infection to a clean flock is to keep it closed or to obtain replacements from sources known to be free of chlamydial infection. Flocks accredited under health schemes that involve annual clinical and serological monitoring are an obvious source of new breeding stock.

If any doubt exists about the status of animals acquired from non-accredited sources, pre-breeding vaccination should be adopted for the new flock entrants and considered for the home-bred group that they will join. Both inactivated and attenuated live vaccines are available. The former are based on whole chlamydial EBs grown in chick embryo yolk sacs or cell cultures and contain an adjuvant. They

give significant, but not complete, protection against field disease and have a role in controlling chlamydial abortion. However, inactivated vaccines comprising mineral oil-based adjuvants can induce local inflammatory responses at the injection site and pose a serious risk to the operator through self-injection. The live vaccine is a chemically induced temperature-sensitive mutant derived from an ovine abortion strain of *C. abortus* [20]. Administered at least 4 weeks before mating, it confers a strong and durable immunity against abortion and, in infected flocks, it reduces shedding of chlamydiae at parturition. As a live vaccine, it requires operator care in handling and administration. Both types of vaccine can be administered up to 4 weeks before breeding, although the inactivated vaccines can also be administered to pregnant ewes but not until at least 4 weeks after breeding. Vaccination should be repeated after 3 years or sooner if circumstances warrant it.

Under experimental conditions, inactivated subunit vaccines derived from detergent-solubilized EBs have proved effective, although relatively expensive to produce. Attempts at developing safer, more stable, lower cost vaccines based on recombinant protein and DNA vaccination technology have been less successful to date, although with improvements in methods of delivery, use of suitable adjuvants and the identification of new candidate protective antigens this should ultimately be an achievable goal [21].

ZOONOTIC IMPLICATIONS

Sporadic cases of respiratory illness have occurred in laboratory staff, abattoir workers and vaccine plant workers handling ovine *C. abortus*, but farmers and others tending infected flocks and cases of abortion apparently do not experience linked respiratory symptoms. In contrast, infected lambing flocks pose a very real threat to pregnant women because of the ability of *C. abortus* to colonize the human placenta. Several cases of chlamydial-induced human abortion and stillbirth with severe and, in one instance, fatal maternal illness are documented. Pregnant women should be advised against working with sheep, particularly during the lambing period. The zoonotic implications of *C. abortus* infections in various animal species have been described recently [2].

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17

Toxoplasmosis and neosporosis

D. Buxton and S.M. Rodger

TOXOPLASMOSIS

Clinical toxoplasmosis in sheep is manifest as abortion and results from a primary infection during pregnancy with the protozoon parasite *Toxoplasma gondii*.

Cause

Toxoplasma gondii is an obligate intracellular parasite and intestinal coccidium of felidae with a worldwide distribution. It has a life cycle that can be divided into two parts; a sexual cycle, restricted to the enteroepithelial cells of cats, which results in the production of oocysts; and an asexual cycle which occurs in a wide range of warm-blooded intermediate hosts, including humans and sheep [1] (Figure 17.1). There are three developmental forms of the parasite, the tachyzoite, the bradyzoite and the oocyst-containing sporozoites. All forms are infectious both to intermediate and final hosts but oocysts result only from gametogony (sexual cycle). The asexual cycle is characterized by an initial phase of rapid replication

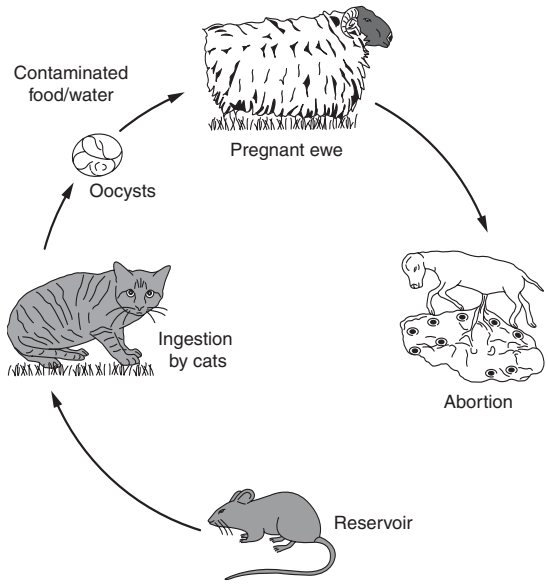

Figure 17.1: Transmission cycle of *Toxoplasma gondii*.

involving tachyzoites and a slower phase of restricted division involving bradyzoites. Each crescent-shaped tachyzoite (about 5 μm by 1.5 μm) can actively penetrate a host cell, where it becomes surrounded by a parasitophorous vacuole in which it multiplies by endodyogeny (two daughter cells form within the mother cell). Multiplication continues until the host cell ruptures, when the organisms are released to parasitize further cells. This process continues until the host dies or, more usually, develops immunity to the parasite. In the latter case, a persistent infection is established, extracellular organisms are thus eliminated, intracellular multiplication slows and tissue cysts containing bradyzoites develop. A small tissue cyst contains only a few bradyzoites but a large one may contain thousands. Cysts are found most frequently in brain and skeletal muscle, and represent the quiescent stage of the parasite within the host. Tissue cysts may break down on occasion to release bradyzoites that may then enter other cells to complete the asexual cycle [1].

Initiation of the sexual cycle occurs when a non-immune cat ingests food contaminated by oocysts or containing tachyzoites or tissue cysts. In the case of the latter, the tissue cyst wall, which is relatively resistant to acid pepsin in the stomach, is dissolved by proteolytic enzymes in the small intestine, and the released bradyzoites penetrate the epithelial cells of the small intestine. The parasite spreads to brain and muscles, where tissue cysts develop (asexual cycle) and simultaneously toxoplasms undergo gametogeny (sexual cycle) in the cat's enteroepithelial cells. Here, in the small intestine (most commonly the ileum), gametocytes develop 3–15 days after infection, giving rise to macrogametes and microgametes. The latter are released to penetrate mature macrogametes, triggering the formation of an oocyst wall around each fertilized gamete. The oocysts (10–12 μm in diameter), each almost filled by the sporont, are then discharged into the intestinal lumen to pass out in the faeces. Sporulation occurs within 1–5 days (depending on aeration and temperature) to produce two ellipsoidal sporocysts, each containing four sporozoites within each oocyst [1]. Thus, during the 4–12 days after ingesting tissue cysts, the cat is capable of shedding millions of oocysts in its faeces, after which it will remain persistently infected but will not normally excrete the parasite again, although stress and illness can trigger the recrudescence of infection. Unrelated illness therefore may lead to the excretion of oocysts in smaller numbers and for a shorter time than in a primary infection. Cats also may become infected by ingesting oocysts or tachyzoites, but in this case only around half of them go on to produce oocysts in relatively small numbers about day 19 or 20 after infection [1].

Susceptible sheep are infected by ingesting feed or water contaminated with oocysts, which are highly resistant and survive for long periods (>500 days) at room temperature in moist conditions. (They are destroyed by a 5 per cent ammonium solution within minutes, 95 per cent ethanol within an hour and 10 per cent formalin within 24 hours, but none of these methods could be used to treat food or water.) In contrast, tachyzoites and bradyzoites do not survive long outside the host and are readily killed by freezing and thawing, desiccation, standard disinfectants and even water.

Clinical signs

Sporulated oocysts ingested by a susceptible pregnant sheep excyst in the small intestine, each able to release the eight sporozoites. As early as 4 days after ingestion, tachyzoites may be found in the mesenteric

lymph nodes, where they multiply and are released into the blood to cause a parasitaemia, which may last from 5 to 12 days, disseminating infection around the body. The cessation of the parasitaemia coincides with the onset of a protective immune response and infection then persists as bradyzoites within tissue cysts. However, in the pregnant ewe, infection may establish in the gravid uterus, where maternal immunological responses are modulated in order to accommodate the fetus (a 'foreign' body or allograft with half of its genes derived from its father).

The ability of the fetus, with its placenta, to recognize and respond to the parasite is negligible in the early stages of gestation, but develops progressively with time so that lambs are born immunocompetent. Toxoplasms initially parasitize the caruncular septa, the maternal tissues of the placentome, before invading the adjacent trophoblast cells of the fetal villi and from there the rest of the fetus [2]. While infection in the latter part of gestation may have no clinical effect, with offspring being born normal but infected and immune, the outcome of infection early in gestation can result in death and resorption of the fetus and may be misdiagnosed as infertility.

In sheep, typical clinical signs of toxoplasma abortion usually result following infection in mid-gestation, with ewes producing stillborn and/or weakly lambs often accompanied by a mummified fetus. Cotyledons on the accompanying placenta(s) will also show lesions visible to the naked eye.

Pathology

Characteristically, the placental cotyledons appear bright to dark red and speckled with white foci of necrosis 2–3 mm in diameter (see Figure 17.2 in the colour plate section), while the intercotyledonary allanto-chorion appears normal [3]. Visible changes in lambs vary, the most obvious being the mummified fetus, typically a shrunken small brown miniature of a lamb with a head resembling that of a bird's skull, together with its own leathery placenta (see Figure 17.3 in the colour plate section). Fetuses dying later in gestation are born in various stages of decomposition, often with clear-to-bloody subcutaneous oedema and a variable amount of clear-to-bloodstained fluid in body cavities. These latter changes tend to indicate that the fetus failed over a period of time but they are not specific to infection with *Toxoplasma*.

The most obvious histopathological changes are the necrotic foci, visible macroscopically, in the cotyledons. Microscopically they appear as large foci of coagulative necrosis, relatively free of inflammatory cells, which may become mineralized with time. Rarely, small numbers of intracellular and extracellular toxoplasms are visible, usually on the periphery of the necrotic lesions or in a villus that is in the early stages of infection [2]. In the fetal brain both primary and secondary lesions develop.

Microglial foci, typically surrounding a necrotic and sometimes mineralized centre (see Figure 17.4 in the colour plate section), often associated with a mild lymphoid meningitis, represent a fetal immune response following direct damage by local parasite multiplication. Toxoplasms are found only rarely, usually at the periphery of the lesions. Focal leucomalacia, seen most frequently in cerebral white matter cores, is also common and is probably due to fetal hypoxia in late gestation (see Figure 17.5 in the colour plate section), caused by the lesions in the placentome preventing sufficient oxygen transfer from mother to fetus. In the liver there may be periportal accumulations of lymphoreticular cells and, on occasions, clusters of similar cells may be seen in the lung. In some cases, lymphoid aggregates have also been recorded in the heart [4].

Diagnosis

A diagnosis of toxoplasmosis as a cause of abortion and neonatal mortality depends not only upon the clinical and post-mortem picture but also on further laboratory investigations. These may include serology, the identification of characteristic histopathological changes in placental cotyledons and lambs' brains, the demonstration of parasite antigen in tissue sections by immunohistochemistry and the demonstration of parasite DNA by means of the polymerase chain reaction (PCR).

Serology

Of the many tests available, the longest established is the dye test [5], which uses live virulent toxoplasma tachyzoites, a complement-like 'accessory-factor' and test serum. When specific antibody acts on the live tachyzoite, it is altered so that it does not stain uniformly with alkaline methylene blue. The test is

expensive to operate and not free from hazard. The indirect fluorescent antibody test (IFAT) [5] gives titres comparable to the dye test and is safer as it uses killed tachyzoites. Many other tests exist and include the direct agglutination test (DAT) [6] and the latex agglutination test (LAT) [7], both relatively rapid and neither requiring sophisticated laboratory equipment. An enzyme-linked immunosorbent assay is ideally suited to screening large numbers of samples in a well-equipped laboratory [8] and various modifications are available. Unless a test specifically designed to detect acute-phase IgM toxoplasma antibody is used, a single positive serum sample only indicates infection of the host at some time in the past, although, as a general rule, a single dye test or IFAT titre of 1/1000 or greater suggests recent infection.

A more certain serological diagnosis of recently acquired toxoplasmosis in sheep therefore depends on the demonstration of a rising titre in paired sera collected 2–3 weeks apart. However, following a toxoplasma abortion, this may not be possible if initial infection occurred some time previously and the antibody titre is no longer rising. The demonstration of toxoplasma antibody in fetal fluids and precolostral lamb serum is a good indication of intrauterine infection with the parasite. With post-colostral samples, it is necessary to demonstrate IgM antibody against *Toxoplasma*, as IgG antibody could represent absorbed maternal colostral antibody.

Immunohistochemistry

Immunohistochemical techniques that allow visualization of both intact *T. gondii* and antigenic debris in tissue sections of aborted materials are convenient and sensitive and have the advantage, when compared with attempts at isolation, of detecting toxoplasma antigen even in decomposed tissues. Immunohistochemical methods such as the Dako EnVision (Dako Ltd, USA), the ABC indirect immunoperoxidase method (Vector Laboratories, USA) and the peroxidase anti-peroxidase (PAP) technique [9] are all equally good.

Polymerase chain reaction

Both viable and non-viable toxoplasms may be identified in tissues with the PCR, using either the P30 or the B1 gene of *T. gondii* as PCR targets in various clinical specimens collected from infected humans. Studies have been carried out also using ovine samples such as aborted placental material and fetal brain, liver and lung from experimentally infected ewes [10, 11]. Detection of *T. gondii* by amplification of the B1 gene would seem to be more sensitive than amplification of the P30 gene, owing to the repetitive nature of the former, of which 25–50 copies are present in the genome of *T. gondii* compared with the single copy of the longer P30 gene. Currently, the technique is not used in routine diagnosis, but the potential of the PCR to identify DNA in paraffin-wax sections from histopathological tissue blocks may broaden its applicability.

Differential diagnosis

Differential diagnosis should seek to eliminate other causes of abortion and neonatal mortality such as enzootic (chlamydial) abortion, campylobacteriosis, listeriosis, border disease and salmonellosis.

Epidemiology and transmission

Epidemiological studies have produced strong evidence indicating that the major source of toxoplasma infection for susceptible sheep is toxoplasma oocysts excreted in cat faeces [12] (Figure 17.1). Susceptible cats becoming infected with *T. gondii* for the first time after ingestion of tissue cysts excrete large numbers of oocysts, which then sporulate and become infective within a few days, remaining so for many months. Thus, infected faeces can contaminate bedding, unprotected stores of hay, concentrated animal feeds, water supplies and pasture. The most important source of infection for cats is probably wild rodents persistently infected with *T. gondii*, as it has been shown that in mice, infection can be passed vertically from generation to generation, although the offspring appear normal [13]. In this way, a reservoir of infection can be maintained in a given location. Vertical transmission in rats occurs much less frequently [14]. Infected birds also are a significant source of infection for cats. On a farm, the cat's territory tends to be centred on farm buildings, although individual male cats may command an area of up to 80 ha (200 acres). Cats acquire infection as a result of hunting so that many will have seroconverted by adulthood. Although less than 1 per cent of a population may be excreting oocysts at any time, infection of

the environment is readily maintained. Environmental contamination with oocysts is thought to be the only significant source of infection for sheep. While vertical transmission of the parasite from persistently infected dams to their offspring *in utero* (endogenous transplacental transmission) has been suggested in a small number of flocks, the weight of evidence from field and experimental investigations indicates that clinical toxoplasmosis is associated with primary infection of non-immune ewes during pregnancy [15]. A long-lasting immunity develops following primary exposure and animals are very unlikely to abort again due to toxoplasmosis in subsequent years.

Prevention, treatment and control

In general, young rather than old cats pose the greatest threat. Toxoplasma oocysts are produced during the initial infection, which occurs as the young cat starts to hunt small animals, although, as already noted, a recrudescence of infection and further oocyst excretion may occur in sick, older animals. Measures to limit the breeding of cats and to maintain healthy adults at numbers sufficient to keep vermin to a minimum, can be useful. While this is good general advice, once an outbreak of toxoplasmosis has started in lambing sheep, there is little that can be done other than to observe sensible precautions by responsible disposal of dead lambs and infected placentas and disinfecting contaminated pens if applicable. Sheep-to-sheep transmission at lambing does not appear to occur to any significant extent in toxoplasmosis. More direct preventive measures include chemoprophylaxis, chemotherapy and vaccination.

Chemoprophylaxis

Research has shown that a significant reduction in toxoplasma-induced perinatal lamb mortality can be achieved by feeding decoquinate during pregnancy [16]. It should be added to the feed to provide 2 mg/kg body weight/day from around mid-pregnancy. Decoquinate is most effective if it is already being fed to susceptible ewes at the time they encounter infection rather than after infection is established. As it does not eliminate the parasite, challenged ewes become persistently infected and develop protective immunity to subsequent challenge. It is not suitable in management systems in which supplementary feed is not given. Monensin also has been shown to be effective when added to the feed (15 mg monensin/head/day) but, because of the low toxic:therapeutic ratio, it is no longer approved for use in sheep.

Treatment

A combination of pyrimethamine and sulfadimidine, well tried in human medicine, blocks folate synthesis, and has been shown to be effective in the treatment of infected sheep [17]. The drug combination, baquiloprim and sulfadimidine, also blocks folate synthesis and has given promising results in a controlled pilot study in non-pregnant sheep (unpublished data).

Vaccination

Following abortion ewes are immune and while to date so-called 'killed' vaccines are not effective, good immunity is induced in sheep by a live vaccine that comprises S48 *T. gondii* tachyzoites [18]. It is currently licensed for use in New Zealand, the UK, Ireland, France, Spain and Portugal, and it induces very good, long-lasting immunity in sheep (and goats) after only one injection and has no measurable fall-off in immunity after 18 months. Non-pregnant, healthy ewes may be vaccinated at any time, apart from the 3-week period before tupping. Other live vaccines, such as that against chlamydial abortion, may be administered at the same time but at different sites, although it is arguably better that such injections are also separated by a couple of weeks. When handling and administering the live vaccine, care must be taken to avoid accidental self-infection.

NEOSPOROSIS

Neospora caninum, which is closely related to *T. gondii*, has been recognized only since the 1980s and, while it appears to be a major cause of fetal loss in cattle [19], it may cause infrequent losses in sheep and goats also.

Life cycle

N. caninum naturally infects a wide range of hosts including cattle, deer, sheep and goats as well as

dogs, coyotes, foxes and wolves. An asexual life cycle, similar to that of *T. gondii*, involving tachyzoites and tissue cysts containing bradyzoites, takes place in these animals, with the sexual life cycle taking place in dogs and coyotes, which produce oocysts that closely resemble those of *T. gondii*. Sporulation can occur within 3 days of excretion in dog faeces, and they will then contain two sporocysts each with four sporozoites [19]. The tachyzoites, which divide by endodyogeny, may be either crescent shaped or ovoid, 3–7 μm long by 1–5 μm wide, depending on their stage of division. The tissue cysts range from 20 to 100 μm in diameter and typically have a clearly discernible cyst wall up to 4 μm thick surrounding a variable number of closely packed bradyzoites and are primarily found in neural tissues (Figure 17.6 in the colour plate section) [19].

Clinical signs

Clinical neosporosis is most important in cattle, in which it is recognized to be a serious cause of abortion throughout the world. Transmission from a persistently infected cow to the fetus is important in the epidemiology of the bovine disease and is responsible for more cases of abortion than ingestion of oocysts produced by dogs. Recrudescence of a persistent infection in the mother occurs during pregnancy, permitting a parasitaemia, which allows tachyzoites to invade the gravid uterus, the placenta and then the fetus. Infection early in gestation, when the fetal immune system is little developed, can be fatal to the fetus with resorption or abortion. Exposure of an older fetus, with its better developed immune system, may result in the birth of a clinically normal but congenitally infected calf [20].

Serological evidence of infection with *N. caninum* in sheep is scant, with reports of three of 660 ewes that aborted in the UK being seropositive and 55 of 597 sheep in São Paulo, Brazil being seropositive [21]. The parasite has been demonstrated in a naturally infected ewe and her offspring, and isolated from another sheep, and neosporosis was identified as the cause in a weak, ataxic lamb, that died when 1 week old [21]. The spinal lesions in that animal were similar to those seen in calves congenitally infected with *N. caninum*. While pregnant sheep have been shown to be very susceptible to experimental infection with *N. caninum* [22], to date, the evidence points to it being an uncommon infection in sheep. *Neospora* has been associated with abortion in goats in the USA and Costa Rica and, while only a few fetuses were lost, there was evidence of seroconversion to *N. caninum* in the Costa Rican herd [19].

ZOONOTIC IMPLICATIONS

T. gondii readily infects man and the proportion of the human population infected with the parasite, dependent on age and the environment, can be 30 per cent or higher. While infection is relatively common, clinical illness is relatively rare. *N. caninum* has not been shown to be zoonotic, although experimental infection of primates has been achieved.

Toxoplasma can pose a serious threat to the unborn human child if the mother becomes infected for the first time while pregnant. The rate of congenital infection varies from one region or country to another, but is usually between one and six per 1000 pregnancies. Other people at risk of developing clinical illness include those who are immunosuppressed, such as tissue transplant patients, victims of AIDS, people suffering from certain types of cancer and those undergoing certain forms of cancer therapy. The very young and very old also may be more susceptible. On occasions, people with no apparent immune deficiency may develop an illness characterized by general malaise, fever and lymphadenopathy. Most human infection appears to result either from exposure to an environment contaminated with toxoplasma oocysts or from ingestion of raw or lightly cooked meat containing toxoplasma tissue cysts. Operator caution is needed when using the live vaccine for sheep.

Tissue cysts

Among the food animals, sheep, goats and pigs, once infected, may remain so for life, with bradyzoites in tissue cysts in brain and muscle. There is less risk of tissue cysts being present in cattle and deer. Free-range poultry meat also may be infected. Cooking of red meat sufficiently to induce a colour change to brown would be expected to kill the parasite, while freezing and thawing will significantly reduce the viability of toxoplasma bradyzoites, if not kill them. Those handling raw meat should wash their hands afterwards. Shepherds, veterinary surgeons, slaughterhouse staff

and butchers, by the nature of their work, may experience greater exposure to the parasite.

Oocysts

Sporulated oocysts may remain infectious for a long period so that flower beds and vegetable plots in which cats have defaecated can present a risk of infection to the vulnerable. It is a sensible precaution to wash all vegetables, whether they are to be cooked or not, before they are eaten. Children's sand pits also may be a source of infection if soiled by cats and should be kept covered when not in use. On the farm, cats may soil hay, bedding and bulk grain stores, and so pose a threat of infection to farm staff as well as stock.

Abortions in domestic stock

Apart from seeking specialist assistance in reaching a diagnosis, common-sense precautions should be taken, including hand-washing and the use of disinfectants. Infected placentas and dead lambs should be disposed of in such a way as to prevent their ingestion by other animals.

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18

Border disease

P.F. Nettleton and K. Willoughby

Synonyms: B disease, 'hairy-shaker' lamb or 'fuzzy' lamb disease, congenital trembles, ovine pestivirus disease

Border disease (BD) is a congenital virus disease of sheep reported in 1959 from the border region of England and Wales and since recorded throughout the world. BD is characterized by barren ewes, abortion, stillbirths, and the birth of small, weak lambs, some of which show tremor, abnormal body conformation and hairy fleeces ('hairy-shaker' or 'fuzzy' lambs). In some outbreaks no hairy-shaker lambs are born, and the disease is difficult to distinguish from other kinds of abortion. Occasionally, losses at lambing time are low, veterinary advice being sought when a group of lambs fails to thrive and the number of scouring and dying lambs is abnormally high.

CAUSE

The cause of BD is a virus (BDV) serologically related to bovine viral diarrhoea virus types 1 and 2 (BVDV-1, BVDV-2) and classical swine fever (CSF) virus, the four viruses being grouped in the genus *Pestivirus* within the family Flaviviridae [1]. They were named after the diseases from which they were first isolated. Traditionally, pestiviruses isolated from pigs have been termed CSFV, those from cattle BVDV and those from sheep BDV. Cross-infection between species occurs readily and viruses are now grouped by their antigenic reactivity and their nucleotide sequences at selected genomic regions. Pestiviruses are enveloped, spherical particles approximately 50 nm in diameter. The genome is a positive single-stranded RNA molecule, approximately 12.5 kb long, having a single open reading frame (ORF) (Figure 18.1) [2, 3]. Virtually all pestivirus isolates from sheep and goats are non-cytopathic in cell culture. Two cytopathic sheep isolates have been described and both contain insertions of cellular sequence within the NS2–3 encoding region, which results in its cleavage to NS2 and NS3 [4]. This is analogous to the similar process in BVDV viruses, which is associated with the development of mucosal disease in cattle. Such cattle are persistently infected with non-cytopathic (NCP) BVDV following infection *in utero*. Mutation of the persisting virus RNA in the region coding for NS2–3

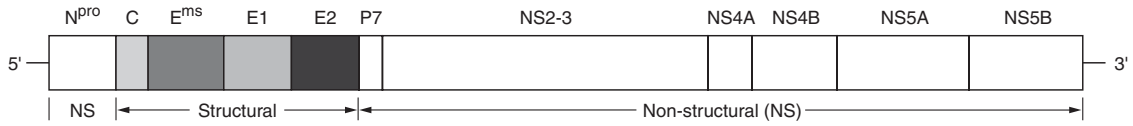

Figure 18.1: Diagrammatic representation of final protein products of the single open reading frame (ORF) of a non-cytopathic BD virus. The ORF is flanked by a 5′non-coding region of 366–72 bases and a 3′non-coding region of 225–73 bases. The ORF encodes proteins processed by viral and cellular enzymes. Of the proteins within the ORF, the first is a non-structural autoprotease N^{pro} followed by the structural C nucleocapsid protein and glycoproteins E^{ms}, E1 and E2, with E2 the immunodominant major envelope protein.The remaining proteins are non-structural [2, 3].

can result in overproduction of NS3, which is correlated with the development of mucosal disease.

Within the four principal species of pestiviruses (BDV, BVDV-1, BVDV-2, CSFV) numerous subgroups are constantly being identified. Genetic sequence analysis has shown that genetic variation is considerable among BD viruses isolated from sheep in Europe so that three subgroups have been identified [5]. A further subgroup has been identified in Tunisian sheep [6], while two further putative novel BDV subtypes have been reported from an Italian goat [7] and a Pyrenean chamois (*Rupicapra pyrenica pyreneica*) [8].

Antigenic analysis of a limited number of pestiviruses using cross-neutralization tests supports genetic analysis and provides evidence for seven major antigenic groups corresponding to BVDV-1, BVDV-2, CSFV, a 'giraffe' isolate and three BDV groups [5]. Comparison of 10 sheep pestiviruses from the UK showed two groups of serologically distinguishable virus isolates, one group related to Moredun BDV and the other to the Weybridge and BVD viruses [9]. These antigenic differences correlate well with genotyping results, since all the Moredun BDV-related isolates type as true BDVs, whereas representatives of the other group all fit into the BVDV-1 genogroup. These results, together with genotyping studies on a total of 38 UK sheep isolates, show that 23 (60 per cent) are true BD viruses and 10 (26 per cent) belong to the BVDV-1 group. A further five isolates (13 per cent) belong to the BVDV-2 group [10]. Continuing surveillance of UK sheep isolates from 25 outbreaks in 2003–4 demonstrated that 80 per cent of outbreaks were due to BD virus and 20 per cent were due to BVDV-1. No BVDV-2 viruses were detected in sheep, although BVDV-2 was detected in cattle in England during this time.

The relevance of strain typing results to vaccine development requires further work. In particular, there is a dearth of cross-protection studies in sheep. The only recorded cross-protection test used field brain material to infect and challenge pregnant ewes [11]. The viruses recovered from those field outbreaks were G1480 (Moredun reference strain), a true BD virus, and B1056, a BVDV-1 isolate. In that thorough experiment, 12 pregnant ewes previously exposed to BVDV-1 were challenged intramuscularly with heterologous BD virus on day 54 of gestation; 11 ewes (92 per cent) had diseased lambs. A further 11 pregnant ewes previously exposed to BDV were similarly challenged with the heterologous BVDV-1 strain; one ewe aborted and five had diseased progeny, i.e. 55 per cent of the ewes had diseased lambs. In contrast, similar-sized groups of immune ewes all had normal lambs when challenged with the homologous virus to which they had been previously exposed.

This result would imply that any BD vaccine should contain at least one representative from the BDV and BVDV-1 groups. The protection between BVDV-2 and these other two sheep-infecting groups will need to be studied. There is evidence that some pestivirus strains elicit significantly broader cross-neutralizing antibodies than others [12], which is likely to be relevant to the design of any future BDV vaccine.

CLINICAL SIGNS

Healthy newborn and adult sheep exposed to isolates from different countries experience only mild or inapparent disease. Slight fever and a mild leucopenia are associated with a short viraemia detectable between 4 and 11 days post-infection, after which serum-neutralizing antibody appears.

However, one French isolate of BDV produced high fever, profound leucopenia, enteritis and death

in 50 per cent of 3–5-month-old lambs [13]. The isolate was recovered from a case of 'Aveyron disease', a disease reported in 1983 among intensively reared milk sheep. One other pestivirus isolate that caused disease in 4–5-month-old lambs was discovered in The Netherlands during investigations into disease in piglets following earlier vaccination of the sows with a live CSFV vaccine. The expected 'Chinese' strain of CSFV could not be demonstrated in the vaccine, but a contaminating pestivirus was shown by its reactivity to be either BVD or BD virus. The contaminant was probably of sheep origin from the lamb kidney cells used to produce the vaccine. Lambs to which the vaccine was administered experimentally developed fever, prolonged leucopenia, anorexia, conjunctivitis, nasal discharge, pale conjunctivae, dyspnoea and diarrhoea, and four of eight lambs died [14].

Generally, clinical signs of BD are seen only following infection of pregnant ewes. While the initial maternal infection is usually subclinical or mild, the consequences for the fetus are serious. Fetal death may occur at any stage of pregnancy but is commoner in fetuses infected early in gestation. Small, dead fetuses may be resorbed or their abortion pass unnoticed, since the ewes continue to feed well and show no sign of discomfort. The observant shepherd may notice brown staining around the perineum, which is readily mistaken for scour. As lambing time approaches, the abortion of larger fetuses, stillbirths and the premature births of small, weak lambs will be the first indication that BD is occurring. During lambing, an excessive number of barren ewes will become apparent, but it is the diseased live lambs that present the main clinical features characteristic of BD. The signs are very variable and depend on the breed, the virulence of the virus and the time at which infection was introduced into the flock. Outbreaks on some farms result in lambs with few clinical features. In other flocks, however, a range of abnormalities will be represented by different lambs.

Affected lambs are usually small, weak and of poor conformation. The limbs are short, fine-boned and knees and hocks may be incapable of extension. The back is short and arched, and the head narrow with doming of the frontal bones and shortening of the mandible. The eruption of incisor teeth may be delayed and pigmentation of these teeth may be obvious. Many lambs are unable to stand unaided, at which time the nervous signs and fleece changes become apparent. When they occur, the nervous signs of BD are its most characteristic feature and take the form of tremor, varying from violent rhythmic contractions of the muscles of the hind legs and back to barely detectable fine trembling of the head, ears and tail. Obvious fleece changes are apparent only in smooth-coated breeds, affected lambs having hairy, rough fleeces due to long hairs rising above the fleece to form a halo, especially over the nape of the neck and upper body. Abnormal brown or black pigmentation of the fleece may also be a feature in BD-affected lambs.

Weak lambs of normal size without typical nervous signs or fleece changes may show a wide range of abnormal behaviour. More obvious lambs of this type have excessively long legs ('camel-legged') a flattened cranium and poorly sprung rib cage; they can stand but wander aimlessly, show little sucking drive and die within 1 or 2 days. Others show head pressing, apparent blindness, nystagmus and gait abnormalities.

With careful nursing, some affected lambs can be reared, although deaths occur at any age. The nervous signs gradually decline in severity as the affected lamb matures, but weakness and swaying of the hindquarters together with fine trembling of the head may reappear at times of stress. Halo hairs are soon lost from the birth-coat, which is replaced by a coarse, kempy fleece (Figure 18.2 in the colour plate section). Affected lambs often thrive badly, make poor weight gains and, under field conditions, many will die before or around weaning time. Occasionally, this is the first presenting sign of disease, when losses at lambing time have been low and no lambs with obvious clinical signs have been born.

PATHOLOGY

Acute infections of lambs and non-pregnant mature sheep with virtually all BDV isolates produce no obvious pathological changes. The exceptional pestiviruses recovered from cases of 'Aveyron disease' and from the CSF vaccine in The Netherlands, however, can cause severe acute infections, with dying sheep showing lesions of haemorrhagic enteritis and fibrinous pneumonia.

The most serious consequences occur when BDV infects susceptible pregnant ewes. Viraemia leads to

an acute necrotizing placentitis, detectable about 10 days post-infection. The placentitis may be severe enough to contribute to the early fetal death and abortion in some cases but, if the pregnancy is sustained, the lesions heal in about 25 days. Virus can cross to the fetus within 1 week of infection and, while the immune response of the ewe rapidly eliminates all virus from the maternal tissues, it has no effect on virus replicating in the fetus. The ultimate outcome of the fetal infection depends on several factors, including the strain and dose of virus, the breed of fetus and its ability to repair damage. The most important factor, however, is the stage of fetal development at which infection occurs. The age at which the fetus gains immunological competence is critical in determining the distribution and persistence of virus, which, in turn, influences the extent of fetal damage. The ovine fetus can first respond to an antigenic stimulus between approximately 60 and 85 days of its 150-day gestation period. The possible fates of fetuses infected before or after this crucial period are summarized in Figure 18.3.

In fetuses infected before the onset of immune competence, viral replication is uncontrolled and fetal death is likely. In experimental infections, deaths of 50–75 per cent of fetuses occur, depending on the virus strain used. In lambs surviving infection in early gestation, virus is widespread in all organs. Such lambs appear to be tolerant to the virus and have a persistent infection, usually for life. Typically, there is no evidence of any inflammatory reaction, and the most characteristic pathological changes are in the central nervous system (CNS) and skin.

At all levels in the CNS there is a deficiency of myelin associated with an increased density of interfascicular glia. The lesions are most obvious in newborn lambs, and the myelin deficiency is thought to result from direct viral action on oligodendrocyte precursors, leaving a deficit of mature myelin-forming cells at critical stages of CNS development [15]. The myelin deficiency has also been attributed to depressed levels of circulating thyroid-gland hormones [16]. In older lambs, myelin defects are less obvious and usually have resolved by 20 weeks of age, while the density of interfascicular glia remains high and cells with swollen nuclei can persist for up to 3 years.

The fleece abnormalities of BD lambs result from an increased size of primary wool follicles and decreased numbers of secondary wool follicles in the skin. This results in a greater-than-normal proportion of large fibres, many of which are medullated, and a reduced number of fine fibres. Although gross fleece changes become less apparent as the BD lamb matures, serial skin biopsies have shown that the follicular alterations are permanent. If fetal infection occurs between approximately 60 and 85 days, when the immune system is developing, the outcome is unpredictable. Some lambs will be born antibody-positive and virus-negative, while others will be viraemic and antibody-negative. Infections at this stage can produce a

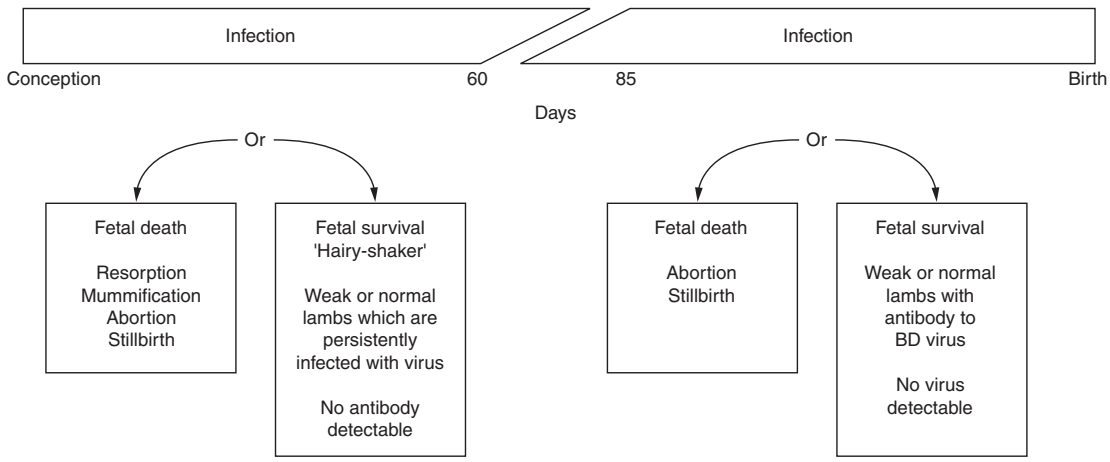

Figure 18.3: Possible outcome of border disease virus infection before and after the fetus gains immunological competence between approximately 60 and 85 days gestation.

violent necrotizing and inflammatory process within the fetal CNS, leading to extensive destruction of the germinal layers of the brain. The ensuing lesions of cerebellar hypoplasia and dysplasia, hydranencephaly and porencephaly result in lambs showing severe nervous signs and locomotor disturbances. Some lambs may show skeletal abnormalities such as arthrogryposis and excessively long legs. The severe destructive lesions appear to be immune-mediated, and lambs with such lesions frequently have high concentrations of serum antibody to BDV.

Fetal infection after approximately 85 days gestation is met by an effective immune system. Fetal death is rare and virtually all lambs will be born apparently normal with demonstrable antibody against the virus. Nevertheless, microscopic lesions can be detected principally in the CNS and consist of a disseminated nodular periarteritis, suggestive of a cell-mediated allergic reaction, affecting small and medium-sized arterioles. These lesions can remain detectable for at least the first year of postnatal life.

The fate of lambs infected in early gestation is variable. Clinically affected lambs have a low chance of survival; many die early in life, while survivors have a poor growth rate and an increased susceptibility to other diseases. Less severely affected lambs and apparently normal persistently infected lambs can survive for years (Figure 18.4).

After colostral antibody to BDV has waned, persistently infected lambs become viraemic with no, or low, titres of BDV-neutralizing antibody. They readily yield non-cytopathic virus from blood and bodily secretions and appear to be immunotolerant to the infecting virus. Normal concentrations of serum protein and immunoglobulins and the ability of the animals to produce antibody to other agents confirm that persistently infected sheep can have a normal immune responsiveness in spite of their specific tolerance. Development of anti-pestivirus antibodies by some persistently infected sheep in later life shows that the immune tolerance may not be absolute.

The mechanisms involved in the establishment and maintenance of persistent BDV infections are complex, and much remains to be learned. An unstable equilibrium appears to be established between the host and the virus. Evidence for breakdown of this equilibrium has been seen in some sheep among groups of persistently infected sheep housed apart from all other animals. Spontaneous development of intractable scour, wasting, excessive ocular and nasal discharges, sometimes with respiratory distress, have been encountered in sheep aged from 2 to 21 months.

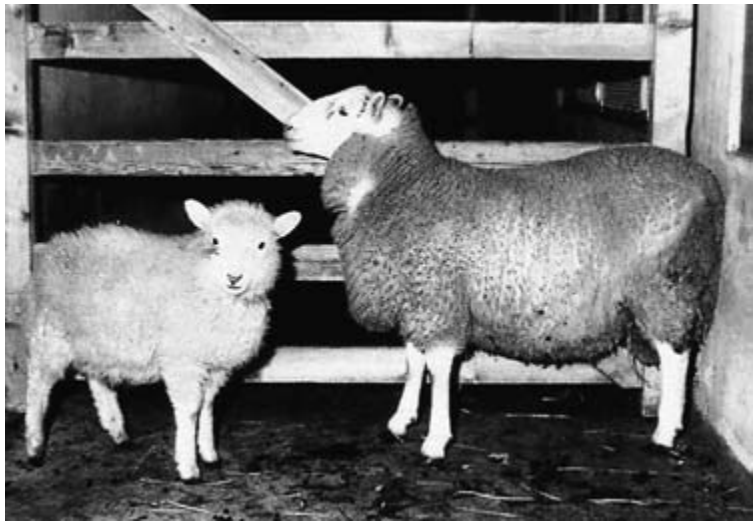

Figure 18.4: Seven-month old Cheviot X lambs both persistently infected with BDV. While one is of normal size and appearance, the other is severely stunted, even though they were born to dams infected with the same virus at the same stage of early gestation.

At necropsy, such sheep can have systemic, chronic, multifocal inflammation including nephritis, myocarditis and pneumonia. More consistent, striking lesions are seen in the gut where there is gross thickening of the distal ileum, caecum and colon, resulting in focal hyperplastic enteropathy. Histologically, there is ulcerative hyperplasia of the mucosa with foci of massive penetration of the muscularis, around which there is mononuclear cell infiltration and necrosis. Cytopathic BDV can be recovered from the gut of these lambs and, with no obvious outside source of cytopathic virus, it is most likely that such virus originates from the lamb's own virus pool. Other persistently infected lambs in the group do not develop the disease. This syndrome, which has also been recognized in occasional field outbreaks of BD [17], has several similarities with bovine mucosal disease [18].

DIAGNOSIS

The diagnosis of BD will present little difficulty if typical 'hairy-shaker' lambs are born. Even so, laboratory confirmation is advisable, since swayback, daft-lamb disease, bacterial meningo-encephalitis, polio-encephalomalacia will have to be considered in differential diagnosis. Placentas and fetuses aborted owing to BDV infection have no distinguishing characteristics so, similarly, laboratory confirmation will be necessary to differentiate BD from the other known infectious causes of ovine abortion. Histological examination of the CNS can confirm BD but should be supported by the demonstration of viral antigen in tissues by specific immuno-staining or virus isolation from blood and tissues. Aborted fetuses are often unrewarding for the demonstration of virus, since they have usually died several days before expulsion. Whenever possible, recently dead or severely affected lambs should be taken to the laboratory. Alternatively, the best tissues to submit are thyroid, kidney, brain, spleen, gut and lymph nodes; fresh for antigen detection and in virus transport medium for virus isolation (Chapter 75). Heart blood for serology and a blood sample from the dam should be collected.

From live newborn 'hairy-shaker' or weak lambs confirmation of infection is based on the demonstration of virus in a heparinized blood sample by antigen enzyme-linked immunosorbent assay or by virus isolation in cell culture. Colostral antibody can mask the presence of virus so that precolostral samples should be collected. To detect the antibody-negative, virus-positive, persistently infected sheep, all animals in a suspected group should be blood-sampled. Alternatively, reverse-transcriptase polymerase chain reaction may be used for diagnosis of persistently infected animals or fetuses [19, 20]. This test is not yet commercially available in the UK for sheep but has several advantages over conventional testing; virus may be detected in the face of colostral antibody, results can be available very rapidly and it is possible to type the virus molecularly which may be important for surveillance. Serological examination of individual sheep for BD antibodies is rarely helpful, but antibody testing of a 10 per cent sample of different age groups of animals can be useful for demonstrating the presence and extent of BDV infection in a flock.

EPIDEMIOLOGY AND TRANSMISSION

Sheep-to-sheep contact is the principal way in which BDV is transmitted, and the most potent source of virus is the persistent excretor. Flocks with no previous experience of the disease are particularly vulnerable. Purchased persistently infected gimmers have been shown to introduce BDV [21]. Persistently infected lambs that reach maturity often have reduced fertility. Females that conceive either abort or produce persistently infected lambs, sometimes over a period of years. Rams usually have small, soft testicles, but can transmit virus in their semen and other secretions. The speed of virus spread in a susceptible flock exposed to one or more persistent excretors will depend on the contact between sheep. At grass, intimate contact at mating time or gathering for any purpose will allow the virus to spread widely, whereas in the summer, with no close contact, virus spread will be slow. Any intensification of husbandry, particularly housing during early pregnancy, increases the risk of an explosive outbreak of BD. In flocks in which the disease is endemic, older ewes are immune and the progeny of primiparous ewes are most commonly affected. Pestiviruses from other domestic ruminants and pigs can also cause BD in sheep [22]. Under natural farming conditions, the most serious

threat comes from cattle, since they are the principal hosts of pestiviruses; serological survey data from several countries indicate that about 70 per cent of mature cattle have antibody to BVD virus, whereas in the same regions the prevalence of pestivirus antibody in sheep is much lower, varying from about 5 to 50 per cent. Furthermore, cattle persistently infected with BVD virus are numerous; surveys in several countries have shown the prevalence of such cattle to be 0.4–0.9 per cent in randomly selected animals and between 1.7 and 10.5 per cent in herds experiencing disease.

Among free-living ruminants, pestiviruses have been isolated from red, roe and fallow deer, and serological surveys in Europe, North America and Africa have shown that many species have detectable antipestivirus antibodies. Where sheep are grazed extensively in contact with free-living ruminants, the possibility of infection cannot be excluded.

One other possible source of BDV infection is a live vaccine contaminated with a pestivirus. Both sheep pox and orf virus vaccines administered to sheep have been incriminated as vectors of BDV infection [23].

CONTROL

The control of BD will depend on the extent of infection in a flock. Sporadic outbreaks can be controlled by removing for slaughter the entire lamb crop and sheep suspected of introducing the disease before the start of the following breeding season. In endemically infected flocks, susceptible animals retained for breeding should be deliberately exposed, while they are not pregnant, to known persistently infected lambs. Close herding for at least 3 weeks preferably indoors is necessary for BDV to spread effectively and exposure should end 2 months before the start of the breeding season.

On farms with no history of BDV, the introduction of new breeding stock needs careful consideration. Ideally, replacement females should be home-bred, and all purchased rams should be blood-tested to ensure they are not persistently infected. Where females are also purchased, the feasibility of blood-testing them should be considered. Recently purchased females should always be mated and kept separate from the rest of the flock until lambing time. Because of the risk of infection of sheep from cattle, it is essential that pregnant ewes are never mixed with cattle.

There is only one commercial vaccine for the control of BDV, and it is not licensed in all countries. It is a killed adjuvanted vaccine, which contains representative strains of BDV and BVD-1 viruses, and which should be administered to young animals before they reach breeding age [24]. Further vaccine development is required, with candidate vaccines being tested for efficacy in pregnant sheep.

BD IN GOATS

BD occurs also in goats. Although there appear to have been few reported outbreaks of natural BD [25], experimental infections of pregnant goats with BDV produces severe placentitis and clinical and pathological signs in the offspring similar to severe BD in sheep. The majority of pestiviruses isolated from goats have been typed as BVDV-1 and may have originated from cattle. One goat isolate from a mixed sheep and goat flock appears to be a novel BD virus [7].

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19

Other infectious causes of abortion

R. Mearns

Various infectious agents – viruses, bacteria, protozoa – can adversely affect normal pregnancy in the ewe, either by targeting the conceptus with little constitutional harm to the dam or by inducing acute systemic illness in the ewe with abortion as a consequence [1]. This chapter gives an account of several bacterial causes of abortion additional to infectious causes covered elsewhere in the book.

SALMONELLOSIS

Several serotypes of *Salmonella* have been associated with deaths and abortion in sheep in all parts of the world. Many animals can be affected in any single outbreak with catastrophic losses but the number of flocks affected annually is small.

Cause

The organisms of the *Salmonella* group are short aerobic Gram-negative rods, and most grow well on laboratory media at 37°C. They belong to the family Enterobacteriaceae and are differentiated within that family by their biochemical and serological reactions. The most common serovar isolated from sheep is *Salmonella enterica* subspecies *diarizonae* serovar 61:kL1,5,7. All other important serovars are from *Salmonella enterica* subspecies *enterica*.

In recent years in Britain, the incidence of *S. typhimurium* has decreased and the three most common serovars after *S. diarizonae* are *S. montevideo*, *S. dublin* and *S. derby* [2]. Individual serotypes of Salmonella may be further subdivided by phage typing, e.g. *S. typhimurium*, and by biotyping, e.g. *S. montevideo*, techniques that are extremely useful in epidemiological studies. For example, over 200 phage types of *S. typhimurium* can be identified and used in tracing the spread of infection between groups of animals and in detecting sources of infection. For *S. montevideo*, some 27 biotypes belonging to two biogroups have been recognized. One biogroup (10di) is predominant in sheep whilst the other (2d) is associated mainly with human, cattle and poultry infections.

Only a few members of the *Salmonella* group are host-specific, and among these are *S. diarizonae* and *S. abortus ovis*.

S. diarizonae accounts for around 60 per cent of all reported *Salmonella* isolated from sheep in the UK and has been the most common serovar isolated since 1998. The role of *S. diarizonae* in clinical disease is unclear as, when isolated from abortion material, there is often concurrent infection with other abortifacients [3]. The organism has been isolated also from diarrhoeic and healthy sheep. Experimental infections do not lead to disease. Sheep may become long-term carriers of *S. diarizonae* in intestinal and reproductive tracts and in the nasal cavity.

S. montevideo has been associated repeatedly with abortion in ewes in Scotland. From 1970 to 1981, a total of 67 incidents was reported, but in 1982 there was a sudden upsurge, mainly in the south-east of the country, where the infection was confirmed as a cause of abortion in 40 flocks [4].

From time to time, other serotypes have become prevalent in sheep in various parts of the world. *S. brandenburg* has been of predominant importance in New Zealand since its first diagnosis in 1996. Since then, *S. brandenburg* has spread via environmental contamination with abortion products, contaminated water, dust from yards where sheep are gathered and by black-backed gulls (*Larus dominicarus*). The same

serotype has also caused abortion in cattle, and enteric disease in horses, goats, pigs, deer and humans. In sheep, it has a high morbidity and high mortality with rapid local spread when introduced to a naïve area. From 5 to 20 per cent of ewes may abort with, on average, a 50 per cent mortality rate. The expelled fetuses are putrid with a cooked appearance, subcutaneous oedema and pale oedematous cotyledons in the placenta. Up to 2 per cent of sheep can be identified as excretors 6 months after aborting. The spread of disease between farms is aided by gulls, which cover a 50-km radius from their nesting sites. Risk factors for abortion are high stocking rates, strip grazing over winter, high fecundity of ewes and outbreaks often following a period of gathering for management tasks. Isolates from different locations and species were indistinguishable until a new 'possibly related' strain was reported in 2004 [5]. An inactivated vaccine is used widely either as a primary course commenced at mating with annual boosters or with the second injection deferred to perceived risk periods. Other important serotypes in New Zealand include *S. typhimurium*, *S. bovis-morbificans* and *S. hindmarsh*.

Clinical signs

Clinical signs of salmonellosis can vary with the serotype, are diverse, and include general systemic and enteric signs as well as abortion. With *S. montevideo*, the clinical picture is very similar to that with *S. abortus ovis*. Abortion is characterized by very little ill health in ewes, and often a vaginal discharge and hollow flanks may be the first sign. In an affected group many ewes may excrete the organism in their faeces without aborting, and the infection can pass through a group of non-pregnant animals without any apparent signs.

In infections with *S. typhimurium*, enteric and systemic signs predominate, although, with in-lamb ewes, a variety of signs will be seen, including some sudden deaths. Affected animals are generally anorexic, and scour profusely. Those that do not die of septicaemia may continue to scour and die from dehydration with sunken eyes and tight skins. Pregnant animals may die of septicaemia before aborting. *S. typhimurium* abortion is often associated with illness in farm workers, farm dogs and other livestock.

S. dublin infection causes disease similar to that produced by *S. typhimurium*, with pyrexia, malaise and diarrhoea as well as abortion. Ewe deaths due to septicaemia are not uncommon. Deaths in neonates may also be a feature with little clinical disturbance in lambs over 1 week old. In one report, 30 of 380 ewes aborted and 23 of them died within 7 days of abortion [6]. Aborted lambs were rotten and decomposed following death *in utero*.

When *S. abortus ovis* is first introduced into a flock up to 60 per cent of ewes may abort with some deaths in ewes and lambs up to 3 weeks of age. The infection then becomes endemic with only sporadic abortion in home-bred ewes lambing for the first time or in bought-in ewes. *S. abortus ovis* infection is now rarely seen in the UK but ranks among the main causes of abortion in some countries of Europe and western Asia.

Pathology

Aborted fetuses and placentae are usually fresh and without macroscopic lesions. Post-mortem findings in ewes are variable. There are lesions of acute metritis and, if abortion or stillbirth has occurred, the swollen uterus usually contains retained placenta, necrotic tissue and serous exudate. The carcass appears septicaemic with an enlarged spleen and generalized congestion of organs. In animals that have not eaten for several days, the gall bladder is distended and the liver swollen and very friable (Figure 19.1). The placenta may be oedematous with haemorrhages in the chorioallantois, enlargement and necrosis of the cotyledons, and multifocal suppurative inflammation. Fetal tissues are usually unremarkable, although there may be multifocal necrosis in the liver and spleen (see Figure 19.2 in the colour plate section). In lambs that are stillborn or survive into the first week of life there are often signs of acute abomasitis and severe enteritis with gross enlargement of the associated lymph nodes. Intestinal contents tend to be very fluid, and inflammatory changes may be detected in the caecum and colon.

Diagnosis

Confirmation of diagnosis depends on the isolation of the causal organisms. In cases of abortion, Gram-negative organisms seen on direct smears made from fetal stomach contents and placentae can be confirmed as salmonellae by direct culture on MacConkey agar. Typical non-lactose-fermenting colonies are present in profuse numbers. Enrichment media, such as selenite

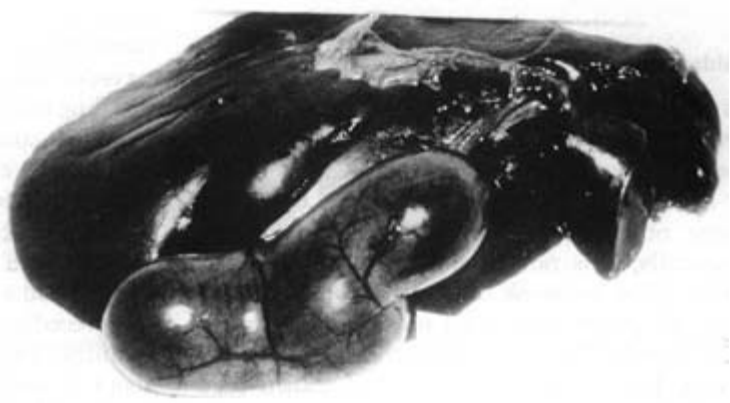

Figure 19.1: Swollen liver from sheep that died of septicaemia due to *Salmonella typhimurium*. Note the grossly enlarged gall bladder.

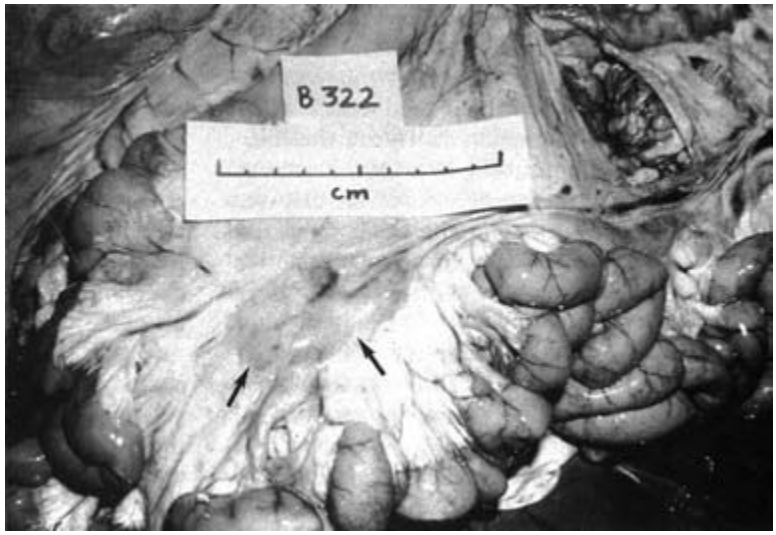

Figure 19.3: The enlarged mesenteric lymph nodes in this ewe are a good site for recovery of salmonellae after faecal shedding has ceased.

broth or Rappaport–Vassiliadis, also can be used but often are not necessary for primary isolation if salmonellae are the cause of abortion.

In flocks with endemic *S. abortus ovis,* culture of vaginal swabs taken not later than 1 week after abortion are most effective, although samples taken up to 4 weeks after abortion are occasionally positive. A polymerase chain reaction (PCR) test is available that identifies the serovar-specific IS200 element.

In enteric and septicaemic cases, isolation of the causal organisms should also be possible by direct culture from organs, faeces, intestinal contents and drainage lymph nodes. Cultures from faeces, intestinal contents and lymph nodes made in a selective liquid medium such as selenite broth are sometimes helpful, as they allow the salmonellae to multiply preferentially over other Enterobacteriaceae. Typical non-lactose-fermenting colonies are then identified by subculturing on to Brilliant Green Agar. In chronic enteric infections, the predilection sites for the bacterium are the posterior mesenteric lymph nodes, which become enlarged (Figure 19.3). The infection

is carried longer in these nodes than elsewhere in the body, and organisms can be recovered from them after faecal excretion has ceased.

When a suspect *Salmonella* has been isolated, serotyping is carried out against O (somatic) and H (flagellar) antigens by slide agglutination tests. In the UK reference laboratories confirm the serotype.

Serology can be used to diagnose abortion due to *S. montevideo* after the event. Marked serum agglutination test responses to H and O antigens peak some 4 weeks after infection. Serological tests are not commonly used for other serotypes in sheep.

Epidemiology and transmission

S. abortus ovis, which is host-specific, is invariably introduced into a flock by an infected subclinical carrier sheep but there are many sources of infection for the other serotypes (e.g. food, water, other animals, wild birds and man). Oral infection probably occurs some weeks before abortions hence the difficulty in trying to establish the source of infection.

In outbreaks of abortion due to *Salmonella* the fetuses and placentae are heavily infected. Organisms excreted in faeces readily contaminate food, water troughs and pasture. Fields containing affected animals become heavily contaminated with organisms, which may find their way into watercourses. Consequently, animals drinking from rivers or streams downstream from such infected fields may become infected.

Wild birds have been incriminated in spreading some serotypes, especially *S. montevideo*, which has been reported frequently as causing abortion in areas frequented by wild geese. However, the evidence remains circumstantial because, as abortions do not occur until several weeks after infection, it is difficult to tell whether geese have infected the sheep or vice versa. It is more likely that foci of infection with *S. montevideo* occur within a flock, and that there is mechanical spread to neighbouring flocks by wild birds. Salmonellae do not appear to multiply and establish in gulls, but may be carried by them from rubbish tips, sewage outfalls and other places where they feed [7]. Sheep troughs could be another source of infection if *S. montevideo* or another serotype is present in a flock. Animals frequently become carriers of salmonellae, especially *S. dublin* in cattle. This serotype is usually introduced to a flock or herd by purchased carriers. Carrier sheep of *S. dublin* have been identified by culture up to 32 months post-abortion [6].

With *S. typhimurium*, sheep tend not to become carriers and, usually, those that survive acute episodes of disease quickly throw off the infection and stop excreting the organism within 6 weeks [8]. Biogroup 10di of *S. montevideo* appears to be associated primarily with sheep, and infected sheep are probably responsible for introducing this serotype into susceptible flocks.

Treatment, prevention and control

By the time salmonellosis is detected, the infection will have spread throughout the group. Nevertheless, isolation of affected animals is worthwhile to limit contamination of the environment and further transmission.

Septicaemic animals can be treated with suitable parenteral antibiotics, and those that scour may require supportive therapy with electrolyte solutions. Often, ewes that abort develop postparturient metritis and injections of long-acting antibiotics at the time of abortion help to prevent this. It is unusual for serotypes other than *S. abortus ovis* to become endemic in flocks in the UK and normally, after an episode of disease, the infection disappears from a flock.

Ewes aborting due to *S. abortus ovis* rarely do so in subsequent pregnancies indicating that protective immunity may follow natural infection. Attempts to protect susceptible animals (replacement stock, new purchases) by mixing them when barren with aborting ewes have had apparent success. Nevertheless, this may produce more carriers and also risks disseminating other concomitant abortifacient infections.

In the face of abortions confirmed or suspected to be due to *S. montevideo*, treatment of all ewes with two injections of long-acting oxytetracycline, 7 days apart, have been purported to reduce the severity of outbreaks although there are no published supporting data.

An inactivated *S. dublin* and *S. typhimurium* vaccine licensed for use in cattle (although not in sheep) has been used in the face of an outbreak to protect ewes yet to lamb [9]. This is useful only when the lambing period is prolonged such as when young ewes lamb as a separate batch later than older ewes (manufacturer's advice).

Where *S. abortus ovis* is endemic, vaccination is the most common preventive procedure. Live vaccines, either attenuated or virulent, provide much better immunity than inactivated ones. Live attenuated vaccines using mutant strains of *S. abortus ovis* (Rv6) and of *S. typhimurium* gave better protection than inactivated vaccines [10, 11] and are safe when used either before mating or during gestation.

CAMPYLOBACTERIOSIS

Outbreaks of abortion in sheep caused by organisms of the genus *Campylobacter* have been reported in many countries since it was first described in 1913. A sporadic cause of abortion, in the UK *Campylobacter* infection tends to occur with increased incidence every 4–5 years within flocks. In recent years it has been the third most common cause of ovine abortion in the UK [12]. Abortion storms in which up to 20 per cent of ewes abort are not uncommon when infection is first introduced into a naïve flock. Campylobacteriosis is the leading cause of diagnosed sheep abortion in New Zealand [13].

Cause

The species involved are *Campylobacter fetus* subspecie*s fetus* and *C. jejuni*. The bacteria are microaerophilic, motile, Gram-negative rods.

Clinical signs

The major manifestation is abortion in the last 6 weeks of gestation and birth of live, weak lambs. Occasionally, there may be mild diarrhoea in ewes prior to abortion due to *C. jejuni*. The timing of infection may be important in determining the extent of abortion; in an experimental model almost 100 per cent of ewes aborted if infected at 105 days of gestation, whereas after infection 3 weeks later only 20 per cent aborted. This may reflect increasing immunocompetence of the fetus [14].

Pathology

No pathognomonic lesions are present in placentae but characteristic grey necrotic foci may be seen in fetal livers. Histopathological features are multifocal hepatic necrosis, bronchopneumonia and non-suppurative inflammation of abomasum and jejunum.

Diagnosis

Diagnosis depends on the demonstration of large numbers of characteristic curved Gram-negative rods in smears from placentae, cotyledons and fetal stomach contents. Confirmation of the species is carried out following culture on special selective media (Skirrows) under microaerophilic conditions.

Immunohistochemistry also can be used on formalin-fixed tissues to demonstrate antigens of *C. fetus* when culture is not diagnostic, for example if fetuses are autolysed or scavenged [15].

Epidemiology and transmission

Transmission is by the faecal–oral route of contaminated feed, water and abortion material. Unlike in cattle, venereal spread does not appear to occur in sheep. The sources of infection for flocks are not fully understood, but carrier sheep are thought to be largely responsible for bringing infection into clean flocks. Primary outbreaks can occur in closed flocks and mechanical transfer of infection to susceptible groups of sheep by farm personnel who have previously handled infected animals can occur. Mechanical transmission by carrion-eating birds can also introduce infection.

Outbreaks of *Campylobacter* abortion appear to be worse when animals are herded in concentrated areas of land, e.g. in bad winters in the UK or in high-density winter grazing in New Zealand.

Treatment, prevention and control

Abortion occurs 7–25 days after infection and spreads rapidly due to the large numbers of organisms in aborted material. Isolation of aborting ewes and disposal of abortion material and contaminated bedding is vital to limit the spread of disease. Often, however, infection has already spread within the flock and little can be done to reduce the losses. Oral and parenteral antibiotics have been used in the face of an outbreak with variable results, and there is little

indication for their use. As the organism is spread mainly by ingestion of contaminated feed and water, susceptible animals should be removed from suspect areas or the stocking density should be reduced.

Ewes have long-lived immunity following infection whether they have aborted or not. This can be used as a method of 'natural vaccination' by mixing ewes that have aborted with non-pregnant replacements. Experimentally, aborted ewes have been shown to excrete the organism intermittently in faeces for up to 42 days post-challenge. However, great care must be taken to ensure that other agents such as *Chlamydophila abortus* are not simultaneously present in a flock, as mixing of replacements in this situation could lead to a chlamydial abortion storm the following year.

Effective killed adjuvanted vaccines have been developed and are used widely in North America and New Zealand [16]. A primary course of two injections, 4 weeks apart pre-mating followed by annual boosters is required to confer protective immunity. Pulsefield gel electrophoresis (PFGE) typing has revealed no major genotypic shift in *C. fetus* subsp. *fetus* over a period of 13 years [17].

In the UK, the disease tends to be sporadic, although some flocks do have outbreaks of abortion due to *Campylobacter* spp every 4–5 years. This may reflect waning immunity of ewes or introduction of naïve replacements to a flock having carrier ewes. The virulence factors and antigenicity of *C. fetus* subsp. *fetus* are dependent on the surface layer proteins which may be potential vaccine candidates [18].

LISTERIOSIS

As well as septicaemia, encephalitis, abomasitis and typhlocolitis, listeriosis can also be manifested by abortion.

Cause

The species of *Listeria* associated with abortion in ewes are *L. monocytogenes* and *L. ivanovii*. *Listeria* spp occur in decaying vegetation and soil, multiplying at pH greater than 5.5. Outbreaks of abortion due to *Listeria* are often associated with feeding of poorly fermented silage.

Clinical signs

Abortion can occur at any stage of pregnancy and as soon as 7 days after infection [19]. Ewes are rarely clinically unwell and overlap of different forms of listeriosis within a flock is relatively rare, perhaps in part due to different serovars involved.

Pathology

Abortion material shows few lesions and autolysis sets in quickly. Occasionally, miliary yellow–white foci of less than 2 mm diameter may be seen in the liver of aborted fetuses. Other occasional findings in the fetus include circular erosions in the mucosa of the abomasum and enlargement of mesenteric lymph nodes [20].

Diagnosis

The organisms (Gram-positive facultative anaerobic bacilli) can be demonstrated in smears and cultured from stomach content of aborted fetuses and placentae.

Epidemiology and transmission

Sporadic cases of *Listeria* abortion may occur under any management and feeding system, but larger outbreaks are usually associated with silage feeding of housed ewes. Epidemiological studies have shown an association between outbreaks of abortion due to *L. ivanovii* and periods of cold, wet weather in silage-fed sheep. Spoiled, incompletely fermented or soil-contaminated grass or maize silage may contain 3000–12 000 *Listeria* organisms per gram. Latent carrier sheep may also introduce infection as *L. monocytogenes* has been found in the faeces of up to 50 per cent of healthy sheep.

Treatment, prevention and control

Treatment of in-contact animals with a suitable antibiotic may reduce losses, but the mainstay of management in the face of an outbreak involves avoiding feeding contaminated silage, or switching to an alternative feed.

Where silage is fed prevention involves use of good quality, fresh silage and daily removal of uneaten silage.

COXIELLOSIS (Q FEVER)

This infection, which may occur in several animal species and in most countries, is mainly of significance as a zoonosis. Recently, its possible use in bioterrorism has been noted [21]. Apparent 're-emergence' of infection in sheep may be due, in part, to improved diagnostic tests [22]. Acute infections in small ruminants can lead to outbreaks of abortion with recurrences over successive years.

Cause

C. burnetii is an aerobic Gram-negative obligate intracellular rickettsial bacterium. It produces a small endospore-like form that is resistant to high temperatures, ultraviolet light and osmotic shock. The spores survive in dust for 120 days, in tick faeces for 586 days and in wool for 12–16 months at 4–6°C. The organism persists in ticks, but unlike most rickettsias it is not dependent on arthropods for transmission.

Clinical signs

In sheep, *C. burnetii* can cause abortion, usually in the last week of pregnancy, and birth of stillborn and weak, live lambs. Generally, the abortion rate is low and may fail to prompt investigation. *C. burnetti* is considered to cause infertility in cattle, but this outcome has never been reported in sheep.

Pathology

Inter-cotyledonary thickening of the placenta with an exudate resembles that seen in *Chlamydophila abortus* abortion. Histopathologically, there is diffuse suppurative inflammation in the chorion without vasculitis, with many organisms in cytoplasmic vacuoles in the chorionic epithelial cells.

Diagnosis

Diagnosis depends on the demonstration of the organism in the placenta and fetus, using staining techniques such as the modified Ziehl–Neelsen (ZN) procedure. The organisms appear as small red coccobacilli, which lie both intra- and extra-cellularly. Care must be taken to differentiate these from *C. abortus* which, unlike *C. burnetii*, is not usually present in the stomach contents of aborted fetuses.

Owing to the high zoonotic risk culture is not normally attempted.

Confirmation of ZN smear findings is by serology or histology. The complement fixation test (CFT) is the Office International des Epizootiques reference test and is highly specific, although sensitivity is relatively poor at 78 per cent. Titres of greater than 1/10 are regarded as indicative of infection with *C. burnetii*. Other serological tests available are enzyme-linked immunosorbant assay (ELISA) and fluorescent antibody test.

Immunohistocytochemistry on formalin-fixed sections of placenta can demonstrate *C. burnetii* antigens.

PCR testing of vaginal swabs, milk or faeces will detect antigen [23]. In addition to diagnosis in the face of abortion, PCR enables investigation of duration of shedding of *C. burnetti*, prevalence of infection and risks of zoonotic spread. However, the relationship between serological response and excretion is unclear as sheep seropositive by ELISA may be negative by PCR, and some detected as excretors by PCR may be negative by ELISA [24]. A combination of PCR and ELISA is optimum for diagnosis and tracking shedding of the organism [25].

Epidemiology and transmission

The organism is shed in vaginal fluids, faeces and milk, and is present in products of abortion. Excretion in vaginal fluids occurs at normal parturitions. Infection via the oropharynx is followed by multiplication in regional lymph nodes and a bacteraemia lasting 5–7 days. The organism localizes in the mammary gland and placenta of pregnant ewes.

C. burnetti spores survive well in the environment and can be transmitted to new hosts by indirect exposure via fomites, such as hay, straw, wool and manure [20] mainly by inhalation. The organism may be airborne and disseminated on wind for more than a kilometre.

Excretion of *C. burnetti* in vaginal fluids peaks at the time of abortion or lambing and may continue for up to 2 months in some ewes. The organism is present intermittently in milk for 8 days post-lambing. Vaginal shedding has been detected in subsequent pregnancies, perhaps due to associated immunosuppression [26]. Antibody titres (ELISA) peak 5 weeks after parturition.

There have been no recent serological surveys of ovine coxiellosis in the UK. In the 1970s approximately 28 per cent of sera examined were positive in the complement-fixation test.

Treatment, prevention and control

In France, vaccine development has been aimed at preventing shedding by small ruminants. An inactivated vaccine for use pre-mating decreases abortion and excretion of the organism in milk and vaginal fluids [27] and is used in infected flocks to reduce the zoonotic risk.

C. burnetti is readily susceptible to treatment with oxytetracycline. In the face of an outbreak treatment does not prevent shedding but may reduce the abortion rate.

LEPTOSPIROSIS

Leptospirosis is a rare cause of sporadic abortion in sheep in the UK. In Northern Ireland the disease appears to be associated with intensively managed lowland flocks and agalactia is also a feature [28].

Several serovars of *Leptospira interrogans* have been implicated in ovine clinical diseases, The most important are *L. pomona,* usually associated with acute haemorrhagic jaundice and mortalities in lambs, and *L. hardjo* which is generally associated with abortion in late pregnancy and agalactia.

Diagnosis is based on demonstration of the organism in the fetus or placenta by culture, immunofluorescence or the microscopic agglutination test (MAT) carried out on fetal fluid. Leptospirosis in not included in routine ovine abortion investigations in the UK.

Serological surveys have indicated *L. hardjo* as the most prevalent serovar in sheep. In 1984 6 per cent of adult sheep in England and Wales were seropositive [29].

Most infections in sheep are believed to be acquired from other animals, especially cattle. However, leptospiruria has been detected in sheep not in contact with cattle, suggesting that sheep may be a maintenance host for *L. hardjo* [30]. Conversely, leptospiruria was not a feature of a flock with evidence of renal infection and a high percentage of seropositive individuals [31]. Experimentally, infection can be transmitted from cattle to sheep and from sheep to sheep, but the low incidence of field disease suggests that infection is usually subclinical leading to seroconversion without disease.

In an outbreak dihydrostreptomycin can be used to treat in-contact pregnant ewes. For susceptible replacements joining a flock with a history of the disease, administration of a reduced (to one quarter) dose of a commercial inactivated leptospiral vaccine can be considered.

VEROTOXIN-PRODUCING *ESCHERICHIA COLI*

Escherichia coli is an unusual cause of abortion and, if cultured from fetal stomach contents or placenta, is often attributed to contamination or autolysis. However, toxin-producing *E. coli* have been implicated in abortions in an exceptionally well managed flock over three successive lambing seasons with suppurative placental inflammation on histopathology and no evidence of any other abortifacient agent (unpublished observations).

Associated clinical signs were 3–10 per cent abortions in the final 2 weeks of pregnancy, pyrexia, anorexia and a dark vaginal discharge [32]. Half of the aborting ewes died of metritis and toxaemia despite treatment with oxytetracycline but survivors subsequently lambed normally.

In each year, *E. coli*, cultured in pure profuse growth from placenta and fetal stomach contents, was consistently typed as 015:Krvc383 with toxin-producing genes identified by PCR. The original source of infection was not determined but persistence within the flock was probably due to healthy carrier sheep. As the *E. coli* genome is highly dynamic, commensal organisms may have acquired

virulence genes by horizontal transfer via mobile genetic elements such as phage [33].

OTHER CAUSES

Other occasional bacterial causes of ovine abortion include *Fusobacterium necrophorum*, *Yersinia pseudotuberculosis*, *Arcanobacterium pyogenes* and *Bacillus licheniformis*. In general, bacterial isolates from stomach contents of fresh, not autolysed fetuses, may be of pathogenic significance. Mycotic abortion is rare in sheep compared to cattle.

ZOONOTIC IMPLICATIONS

Most of the conditions considered in this chapter can cause human illness of varying severity, emphasizing the need for awareness and precautionary measures in cases of ovine abortion and in handling affected placentas and fetuses. The lambing season presents occupational hazards for handlers working under conditions in which it is difficult to maintain appropriate standards of hygiene and for veterinary surgeons and laboratory workers who may be handling infected animals and diagnostic samples. Pregnant women should not work with lambing sheep nor handle clothing worn by persons so involved.

While *Salmonella abortus ovis* is not regarded as pathogenic for humans, other serotypes of *Salmonella* involved in sheep abortion are zoonotic and thus early identification of the specific serotype involved in outbreaks of salmonella abortion is necessary to avoid risks to human health. Diarrhoea, abdominal pain and fever are common results of infection.

Campylobacter jejuni has a low infective dose and is capable of causing bouts of diarrhoea, particularly in young, debilitated or immunocompromised individuals. Infection with *Listeria monocytogenes* is a particular hazard for pregnant women and the immunocompromised (see also Chapter 37). Both *Leptospira hardjo* and *L. pomona* can cause an influenza-like illness with fever, headaches, muscle and joint pains.

Human Q (query) fever results from infection with *Coxella burnetti*. Acute infection presents as an influenza-like illness, while chronic infection manifests as hepatitis, endocarditis, osteomyelitis and lymphadenitis. A post-Q fever fatique syndrome is also recognized. Zoonotic transmission is mainly by aerosol but can also occur by drinking raw milk or dairy products. The infective dose is low and seasonal outbreaks coinciding with lambing time are noted.

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20

Brucella melitensis infection

G. Castrucci

Brucella melitensis is the only species of the genus *Brucella* that ordinarily infects sheep and goats. In sheep, sporadic infections by *Brucella abortus* are also observed. An epidemiological investigation of 550 strains of *Brucella* from all over the world has shown that the brucellae predominantly affect one species: *B. abortus* for cattle, *B. melitensis* for sheep and goats, and *B. suis* for pigs [1]. However, the specificity is not absolute, and cattle, sheep and goats may be infected with *B. abortus* or *B. melitensis*.

CAUSE

The genus *Brucella* consists of six species: *B. melitensis*, *B. abortus*, *B. suis*, *B. neotomae*, *B. ovis* and *B. canis*. The first three are further subdivided into three, eight and five biovars, respectively.

From an investigation conducted on the genomes of 51 strains of *B. melitensis*, all the strains appeared to be very similar, so that it seems justifiable to consider them as a single species, which, for priority reasons, could be called *B. melitensis*.

The members of the genus are pleomorphic, often of cocco-bacillary shape. They grow in media containing trypticase-soy, are non-motile, produce catalase and do not ferment glucose or lactose or liquefy gelatin. The identification of the brucellae can be confirmed by phage typing and oxidative metabolism.

Recently, a method has been described which allows a rapid identification of *Brucella* spp [2]. The method is based on nucleic acid amplification, a real-time polymerase chain reaction (PCR) assay in a multiplex format that will permit the confirmation of bacterial isolates as *Brucella* spp, or *B. abortus*, or *B. melitensis* within 2–3 hours.

CLINICAL SIGNS

The main route of entry of the bacteria is the nasopharynx. However, the cutaneous route must not be excluded. The bacterium spreads via the lymphatics and is arrested in the lymph nodes. In animals resistant to infection, the brucellae are killed by macrophages, the active cells of the immune system, with the intervention of the antibodies and lymphocytes. In susceptible animals, on the other hand, the bacterium survives phagocytosis and replicates inside cells. Following lysis of the phagocytic cell, the brucellae are liberated and infect other cells. Bacteraemia may eventually develop. In fully susceptible pregnant and non-pregnant animals, *B. melitensis* cells are present in blood for 30–45 days after infection. In virgin females, from endemically infected areas, bacteraemia is rare and seen only in a small number of animals.

In pregnant animals, the bacterium enters the uterus, where it reproduces in the placenta and fetal tissues, inducing an infective state not necessarily followed by abortion. The percentage of aborting animals varies according to circumstances.

In non-pregnant animals, *Brucella* can cause a chronic infection, which is of epidemiological importance because, after an initial serological reaction in the animal, the infection becomes non-apparent thus creating problems in diagnosis. In non-pregnant ewes, *B. melitensis* is not excreted from the vagina. However, in pregnant animals, excretion starts at the time of delivery or abortion and may last for months.

Following infection with *B. melitensis*, the mammary gland is often colonized, and infection of the udder interferes with the production of milk, thus reducing or arresting milk output. Brucellae are not always excreted during lactation, although it is

believed that infection of the udder is the means by which infection persists to the following pregnancies. Excretion of the bacteria may last for as long as 180 days after delivery or abortion.

DIAGNOSIS

Abortion of an infective nature may be suspected on the basis of history and clinical examination, especially when several ewes are involved. However, only bacteriological and serological tests may confirm the presence of *B. melitensis*.

Bacteriological methods

Microscopic examination can be used for materials in which large numbers of brucellae are suspected, such as, for example, the placenta, stomach content of the fetus as well as its lungs and liver and the vaginal discharge in the case of abortion.

When smears are stained by the methods of Machiavello, Stamp or Këster, the brucellae are coloured red. Immunospecific staining with IgG conjugated with fluorochrome may also be used.

Isolation of the bacterium in culture requires the selection of samples, especially those from abortions, integrating them with those from lymph nodes, udder, uterus, seminal vesicles, accessory glands, testicle, epididymis and other organs that have macroscopic lesions. Blood is collected in a 2 per cent w/v sodium citrate solution. Freezing and thawing of blood samples helps release intracellular brucellae.

Blood samples and tissue homogenates are added to agar-serum glucosate. If low numbers of bacteria are foreseen, or if antibiotics have been added, it is advisable to enrich the culture with agar-blood or agar Border–Gengou. If contaminating bacteria are present in the sample, the use of selective media is advisable.

When the Rev-1 vaccine is being used, it will be necessary to distinguish the vaccinal strain from the virulent *B. melitensis*. The Rev-1 has a low virulence for the guinea-pig, which, if inoculated subcutaneously with a 10^3 dose of *Brucella* organisms, gives negative cultures from the spleen 3–5 months after inoculation, whereas virulent *B. melitensis* induce an infection lasting 6–12 months.

Serological procedures

Standard agglutination test (SAT)

This test, which is widely employed for sheep and goats, is limited by the possibility of negative or suspicious results in chronic brucellosis. The SAT may be influenced by Rev-1 and other antigens, and the response can be variable even in the same animal. For these reasons, the SAT must be used only as a screening test and, in cases in which a low titre is found, additional methods are necessary.

Rose Bengal test

This test uses stained buffered antigen. It is a cheap and convenient test, but its dependability can vary according to the different sensitivities of the antigens used. With a 10 per cent antigen concentration, a higher specificity is obtained, although with a lower sensitivity compared with SAT. If a concentration of 5 per cent is used, a large number of infected animals is detected.

On the other hand, it has been proved that an increase in the volume of sera to be tested also improves significantly the sensitivity of the test [3].

Complement fixation

This is the method of choice in chronic infections and for the differentiation of serological reactions between vaccinated and infected animals. Its employment is also suggested for the control of blood samples of animals belonging to infected flocks, for which the SAT has given negative or dubious results.

Other serological tests

Several procedures have been suggested to supplement or possibly replace the above tests. They are the mercaptoethanol test, indirect haemolysin test, Coomb's antiglobulin test, radioimmunoassay, enzyme-linked immunosorbent assay (ELISA) and gel diffusion test.

In the last few years, several attempts have been made to standardize a test that eventually could clearly

differentiate an antibody response of infected sheep from Rev-1-vaccinated sheep. Apparently, the goal has been reached by testing sheep sera in an ELISA with partially purified *Brucella*-cytosoluble 20-kDa protein, which seems to have the potential for detecting *B. melitensis*-infected ewes and their differentiation from *B. melitensis* Rev-1-vaccinated ones [4].

Cross-reactions

All the methods mentioned previously may be influenced by heterospecific antibodies in serum.

Cross-reactions have been demonstrated between *Brucella* strains in the 'S' phase and bacteria belonging to other genera, viz. *Francisella tularensis*, some serotypes of *Escherichia coli* (0:116–0:157), *Pseudomonas maltophila*, serotypes of *Salmonella*, and, above all, serogroups 0:9 and 0:16 of *Yersinia enterocolitica*. Investigations have shown that *Y. enterocolitica* may infect sheep. Cross-reactions may thus create problems in those areas in which an eradication programme is being applied.

Allergic reactions

Many substances for the allergic diagnosis of brucellosis have been produced through the years. Among these, the 'Mirri' allergen has been widely employed in Italy. INRA allergen is extensively used nowadays [5]. It is a product rich in proteins, lacking in lipopolysaccharides, prepared from a strain of *B. melitensis* (B 115 in rough phase). This allergen induces neither local nor generalized reactions in non-sensitized animals, but gives cutaneous reactions in animals sensitized either by infection or vaccination with members of the genus *Brucella*. The INRA allergen neither sensitizes nor induces an increase in the titre of antibodies, and thus will not interfere with allergic or serological tests.

In sheep, 0.5 ml of INRA allergen is inoculated intradermally in the lower eyelid. Reading is done after 48 hours from the front of the animal, in order to have the untreated eye as a control. The method is suggested for screening a large number of animals, thus eliminating the time-consuming collection of blood samples. An increment in capillary permeability of the product is obtained by adding hyaluronidase to the allergen. This causes a quicker and more intense reaction, allowing a reduction in the allergic response in those sheep with a doubtful positive reaction [6].

EPIDEMIOLOGY AND TRANSMISSION

Geographical distribution

B. melitensis infection in sheep and goats is prevalent in Mediterranean and Middle Eastern countries, particularly Iran, and tends to spread eastwards to southern regions of the former USSR, Mongolia and northern China. It is present in different countries in Africa, in southern India and in parts of Latin America. In Europe, the infection is absent north of the 45th parallel. It is absent as well from USA, South-east Asia, Australia, New Zealand and the Pacific Islands.

Transmission

Brucellosis of small ruminants affects sexually mature individuals with abortion, most important in the later stages of pregnancy. Sexually immature animals are resistant. The receptivity of ewes to *B. melitensis* varies according to the breed. Maltese sheep are resistant, while the Awassi breed of the Middle East is quite susceptible.

The spread of an infection from country to country or within the same country generally follows the transfer of infected animals. After the Second World War, a vast movement of sheep and goats took place in Europe, contributing to the spread of the infection. Brucellosis is also transmitted from farm to farm through wild animals and dogs responsible for carrying around aborted fetuses. Mixing herds at pasture and keeping the animals in shelters during the night, particularly if in such areas parturition takes place, represent major factors for transmission of the infection. Cattle can also be infected from sheep. Dogs and rodents in contact with infected animals may acquire infection, but this mode of transmission is of little importance from an epidemiological point of view. In the transmission cycle, insects and ticks may also be involved.

The phenomenon of latency, so common in cattle, has been confirmed also in *B. melitensis*-infected sheep [7], even if it seems that the latently infected

ewes rarely transmit the infection to their lambs. However, in spite of the low frequency of transmission, the existence of such latent infections greatly increases the difficulty of eradicating brucellosis.

Material from an abortion represents the main source of transmission, with the excretion of enormous numbers of bacteria; the placenta, the fetuses and the fetal fluids are highly infective. After delivery or abortion, the excretion of brucellae in the vaginal discharge continues for about 3 weeks but may last up to 2 months. Therefore the soil where deliveries take place becomes massively contaminated. The number of brucellae excreted in milk is generally not relevant for sheep-to-sheep transmission, but is important for the transmission of the infection to humans.

The nature of the material that is contaminated by the brucellae is of some importance. Sand and straw used for bedding may absorb a considerable number of bacteria. Impervious material, such as concrete, keep bacteria on the surface and animals may therefore become infected through inhaling the contaminating micro-organisms.

It is a general belief that the male does not play an important role in the epidemiology of brucellosis. It is possible, however, that it may transmit the infection through mechanical means. In males, the infection may affect the reproductive organs and, quite often, orchitis develops.

The resistance of *Brucella* in the environment is not easily determined because the conditions in which the bacteria may be found are very variable. The organism can survive in dust from 3 to 44 days, on sterile surfaces for 20 days and in tap water for 30 days. Resistance in wooden houses and on the floor of shelters is about 4 months. In pastures exposed to the sun, survival is up to 15 days, while in the shade it is 35 days. *B. melitensis* is killed by pasteurization, and it is sensitive to common disinfectants.

TREATMENT, PREVENTION AND CONTROL

The widespread distribution of the bacteria in the body and their ability to survive inside cells render chemotherapy ineffective.

To date, little has been accomplished with the control and eradication of brucellosis in small ruminants. The best scheme to follow is the identification and culling of infected animals. Prophylactic campaigns aimed at eradicating the disease have been successful in the most advanced European countries but have fallen short of this aim in developing countries.

Three strategies are available:*

- Vaccination as a preliminary intervention.
- Vaccination associated with culling of infected animals.
- Identification and culling of infected animals with no vaccination.

Where the level of infection is not known, a trial investigation is necessary before selecting the most appropriate prophylactic method. However, the following general sanitary measures are considered to be of some beneficial effect in controlling the disease:

- Introduction of new sheep or goats to the herd should be rigorously controlled, and mating should also be with animals from non-infected herds.
- Strict isolation is necessary on the introduction of animals susceptible to brucellosis.
- The mixing at markets or at pasture of healthy animals with infected animals or those of unknown status must be avoided.
- Periodic clinical and serological examination of rams selected for mating is necessary.
- Precautions should be taken during transit of sheep to avoid infection.

The main concern of the authorities must be to curb transmission through the elimination of all sources of infection, employing mandatory identification of the infection sites and enforcement of all measures aiming at maintaining the health status of the area. Cases of abortion must be reported and confirmed by serological and allergic tests. Infected animals must be culled, and their owners compensated by the competent authority. It should be emphasized that any measure taken toward reducing the incidence of abortion and uterine infection contributes by reducing the number of *B. melitensis* cases available for transmission.

If possible, lambing should take place in isolation. Destruction of infected materials, i.e. by incineration,

* Vaccination is not allowed in Member States or regions of the European Union in which official brucellosis-free status (*B. melitensis*) has been achieved or is being sought.

as well as disinfection of the areas where deliveries take place, is necessary. Attendants must disinfect hands after handling infected material. It is also important that milking be performed with the maximum hygiene.

The use of vaccine is useful only as an addition to the rules mentioned, as the immunizing level given by the vaccine is never complete. The persistence of virulent brucellae in vaccinated sheep may cause a chronic infection that goes undetected, but contributes to the dissemination of infection.

In sheep, two vaccines are generally used: the Rev-1 strain of *B. melitensis*-attenuated vaccine [8]; and the H-38 *B. melitensis*-inactivated vaccine [9].

Rev-1 vaccine

Rev-1 is a non-dependent inverse mutant of a streptomycin-dependent strain of *B. melitensis*. Even though it is used as an attenuated vaccine, it retains a residual pathogenicity that may cause abortion if inoculated into pregnant ewes, as well as excretion of the organisms in milk if inoculated during lactation. Apart from these inconveniences, Rev-1 has advantages, as it is stable, is not transmissible to other sheep and provides long immunity. Vaccinated animals produce antibodies that can be demonstrated by serological tests. Complement-fixing antibodies disappear 6–8 weeks after vaccination, in most cases. For this reason, Rev-1 is employed only in young animals before they reach their reproductive age. However, in some instances, the vaccine is also administered to adult ewes, in which, in order to reduce the likelihood of abortions and excretion in the milk, a reduced dose of the vaccine and subconjunctival administration have been used. Considering the fact that subcutaneous administration confers a longer persistence of antibodies and the obvious difficulty in performing two separate vaccinations, it has been suggested that the animals should be vaccinated only once, intraconjunctivally, with a 10^9 dose of vaccine [10].

Following Rev-1 vaccination, the bacteria are disseminated widely, followed by their localization in the prescapular lymph nodes on the side of inoculation, with a possible spread to the cranial lymph nodes. In most cases, the organism disappears after 3 months [11]. Rev-1 induces a very efficient immunity, lasting more than $2^1/_2$ years.

A deletion mutant of strain Rev-1 was recently obtained which, according to the results of tests conducted in mice, would allow serological differentiation between infected and vaccinated sheep [12]. The deleted gene codes for the periplasmic protein BP26, the immunodominant antigen in the serological response of *B. melitensis* in sheep. The authors suggest, if proven safe and effective in the target species (sheep), the use of this Rev-1 bp26 deletion mutant as a vaccine for the eradication of *B. melitensis* infection in sheep. Several other prospective candidate vaccines are also under investigation [13].

H-38 vaccine

H-38 vaccine is produced by a virulent strain of *B. melitensis* biotype 1, inactivated with formaldehyde and suspended in adjuvant oil (Arlacel A). One dose containing 3×10^{11} bacteria induces good protection which lasts 15 months. As it is an inactivated vaccine, it may be used in pregnant and lactating animals. Unfortunately, two disadvantages have been reported: the antibody response develops more slowly compared with Rev-1, and H-38 frequently causes a local reaction at the inoculation site, which can be severe. Moreover, as the characteristics of the vaccine may change from one batch to the other, the use of H-38 has been limited.

ZOONOTIC IMPLICATIONS

Brucellosis in humans is characterized by fever, chills, night sweats and great weakness. Most of the infections caused by *B. melitensis* are contracted from the drinking of raw, infected milk or from eating dairy produce such as certain cheeses made from sheep milk. Infections are also derived from direct contact with infected secretions and excretions of sheep and goats. Infection in humans is sometimes referred to as Malta fever.

B. melitensis is particularly infective to man and, since milk-producing ewes are more receptive than sheep raised for slaughter, milk sheep provide a higher risk for human infection.

The possible pathogenicity of Rev-1 for man has to be considered. Experimental inoculation of Rev-1 in human volunteers has produced clinical signs. Veterinarians may infect themselves while vaccinating

with Rev-1. However, at the beginning of vaccination campaigns, the personnel involved have a distinct chance of being exposed to infected animals. Also, particular caution must be taken by laboratory technicians producing the bacterium for the vaccine.

ACKNOWLEDGEMENTS

This chapter is based essentially on that written by the late Professor V. Cilli for the Italian edition of *Diseases of Sheep* (Esculapio, Bologna, 1986). I am grateful to Dr Filippo Castrucci for his contribution in translating the original article and to Professor Franco Frigeri for his help in preparing the manuscript and the bibliography.

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21

Ulcerative balanitis and vulvitis

A. Greig

Ulcerative conditions of the external genitalia of sheep have been described in a number of countries since the early 1900s. Four different entities are recognized, namely venereal orf, caused by a *Parapoxvirus*, enzootic posthitis caused by a diphtheroid bacterium [1], a mycoplasma-associated vulvovaginitis [2] and an ulcerative balanitis and vulvitis condition seen in Australia [3] and the UK [4] for which no consistent agent has yet been identified. The isolation from one of three ewes with vulvovaginitis of a human mycoplasma, *M. fermentans*, together with recovery from all three ewes of *Histophilus ovis* and *Arcanobacterium pyogenes*, both previously associated with ovine reproductive disease, is a novel finding of uncertain significance [5].

VENEREAL ORF

Cause

The *Parapoxvirus* of orf or contagious pustular dermatitis may infect the skin in many areas, including the genitalia of ewes and rams (see Chapter 42).

Clinical signs and diagnosis

The disease is manifest by ulcerative lesions on the prepuce, penis and vulva. Generally, orf lesions are present on the skin of other areas, and are usually proliferative and pustular. Diagnosis is based on clinical signs with confirmation by electron microscopy, by immunodiffusion or by recovery of virus in cell culture.

ENZOOTIC POSTHITIS

Synonyms: pizzle rot, sheath rot, balanoposthitis.

This condition principally affects castrated male sheep with the greatest recorded prevalence being in Australia [1], where up to 40 per cent of the group may be affected, although a small outbreak of posthitis has been seen in wethers [6] and ram lambs in the UK.

Cause

Corynebacterium renale or a related urease-producing diphtheroid is considered to be the microbial pathogen involved, but several dietary factors, viz. high protein diets, oestrogens in the feed or alkaline drinking water, are considered essential for the severe condition to develop. Ulceration is thought to be caused by the release of ammonia following hydrolysis of urinary urea by the organism.

Clinical signs and diagnosis

Initial lesions are small, ill-defined areas of superficial necrosis affecting the skin of the preputial ring, which subsequently may extend internally to affect the lining of the prepuce and the penis. As the internal condition worsens, the prepuce swells and becomes more pendulous and the animal becomes anorectic and lies down frequently.

Without treatment, stenosis of the preputial orifice can occur. Similar ulcerative lesions affect the ewe's vulva and posterior vagina, and resolution may

lead to distortion of the genitalia. Diagnosis is based on clinical signs and the recovery of urease-producing *Corynebacterium* spp from the lesions.

Treatment

Topical treatment with antiseptics coupled with systemic antibiotic therapy and removal of, or from, the dietary factors associated with this condition lead to resolution.

MYCOPLASMA-ASSOCIATED VULVOVAGINITIS

The primary report of the vesicular form of this condition was in ewes in late pregnancy in Australia [2]. Subsequent reports are generally of mycoplasma-associated granular vulvitis.

Cause

A mycoplasma closely related to *Mycoplasma mycoides* subspecies *mycoides*, subsequently called *Mycoplasma* spp 2D, was isolated but failed to reproduce the condition in experimental transmission studies. Subsequently, a ureaplasma was implicated in an outbreak of vulvitis in Northern Ireland, and vulvitis and vaginitis with ulceration of the vestibule was evident in some cases following experimental transmission [7].

Clinical signs and diagnosis

Congestion and oedema of the vulva with scabs on the lower commissure and small vesicles and plaques on the posterior floor of the vagina are seen. Initially, ewes in late pregnancy were affected, but the condition persisted in the Australian flock for 6 months.

Clinical appearance and microbiological examination are the basis of diagnosis. However, recovery of mycoplasmas or ureaplasmas from the lesion cannot, by itself, be regarded as confirming the condition, since these agents are considered commensals of the lower reproductive tract. Further investigation and classification of these agents into pathogenic and non-pathogenic strains or serotypes will be needed before lesions can be attributed to an isolate.

Treatment

Long-acting tetracycline, which successfully eliminated ureaplasmas from 84 per cent of ewes [8], would probably be equally effective against *Mycoplasma* spp.

ULCERATIVE BALANITIS AND VULVITIS OF UNKNOWN AETIOLOGY

Cause

Despite exhaustive examinations for viruses and bacteria, no infectious agent has been consistently isolated, and transmission studies with scab material have failed to reproduce the condition [3].

Clinical signs

Frequently, the first sign is the presence of blood on or around the vulva of a number of ewes from around 18–20 days after the start of mating. Swelling of the vulva with ulceration confined to the skin of the vulva can affect up to 30 per cent of the ewes (see Figure 21.1 in the colour plate section). In rams, the primary lesion is a sharp-edged, deep ulcer on the glans penis (Figure 21.2), which can involve the whole glans,

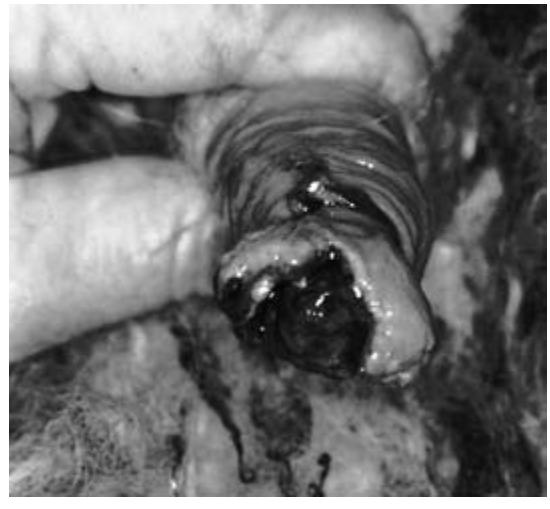

Figure 21.2: Ulceration of the glans penis; cause unknown.

including the vermiform appendix. In the acute stage, a blood clot fills the ulcer, which, if removed, causes copious haemorrhage. This is the likely source of the blood on the wool around the vulva of ewes. Surprisingly, libido is largely unaffected. In a few cases, the preputial orifice is also affected with scabs, while others show a diffuse inflammation of the prepuce with profound oedema. Generally, the whole working ram stud is affected in an outbreak.

Treatment

Affected rams should be removed from the ewes, rested and treated with local and parenteral antibiotics until the penile lesions heal, when they can be returned to the ewe flock. Despite the severity of lesions in both the ewes and the rams, the subsequent lambings are seldom disrupted to any degree.

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Part V
Diseases of the alimentary system

22

Diseases of the oral cavity

A.L. Ridler and D.M. West

Oral lesions are present in many systemic diseases of sheep, including foot-and-mouth, bluetongue, orf, ulcerative dermatosis and sheep pox. However, clinical examination of these cases usually reveals other generalized signs. This chapter covers only those conditions in which the lesions are limited to the oral cavity [1].

Diseases of the teeth and their supporting soft tissues are common and, in many parts of the world, are the main reason for culling otherwise healthy breeding ewes before the end of their natural reproductive life, with consequent increased flock-replacement costs.

Dental conditions include developmental and composition abnormalities, diseases resulting in premature incisor or cheek-tooth loss, excessive and/or irregular tooth wear, deformities of the mandible and problems of occlusion or bite.

INCISOR LOSS

Synonyms: broken mouth, periodontal disease, paradontal disease

Premature incisor loss is recognized as a significant dental problem throughout the world. Abattoir surveys in Britain have found incisor loosening or loss in 60–70 per cent of cull ewes [2]. However, broken mouth is not evident in all flocks; some are completely free of the disease while in others it may appear in animals at any age between 3 and 8 years, the prevalence within the flock varying from 5 to 70 per cent. There are no precise figures for the proportion of flocks affected in Britain, although epidemiological returns and flock-recording schemes suggest that it may be over 50 per cent. Incisor loss is also a common problem in many areas of Australia and New Zealand.

The economic importance of incisor loss depends on its incidence, flock management and farm type. On hill and upland farms where grazing is poor, broken-mouthed ewes are culled at a young age because they may be unable to maintain body condition. The consequent high replacement costs are compounded by the low sale value of broken-mouthed ewes (up to 30 per cent less than age-matched sound-mouthed animals). On lowground farms, pasture conditions often allow maintenance of body condition and production despite incisor loss. In New Zealand, broken-mouthed ewes have been shown to be capable of live-weight performances identical to sound-mouthed ewes, provided their herbage allowances are some 30 per cent higher and they do not have to compete for it [2].

Clinical signs

When tooth loosening and loss have occurred, the diagnosis of broken mouth is straightforward, but early signs can be identified by careful examination of permanent incisors and their gingivae.

Gingivitis, characterized by gingival oedema, patchy reddening of the gingival margin and capillary fragility is evident in all sheep. In animals that remain free of broken mouth, this may persist throughout life but remain slight and difficult to see. In flocks in which broken mouth develops, the gingivitis worsens soon after incisor eruption. The severity of this chronic gingivitis fluctuates with time, periods of quiescence being interspersed with episodes of acute inflammation, when pus may be expressed from the gingival sulcus and the whole gingival margin is affected [3]. Repeated bouts of acute gingivitis lead to fibrosis of the gingival margin, which becomes thickened and irregular. On occasion, outbreaks of acute necrotic

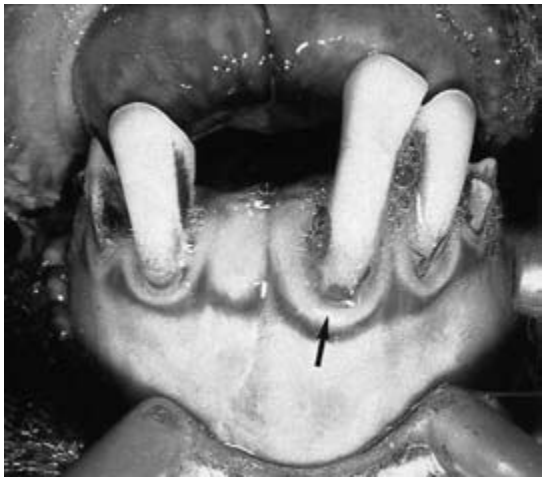

Figure 22.1: Typical broken mouth. Note a central incisor has been lost and gaps have formed between the teeth, which appear long and the gingiva around the teeth is swollen (arrow).

gingivitis and traumatic damage to the gingiva are recognized in flocks developing broken mouth, but are not consistent findings and are considered to be separate aggravating conditions. Gingivitis is also recognized around deciduous teeth, and it has been suggested that this is more severe in flocks with a high incidence of broken mouth.

As a result of the gingivitis, recession of the gingival margin occurs, which may make the tooth crowns seem very long (Figure 22.1). Irregular incisor crown wear, progressing to peg-like teeth, is a common adjunct to gingivitis, especially late in the condition, but it should be noted that neither long crowns nor irregular wear are necessary precursors to tooth loss. Grass impaction around long incisors is a further common finding and is considered an aggravating factor. The time from the appearance of severe gingivitis to tooth loss may span as little as a year in some flocks, but may extend over 3–4 years in others.

Pathogenesis

A complex microbial population develops within the gingival sulcus as soon as each tooth erupts into the oral cavity, which becomes associated with a localized inflammatory response in the sulcal wall and associated lamina propria. On farms free of broken mouth, the amount of subgingival plaque is small and the inflammatory response localized, but, where broken mouth occurs, the amount of plaque and its morphological complexity increases and the local inflammatory response intensifies. Up to 18 months prior to tooth loss, gingivitis progresses to periodontitis, which may involve all the supporting periodontal ligament and even alveolar bone.

The morphology of the periodontal ligament and surrounding alveolar bone are designed to accept the normal mechanical grazing forces applied to the incisors. Episodes of gingivitis and periodontitis are associated with deepening of the sulcus around the tooth to form pockets [4] and the destruction of collagen within the periodontal ligament. In time, this destroys the functional integrity of the periodontium, particularly on the lingual aspect of the incisors, and makes the tooth liable to loss from normal grazing/feeding forces [5].

Similarities are recognized between the pathogenesis of broken mouth and periodontal disease in man. Immune responses to subgingival plaque antigen components appear to be of primary importance to man and sheep, although no specific plaque antigen or single bacterial species has been implicated. *Bacteroides* spp and *Fusobacterium* spp isolated from sheep with periodontitis are similar to those implicated in human periodontal disease. The current understanding is that one or more of a number of periodontopathic micro-organisms are associated with the induction of a range of host responses resulting in tissue destruction.

Causes and epidemiology

Although the basic pathogenesis of broken mouth is recognized, the reasons for the variable incidence of periodontal disease between flocks remains unclear. Over the years, several suggestions have been made, but the evidence for any one is, at best, inconclusive. An imbalance in the nutritional ratio of calcium to phosphorus, or lack of calcium and an effect on the bony tooth supports, once the commonest hypothesis, has been discounted [6]. Improved pasture management (reseeding and liming), tough diets (root crops), excess wear on cheek teeth, faulty occlusion, high levels of oestrogen in the diet and problems in hogg nutrition (overwintering on root crops) are further unsubstantiated proposals. Good protein and

trace element nutrition may also influence the health of the periodontium, its repair, nutrition and defences. Broken mouth is a multi-factorial problem that may be unique to each farm and, while no evidence is available to link any of the above factors to its pathogenesis, the possible association between broken mouth and management should be kept in mind in any investigation.

Treatment and control

There is no treatment or control for broken mouth. Control through management changes has been singularly unsuccessful. A number of dental surgical procedures have been tried, including metal splints to aid tooth support and crown bite correction by the use of an industrial grinder. Neither procedure has been able to prove its economic worth or long-term efficacy, and the latter technique has been banned on welfare grounds in the UK and New Zealand.

Advice on the control or prevention of broken mouth must be empirical and with little guarantee of success. In the first instance, the real rather than perceived importance of tooth loss to the flock should be assessed in the light of current concepts of dental adaptability to different pasture conditions. Changes in farm policy to allow casting to a separate flock maintained under slightly better conditions, although leading to higher management costs, may allow the production of two or more extra crops of lambs while reducing replacement costs and minimizing the losses from poor sale prices. Practical management alternatives for any factor that may aggravate tissue damage around teeth, such as root feeding, should be examined and changes initiated on a trial basis to assess their significance [4].

INCISOR WEAR

In New Zealand and Australia, excess incisor wear, in which the incisors are worn down to gum level before 3–4 years of age, is common, affecting up to 30 per cent of flocks in some districts [7]. This has a significant impact on farm profitability owing to the proportion of replacements required. The rapid wear of permanent incisors is also reflected in the temporary incisors of young sheep on the same properties (Figure 22.2). In Britain, what little information is available suggests that incisor wear is only significant in some localized areas where sheep are grazed on marginal sandy pasture.

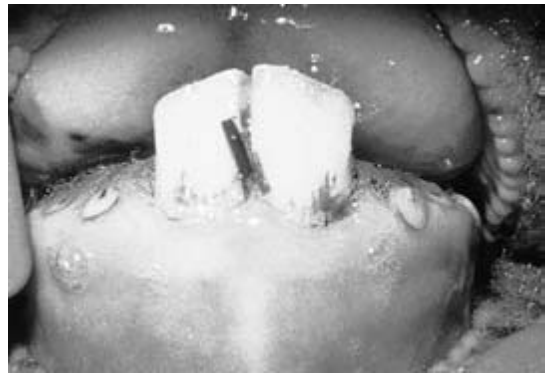

Figure 22.2: Excessive wear: the temporary teeth are worn to gum level and the recently erupted permanent central incisors show signs of early wear.

Many contributing aetiologies have been suggested for excessive incisor wear, but abrasion due to soil ingestion at the time of prehension generally has been accepted as the most important. However, the clinical picture of wear is not always typical of shortening due to abrasion at the occlusal surfaces. Incisor teeth do not simply shorten, but also dissolve and wear from the sides, so that the incisors are eventually reduced to 'pebbles'. It has been proposed that acids in soils may dissolve teeth, and studies *in vitro* have demonstrated that sheep dentine demineralizes in buffered sodium lactate solutions containing calcium and phosphate ions at pH levels within the range commonly reported for herbage and soils [8]. This solubilization hypothesis has yet to be fully tested *in vivo* but, in conjunction with physical abrasion, could account for the excessive rates of incisor teeth wear observed in many flocks.

DEFECTIVE ENAMEL FORMATION

Developmental defects of enamel in the permanent incisors of sheep result from some disturbance to the activity of ameloblasts during tooth development. These defects include enamel hypoplasia seen as

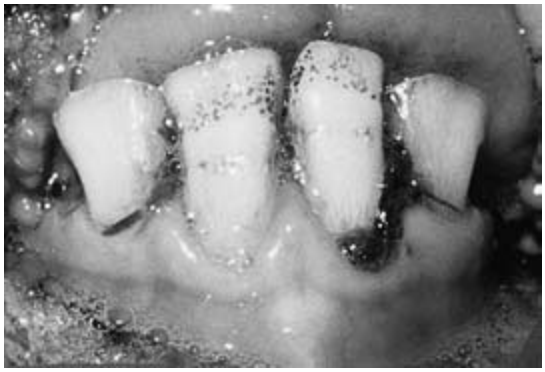

Figure 22.3: Enamel hypoplasia shown as two horizontal lines of enamel pitting on the central permanent incisor teeth.

small, shallow pits, no larger than 1–2 mm across (Figure 22.3) and enamel opacities in which there is a change in translucency of the enamel. Enamel defects are seen most often in recently erupted central permanent incisors, which develop in the jaw of growing lambs between 5 and 14 months of age and are fully formed before eruption at 15–18 months. Enamel defects of human permanent teeth are common and many factors which interfere with normal amelogenesis have been described. In sheep, defective enamel formation has been associated with feeding young sheep grain diets high in phosphorus and low in calcium, parasitism, undernutrition resulting from drought conditions and excessive fluorine ingestion. Infecting lambs with worm burdens, especially *Trichostrongylus* spp nematodes, has reproduced enamel defects similar to those observed on farms [9]. The disappearance of enamel hypoplasia after appropriate nematode parasite control programmes have been implemented suggests this may be an important cause of enamel defects in grazing sheep.

On its own, enamel hypoplasia is of little concern unless excessive incisor tooth wear is also present in sheep on the same farm.

CARIES

Caries result from bacterial action on the incisor enamel and are characterized by the development of deep holes, most frequently at the neck of the deciduous incisors at gum level (Figure 22.4). The incidence within a flock is high, but few outbreaks are reported because malnutrition, the only significant consequence of caries, occurs only under specific conditions of management. Crowns, weakened by caries, snap off at gum level, leaving a ragged stump; hoggs fed on root crops during winter are unable to bite into hard roots, and body condition deteriorates as a result. Histologically, the condition in lambs resembles that of man and, like that condition, is probably associated with diets high in soluble carbohydrate (e.g. concentrate feeding and some strains of root crops). Caries in adult teeth have been reported but are unlikely to be of clinical significance. The effects of caries are readily reduced by the addition of hay to the affected lambs' diet [10].

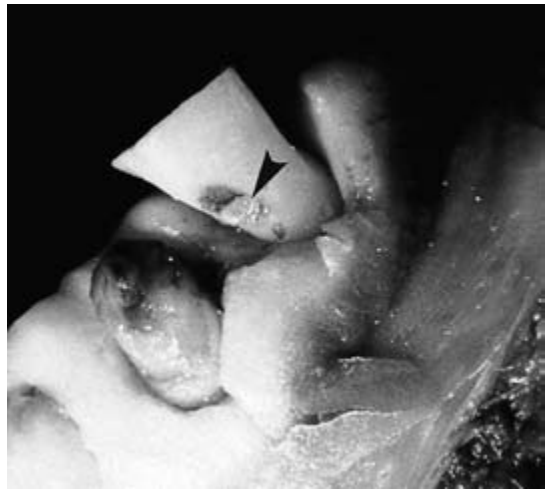

Figure 22.4: Dental caries on an incisor tooth (arrow). Brown staining may surround the pit in the enamel.

FLUOROSIS

Dental fluorosis is often the only visible evidence of fluoride intoxication since skeletal lesions may remain inapparent. Fluoride interferes with the normal deposition of mineral in developing teeth, and its effects therefore depend on the age of the animal and duration of exposure. The clinical signs appear long after fluoride intoxication. Pitting of the enamel may be extensive, and the enamel discoloured and chalky, with affected teeth wearing much faster than normal. Where exposure is brief, the resulting dental lesions may be limited to an irregular groove around a single

pair of teeth, which, in time, may be mistaken for carious pitting of enamel.

Fluorine toxicosis may follow industrial contamination or the application of certain rock phosphate fertilizers to pasture (Chapter 57). The diagnosis depends on a history of access to contaminated pasture and the analysis of feedstuffs and water. Blood levels of fluorine may rise above the normal level of 0.2 mg/dl and fluorine in urine may also be elevated above 6 ppm, but these are not consistent features. There is no treatment apart from removing the flock from the contamination so as to prevent further lesions from developing.

TOOTH DISCOLORATION

Discoloration of the tooth crown is a regular condition in sheep. Brown pigmentation of the incisor surface, most pronounced at the gum margin and disappearing towards the incisive edge, is quite normal and is due to colouring of the porous cementum that covers the complete incisor crown at eruption. A thicker mineralized deposit over enamel, calculus, is seen occasionally, but is more common round cheek teeth, where it may be brown or black and occasionally have a metallic sheen. In large quantities, it may aggravate local gingivitis but otherwise has no clinical significance.

ANTERIOR SWELLING OF THE MANDIBLE

Localized unilateral swelling of the anterior portion of the horizontal ramus of the mandible below the incisors in 2–4-year-old ewes occasionally may become a flock problem. Clinically, the swellings are bony and involve the incisor supports, one or more incisors always being displaced or missing. Histologically, the swellings are sterile, fluid-filled spaces lined with stratified epithelium, lying within a thin shell of alveolar bone (Figure 22.5). In most cases one, or more, permanent incisors is found in close apposition to, or within, the cyst wall. The term 'dentigerous' cyst, meaning 'containing a tooth', is commonly used to describe these swellings, but 'odontogenic' cyst has been suggested as more scientifically correct, as the cysts arise from epithelium associated with the formation of teeth. The cause of these so-called dentigerous cysts

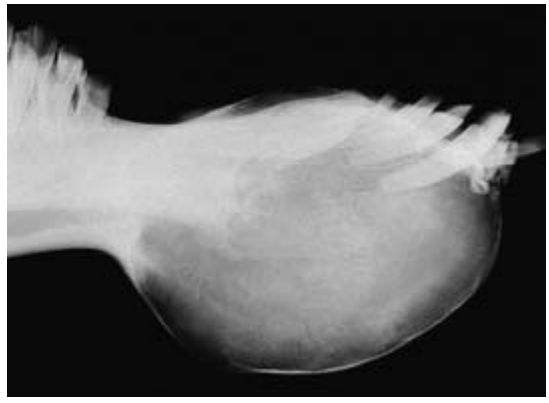

Figure 22.5: Radiograph of anterior mandible showing an odontogenic cyst.

is unknown. Their sporadic occurrence within a flock, especially flocks with excessive wear of temporary teeth, suggests that they may be an extreme form of malpositioning and maleruption. It has been proposed that they arise as a result of abscessation of periodontal tissues during the development and eruption of permanent incisors, followed by partial recovery and cyst formation from displaced epithelial fragments, but this suggestion requires confirmation [11].

DISEASES OF THE CHEEK TEETH

Diseases of the molar and premolar teeth have received little attention owing to the difficulty of clinical examination. However, abattoir surveys have shown that dental diseases of the cheek teeth are common in ewes and, on occasion, may become severe flock problems.

Clinical signs

Diseases of the cheek teeth may become apparent only when ewes develop excessive weight loss or pregnancy toxaemia. Otherwise, a picture is seen of slow deterioration in condition of a few ewes, swelling over the cheeks, occasional halitosis and some dribbling of rumen liquor during cudding. External palpation of the mandible may show hard bony swellings in the region of the cheek teeth of some ewes, occasional fistulae to the ventral aspect of the mandible and the smoothing out of cheek-tooth outlines in others due to impaction of grass between the teeth. Actual loss of

mandibular or maxillary cheek teeth may also be palpated through the cheeks.

Causes and pathogenesis

It is probable that chronic gingivitis and periodontitis are aggravated to more acute forms by farm-specific environmental factors. These factors have yet to be examined but, from evidence in other species, are likely to include fodder consistency, anatomical features precipitating grass impaction, and soil/herbage conditions that lead to trauma of the gums, gingiva and sulci. Similarly, many other syndromes appear to be secondary complications of an acute periodontitis. Thus, if the alveolar bone is sufficiently rarefied by alveolitis, then abscessation and fistulation to the ventral aspect of the jaw can ensue. In other cases, all the ligamentous support to a molar tooth may be destroyed but, because of its length, the tooth is not shed. Such teeth are likely to wear unevenly and rapidly, giving an irregular grinding table and shorter tooth. However, worn short teeth may be lost, leading to overgrowth of the opposing tooth into the empty socket and ulceration and lacerations of the tongue, gums and cheek. The first and second premolars have relatively short roots and are often lost as a result of chronic periodontitis, without need for excess wear. Packing of food material into pockets and sockets gives a severe halitosis and will exacerbate any infection still present.

There is no known cause for these conditions. A pathogenesis similar to broken mouth may be a possibility in many cases, but secondary acute bacterial infections are probably required to induce periostitis and fistulation. Once molar disease becomes severe enough to induce clinical disease, the condition is irreversible and affected ewes usually are destroyed.

MALOCCLUSION

Correct occlusion, the meeting of the incisors within 1–3 mm of the front of the upper pad, is considered important in the selection and breeding of rams and ewes. Severe overshot or undershot jaws, in which there is greater than 5 mm discrepancy between the incisors and the front of the upper pad, are considered to be inherited, but there is no evidence that occlusion within these extremes is controlled genetically or has a marked effect on broken mouth or other dental disease. Occlusion is a complex measurement involving the relative lengths of maxilla and mandible, anatomy of the temporomandibular joint, angle of the incisor in the jaw, length of incisor and, most importantly, ligamentous damage resulting from periodontitis. The last three are acquired traits, which change through life, thus the heritability of malocclusion is likely to be low.

An undershot jaw later associated with an inability to close the mouth occasionally develops in hoggs. This is a rachitic syndrome, which is rare now owing to improved sheep nutrition (Chapter 53).

SOFT TISSUE LESIONS OF THE ORAL CAVITY

Vesicles and erosions on the dental pad, gums, lips and tongue may occur in foot-and-mouth disease (FMD). Lip and gum lesions have also been described in sheep from the UK and New Zealand that did not have foot-and-mouth disease [12–14]. Typically, these lesions presented as discrete fresh or healing ulcers on the gums, usually located in the midline ventral to the incisor teeth. The ulcers had a raised edge, giving them a crater-like appearance. In a New Zealand abattoir survey, 3–4 per cent of the nearly 8000 adult ewes examined had oral lesions [14]. Following eradication of foot-and-mouth disease from the UK in 2001, a survey of 20 000 sheep heads found a 1 per cent prevalence of idiopathic oral lesions variously affecting the lower and upper gums, dental pad, hard palate and tongue. A well-illustrated review of the lesions and their differentiation from those of foot-and-mouth disease is available [15]. The lesions have been postulated to be associated with trauma, or with grazing sparse, rough or short pasture, the provision of feed or salt blocks, and the use of feeding troughs with sharp edges.

Damage to the soft tissues at the back of the oral cavity, and subsequent abscess formation, can occur following drenching gun injuries, penetration of foreign material such as grass seeds into the cheeks or tonsillar fossae, or external penetrating wounds. Typical signs include inappetance, dribbling of rumen liquor around the lips during cudding (cud-staining) and loss of body condition.

BACTERIAL INFECTIONS OF THE ORAL CAVITY

Orf lesions in lambs can become contaminated with secondary bacterial invaders, especially *Fusobacterium necrophorum*, giving a necrotic stomatitis, which can be fatal. In fact, necrotic stomatitis can be a sequel to any lesion that disrupts the integrity of the oral mucosa.

Flock outbreaks of actinobacillosis due to *Actinobacillus lignieresi* occur sporadically. Lesions consist of multiple fibrotic nodules in the subcutaneous tissues of the cheek, lips, nose and throat. These nodules progress to fibrous sinuses that fistulate to the oral cavity or outside, often releasing thick, odourless, adherent, green-yellow pus. Similar granulomatous lesions can occur also with *Arcanobacterium* (*Actinomyces*) *pyogenes*, although with pus that is foul-smelling, more fluid and yellow. Specific diagnosis depends on the isolation or demonstration of the causal organism. The conditions can be treated with streptomycin intramuscularly but are best prevented by limiting the occurrence of oral lacerations and wounds.

TUMOURS

Oral tumours in sheep are rare. Squamous cell carcinomas occasionally occur with invasion into surrounding tissues, but only fibrosarcomas of the jaw have been reported frequently enough to become flock problems. These are discussed in Chapter 58.

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23

Clostridial diseases

C.J. Lewis

Clostridial diseases of sheep have been recognized clinically for over 200 years, but not until the end of the nineteenth century did their bacterial nature start to be unravelled, a process that continued over the next 50 years [1]. Even during the 1990s, new information came to light as the importance of *Clostridium sordellii* as a cause of abomasitis and enteritis in all ages of sheep was established [2].

In general, the clostridia associated with disease have a ubiquitous distribution in the environment, particularly in soil. They occur also in small numbers in clinically unaffected animals. In most cases, disease is precipitated by so-called 'trigger factors', ranging from changes in management to traumatic damage to organs or parasitic activity. Such factors facilitate rapid multiplication of the organism, with toxin production and occasional invasion of tissues. Usually, the course of clostridial disease is so rapid that the animal is moribund or dead before treatment can be considered, but with tetanus and botulism the animal may survive for several days. In the case of valuable individuals, aggressive intensive therapy of clostridial disease can be successful on occasions. Most clostridial diseases are amenable to prevention by vaccination.

CAUSE

The clostridia are anaerobic rod-shaped organisms with rounded ends. They vary from 3 to 10 μm in length and from 0.5 to 1.5 μm in width. Some species are motile and all have the ability to form spores, each with its definitive morphology. Clostridia are Gram-positive but pleomorphism can occur and, in old cultures, they often stain Gram-negative. The clostridial diseases of sheep can be grouped broadly according to the systems or organs involved:

- affecting the alimentary system (the enterotoxaemias)
- affecting the parenchymatous organs
- causing myonecrosis and toxaemia
- causing neurotrophic disorders.

Some clostridial species produce several distinct diseases, which fall into more than one category. Table 23.1 lists those clostridia that cause or contribute to disease in sheep.

Strict anaerobic techniques and a variety of media are necessary for successful laboratory culture of clostridia, particularly when more than one member of the group is present, since some members can be favoured and mask the presence of others. Once isolated, the identity of the individual is confirmed either by gas-liquid chromatography (GLC) or by fluorescent antibody techniques (FAT). In the case of *C. perfringens*, polymerase chain reaction (PCR) techniques are available to confirm the identity of various types. PCR is rapidly replacing enzyme-linked immunosorbent assay (ELISA) procedures, which, in turn, have superseded both mouse inoculation and guinea-pig skin neutralization tests for diagnostic purposes.

THE ENTEROTOXAEMIAS

This group of diseases is caused by the various types of *C. perfringens*. In the UK, they are the commonest clostridial diseases to affect sheep. The five distinct types of *C. perfringens* are distinguished by their toxicological properties (Table 23.2). Types B, C and D are of major importance to sheep, while type A is occasionally pathogenic.

The toxins of major importance are alpha, beta and epsilon; the significance of others (iota and

Table 23.1: Clostridial diseases of sheep

Organism	Associated disease	Comment
Enterotoxaemias		
C. perfringens A	Enterotoxaemia	Rare
C. perfringens B (beta and beta 2)	Lamb dysentery and haemorrhagic enteritis	Lambs less than 21 days, UK and Europe Not Australia and New Zealand, Haemorrhagic enteritis world-wide
C. perfringens C		
Subtype 1	Struck	Adult sheep. Uncommon in UK. South Africa and Australia
Subtype 2	Necrotic enteritis	USA only
C. perfringens D	Pulpy kidney	All ages and cosmopolitan
C. novyi B	Black disease	Generally in adults. Cosmopolitan.
C. haemolyticum (*C. novyi* type D)	Bacillary haemoglobinuria	Sporadic, UK and Ireland
C. sordellii	Abomasitis and toxaemia	UK, New Zealand, all age groups
C. septicum	Braxy	UK, Scandinavia, mainly weaned lambs and shearings in autumn
Myonecrosis and toxaemia		
C. chauvoei	Blackleg (blackquarter) Post-parturient gangrene (also malignant oedema)	Cosmopolitan
C. septicum	Malignant oedema	Rare in Europe
C. novyi A	Big head, malignant oedema	Rams and particularly hot arid conditions
C. perfringens A	Malignant oedema	Rare
C. sordellii	Malignant oedema	USA and New Zealand
Neurotropic disorders		
C. perfringens D	Focal symmetrical encephalomalacia	Cosmopolitan
C. tetani	Tetanus	Mainly lambs, cosmopolitan
C. botulinum C and D	Botulism	South Africa and Australia, particularly under drought conditions. UK from poultry litter (type C)

Table 23.2: Distribution of toxins among different types of *C. perfringens*

	Major lethal toxins		
Type	α	β	ε
A	+++	–	–
B	+++	+++	+++
C	+++	+++	
D	+++		+++
E	+++		

theta) in the disease process remains unclear [3]. The recently described beta2 toxin requires further evaluation as to its role in the disease process. The principal ovine diseases caused by *C. perfringens* are lamb dysentery and haemorrhagic enteritis, struck and pulpy kidney. On occasions, type A has been held responsible for haemorrhagic enteritis, haemolytic disease or gas gangrene. Other diseases in the enterotoxaemic category are abomasitis and toxaemia (*C. sordelli*) and braxy (*C. septicum*).

Lamb dysentery

The disease is confined to lambs under 3 weeks of age, usually occurring towards the end of the lambing period, and is caused by *C. perfringens* type B. In an outbreak, the disease initially affects lambs of 1–4 days but as it progresses lambs of 2–3 weeks become affected. Losses can be severe, with 20–30 per cent of lambs dying. Death usually occurs 2–12 hours after

the onset of symptoms, but in older lambs can be delayed for up to 2–3 days.

Clinical signs, pathology and diagnosis

The first sign of disease is sudden death of apparently strong lambs within the first few days of life. Lambs that have been well-mothered and are feeding well cease sucking, start to bleat continuously and have a tucked-up appearance with obvious abdominal pain. Prostration and death, with or without central nervous signs, occur within a few hours [4]. Dysentery may or may not be a feature.

The causative bacteria are ingested from soil and faecal contamination of the udder. Under ill-understood conditions, they proliferate, attach to the intestinal epithelial cells and produce large quantities of beta and epsilon toxins. The age incidence may be explained by the observation that the beta toxin is highly sensitive to and is inactivated by trypsin. Colostrum contains a potent trypsin inhibitor. Beta toxin is necrotizing and initially damages the microvilli leading to the destruction of the epithelial cells and the production of haemorrhagic enteritis with ulceration of the intestinal mucosa.

An initial diagnosis can be made on history, clinical signs and necropsy findings. A more definitive diagnosis can be made by the demonstration of the specific toxins by the ELISA methods for the detection of both beta and epsilon toxins [5]. The beta2 toxin has also been incriminated as a cause [6]. As these tests are highly sensitive, the results must be reconciled with the gross necropsy findings. A haemorrhagic enteritis (see Figure 23.1 in the colour plate section) with attendant excess of sanguineous serous fluid in the abdominal cavity are the major findings. The lesions occur as a localized area of necrosis. The intestinal mucosa is congested and dark red with ulcers up to 2.5 cm in diameter penetrating deeply into the serosa, predominantly in the ileum. In older animals that have survived for a few days, focal symmetrical encephalomalacia (FSE) may be present owing to the action of the epsilon toxin. Anaerobic culture of intestinal contents can yield almost pure cultures of *C. perfringens*. Confirmative identity is made by GLC or by PCR techniques.

Epidemiology, treatment and control

The disease occurs in the UK, Europe and South Africa, but is absent from New Zealand and Australia, and is rare in North America. Lamb dysentery is more common in hill areas and more prevalent in cold, wet springs, when ewes are confined to small yards or pastures, which quickly become contaminated with faeces. Frequently, it is the more voracious single lamb born to a high-yielding ewe that is most likely to succumb.

Treatment is not practical because of the peracute nature of the disease. Prevention includes management to prevent overcrowding and contamination of limited lambing yards, and the provision of shelters for ewes and lambs. Once the disease has been diagnosed, administration of hyperimmune serum to all lambs under 3 weeks and all those subsequently born is effective, although expensive and time-consuming. The ideal method of prevention is by vaccination of the ewe.

Struck

The disease occurs in adult sheep at pasture and is caused by *C. perfringens* type C. In the UK, struck is observed most frequently in spring in adults. In recent years, it has been associated also with deaths in 6–8-month-old finishing lambs. As with the other enterotoxaemias, animals in best condition are most likely to be afflicted. Early reports suggested a relationship with the ingestion of liver fluke metacercariae. Other factors include abrupt changes in diet and sudden concentrate feeding.

Clinical signs, pathology and diagnosis

The first indication is the finding of dead sheep, usually recumbent on their sternum. If found alive they are dull, resent being moved and may be lame. Losses are usually sporadic. The bacteria are ingested from the soil or may be present in small numbers in the intestinal tract of a normal sheep. Their rapid multiplication in the intestinal tract leads to the production of large quantities of beta toxin, the necrotizing effect of which results in extensive desquamation and destruction of intestinal epithelial cells.

As with lamb dysentery, initial diagnosis is made on history, lack of clinical signs and necropsy findings. Both epicardial and thoracic effusions are present and the abdominal cavity frequently contains large quantities of pale pinkish fluid. Haemorrhagic enteritis affects areas of jejunum and ileum but few,

if any, ulcers are present. If necropsy has been delayed for a few hours, the muscles reveal fairly marked putrefactive changes.

Beta toxin may be demonstrated in the gut contents but must be reconciled with gross necropsy findings and history. *C. pefringens* may be isolated by anaerobic methods from the gut contents and its identity confirmed by GLC or PCR.

Epidemiology, treatment and control

In the UK, struck is the rarest of the enterotoxaemias, being reported most frequently from Kent, Yorkshire and Suffolk. It has a global distribution and, in the main, affects adult sheep. *C. perfringens* type C subgroup 1 produces the classic struck, and subgroup 2 causes a necrotic enteritis of young lambs in North America [7].

Treatment is not practical because of the peracute nature of the disease. Control includes avoiding abrupt changes to lusher diets, and the removal to barer pastures once a case has been confirmed. Prevention is by vaccination.

Haemorrhagic enteritis

This sporadic condition affects lambs in the first few days of life, is caused by *C. perfringens* types B (either beta or beta2 toxin) or C, and differs from lamb dysentery only in the gross pathology and being marginally less acute, affecting lambs up to 3 weeks of age.

Clinical signs, pathology and diagnosis

Frequently, lambs are found *in extremis*, having ceased to suck; they shiver, tend to separate from their dams and may or may not exhibit abdominal pain. Death supervenes after a period of recumbency and is usually peaceful. Blood-stained fluid faeces are usually passed terminally. At necropsy, a generalized haemorrhagic enteritis is present but lesions are more commonly confined to the jejunum and ileum.

Distinct necrotic ulcers are not a feature. In severe cases, free blood may be present in the lumen of the intestine. The mesentery is hyperaemic, mesenteric lymph nodes oedematous and sometimes haemorrhagic. Serous membranes may contain ecchymotic haemorrhages, but this is a variable feature, as is the presence of an excess of straw-coloured pericardial fluid. These changes are suggestive of haemorrhagic toxaemia, as is the history. The demonstration of either beta or beta and epsilon toxin in gut contents, although not confirmative, helps to sustain the diagnosis. Isolation of *C. pefringens* B or C by anaerobic methods and confirmed by GLC or PCR techniques further establishes a diagnosis.

Epidemiology, treatment and control

The condition is caused by *C. perfringens* type B or C subtype 1 in the UK, while in America subtype 2 is incriminated. The bacteria are ingested from soil, contaminated wool and teats of the ewe. Insanitary conditions and dirty ewes lambing extensively or in houses predispose to haemorrhagic enteritis. Under ill-understood conditions, the organism proliferates in the intestinal tract leading to the production of large quantities of toxin. The peracute nature of the disease precludes treatment. Vaccination and attention to hygiene are the keys to control, although present vaccines do not contain the beta2 toxoid.

Pulpy kidney

Enterotoxaemia caused by *C. perfringens* type D is known as pulpy kidney disease and is by far the most widespread and important of all the diseases caused by *C. perfringens*. Usually, it is encountered in lambs of 4–10 weeks of age and in finishing lambs of 6 months and older. Adults can be affected sporadically but, on occasion, losses can be as high as 10 per cent. Rams, in particular, appear susceptible as they are prepared for mating.

Clinical signs, pathology and diagnosis

The disease is peracute, with death intervening often less than 2 hours after the onset. Most cases are found dead, usually in lateral recumbency. Those observed alive exhibit ataxia progressing to recumbency with opisthotonos and convulsions with or without nystagmus. In rare cases of animals surviving for a short period, there is a profound dullness and diarrhoea is a marked feature.

Initial diagnosis is made on history, usually a lack of clinical signs and necropsy findings. Carcasses are usually in good condition but, if necropsy is delayed, there is rapid decomposition with a marked purple

discoloration of the skin and usually signs of an early toxaemia, with congested blood vessels and discoloration of the muscles. There is an excess of straw-coloured pericardial fluid with or without large fibrin clots, and both pericardial and endocardial haemorrhages are present. Pulmonary oedema is common. The abdominal cavity contains blood-tinged fluid, and haemorrhages may be seen on the viscera (see Figure 23.2 in the colour plate section). There is a patchy congestion with or without haemorrhages of the intestinal mucosa. The kidneys are characteristically soft and pulpy and, if held under a stream of water, the parenchyma washes away leaving the frond-like cortical stroma. If the bladder contains urine, a glycosuria will be present.

Intestinal contents will be positive for epsilon toxin but negative for beta toxin. Anaerobic cultures will yield *C. perfringens*, usually in pure culture. These can be confirmed as *C. perfringens* type D by GLC or PCR techniques. A diagnosis can be established by histology of the brain, in which a characteristic focal symmetric encephalomalacia is seen.

Epidemiology, treatment and control

C. perfringens D is a natural inhabitant in small numbers of the intestinal tract of sheep as well as of soil contaminated with faeces and has a global distribution. In the UK, it accounts for most sheep fatalities due to clostridial infection. Outbreaks of pulpy kidney are associated with a sudden change of diet or the introduction to a higher plane of nutrition, particularly when concentrates containing large amounts of easily fermentable carbohydrates are suddenly introduced. Greedy feeders and the best lambs usually succumb. Losses can reach 10–15 per cent in severe outbreaks.

Under favourable conditions in the gastrointestinal tract, particularly in the presence of starch granules or carbohydrates, the bacteria multiply rapidly, producing the non-toxic protoxin, which is converted to the lethal necrotizing epsilon toxin by the action of trypsin. Large quantities of toxin must accumulate in the intestinal tract before it can be absorbed [8]. The toxin first increases the permeability of the intestinal mucosa and thereby facilitates rapid absorption. Initially, stimulation, followed quickly by depression, of the central nervous system occurs. Vascular endothelium degenerates and intracellular oedema as well as necrosis occurs within the brain. In addition, mobilization of hepatic glycogen results in marked hyperglycaemia and attendant glycosuria. The damage to the vascular endothelium is also responsible for the protein-rich effusions that occur. Treatment is impracticable as death is so rapid. Even if cases are observed alive, the damage to the various body systems is so great that they are by then irreversible. Work in New Zealand [9] suggests that vaccination in the face of an outbreak will reduce further losses markedly, while control of enterotoxaemia consists of reducing the factors that can precipitate disease. Diets should not be changed suddenly and, when feeding concentrates, a slow introduction is essential. If cereals are fed, then whole grain should be used to prevent too rapid passage from rumen to the abomasum and jejunum. Because *C. perfringens* type D is ubiquitous in its distribution, control measures will only reduce but not prevent the disease. As with all the other enterotoxaemias, the disease is best prevented by vaccination.

Enterotoxaemia and fatal haemolytic disease

C. perfringens type A has been held responsible for cases of enterotoxaemia in sheep in Europe and Australia and haemorrhagic disease of lambs in the UK and USA. *C. perfringens* type A is a constant and regular inhabitant of the intestinal tract of sheep. Some spore-forming *C. perfringens* type A produce a large quantity of alpha toxin, which possesses phosphorylase C and sphingomyelinase activity, both of which are associated with haemolysis.

The clinical signs and pathology are similar to those observed in haemorrhagic enteritis associated with *C. perfringens* B. Diagnosis depends on clinical signs and the failure to demonstrate beta and epsilon toxins in the intestinal content whilst demonstrating large quantities of alpha toxin. If the ELISA is relied on and the intestinal contents unpreserved over a considerable time, the beta toxin may be denatured and only the more stable alpha detected, thus leading to confusion as to the true cause of the haemorrhagic enterotoxaemia. The role of *C. perfringens* type A in sheep disease still requires elucidation.

Braxy

Braxy or bradshot is a disease of autumn and winter, and is most commonly encountered in lambs born in

the previous spring, or in shearlings. On some occasions, losses can occur at lambing time if ewes are turned out on to frosted pastures. It is caused by *C. septicum*, which produces four toxins.

Clinical signs, pathology and diagnosis

Animals are usually found dead but, if encountered early, they are depressed, febrile and show abdominal pain. Recumbency and death soon follow.

A marked feature of braxy is the very rapid putrefaction of the carcass. Necropsy reveals a severe abomasitis with areas of oedema, congestion and ulceration of the mucosal surface. Epicardial haemorrhages may be present.

An initial diagnosis can be made on history, age of the animal and necropsy findings. Definitive diagnosis is achieved by the demonstration of large numbers of Gram-positive rods in the affected areas of the abomasum. Anaerobic cultures taken from the affected areas produce characteristic spreading colonies, which can be confirmed as *C. septicum* by FAT [10].

Epidemiology, treatment and control

Braxy is encountered in countries with cold winters, such as the UK, Iceland and the Nordic countries, and other upland areas of Europe. The disease has been recorded in South Australia. *C. septicum* is found in small numbers as a natural inhabitant of the intestinal tract of sheep. It is also widely distributed on faecal-contaminated pastures. Ingestion of frozen forage appears to precipitate a primary abomasitis that is colonized by *C. septicum*, which then releases its powerful toxins. The toxins first damage the mucosal surface, which facilitates their rapid absorption and results in a toxaemia. Treatment is not an option, but braxy can be prevented by vaccination.

Abomasitis and toxaemia

Abomasitis in lambs aged 4–10 weeks and toxaemia of older lambs and adults can be caused by *C. sordellii*. The condition has been described in the UK [2] and reported from New Zealand and Australia. *C. sordellii* is 2–6 μm long and 0.6–1 μm wide.

Sporulation takes place readily and spores, round or cylindrical, do not distend the outline of the rod. Two toxins are produced, haemolytic (HT) and lethal toxin (LT), the latter being strongly cytotoxic.

Clinical signs, pathology and diagnosis

In lambs of 4–10 weeks, sudden death is the predominating feature. If seen shortly before death, the lambs appear bloated, move away and lie alone. The condition is seen almost exclusively in intensively housed or heavily creep-fed lambs. In older lambs and ewes, sudden death is again the predominating sign. If observed before death, affected sheep are recumbent, with the chin resting on the ground, but death occurs rapidly.

In young lambs, the outstanding and consistent feature post-mortem is a displaced and distended abomasum lying across the body cavity immediately distal to the xiphisternum (see Figure 23.3 in the colour plate section). The serosal surface is grey and glistening with marked sub-serosal oedema and emphysema. Opening the abomasum reveals up to 1 cm of oedema and emphysema between serosal and mucosal surfaces, the folds are oedematous and gastrorrhagia is a regular feature. In contrast, the condition in older lambs and adults is less spectacular. On occasions, there may be an intense abomasitis with a degree of oedema but emphysema is not present. Sometimes there is a typhilitis with petechial and ecchymotic haemorrhages between serosal and mucosal surfaces of the caecum. On other occasions, few necropsy signs are present. In young lambs, the gross necropsy findings are characteristic while, in older animals, the varying signs and rapid putrefaction are suggestive of a clostridial condition. Where lesions occur, direct smears reveal many Gram-positive rods. When further stained by an indirect FAT, the rods fluoresce. Anaerobic cultures produce greyish white colonies with a variable border of haemolysis. GLC analysis or indirect FAT of the colonies confirms their identity. In very fresh carcasses, *C. sordellii* can be isolated only from the abomasum but, in older carcasses, isolation can be made from abomasum, intestinal tract and liver.

Recently, *C. sordellii* has been incriminated in periparturient disease. Lambs die *in utero* at full term, even if removed promptly and aggressive therapy is instituted the ewe almost invariably dies [11].

Epidemiology, treatment and control

In ewes, deaths are sporadic and most have been recorded soon after lambing, usually associated with

a change in diet. Several strains of *C. sordellii* have been identified, but no relationship between necropsy signs and strains has been established.

Unlike other clostridia, *C. sordellii* does not appear to be a natural inhabitant of the intestinal tract of sheep but is widely distributed in the environment. The reason for its sudden multiplication in the abomasum is obscure, but could be associated with the increased passage of carbohydrates. Cases are encountered more frequently in sheep grazing in new leys and root crops, suggesting that disturbance of the soil for cultivation may have allowed spores to rise to the surface.

Treatment is not an option but vaccines are now available.

DISEASES OF PARENCHYMATOUS ORGANS

The liver and kidney are particular targets for *C. novyi*, which, like *C. perfringens*, is represented by several types, of which A, B and D occur in sheep, and its classification into types depends on the toxins and enzymes produced (Table 23.3). In sheep, *C. novyi* is responsible for black disease and bacillary haemoglobinuria.

Black disease

Black disease or infectious necrotic hepatitis, caused by *C. novyi* type B, is confined to the wetter areas of the UK [12] and other sheep-rearing areas of the world, in particular New Zealand and Australia. Disease usually occurs in 2–4-year-old sheep, generally in late summer and autumn.

Clinical signs, pathology and diagnosis

Clinical signs are rarely observed. Affected sheep rapidly become dull, unsteady, collapse and die peacefully, usually within a very short time of onset. Most outbreaks affect about 5 per cent of sheep but, in severe outbreaks, mortality can reach 30 per cent.

A marked post-mortem feature of black disease is very rapid decomposition of the carcass and darkening of the skin, hence the name (see Figure 71.3 in the colour plate section). Subcutaneous blood vessels are engorged, and there is frequently oedema in the fascia between the abdominal muscles and in the axilla. A constant feature is excessive effusion of fluid in the thoracic and abdominal cavities, the latter frequently blood-tinged. There is an excess of pericardial fluid but fibrin clots are rare. Varying degrees of endocardial and epicardial haemorrhages may be present. The liver is usually congested and very dark with areas of necrosis up to 3 cm in diameter and surrounded by a zone of hyperaemia. These are frequently deep in the tissue and not always visible on the surface. A frequent additional finding is evidence of recent fluke migration [13]. In cases in which other hepatic insults have occurred, the liver is often shrunken, congested and solidified.

Diagnosis is based on history and necropsy findings. In addition, smears from the liver reveal large numbers of large Gram-positive rods. Confirmation is by FAT techniques. Care has to be exercised, since *C. novyi* B is a common normal inhabitant of the ovine liver and the demonstration of *C. novyi* B by FAT alone cannot constitute a definite diagnosis as the organism multiplies rapidly in cadavers. Confirmation can be by the demonstration of both beta lecithinase and the lethal alpha toxin in the abdominal effusions. *C. novyi* is one of the most fastidious of all clostridia to culture and requires freshly prepared pre-reduced media with rapid inoculation of the plates.

Table 23.3: Distribution of toxins and enzymes of *C. novyi* types affecting sheep

	α	β	γ	δ
C. novyi type	Necrotizing lethal	Lecithinase haemolytic necrotizing lethal	Lecithinase haemolytic necrotizing	O-labile haemolysin
A	+++	−	+	+
B	+++	+	−	−
C	−	+++	−	−

Epidemiology, treatment and control

The organism is found in the normal livers of sheep, and migrates from the alimentary tract via the lymphatics. The intervention of a necrotic process causes the organism to proliferate and produce large quantities of lethal toxins. The commonest cause of liver necrosis is the passage of young migrating immature liver flukes. Other insults may also precipitate disease. Black disease is most prevalent in years when sheep ingest large numbers of fluke metacercariae. *C. novyi* type B spores are prevalent in soil in areas where the disease occurs. Environmental conditions favouring fluke infestation also favour survival of *C. novyi* B spores. Soil can become heavily contaminated by faeces or by rotting unburied carcasses [14]. Disease can be spread from premises by the movement of sheep carrying hepatic spores. Black disease is not amenable to treatment. Control of liver fluke will help to reduce but not to eliminate the disease. The availability of effective, inexpensive flukicides has done much to reduce the incidence of fluke. Yet, when favourable climatic conditions prevail for fluke and their host snails, the incidences of both fluke and black disease increase. Vaccination is the sure way of control.

Bacillary haemoglobinuria

Bacillary haemoglobinuria or redwater disease occurs in adult sheep. It is not a common condition and is caused by *C. haemolyticum* (*C. novyi* type D), which is morphologically identical to *C. novyi* type B. The disease occurs sporadically in the UK as well as in the wetter areas of western South America, but probably is under-diagnosed owing to its sporadic nature and difficulty in differentiating it from black disease without laboratory facilities.

Clinical signs, pathology and diagnosis

Bacillary haemoglobinuria is characterized by sudden onset of a high fever and production of dark-red urine. Eventually, the animal becomes jaundiced, recumbent and dies peacefully over a period of 2–3 days.

A feature of deaths due to bacillary haemoglobinuria is the rapid onset of rigor mortis and the red staining of the perineal wool. The degree of jaundice varies as does the degree of thoracic and peritoneal effusion. The characteristic lesions are large pale infarcts in the liver, varying from 5 to 20 cm in diameter and either single or multiple. Infarcts are surrounded by dark-red zones of hyperaemia. Haemorrhage in the substance of the kidneys is a variable finding, but the urine in the kidney and bladder is invariably red.

History, gross necropsy findings and the demonstration of large Gram-positive rods in the liver provide a basis for diagnosis, with confirmation by FAT techniques. Much care must be exercised in interpretation, since *C. haemolyticum* is a normal inhabitant of the liver, and cross-reaction with *C. novyi* type B can occur. Confirmation can be obtained by demonstration of only beta lecithinase in the peritoneal effusions. *C. haemolyticum* is even more fastidious to culture than *C. novyi* type B [9]. Work in New Zealand suggests that bone marrow of cadavers is probably the most rewarding source. Definitive diagnosis is by culture and GLC confirmation that the isolate is *C. haemolyticum*.

Epidemiology, treatment and control

The organism can be found in the liver of normal sheep and in soil contaminated by sheep faeces. Whilst migrating young fluke may be a precipitating cause, other hepatic insults such as telangiectasis, necrobacillosis caused by *Fusobacterium necrophorum necrophorum* or invading *Cysticercus tenuicollis* are considered precipitating factors when disease occurs in areas devoid of fluke infestation.

Control of liver fluke, and the regular worming of sheep dogs to reduce *C. tenuicollis* infestation, will reduce incidence, as will other measures to prevent hepatic insults to sheep. Vaccination is the control method of choice.

MYONECROSIS AND TOXAEMIA

This group of diseases most commonly results from contamination of wounds or open surfaces and may affect sheep of any age. Shearing injuries, docking wounds, damage at assisted lambing and undressed navels are common points of entry.

Blackleg, post-parturient gangrene and navel-ill

These diseases are found wherever sheep are farmed and are caused by *C. chauvoei*, which produces four

very powerful toxins: alpha (oxygen-stable haemolysin), beta (deoxyribonuclease), gamma (hyaluronidase) and delta (oxygen-labile haemolysin).

Clinical signs, pathology and diagnosis

Clinical signs depend on the site of infection. When muscles of the limbs or back are involved, there is a marked febrile reaction, initially the gait becomes stiff and is soon followed by refusal to move. Recumbency and death rapidly intervene [4]. In post-parturient gangrene a sanguineous discharge is present at the vulva and the perineum may be swollen and oedematous. Crepitus is absent. Navel infection in lambs leads to rapid death. Sheep with cardiac myositis are usually found dead [15]. In the case of shearing wounds, extensive local lesions occur with oedema and necrosis of underlying tissues, including muscles. The affected sheep is very dull with a high temperature.

A characteristic of death due to *C. chauvoei* is the very rapid bloating and decomposition of the carcass. The bacterial toxins cause a severe necrotizing myositis, which is evident at necropsy. The carcass is almost invariably bloated and there is often considerable yellow gelatinous or blood-stained oedema in the subcutaneous tissues and around the lesion. In blackleg, the lesion can be extensive or quite small, deep in the muscle masses, with only a limited amount of emphysema.

Infected wounds are characterized by subcutaneous swelling with oedema but little emphysema. Lesions may extend inwards to affect underlying muscle masses. In post-parturient gangrene, the lesion involves the wall of the vagina and sometimes the uterus, and can extend to the muscles of the hind leg. Oedema and intensive congestion are the consistent findings. On rare occasions when infection has preceded parturition, the dead lambs are oedematous and emphysematous. In cardiac myositis, there is a haemorrhagic pericardial infusion in which fibrin clots may be present and lesions are detected in the cardiac muscle, which is dark, congested and oedematous.

Diagnosis is based on necropsy findings. The demonstration of large numbers of Gram-positive rods at the margins of the lesion adds weight to the diagnosis. Further staining using FAT or PCR techniques will confirm the diagnosis. Anaerobic culture for *C. chauvoei* is not as difficult as *C. novyi*, provided appropriate culture medium is used. Confirmation that the colonies are *C. chauvoei* is by FAT methods or by GLC.

Epidemiology, treatment and control

C. chauvoei spores survive well for very long periods in the soil. In sheep, most of the clinical signs can be attributed to contamination. In the case of cardiac myositis and blackleg, the portal of entry is probably through the alimentary mucosa after ingestion of contaminated foodstuffs. The exact mechanism by which the latent bacteria are simulated to growth and toxin production is unknown.

Except in very valuable animals, treatment is not normally an option. Good hygiene at lambing time and scrupulous cleanliness if lambings are assisted help to reduce the incidence. Skilled shearers and routine disinfection of combs and cutters also reduce the number of contaminated shearing wounds. Management should endeavour to exclude sheep from known affected pastures during summer and autumn. Vaccination is the ideal way to prevent disease.

Malignant oedema

Malignant oedema is an acute, rapidly fatal condition, following contamination of wounds with various members of the genus *Clostridiu*m, including *C. perfringens* type A, *C. septicum*, *C. novyi* type A and *C. sordellii* (for *C. chauvoei* involvement see blackleg).

Clinical signs, pathology and diagnosis

A specific condition known as swelled head or big head occurs in rams of 6 months to 2 years of age, particularly when run together as a group. Initially, swelling under the eyes rapidly involves the whole head and upper neck. The animal is febrile, dull, anorexic and soon dies. There is oedema and swelling at the site of infected wounds, and crepitus may or may not be present, depending upon the organism involved. If the lesion is close to muscle groups, lameness may ensue. The animal is febrile and dull, and death occurs within 1–2 days.

The pathological features depend to a degree on which clostridial organism is involved. Usually the primary lesion is a cellulitis, which is followed by a typical emphysematous and oedematous gas gangrene or a generalized toxaemia. In the latter cases, there is often subcutaneous oedema with sanguineous effusions into body cavities.

In the living animal the clinical signs are virtually pathognomonic, except that swelled head could be

confused with acute extensive orf. In fatal cases, necropsy will indicate a toxaemic carcass but specific cellulitis with comparatively little muscle involvement. Blood-tinged fluid or gelatinous clear fluid with or without gas will be present. Impression smears will reveal large numbers of Gram-positive rods. FAT techniques can be used to differentiate *C. chauvoei*, *C. septicum* or *C. novyi* type A, and an indirect FAT will identify *C. sordellii*. Anaerobic cultures aided by FAT, GLC or PCR techniques will confirm the identity of the organism responsible for this condition.

Epidemiology, treatment and control

Malignant oedema is caused by the contamination of wounds by soil containing the spores of the appropriate clostridia. Deep wounds and, on occasions, intramuscular injection sites are ideal for the proliferation of clostridia. Except in the case of young rams, the condition is sporadic and seen most commonly in Australia and New Zealand and other dry dusty areas, but occurs also wherever sheep are kept in unhygienic conditions.

Unlike most clostridial conditions, heavy doses of antibiotic in the very early stages aided by local debridement can halt the process. Recovery is slow, and often, particularly in the case of rams, disfigurement is permanent. Control is by good hygiene, experienced shearing and avoidance of heavily contaminated handling yards. In the case of ram studs, management practices to reduce fighting and the avoidance of known contaminated pastures reduce the incidence.

NEUROTROPIC DISORDERS

Tetanus, botulism and focal symmetrical encephalomalacia are clostridial diseases in which the nervous system is affected.

Tetanus

Tetanus is highly fatal, particularly in lambs but also in adult sheep, and is caused by *C. tetani*. The condition has a global distribution. Unlike other clostridia, the causative organism is a slender motile rod 3–6 μm long and 0.3–0.6 μm wide. Spores form terminally, and distend the outline of the rod, producing the so-called drumstick effect.

Clinical signs, pathology and diagnosis

Contamination of docking and castration wounds in lambs when the procedures are carried out in heavily contaminated yards is the commonest cause of disease. Frequently, large numbers of lambs will succumb. It has been recorded also in lambs castrated by the rubber ring method. In adult sheep, sporadic cases occur when puncture wounds are contaminated by soil containing spores.

The first signs are a generalized stiffness followed by a more marked stiffness of the hind limbs. Eventually, the animal becomes recumbent and tetanic convulsions steadily increase in severity and frequency, with opisthotonos in the terminal stages. Death occurs 4–7 days after the onset of signs due to respiratory failure. Clinical signs are often not presented until 3–10 days after the initial wound infection, while, in adults, up to 3 weeks may elapse before signs appear.

Proliferation and then autolysis of the bacteria are necessary before a powerful neurotoxin, tetanospasmin, is released. The toxin reaches the central nervous system by passing-up peripheral nerve trunks. The exact mechanism by which the toxin exerts its effect on the nervous tissue is still subject to debate. Diagnosis is based almost exclusively on clinical signs. Frequently, the delay in the development of signs after the time of initial infection prevents identification of the site of infection. If a site can be identified, strict anaerobic bacteriological techniques can be employed to isolate the organism.

Epidemiology, treatment and control

Soil is contaminated by faeces containing the spores of *C. tetani*, which can survive for many years. Moreover, they are resistant to many disinfectants and even to steam cleaning. Once a wound has been contaminated, the spores lie dormant until conditions are favourable for vegetative growth.

In acute recumbent cases, treatment is unrewarding. In the early stages, large doses of antisera and antibiotic with debridement of the site of infection can halt the progress of the disease, but eventual recovery is slow. Such treatment is very expensive and would be reserved for valuable animals. Good hygiene at docking and castration time, and the provision of clean

yards, help to reduce challenge. Vaccination is the ideal method of control.

Focal symmetrical encephalomalacia

This sporadic disease of both sucking and weaned lambs and of adult sheep is caused by intestinal absorption of small quantities of epsilon toxin of *C. perfringens* type D.

Clinical signs, pathology and diagnosis

Separation from the flock, wandering and anorexia are the commonest signs. Not infrequently, it is the animals in poorer bodily condition that are affected. Eventually, the sheep becomes ataxic and lies quietly in lateral recumbency. Nystagmus may be present but irregular, and eventually paddling and convulsions occur. The sheep is unable either to eat or drink and slowly drifts into a coma and dies.

Usually, no gross necropsy findings are present, lesions being confined to histological changes in the brain [16]. The main characteristics are perivascular oedema and haemorrhages in the cerebral cortex, corpus striatum, thalamus, mid-brain and cerebellum white matter as well as the peduncles. Malacia is focal and sharply demarcated from surrounding tissue. The changes involve vacuolation of the neuropil, pyknotic glial nuclei and degeneration of the neurones. The degree of macrophage infiltration is greatest in long-standing cases. Diagnosis is made entirely from brain histology.

Epidemiology, treatment and control

C. perfringens type D forms part of the normal flora in the intestinal tract and produces small quantities of toxin. Together with the bacteria, this is kept to very low levels by the movement of the ingesta through the alimentary tract. However, any ruminal stasis or slowing of the intestinal tract will allow an increase in toxin absorption and mucosal permeability. Additionally, different strains of *C. perfringens* type D produce varying quantities of protoxin. In weaned lambs and adults, there is often a history of movement to fresh pasture or the administration of anthelmintics. Morbidity is usually low with only individuals affected. No effective treatment or control strategy is available. Vaccination is the ideal method of prevention.

Botulism

Botulism is caused by the toxins produced in foodstuffs by *C. botulinum*. Of the seven toxigenic types of *C. botulinum*, only C and D affect sheep and, of these, type C is by far the commoner. *C. botulinum* is a large Gram-positive rod 4.0–16 μm long and 0.6–1.2 μm wide. Large subterminal spores distend the outline of the rod. The spores are extremely resistant and survive in the environment for many years.

Clinical signs, pathology and diagnosis

Sheep do not exhibit the typical flaccid paralysis of other species until the final stages of the disease. Initially, there is some stiffness, incoordination and excitability with a characteristic bobbing of the head. Later there is salivation, nasal discharge and finally abdominal breathing followed by rapid death. The neurotoxins produce a functional paralysis without any histological or gross pathological changes. Diagnosis is essentially by clinical signs. Frequently, the offending foodstuffs have been totally consumed before an outbreak occurs. The detection by FAT of *C. botulinum* in intestine and liver can be suggestive that the condition being investigated may be botulism but cannot confirm it.

Epidemiology, treatment and control

Botulism has been recorded sporadically from many countries. Major outbreaks occur in countries such as South Africa and Australia, where sheep are extensively grazed and suffer phosphorus and protein deficiency. Outbreaks occur more frequently in drought conditions. In the UK, outbreaks have been associated with the grazing of pasture recently top dressed with poultry house litter and containing the carcasses of casualty birds [17]. The feeding of rodent-contaminated, poorly fermented big bale silage is also hazardous. Botulinum C and D toxins survive for very long periods in carrion and foodstuffs. Restricted access to carrion-contaminated foodstuffs aids control. In areas such as South Africa and Australia, where the condition is endemic, control is by specific vaccination.

VACCINATION AGAINST CLOSTRIDIAL DISEASES

Clostridial diseases are ideal candidates for control by vaccination. Modern vaccines are based on the purified toxoids of the lethal toxins causing disease. Fortunately, these toxoids are very antigenic. The exception is *C. chauvoei*, for which it is necessary to include cellular material to achieve solid immunity. While monovalent vaccines can be used for specific conditions, there has been an increasing tendency to produce polyvalent vaccines to provide comprehensive protection either for a specific age range of sheep or for a specific geographical location. Prevention of lamb dysentery, haemorrhagic enteritis and tetanus depends upon high levels of protection soon after birth. This is not achievable by vaccination of the lamb. No clostridial antibodies are passed to the developing fetus by way of the maternal blood supply. Fortunately, the ewe concentrates antibody in her colostrum during the last 13 days of pregnancy, a process that requires high levels of circulating antibody. Thus, the principle of clostridial vaccination is to protect the breeding ewe systemically and her offspring via colostral antibody. Toxoid vaccines are inactivated and, to achieve maximum response, two doses are required 4–6 weeks apart. This is frequently referred to as the primary course. Antibody levels wane over the course of a year and annual revaccination is required. In breeding sheep, this booster dose is best administered about 4 weeks before lambing to maximize protection to the lambs and afford up to 12 weeks protection [18]. Circulating maternal antibody does not appear to interfere with the response to primary vaccination in the lambs themselves.

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24

Mycobacterial infections

J.M. Sharp

Sheep are affected by two chronic diseases that are caused by mycobacteria. The most common of these is paratuberculosis (*synonym*: Johne's disease), a chronic enteritis of all ruminants that is widely distributed throughout the world. More rarely, tuberculosis has been reported in sheep.

JOHNE'S DISEASE

Johne's disease is a chronic enteritis of all ruminants that is widely distributed throughout the world. It is economically important as a result not only of clinical disease but also of reduced productivity during the prolonged preclinical stages. Although Johne's disease was described first over 100 years ago and the causal organism identified, it remains a problem today because current diagnostic tests and vaccines have been unable to deliver totally effective control. Johne's disease is also known to affect a wide range of ruminant wildlife species, as well as some monogastric species, which raises the prospect of interspecies transmission and sylvatic reservoirs, which would further complicate the development of effective control strategies.

Cause

The aetiological agent of Johne's disease is the bacterium *Mycobacterium avium* subspecies *paratuberculosis* (*M.a. paratuberculosis*) (syn. *M. johnei*). This bacterium is aerobic, non-motile, weakly Gram-positive and acid-fast, 1–2 μm long and 0.5 μm wide. It is a slow-growing organism with fastidious requirements for growth on artificial media, in particular an exogenous source of mycobactin, an iron-scavenging siderophore. The bacteria form small, raised, dull-white, rough colonies. All ovine strains of *M.a. paratuberculosis* are difficult to culture, particularly a pigmented strain of the bacterium that is unique to sheep, and colonies may be visible only after incubation for 9 months.

Sequencing of the entire genome of *M.a. paratuberculosis* has confirmed its close relationship to other mycobacteria, particularly *M. avium* [1] and all isolates are closely related, both antigenically and genetically. Improved understanding of the molecular biology of *M.a. paratuberculosis*, combined with new molecular technologies, has allowed differentiation of *M.a. paratuberculosis* from other members of the *M. avium* complex [2, 3] and differentiation of strains of *M.a. paratuberculosis* from different hosts. In some countries, e.g. Australia, the genetic markers are specific to isolates from sheep or cattle [4, 5], but in other countries there is no clear relationship [6].

M.a. paratuberculosis can be widely dispersed in the environment and is capable of surviving there for long periods of time, probably as a result of its impermeable cell wall. *M.a. paratuberculosis* will survive in faeces, soil [7] and water for many months [8, 9].

Clinical signs

There are no specific clinical signs of Johne's disease in sheep, which should be considered as part of the 'thin ewe syndrome'. It presents principally as a chronic progressive loss of body condition as a result of protein malabsorption and consequent muscle wasting. Although the disease is always chronic, there is considerable variation in its the course in sheep. In some instances, the disease progresses relatively rapidly, with the interval between the appearance of wasting and death measured in months. In other cases, after the initial loss of condition, there may be no clinical deterioration for long periods. Unlike in cattle, diarrhoea is not a feature of Johne's disease in sheep. This is probably due to the sheep's

ability to reabsorb water in the large intestine. In advanced cases, the faeces may become soft and unformed.

Pathology

The principal pathological changes centre on the intestine and the related lymphatic and lymphoid tissues, although, in advanced cases, pathology associated with cachexia may be present. It must be emphasized that gross changes in sheep with Johne's disease are often difficult to detect, and do not resemble those caused by the disease in cattle [10]. In advanced cases, there is wasting, with gelatinous atrophy of fat depots and serous effusion into body cavities. In the intestine, macroscopic changes principally affect the terminal ileum, but may extend to involve the jejunum and colon. The ileum may be thickened and may feel doughy when handled, but more usually the only visible change in the lining is a slight fleshy or velvety thickening, or a faint granularity of the surface, perhaps with slight congestion (see Figure 24.1 in the colour plate section). These subtle changes may be overlooked in a cursory examination. Occasionally, there may be a tendency for the mucosa to form fissures when bent over the fingers. Where infection with pigmented strains occurs, the mucosal lining takes on a pathognomonic bright-yellow colour, due to the presence of pigmented *M.a. paratuberculosis* in the lamina propria.

The afferent lymphatic vessels in the mesentery may be thickened and convoluted, and contain numerous small (1–4 mm) whitish nodules, which may be caseous or even calcified. Similar nodules or white flecks may be seen on the peritoneal surface of the ileum, or the cut surface of the intestinal wall or the mesenteric lymph nodes. The latter are almost invariably enlarged and prominent at necropsy.

Although all clinical cases present as an afebrile, chronic wasting, the severity of the signs is unrelated to the extent of the pathology. Two distinct types of pathology are apparent, based on the abundance of mycobacteria and cellular infiltrate [11–13]. The more common form, known as lepromatous or multibacillary, is characterized by numerous acid-fast *M.a. paratuberculosis*, packing the cytoplasm of the many large macrophages that infiltrate the mucosa in all cases, forming extensive, diffuse sheets. Lymphocytes and granulocytes are present in much lower numbers. Occasional multi-nucleate, Langhan's-type giant cells may be seen. These changes cause marked thickening of the intestine.

The less common form, known as tuberculoid or paucibacillary, comprises approximately 30 per cent of cases. Thickening of the intestinal wall is less prominent and may be difficult to distinguish from unaffected gut. It is characterized by a more marked lymphocytic infiltrate with scattered, small focal granulomata and giant cells. Lesions may exhibit caseation, calcification or fibrosis, the resultant nodular lesions being visible macroscopically. This tendency for lesions to undergo caseation or calcification is an important point of differentiation from the lesions of Johne's disease in cattle. Acid-fast *M.a. paratuberculosis* are sparse or undetectable in tuberculoid lesions, and are usually absent from caseous or calcified foci.

The two types of pathology in Johne's disease correlate with different host responses to the bacterium. Sheep with multibacillary disease have a strong antibody response but a weak, or absent, cell-mediated immunity (CMI), as indicated by poor skin hypersensitivity and predominant Th2-like cytokines (IL4 and IL10) [11, 12]. Electron microscopy indicates that *M.a. paratuberculosis* appear to be able to multiply in epithelioid cells in these lesions. Animals with paucibacillary disease show a strong CMI response and strong skin hypersensitivity, poor or absent antibody response, and predominant Th1-like cytokines (IL-2 and IFN-γ). In these lesions, the bacteria appear to degenerate in epithelioid macrophages. Thus, the tuberculoid form of Johne's disease appears to reflect the ability of the host to reduce the weight of intestinal infection.

Diagnosis

Johne's disease can occur in any sheep over about 1 year of age. However, there are no specific clinical signs, and Johne's disease must be differentiated from other chronic wasting diseases in adult sheep. It is important to recognize that the interplay between bacterium and host is complex and dynamic.

The initial site of infection is believed to be the Peyer's patches in the terminal ileum. Bacteria are taken up by M cells covering the patches [14] and infection becomes established in the associated underlying lymphatic tissue. After this initial phase of infection, a number of different sequelae occur, the outcome of

which are related to the ability of the host to mount an effective cell-mediated immune response as well as the dose of the initial infection, as a heavy initial infection is more likely to be overcome than a light one. The predominant early immune response is cell-mediated and antibodies are undetectable. During the late stages of disease, the type of host response correlates with the type of pathology. It is clear, therefore, that no single diagnostic test is adequate.

In an individual sheep, ante-mortem diagnosis, particularly during the preclinical stages, is unreliable because all available diagnostic tests suffer from poor sensitivity and/or poor specificity. Finding clumps of acid-fast organisms in the faecal smears may indicate infection, but a negative result does not rule out the possibility of *M.a. paratuberculosis* infection. Faecal culture is of some diagnostic value, but the prolonged growth period and overgrowth of the cultures with contaminants has limited its usefulness. However, recent improvements in culture media and application of new liquid culture technologies has led to great improvements [15, 16]. Direct examination of faeces by newer molecular techniques, such as polymerase chain reaction (PCR), may be useful for examination of tissues collected at necropsy but generally has not been successful with faeces owing to the presence of inhibitors . Biopsy of the terminal ileum and ileo-caecal lymph nodes may be a useful diagnostic aid as the biopsied tissues can be examined histologically, cultured or examined by PCR for the presence of *M.a. paratuberculosis*.

Post-mortem diagnosis is more reliable. The gross appearance of the intestines may be indicative and diagnosis of the pigmented form of Johne's disease presents few problems at necropsy. The bright-yellow pigmentation of the ileum and the presence of enormous numbers of acid-fast bacilli in smears from the intestinal erosions or enlarged mesenteric lymph nodes provide confirmation of the diagnosis. In most cases, histological examination of intestinal tissues and mesenteric lymph nodes is very important, especially since macroscopic thickening of the intestines or enlargement of the mesenteric lymph nodes are not consistent features. Culture of these tissues, or analysis by PCR, is helpful if considered in conjunction with histology, as it is possible to detect *M.a. paratuberculosis* in these tissues in the absence of lesions sufficient to cause disease. Although current immunological tests such as agar gel immunodiffusion and ELISA cannot accurately or reliably diagnose Johne's disease in individual sheep, they have been useful for diagnosis on a flock basis. Further improvements in these techniques will undoubtedly follow the identification of specific antigens in *M.a. paratuberculosis* and be incorporated into these assays [17]. Immunological diagnosis of Johne's disease in a flock can be supported by post-mortem examination of a wasting sheep, as described above.

Epidemiology and transmission

Johne's disease has a global distribution, although documentation of the disease in sheep is less well recorded than in cattle. The disease is endemic in many countries and its introduction into Iceland by five rams from Germany in 1933 and subsequent spread to sheep and cattle has been well documented. The introduction of Johne's disease to New Zealand and Australia appears to be more recent and has been the subject of great efforts at control in Australia. The principal route of infection is oral–faecal transmission, but infection also can be acquired *in utero* if disease in the ewe is advanced. *M.a. paratuberculosis* is excreted in the faeces of infected sheep at all stages of the disease, including preclinical, although usually in larger numbers once clinical signs have developed. Age at initial infection is an important predisposing factor. If sheep become infected as lambs, they are more likely to develop clinical signs, whereas if infected as adults, overt disease is less common. Re-infection does not influence the course of the disease, and it is the initial infection that determines the final outcome. Experimentally, as few as 1000 *M.a. paratuberculosis* will cause infection in lambs. Other reported risk factors include a variety of environmental and genetic factors, e.g. soil type, restricted diet, stress and breed.

M.a. paratuberculosis has a wide host range that includes not only domestic ruminants but also wild ruminants and some monogastric species, notably wild rabbits [18–20]. The relatively high prevalence of infection with *M.a. paratuberculosis* in these species and, particularly, rabbits suggests that they could have a role in the epidemiology of these infections [21]. This notion is further supported by studies showing that infection in rabbits appears to be transmitted horizontally and vertically, and therefore may contribute to maintaining infection in these populations in the absence of a ruminant host [22].

Control

There is no treatment for Johne's disease and control must be attempted at the flock level by means of flock management, removal of affected animals, vaccination or combinations of these.

Johne's disease as a flock problem tends to be cyclic; the disease often appears then disappears after a few years in the absence of control measures. Infected animals are obvious sources of infection for uninfected flockmates and ideally should be removed. However, because detection of Johne's disease in individual sheep during the preclinical stages is unreliable, any detection and cull policy is confined to removal of wasting sheep. Lambs born to ewes that develop the disease should not be retained in the flock as they are most likely to be infected either from their dams' faeces or *in utero*.

Vaccination with *M.a. paratuberculosis*, with or without adjuvant, has provided practical benefits in the control of paratuberculosis in sheep. Although vaccination does not prevent infection, it reduces the number of bacteria that are excreted, as well as the number of sheep that develop pathology and clinical illness [23–25]. However, it is important to recognize that some vaccinated sheep may shed large numbers of bacteria in their faeces and, therefore, vaccination must be used in conjunction with other managemental interventions. Accidental self-inoculation of oil-adjuvanted vaccine has been reported to cause extensive cellulitis or ulcerative lesions that required surgery [26], emphasizing the need for operator care in administration.

Vaccines prepared for use in cattle are suitable for sheep. Vaccination generally is used in lambs up to about 3 months of age and revaccination is not advised, since this causes severe local reactions in sensitized animals. As vaccination causes allergy to avian and mammalian tuberculin and antibodies detectable by several tests, including the complement fixation test for Johne's disease, it cannot be used in flocks that require certification for export. It is not known if it is possible to vaccinate against the pigmented form of Johne's disease.

Traditionally, the choice of control strategy has been influenced by factors such as the commercial value of the animals involved and whether Johne's disease is endemic in the flock or area. However, the wide host range and potential existence of wild-life reservoirs raises the possibility of interspecies transmission of *M.a. paratuberculosis*. If the bacterium can establish a cycle of infection involving several host species, current control strategies employing a detection and cull policy, or destocking and running other livestock on the premises, may prove ineffective in the long term, and vaccination may be a more acceptable approach.

TUBERCULOSIS IN SHEEP

Tuberculosis is rare in sheep, despite their susceptibility to infection with both *Mycobacterium bovis* and *M. avium*. This may be because few opportunities occur for infection rather than to any innate resistance and recent occurrences have been in sheep in contact with tuberculous cattle [27–30].

Clinical signs

Usually there are no specific clinical signs and many more subclinically infected sheep are found at necropsy than might be expected [28]. Signs of chronic bronchopneumonia have been reported in infected flocks.

Pathology

Tuberculous lesions usually show similar morphology and distribution to those in cattle. They have been reported predominantly in the thoracic organs and comprise encapsulated nodules, containing caseous or calcified material, in the lungs and enlarged mediastinal lymph nodes. Microscopic examination of these nodules reveals central caseation and calcification surrounded by epithelioid and Langhan's giant cells. The fibrous capsules are infiltrated by lymphocytes and plasma cells. Acid-fast organisms can be detected but only in small numbers.

The lesions of avian tuberculosis in sheep are less progressive and, where gross lesions are present, take the form of fibrotic nodules in the lymph nodes.

Diagnosis and control

Macroscopic lesions may be incidental findings at necropsy in individual animals. Diagnosis of tuberculosis should be confirmed by histological examination and/or the isolation of either *M. bovis* or

M. avium in culture to differentiate from lesions of visceral *Corynebacterium ovis* infection and possibly *Muellerius capillaris*. The prevalence of infection in a flock is best achieved by the comparative tuberculin test, using mammalian and avian tuberculins. It should be remembered that in most countries tuberculosis is a notifiable disease. Control of the disease would depend on the local legislation but, in any circumstances, slaughter of an infected flock and restocking after suitable disinfection of the premises is desirable.

Although mixed grazing with cattle and sheep has a place in pasture management and helminth control, the practice may be unwise under circumstances where cross-infection with mycobacterial infections could take place.

ZOONOTIC IMPLICATIONS

Animals affected by tuberculosis are well-recognized as a zoonotic threat, and the disease is notifiable in many countries.

More recently, a speculative link has been rekindled between the human chronic enteritis, Crohn's disease, and Johne's disease in animals. Crohn's disease shares a number of pathological features with paratuberculosis, which has prompted a search for *M.a. paratuberculosis* in the human disease. *M.a. paratuberculosis* may be detected in higher proportion of resected gut lesions from Crohn's patients by PCR and culture, and serum antibodies to *M.a. paratuberculosis* can be demonstrated in these patients. However, considerable controversy surrounds the debate on the aetiological significance of these findings and there is no consensus view at present [31].

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25

Other enteric conditions

R.C. Gumbrell

This chapter describes several enteric diseases not considered elsewhere. It includes infectious diseases (campylobacteriosis and yersiniosis) and non-infectious diseases (carbohydrate engorgement, bloat and redgut) whose prevalence varies from country to country, depending on location, husbandry and exposure to infection. Generally, they are acute diseases and, in some closely controlled situations, can cause significant loss, usually by death. A wide range of unusual diseases of the gastrointestinal system is also briefly described.

INFECTIOUS DISEASES

Campylobacter enteritis

Synonyms: winter scours (New Zealand), weaner colitis (Australia)

This enteritis, with a high morbidity (80 per cent) but usually low mortality, was first reported in New Zealand in 1955. Since then, outbreaks have been reported in weaned lambs in Britain, Australia and New Zealand [1].

Cause

Campylobacter-like organisms, variously identified as *C. coli, C. jejuni* and *C. ovicolis*, have been cultured from gut contents and/or faeces of affected sheep using enrichment media such as Skirrow's. However, similar organisms are also found in the gastrointestinal tract and faeces of healthy sheep. Both pastured and concentrate-fed animals have been affected. A *Campylobacter*-like organism has been seen adhering to the superficial epithelium of the colon. The disease has been reproduced in sheep by inoculating them with cultures of *Campylobacter* spp. isolated from affected animals [2].

Clinical signs, pathology and diagnosis

The features of this disease are a persistent watery diarrhoea, often in otherwise healthy animals. Some animals show depression, rapid collapse and death. In most, the large intestine is fluid-filled and shows superficial mucosal congestion with erosions. The rest of the gastrointestinal tract is affected similarly but to varying degrees. Nephrosis has been reported in New Zealand outbreaks [3].

The disease may be diagnosed by culturing *Campylobacter* from the faeces of live animals and colon contents of dead animals on *Campylobacter*-selective media such as Skirrow's. The epidemiology and gross pathology are also characteristic.

Epidemiology, treatment and control

Campylobacter spp. cause disease in many animals but the enteric infections of wild and domestic animals and birds associated with *C. coli* and *C. jejuni* are not well understood. It is possible that there are reservoirs of infection in wildlife and in carrier animals. Changes in the normal gut flora, brought about by changes in food, particularly at weaning, increases in stocking density and other management factors, may be precipitating factors.

Injections of long-acting tetracyclines have been reported to control outbreaks, as have oral sulfonamides, for example, sulfadimidine at 150 mg/kg.

Yersiniosis

Cause

Yersinia enterocolitica and *Y. pseudotuberculosis* occasionally cause disease in sheep, usually young sheep,

as well as in other farm animals. Strain variations of both bacteria are recognized. Yersinosis has been reported particularly in sheep in Australia [4–6] and New Zealand, where morbidity can reach 90 per cent and mortality 7 per cent. More recently, outbreaks have been reported in China [7] and in Scotland [8].

Enteric infection with these organisms appears to be the result of heavy infection pressure, particularly on young animals (under 1 year) that are predisposed by other factors such as cold weather, starvation, change of diet and management procedures.

Clinical signs, pathology and diagnosis

Chronic ill-thrift, wasting, with or without diarrhoea, which may contain mucus and/or blood, are the usual signs.

On necropsy, the contents of the intestines are liquid and there may be thickening of the intestinal mucosa, with enlarged oedematous mesenteric lymph nodes. Suppurative erosive enterocolitis is present with micro-abscesses containing prominent colonies of Gram-negative cocco-bacilli. Similar abscesses may be present in the nodes.

Culture of the organism from faeces and/or gut contents using selective media provides a diagnosis. The pathology of the intestines and nodes is also pathognomonic.

Epidemiology, treatment and control

Y. pseudotuberculosis is a common inhabitant of the intestine of many domestic and wild animals and birds. Clinically normal animals can be infected and show no disease but can perpetuate the infection in the flock, causing environmental contamination. *Y. enterocolitica* is usually carried in a flock and rarely causes disease.

The organisms are sensitive to a wide range of antibiotics that may be used for treating sheep. Good nutrition and the avoidance of stress are important in preventing disease.

NON-INFECTIOUS CONDITIONS

Acute carbohydrate engorgement

Synonyms: rumen overload, lactic-acid poisoning, acidosis, founder, grain overload

Cause

This condition is caused by the sudden ingestion of carbohydrate-rich feed such as grain or, less commonly, other carbohydrate foods such as apples, potatoes or bread. The sudden supply of large amounts of carbohydrate causes the rumen micro-organisms to produce large quantities of lactic acid, which decreases the rumen pH, so destroying cellulolytic bacteria and protozoa. The rumen osmolality increases, drawing in water from the systemic circulation, causing haemoconcentration and dehydration [9].

Clinical signs, pathology and diagnosis

The onset of clinical signs is quicker with ground feed than with whole grain. Sheep are depressed, stagger and some will become recumbent within 24–48 hours. They refuse to eat and many grind their teeth.

Diarrhoea may be present and kernels of grain may be found in the faeces. There is severe dehydration, and laminitis may develop in some long-standing cases.

Rumeno-reticular contents are thin and porridge-like with an odour suggestive of fermentation. The cornified epithelium of the wall of these organs is readily removed, exposing a dark haemorrhagic surface. Abomasitis may be present. Fungal infections, rumenitis and abomasitis may follow the acute disease.

The history and clinical signs are important. The pH of the rumen contents is usually less than 5 in cases of acute carbohydrate engorgement (the values increase after death), and microscopic examination will show only bacteria and dead protozoa.

Epidemiology, treatment and control

This is generally a flock problem, associated with sudden access to whole grain, usually when grazing stubble, ground grain or other carbohydrates.

Treatment is difficult. Aluminium hydroxide powder, 1–2 g given by mouth as an aqueous suspension, 2–3 times daily, is considered the treatment of choice. Sodium bicarbonate, 40–60 g, given orally as an aqueous suspension 2–3 times daily is a common treatment but can cause bloat and/or alkalosis. In particularly valuable sheep, intensive parenteral fluid therapy can be used. Suitable therapy is 1–2 litres of

glucose saline with 10 ml of 2.5 per cent sodium bicarbonate solution IV, plus intramuscular injection of long-acting tetracycline, 20 mg/kg. Removal of remaining carbohydrate by rumenotomy should be considered.

Access to feeds with large amounts of carbohydrate should be prevented and the introduction of hard feeds controlled to prevent engorgement and allow the rumen microflora to adapt to the new diet.

Bloat

Bloat is the distension of the rumeno-reticulum, usually caused by special feeding conditions. It is unusual in sheep, probably because they graze lower-quality pastures than cattle and also because they tend to be pastured continually.

Cause

Bloat results from the ingestion of succulent legumes, which are readily broken down to produce, with the gases of fermentation, a stable froth. This prevents eructation and leads to a rapid build-up of gas in the rumeno-reticulum. Death is caused by acute neural, respiratory and cardiac stimulation.

Clinical signs, pathology and diagnosis

The signs are those of a distended abdomen, particularly on the left side, with staggering, recumbency and death. The rumen is grossly distended, and there is congestion of mucosal and submucosal areas of the cervical oesophagus.

History and necropsy findings provide the basis for a diagnosis.

Treatment, prevention and control

Treatment depends on releasing pressure by rumenotomy and dosing with froth-reducing agents. Prophylactic use of froth-reducing agents, e.g. 2 g poloxalene per day in feed or molasses blocks, 0.5–1 litre of mineral oil or 10 g of poloxalene per 45 kg, are suitable treatments and will prevent the condition developing, as will limiting access to dangerous pastures [10].

Redgut

Synonyms: intestinal volvulus.

This is a sporadic and fatal condition of ruminating sheep, characterized by sudden death and reddened small and large intestines.

Cause

Sheep grazing legumes or other readily fermentable crops have a high rate of passage of ingesta through the rumeno-reticulum, which decreases in size. As fermentation in the large intestine increases, that organ increases in size. The intestinal mass becomes displaced up to 180° clockwise when viewed from the ventral surface. This is an unstable position and, in a few sheep, there is a further sudden torsion of the intestinal mass causing rapid death [11].

Clinical signs, pathology and diagnosis

Sudden death without any premonitory signs is usual. On post-mortem examination, the intestinal mass has rotated 180–360° clockwise (see Figure 25.1 in the colour plate section). The intestinal reddening starts 100–120 cm distal to the pylorus and ceases at the terminal colon. In a very few animals, there is little or no intestinal displacement.

Diagnosis depends on history and necropsy findings.

Epidemiology, treatment and control

The disease usually occurs about 3 weeks after grazing lush pastures, particularly of legumes. Losses are usually sporadic but may be as high as 20 per cent.

This problem can be prevented by not allowing sheep to graze dangerous pastures, or by intermittent grazing of these pastures.

OTHER CONDITIONS

Congenital conditions

Atresia of the intestine, sometimes of the ileocaecal junction, occurs very occasionally. Atresia ani is more common. All produce distension of the abdomen in lambs up to 7 days old with a lack of faeces. Surgical correction of atresia ani is possible, but anal constriction may occur later in life.

Rumenitis

Bluetongue (Chapter 60) and peste des petits ruminants (Chapter 61) can cause severe rumenitis with ulceration, but the usual cause of rumenitis in temperate regions is the excessive feeding of rapidly fermentable carbohydrates, usually grain. The lactic acid produced causes inflammation and predisposes to local infections.

Ruminal impaction

If there is severe water deprivation or 'vagus indigestion' (associated with achalasia of the reticuloomasal sphincter), rumen fermentation stops and the animal does not cud. Ketonaemia may be present. Impaction by foreign material, such as cloth, also causes ruminal impaction.

In these conditions, the rumen is distended with grey, soggy or dry fibrous contents.

Traumatic reticulitis

Sharp objects, such as a piece of wire or a needle, can penetrate the reticulum, causing local peritonitis. The object may penetrate the diaphragm and cause pericarditis. Treatment is by immobilization of the affected animal for 3–5 days, along with administration of parenteral antimicrobials, e.g. 2–10 mg/kg oxytetracycline daily or trimethoprim (40 mg) + sulfadiazole (200 mg), 15–24 mg/kg daily.

Abomasal bloat, haemorrhage and ulcers

This syndrome has been described mainly in 3–4-week-old lambs, both naturally and artificially reared, in Norway [12] as well as in New Zealand and other countries. In the latter instances it appears more common in lambs fed warm milk replacement diets, particularly when they drink large quantities at infrequent intervals [13]. The Norwegian researchers report it as the primary cause of death in 6–13 per cent of the dead lambs examined. These and other workers have also implicated the bacteria *Sarcina ventriculi* and *Clostridium sordelli* (Chapter 23) in this condition. It is thought that bacterial proliferation results in gas production with resultant bloat and also ulceration and haemorrhage. Lambs show gross abdominal distension about 1 hour after feeding and die quickly from asphyxia and heart failure. Reported treatments include surgical relief of abomasal gas and oral antibiotics. Methods of prevention include feeding the milk replacer cold and very low levels of formalin in the feeds.

Abomasal impaction

Adult Suffolk ewes in the USA are reported to develop abomasal impaction or dilation and emptying defect. Ewes are usually in late pregnancy or recently lambed and show progressive weight loss and eventual death. The abomasum is enlarged with dry doughy contents. A similar condition occurs in lambs weaned prematurely on to pelleted rations with a milk replacer. They develop pot bellies, pine and die after a few weeks. The abomasum is impacted and distended and the rumen is filled with sour undigested milk substitute.

Abomasal ulceration

This condition occurs in several infectious diseases, notably peste des petits ruminants (Chapter 61) and systemic pasteurellosis (Chapter 32). It can also occur following mechanical abrasion with sand and/or wire fragments and in poisoning with corrosive substances such as arsenic, mercury or zinc. Other non-specific toxins, possibly mycotoxins associated with mouldy feed, may also produce abomasal ulceration. The condition is occasionally seen in young suckling lambs when the ewe's milk supply has partially or completely failed and the lamb has been forced to eat fibrous feed. The ulceration can include consequential fungal infection and may lead to abomasal rupture and death.

Intestinal adenocarcinoma

An abnormally high rate of small intestinal adenocarcinoma has been recorded in Iceland, New Zealand, Norway and elsewhere in Europe and in Australia. In New Zealand, there was a higher incidence in British breed ewes (0.9–1.5 per cent) compared with Corriedale and Merino ewes (0.2–0.4 per cent), and a higher rate in sheep pastured on feed sprayed recently with phenoxy or picolinic herbicides.

Intestinal obstruction

This is unusual in sheep, but some 'outbreaks' have been associated with heavy infestations of nodular worm (*Oesophagostomum columbianum*), with a high incidence of intussusception in travelling sheep (cause not known) and with abomasal phytobezoars in South African sheep.

Intestinal torsion in milk-fed lambs

Intestinal torsion occurs in milk-fed lambs, usually being bottle-fed. It involves the intestinal mass, is similar to redgut and may occur also in sucking lambs with access to hard feeds of various types, again with lesions similar to redgut.

A predisposing factor may be the small size of the undeveloped rumeno-reticulum in lambs before weaning, plus the infrequent feeding of large amounts of milk in bottle-fed lambs or the ingestion of cellulose-containing feeds in sucking lambs. Both of these pass to the intestines, increasing the size of the large intestine and causing intestinal displacement with the potential for further intestinal torsion and death.

Regional ileitis

Sporadically, lambs up to 6 months old in the UK, New Zealand and the USA, and up to 3 months in The Netherlands and Norway, may have regional ileitis, usually in the terminal region. Clinically affected lambs may stretch and show ill-thrift and intermittent diarrhoea. On necropsy, areas of the ileum show hyperplasia with plaque-like thickening. Islands of hypertrophied mucosa are present in the submucosa with associated inflammation.

Rectal prolapse

Prolapse of the rectum (Chapter 14) may occur as a consequence of diarrhoea, particularly in coccidiosis. It has also been reported in sheep grazing pastures such as subterranean clover, which contain oestrogen-like compounds. It also occurs in feedlot lambs, where it is considered to be associated with several factors including straining from coughing, proctitis and cystitis, and intra-abdominal distension from overfilling of the rumen, fat and prolonged recumbency.

ZOONOTIC IMPLICATIONS

Many *Campylobacter* spp, including *C. jejuni*, can cause human enteric disease. Appropriate hygiene is important for people handling infected animals.

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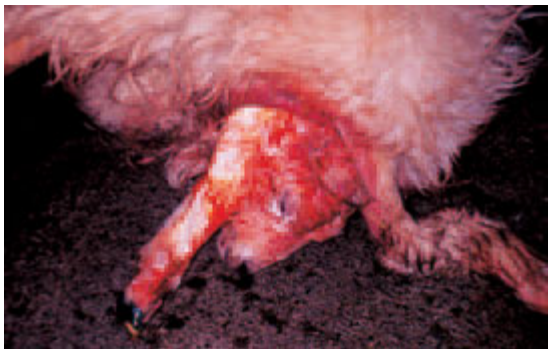


Figure 11.1: Dystocia – head swollen and one leg retained in a small Welsh ewe.

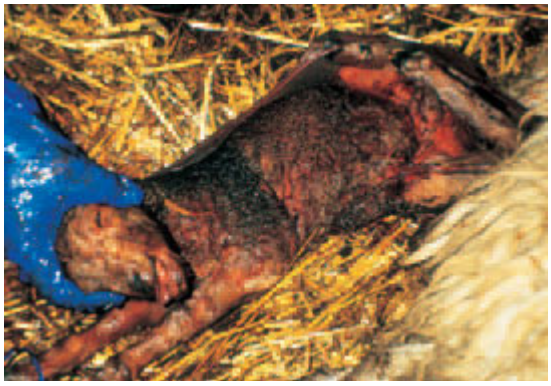


Figure 11.2: Dystocia – delivering a dead lamb. The eyes are sunken, indicating death some hours previously. The body is covered in meconium, an indicator of fetal distress.

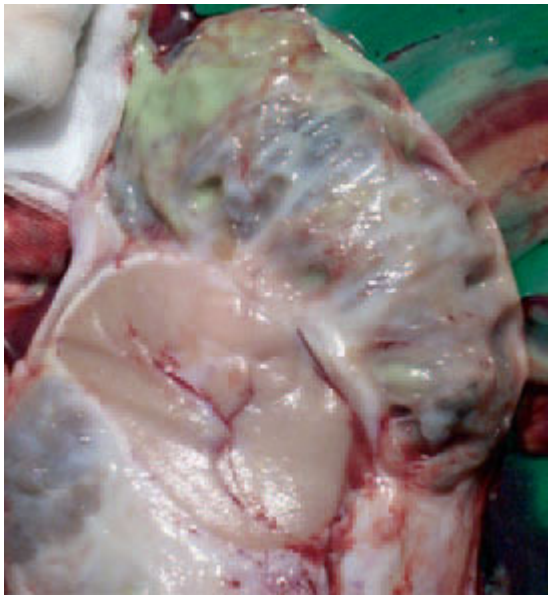


Figure 13.8: Epididymitis: section of affected body and head of epididymis.

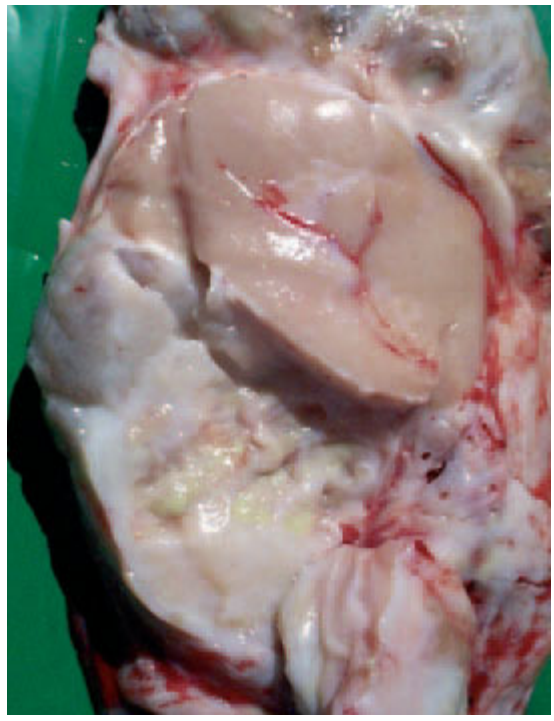


Figure 13.9: Epididymitis: section showing affected tail of epididymis.

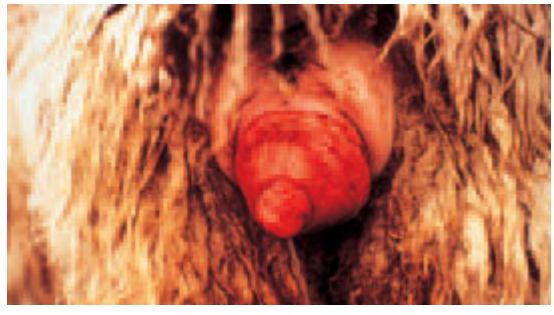


Figure 14.1: Vaginal prolapse antepartum, showing a portion of the cervix.

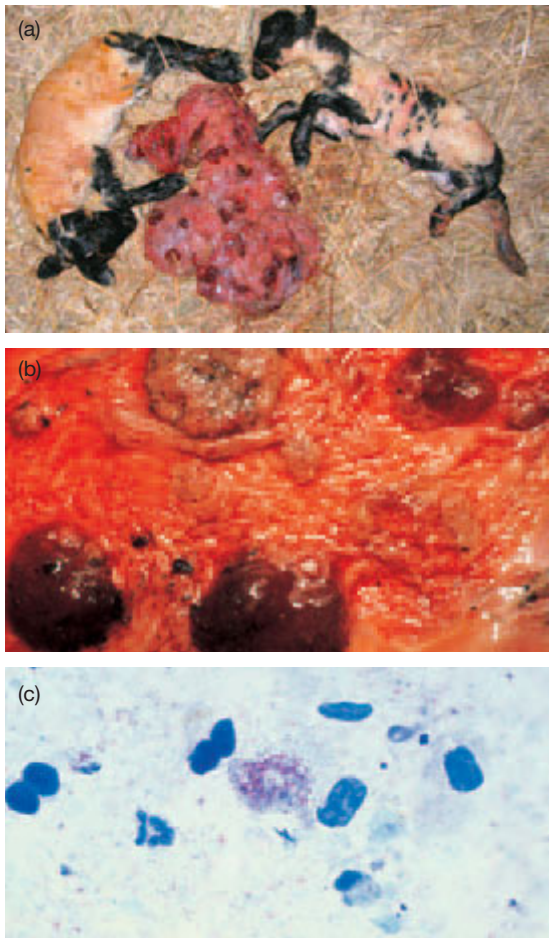


Figure 16.2: Chlamydial abortion: (a) lambs aborted in late pregnancy and placentas showing typical necrotic changes; (b) detail of necrotic cotyledon and corrugated thickening of adjacent chorion; (c) smear of affected cotyledon, stained by modified Ziehl–Neelson, showing a clump of reddish chlamydial elementary bodies in a blue background of cells and debris.

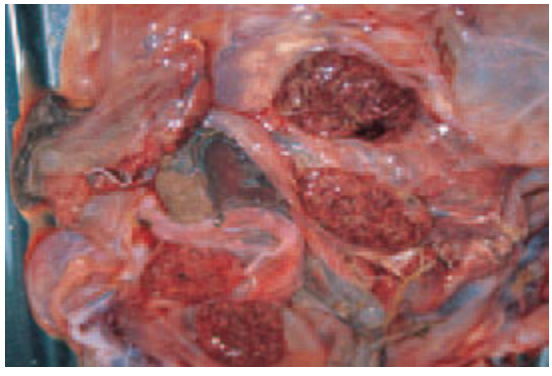


Figure 17.2: Placenta from a case of *Toxoplasma* abortion. Note the characteristic white spots on the dark red cotyledons.

Figure 17.3: Chocolate-brown mummified fetus inside its placenta, alongside a live lamb from the same ewe.

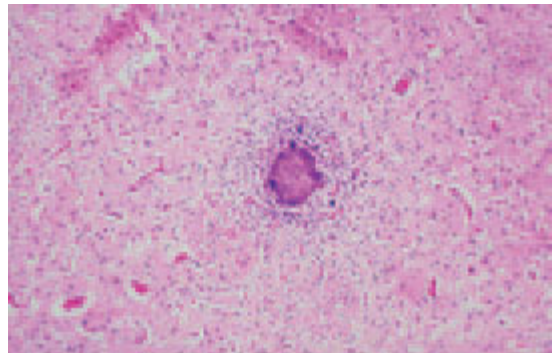


Figure 17.4: Lamb cerebrum from a case of *Toxoplasma* abortion. The central inflammatory focus is characteristic of intrauterine toxoplasmosis and shows a mineralized necrotic area surrounded by microglia and other mononuclear inflammatory cells. The lesion is the result of local multiplication of tachyzoites causing tissue damage and a fetal immune response.

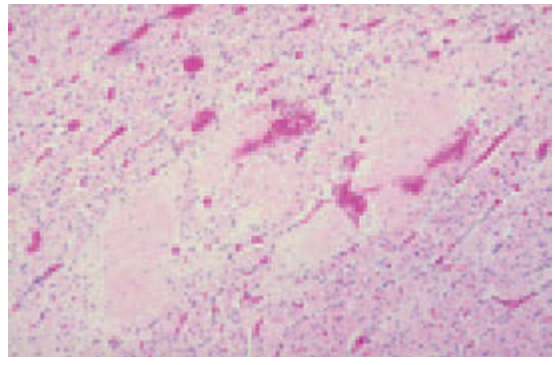
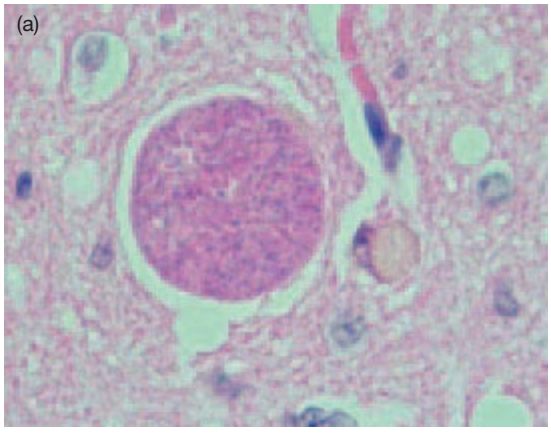


Figure 17.5: Cerebral white matter of a lamb in a case of *Toxoplasma* abortion. A large area of degeneration (focal leucomalacia) characterized by a loss of glial nuclei runs diagonally across the figure. Note also the small associated haemorrhages. These changes are the result of fetal hypoxia in late gestation.



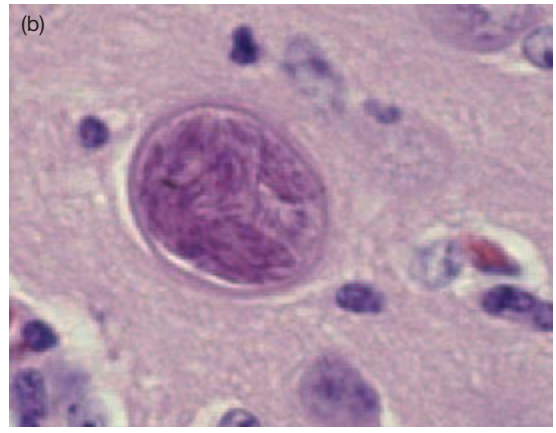


Figure 17.6: (a) A *Tomoplasma gondii* tissue cyst in the brain. Note the numerous bradyzoites enclosed within a thin cyst wall that is difficult to discern. The surrounding white space is an artefact, due to shrinkage during processing. (b) A *Neospora caninum* tissue cyst in brain. Note the characteristic thick cyst wall surrounding bradyzoites of similar size and appearance to those of *T. gondii*. The white space inside and outside the cyst wall is an artefact, due to shrinkage during processing. Both (a) and (b) are stained with haematoxylin and eosin.

Figure 18.2: Lamb with an abnormally hairy fleece due to *in utero* infection with BDV.

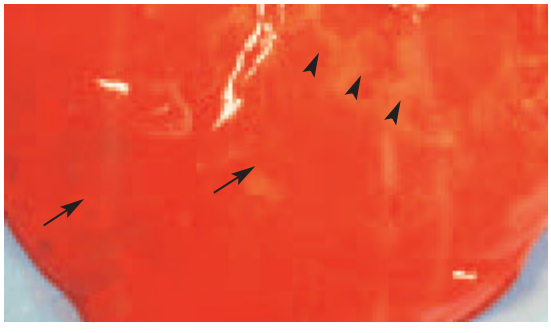


Figure 19.2: Liver of fetus aborted from a *S. abortus ovis* infection (confirmed by bacterial isolation) showing ring-shaped foci of necrosis, either isolated (arrows) or confluent (arrow heads). These lesions are very similar to those described for *Campylobacter* spp. abortion. (Courtesy of L. Cuevo, SIMA, Derio, Spain.)

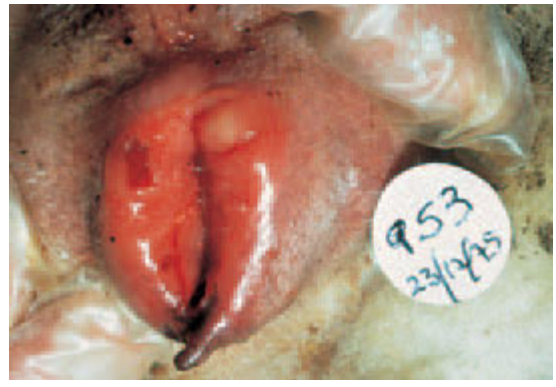


Figure 21.1: Swelling and ulceration of the vulva; cause unknown.

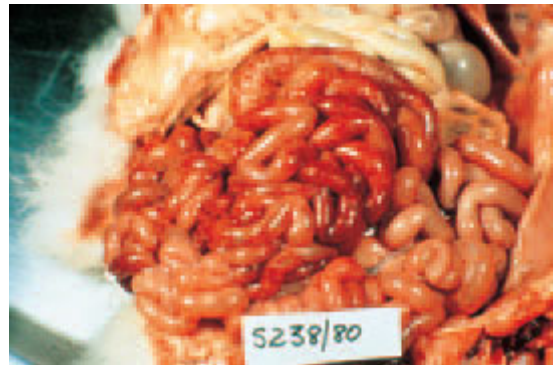


Figure 23.1: Congested small intestine in a lamb with lamb dysentery. (Courtesy of the Veterinary Laboratories Agency, Carmarthen.)

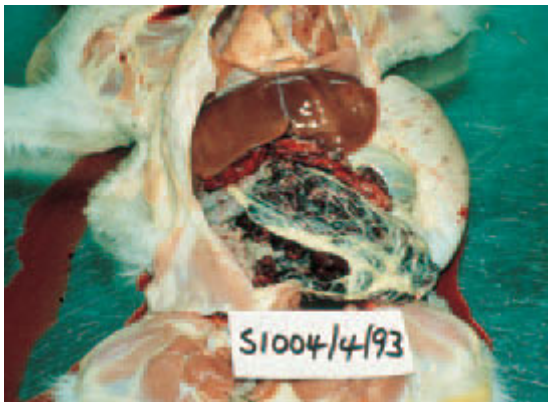


Figure 23.2: Acute pulpy kidney disease in a 3-month-old lamb. Note congestion of the small intestine and haemorrhages on the abomasum. (Courtesy of the Veterinary Laboratories Agency, Shrewsbury.)

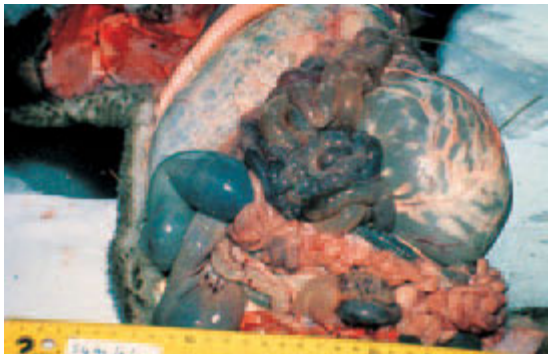


Figure 23.3: *C. sordellii* infection in a 2-month-old lamb, showing typical abomasal displacement. (Courtesy of the Veterinary Laboratories Agency, Shrewsbury.)

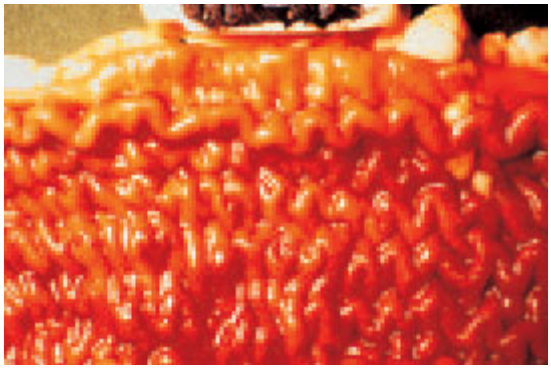


Figure 24.1: Thickening and pigmentation of the small intestine in paratuberculosis.

Figure 25.1: Redgut showing typical intestinal rotation.

Figure 28.1: *Fasciola hepatica* adults [1]. (Courtesy of Novartis Animal Health.)

Figure 28.5: Black disease, ovine liver [1].

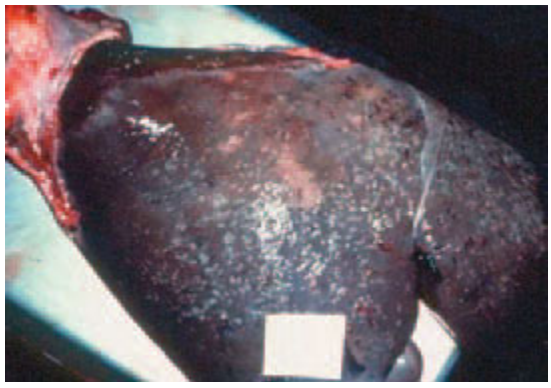


Figure 28.6: Acute fasciolosis, ovine liver.

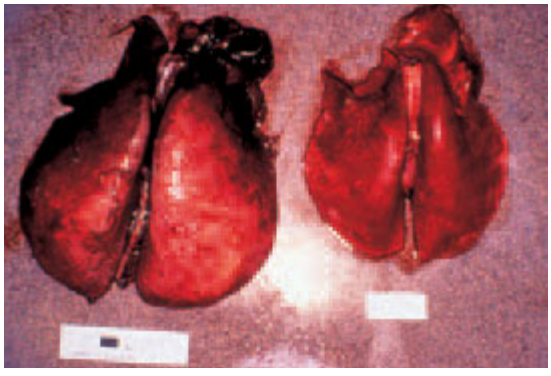


Figure 31.1: Maedi lung (left) compared with normal lung (right). Note the increased volume.

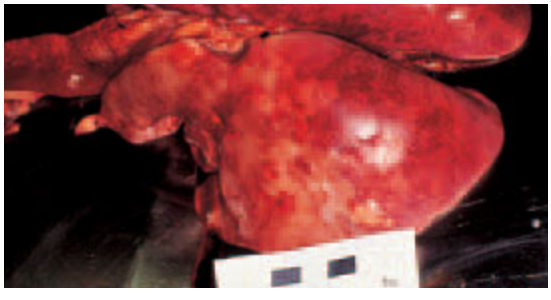


Figure 31.2: Advanced case of maedi, showing mottled appearance of the lung surface.

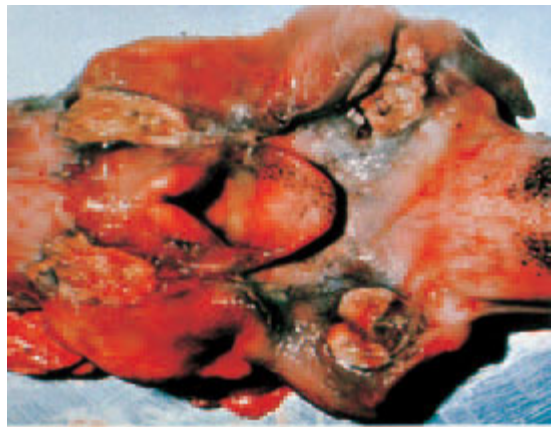


Figure 32.5: Necrotic lesions in the pharynx and tonsillar crypts of a sheep with systemic pasteurellosis. (Photograph reproduced by kind permission of the Editor, *Journal of Medical Microbiology*.)

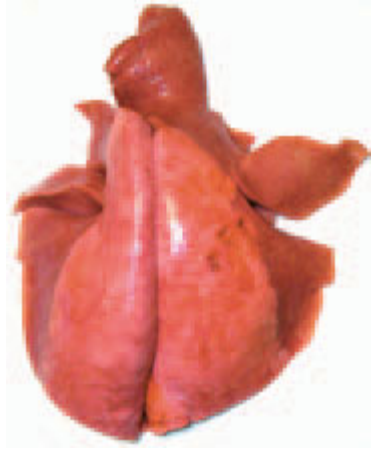


Figure 33.1: Atypical pneumonia from which *Mycoplasma ovipneumoniae* was isolated. Red consolidation seen in apical and cardiac lobes.

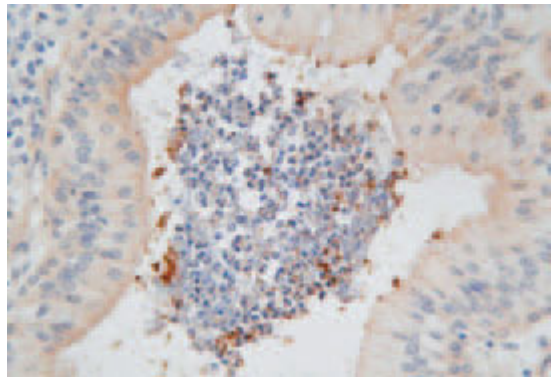


Figure 33.2: Immunohistochemistry of *Mycoplasma ovipneumoniae* infection showing specific brown staining of antigen in the bronchiole airways.

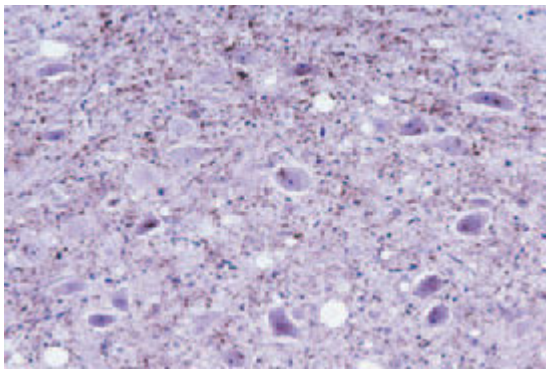


Figure 35.2: Accumulation of disease-associated PrP in the dorsal motor nucleus of the vagus of a sheep clinically affected with natural scrapie. Immunolabelled deposits are both intracellular and extracellular, and vacuolation of the neuropil can also be observed. Immunohistochemistry with PrP mAb R145 and haematoxylin counterstaining.

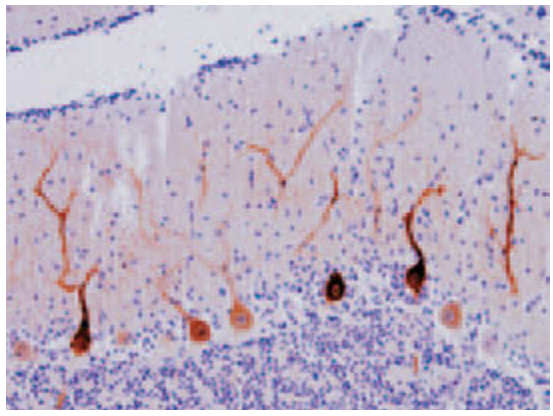


Figure 36.1: Immunohistochemical labelling of a section of cerebellum showing intense brown staining of louping-ill antigen, counterstained with haematoxylin.

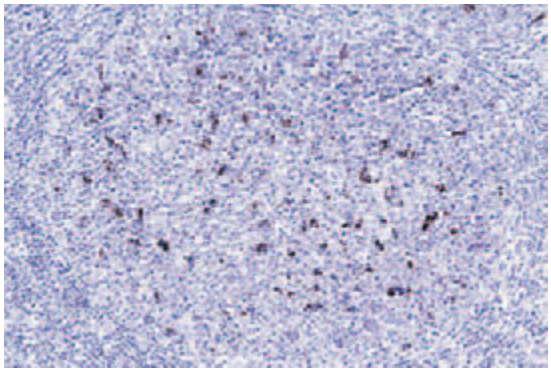


Figure 35.3: Accumulation of disease-associated PrP in the palatine tonsil of a sheep clinically affected with natural scrapie. Immunolabelled deposits are in the cytoplasm of tingible body macrophages located in the light and dark zones of a lymphoid follicle. Immunohistochemistry with PrP mAb R145 and haematoxylin counterstaining.

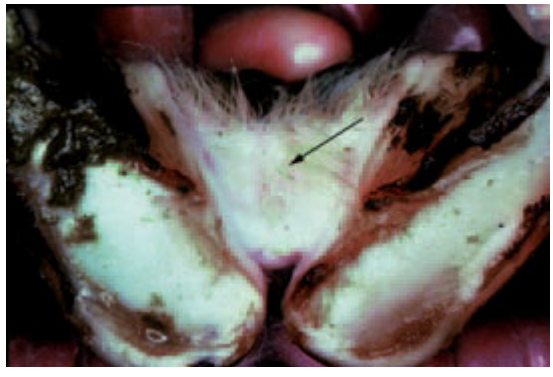


Figure 39.1: Normal interdigital skin (IDS) in hoof of Merino. Note the covering of hair.

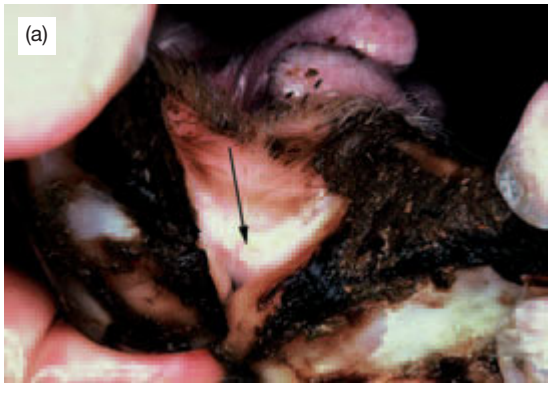
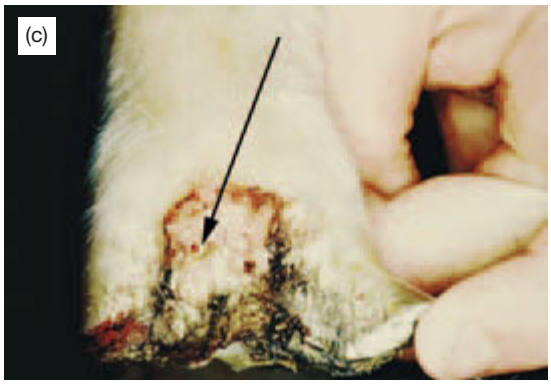
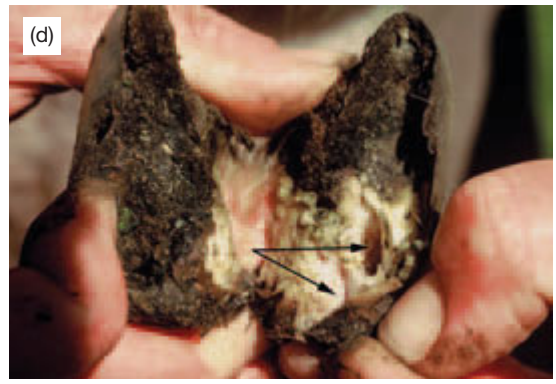
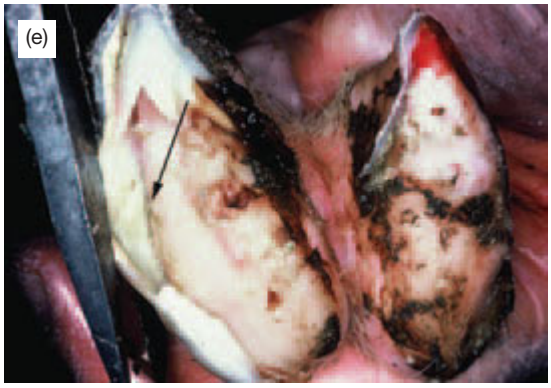


Figure 39.2: Foot-root: examples of increasing severity (see Table 39.1).
(a) Score +: minor inflammation of IDS. Erosion of *stratum corneum* (arrow). (b) Score ++: intense inflammation of IDS. Initial separation of soft horn of heel (arrows).







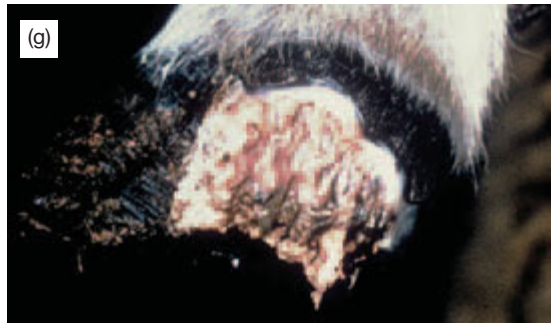


Figure 39.2: Foot-root: examples of increasing severity (see Table 39.1).
(c) Score ++: verrucose form. (d) Score +++: persisting inflammation of IDS. Established separation of soft horn of heel (arrows). (e) Score ++++: separation of hard horn of toe and abaxial walls (arrow). (f) Score ++++: foot trimmed to reveal necrotic surface of soles and advancement of infection to laminae of abaxial walls (arrows). (g) Score ++++: necrotic laminae of abaxial wall.

Figure 39.3: Contagious ovine digital dermatitis (CODD) showing haemorrhagic necrosis of laminae underlying hard horn of abaxial wall. (Courtesy of K.A. Abbot.)

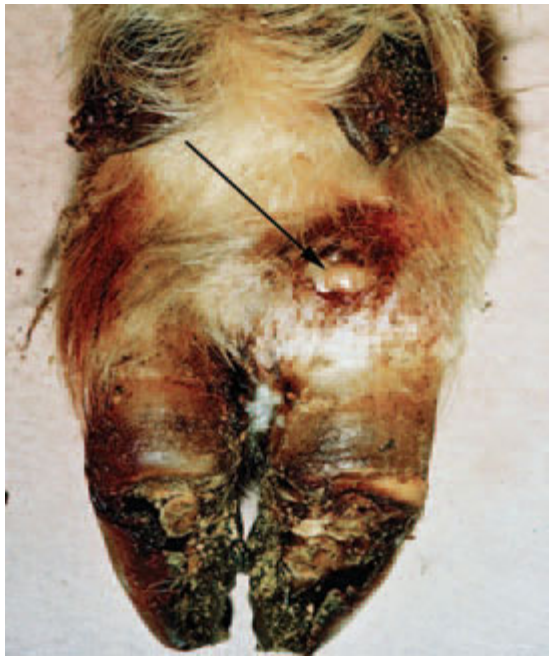


Figure 39.4: Heel abscess. Note discharging sinuses (arrow).

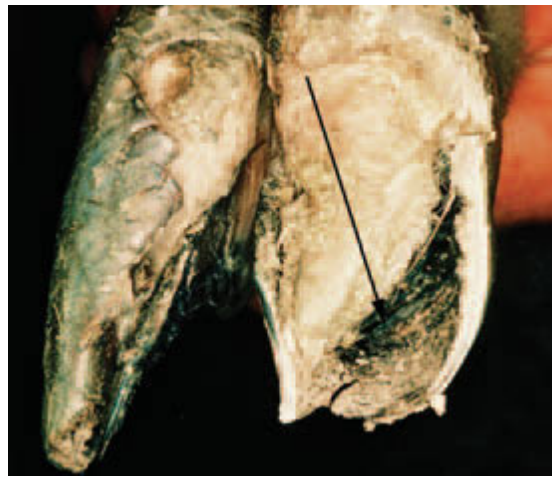


Figure 39.5: Shelly toe. Foot trimmed to show cavity between double abaxial walls.

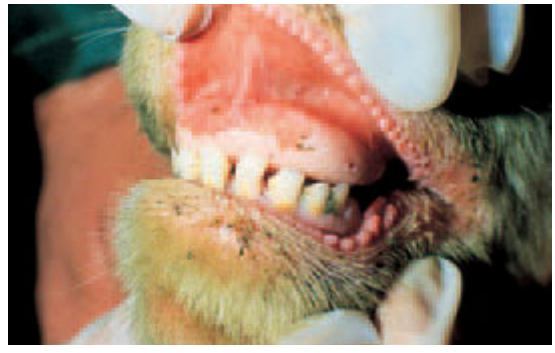


Figure 40.1: Foot-and-mouth disease. Two-day-old erosion along dental pad.

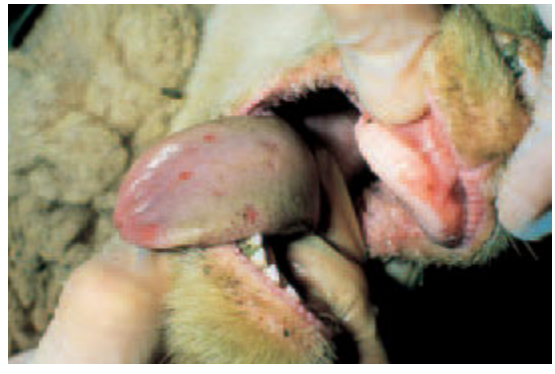


Figure 40.2: Foot-and-mouth disease. Two-day-old multiple erosions on the dorsum of the tongue and erosion of the dental pad.

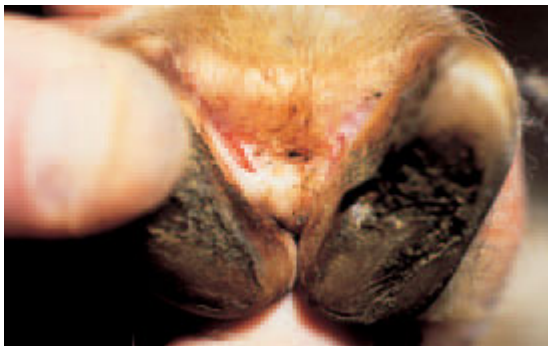


Figure 40.3: Foot-and-mouth disease. Ruptured vesicle within the interdigital space.

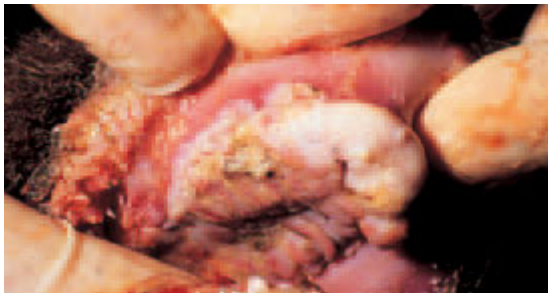


Figure 42.1: Orf lesion on the hard palate of a lamb.

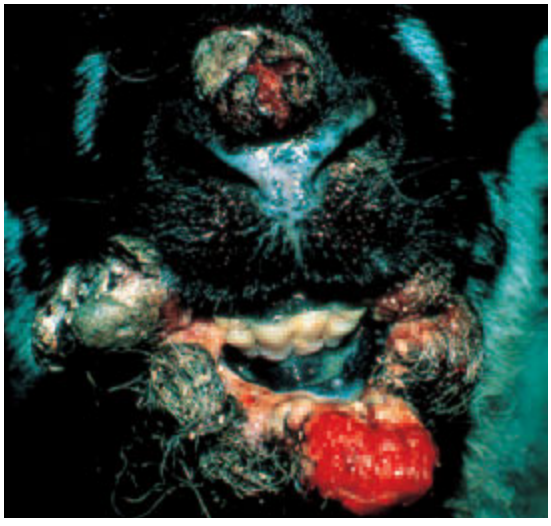


Figure 42.2: Orf: wart-like growths on the lips and nose.

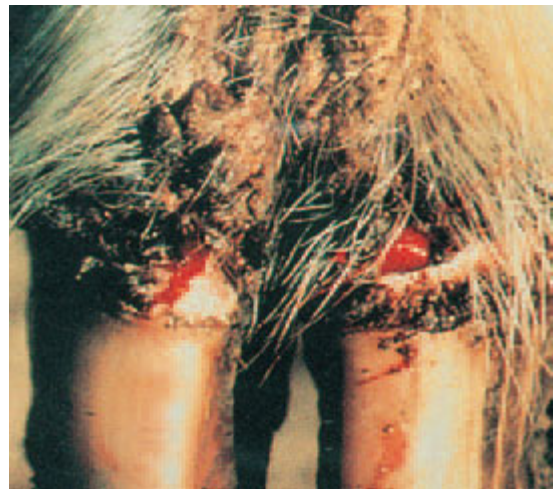


Figure 42.3: Orf lesions affecting the coronary band and interdigital area.

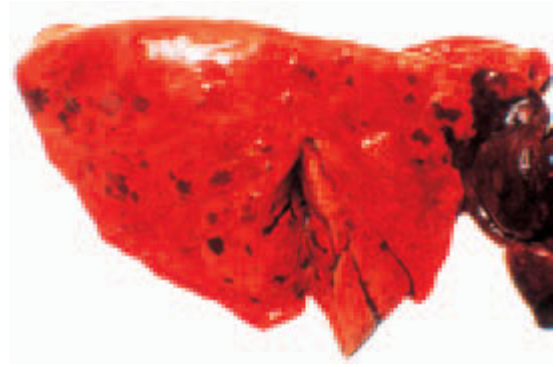


Figure 43.2: Reddish-brown lesions of sheep pox in the lung.

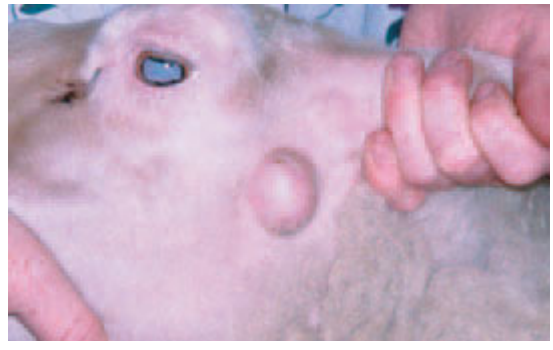


Figure 44.1: Caseous lymphadenitis: abscess in the parotid lymph node.

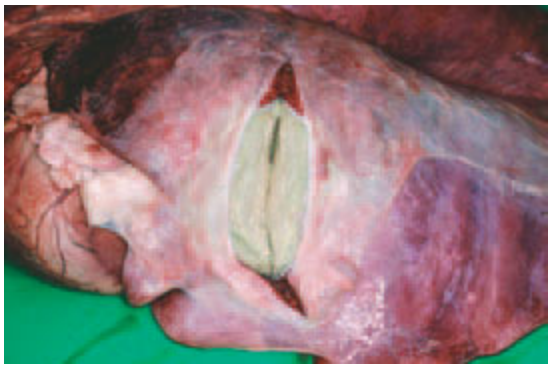


Figure 44.2: Large caseous lymphadenitis abscess in the lung parenchyma.

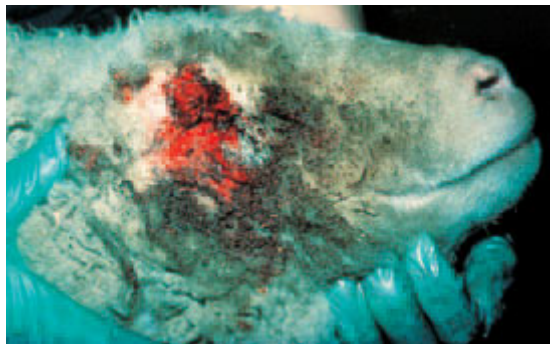


Figure 45.1: Staphylococcal dermatitis of the face.

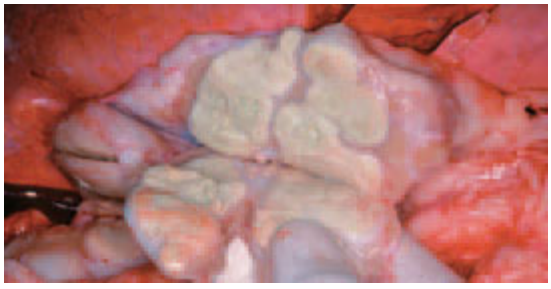


Figure 44.3: Caseous lymphadenitis: abscesses in the mediastinal lymph node chain.

Figure 46.1: Dermatophilosis, showing dry scabs in the wool fibres.

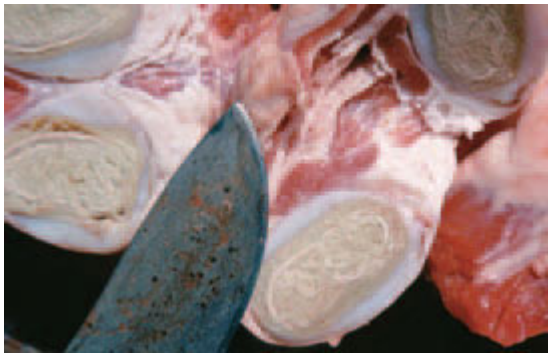


Figure 44.4: Cross-section of caseous lymphadenitis abscess showing distinctive onion ring structure.

Figure 46.2: Dermatophilosis in a lamb

Figure 49.2: Bog asphodel (*Narthecium ossifragum*).

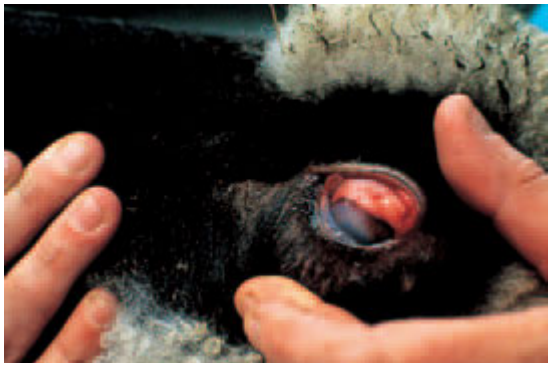


Figure 50.1: Keratoconjunctivitis, showing congestion of scleral blood vessels and keratitis.

Figure 54.3: Merino lamb exhibiting excessive lachrymation as an early clinical sign of cobalt deficiency [46].

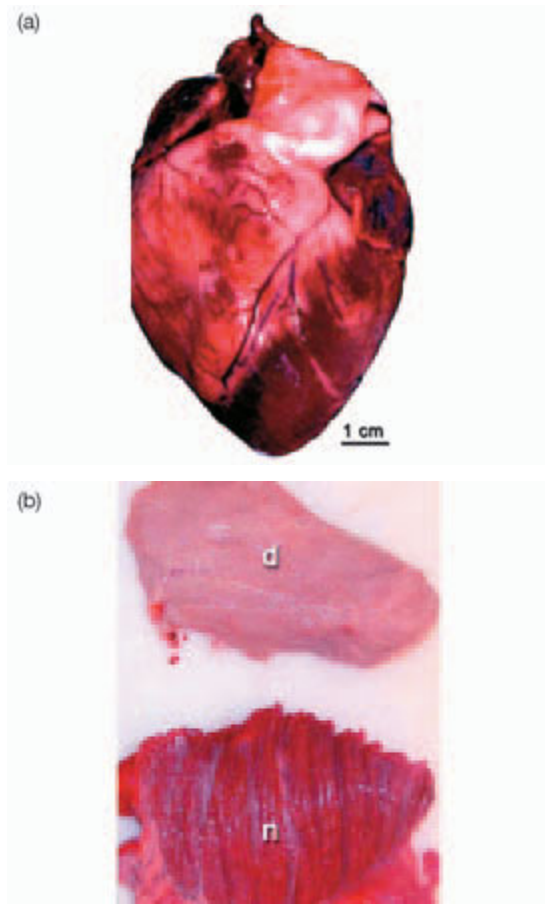


Figure 54.6: White muscle disease. (a) Heart from an affected lamb. (b) Skeletal muscle from WMD-affected (d) and normal healthy animals (n). (Both illustrations reproduced courtesy of Dr Alfonso Lopez, Atlantic Veterinary College, University of Prince Edward Island, Canada.)

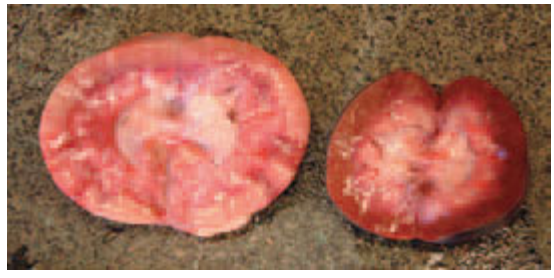


Figure 55.1: An enlarged, soft and pale kidney with an expanded cortex from a 6-week-old lamb with nephrosis, with a normal kidney from a similar aged lamb for comparison.

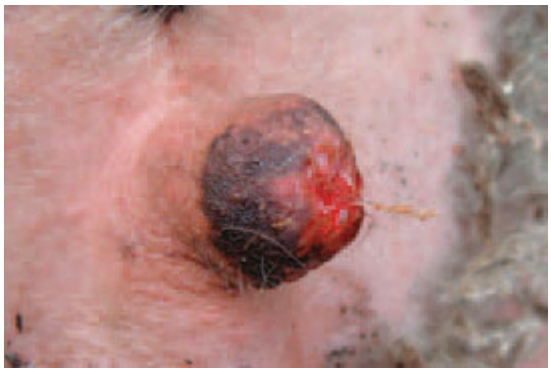


Figure 55.3: Swelling of the prepuce, dribbling of urine and rice grain-like struvite crystals on the preputial hairs.

Figure 55.4: Swelling and necrosis of the vermiform appendage of a Bluefaced Leicester ram, associated with the presence of struvite calculi.

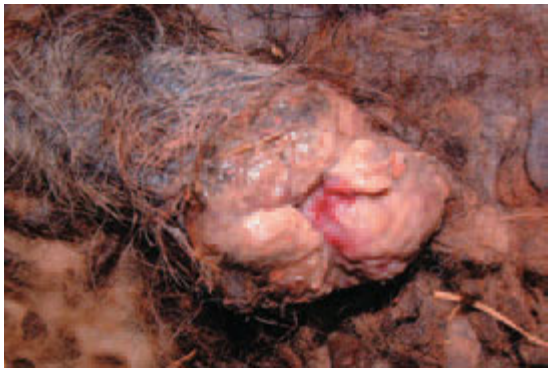


Figure 55.6: Ulceration and necrosis of the prepuce, with accumulation of exudate and staining of the surrounding wool associated with posthitis.

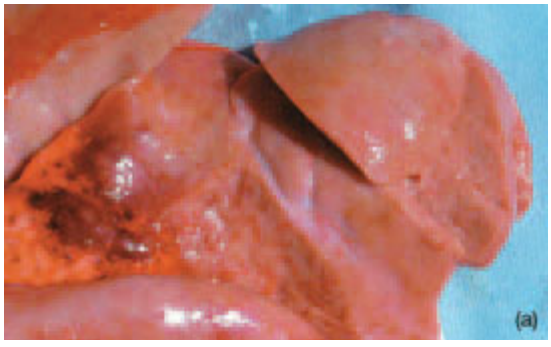
Figure 56.1: Lupin. (Courtesy of Royal Botanic Garden, Edinburgh.)

Figure 56.2: Yew. (Courtesy of Royal Botanic Garden, Edinburgh.)

Figure 56.3: Foxglove. (Courtesy of Royal Botanic Garden, Edinburgh.)

Figure 56.4: Fat hen. (Courtesy of Royal Botanic Garden, Edinburgh.)

Figure 56.5: Hogweed. (Courtesy of Royal Botanic Garden, Edinburgh.)



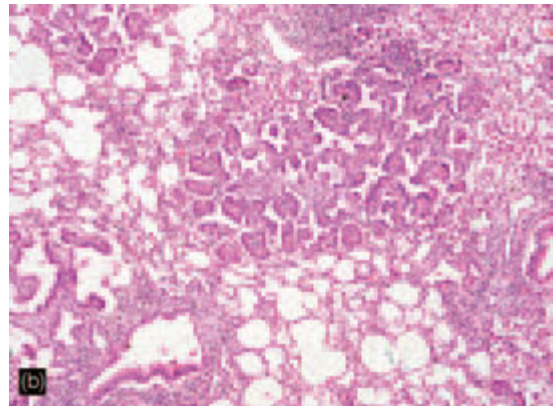
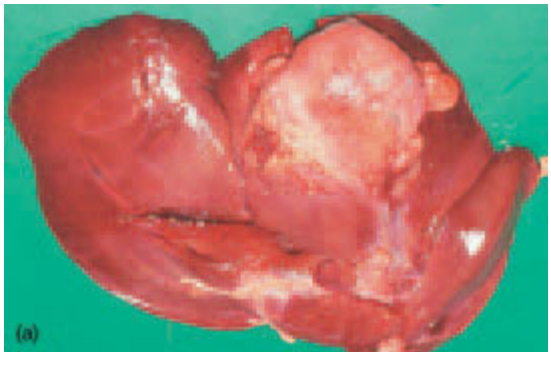


Figure 58.1: (a) Macroscopic appearance of consolidated neoplastic lung in ovine pulmonary adenocarcinoma (OPA). (b) Histopathological appearance of typical early focus of neoplastic alveoli in OPA.



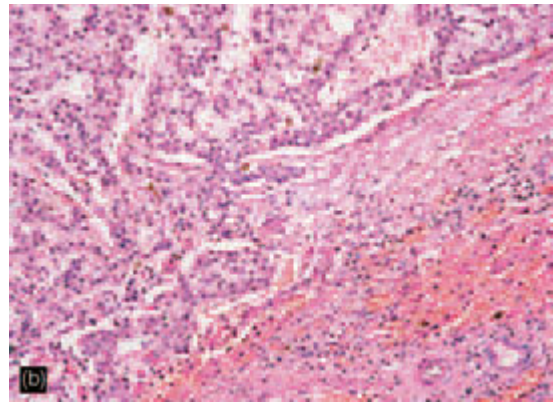


Figure 58.2: (a) Visceral surface of the liver from a 2-year-old ewe with a hepatocellular tumour. (b) Histopathological section of a hepatocellular carcinoma with compressed and congested hepatic sinusoids forming a compression capsule.

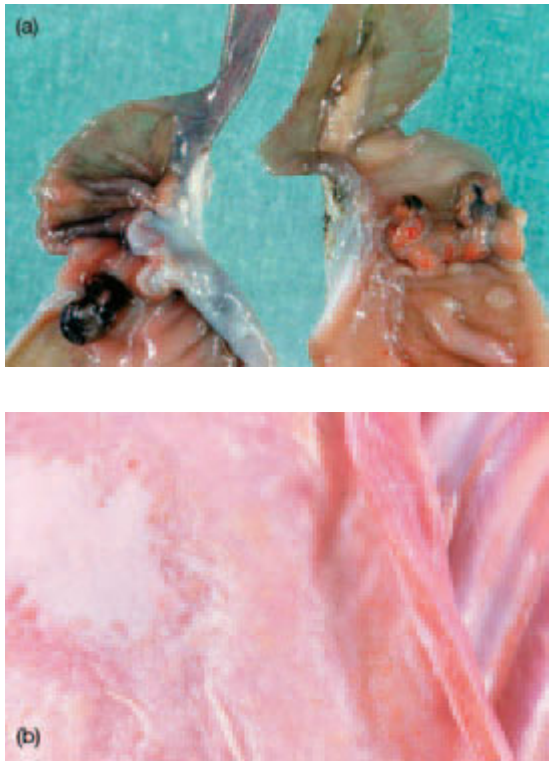


Figure 58.3: (a) Polypoid and stenosing adenocarcinomas in the small intestine of an adult Blackface ewe that had grazed bracken and presented with ascites. (b) Transcoelomic metastasis with scirrhous (fibrosing) peritonitis in a sheep with intestinal adenocarcinoma.

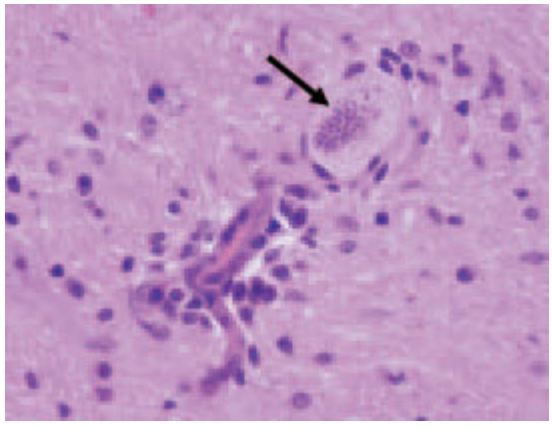


Figure 59.1: The arrow identifies a *Sarcocystis tenella* meront (schizont) in an endothelial cell in the brain of a lamb recently infected with sporocysts.

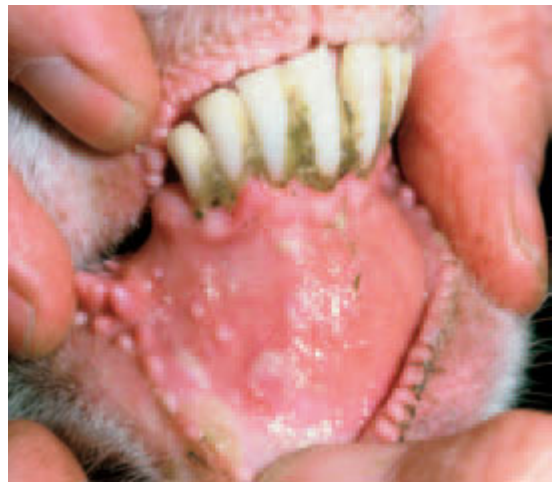


Figure 61.2: Peste des petits ruminants. Necrosis on lower gum.

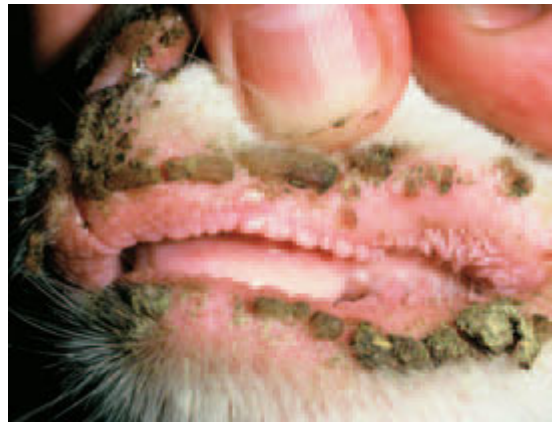


Figure 61.3: Peste des petits ruminants. Discrete sit-fast scabs along muco-cutaneous junction, usually seen in convalescent animals.

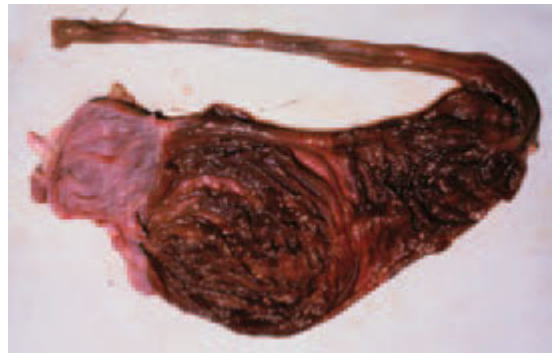


Figure 61.4: Peste des petits ruminants. Slight omasal engorgement, contrasting with very severe engorgement of the abomasum and proximal duodenum

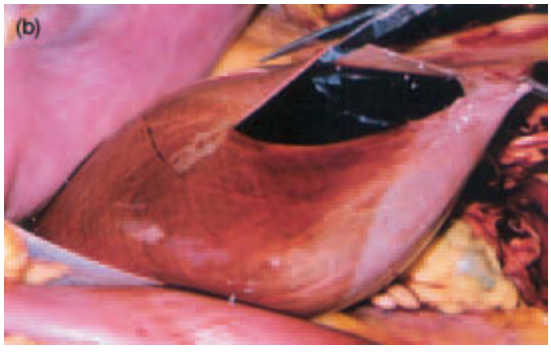


Figure 69.2: Chronic copper poisoning: (a) icteric liver; (b) dark urine.

Figure 71.3: Gas gangrene caused by *Cl. septicum*. Note the rear limb with dark colour and subcutaneous gelatinous oedema. In contrast see the healthy fore limb.

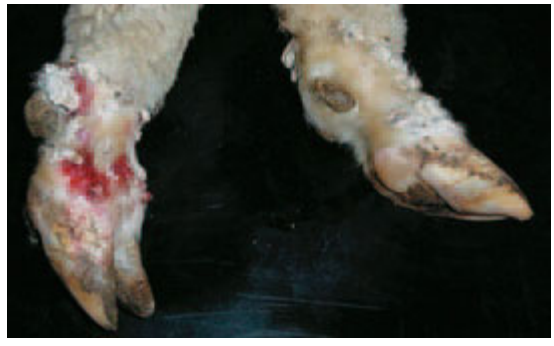


Figure 71.5: Strawberry foot root caused by a severe *Dermatophilus congolensis* infection.

Figure 71.2: *Brucella ovis*. Notice the enlargement of the left epididymis, mainly its tail, the atrophy of the left testicle and the severe fibrosis of tunica vaginalis.

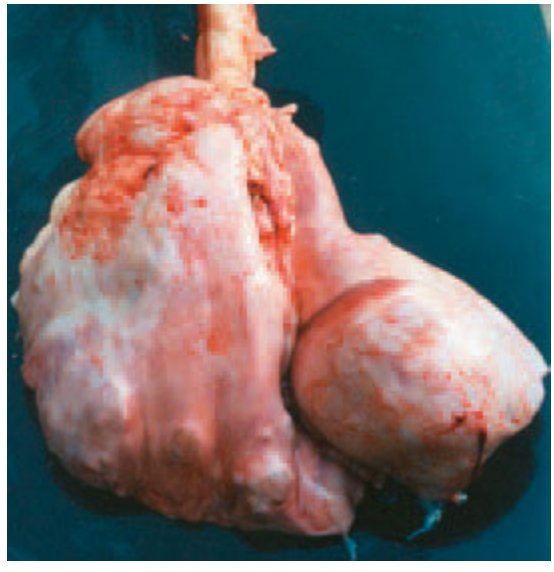


Figure 71.7: Pneumonia associated with *Erysipelothrix rhusiopathiae*. Lungs deformed by several abscesses full of greyish pus.

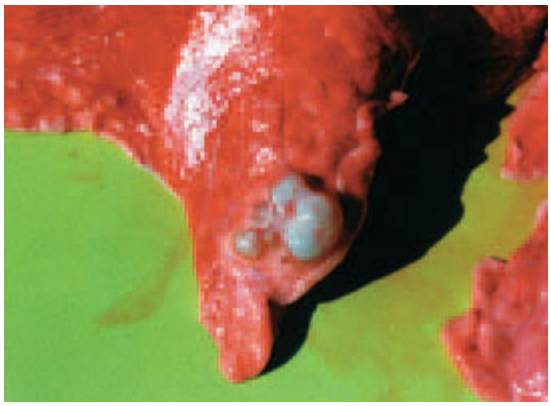


Figure 71.8: Hydatid disease due to *Echinococcus granulosus.* Note the cysts in the pulmonary parenchyma.

Figure 71.9: *Astragalus pehuenches,* the cause of locoism in sheep and horses in Patagonia.

Figure 71.11: (a) *Poa huecú* and (b) *Festuca argentina*, plants which produce intoxication in sheep.

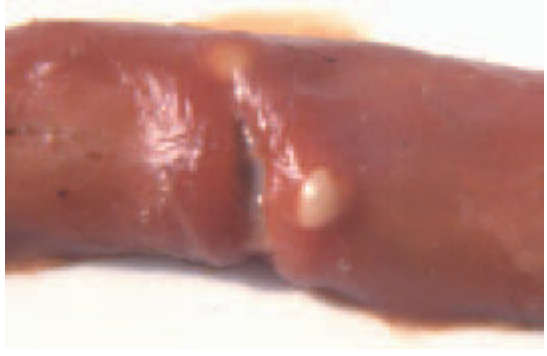


Figure 71.13: Rice grain-like cyst of *Sarcocystis ovifelis* (*S. gigantea*) in the oesophagus of a sheep.

26

Cryptosporidiosis and coccidiosis

S.E. Wright and R.L. Coop

CRYPTOSPORIDIOSIS

Cryptosporidium is a protozoan coccidian parasite belonging to the phylum *Apicomplexa* and is recognized as being of veterinary and medical importance. At least seven valid named species are found in mammals: *C. muris*, *C. felis*, *C. canis*, *C. wrairi*, *C. andersoni*, *C. hominis* and *C. parvum*, the latter species being the cause of neonatal diarrhoea in lambs and calves and responsible for zoonotic infections [1]. *C. hominis*, found almost exclusively in man, was originally referred to as type I *C. parvum*, but is now thought of as a separate species. *C. parvum* was first described in 1912 in the small intestine of mice, but only in 1971 was it associated with an outbreak of bovine diarrhoea. The first reports of human *Cryptosporidium* infections in 1976 and the subsequent findings of severe protracted diarrhoea as a result of infection in immunocompromised patients with AIDS increased awareness and generated global research on this parasite.

Cause

C. parvum primarily infects the small intestine and is transmitted directly between hosts by the faecal–oral route via the sporulated oocyst (average 4.5–5.5 μm in diameter), which is infective to a wide range of species of mammals. Ingestion of thick-walled oocysts is followed by excystation and release of sporozoites which invade the intestinal enterocytes and undergo rapid asexual multiplication (schizogony). Type I meronts can recycle by releasing merozoites, which invade new enterocytes. Sexual multiplication (gametogony) results in the formation of zygotes, most of which form thick-walled sporulated oocysts that pass out in the faeces as the resistant infective stage. A smaller proportion of zygotes (approximately 20 per cent) form thin-walled sporulated oocysts, which rupture in the small intestine; the released sporozoites invade other enterocytes (autoinfection).

These multiplicative stages (type I meronts and thin-walled oocysts) and sporulation within the host ensure rapid colonization of the epithelium of the small intestine when the intake of *C. parvum* oocysts is low.

Clinical signs

C. parvum infection in neonatal lambs (4–10 days old) causes a profuse watery diarrhoea which, in well-fed animals, often persists for 5–7 days before abating and the lambs then recover. The scouring often leads to dehydration, inappetence, abdominal tension and lethargy. Diarrhoea frequently coincides with the onset of oocyst shedding 3–7 days after ingestion of oocysts, and peak output can range from 10^5 to 10^7 oocysts per gram of faeces. Oocyst excretion may persist for several days after clinical signs have diminished [2]. In severe cases, lambs may die within 2–3 days of the onset of diarrhoea and, in these outbreaks, *C. parvum* is often associated with other enteropathogens. During a clinical infection, total parasite production may exceed 10^{10} oocysts, excreted over a 7–10-day period. Adult sheep can undergo non-clinical infection, shedding low numbers of oocysts, particularly around parturition, thus acting as a reservoir of infection for the susceptible lamb crop.

Pathology

C. parvum parasitizes the distal jejunum and ileum of lambs, although infection can sometimes progress into the caecum, colon and rectum. Gross findings are yellow–brown, watery intestinal contents and gas

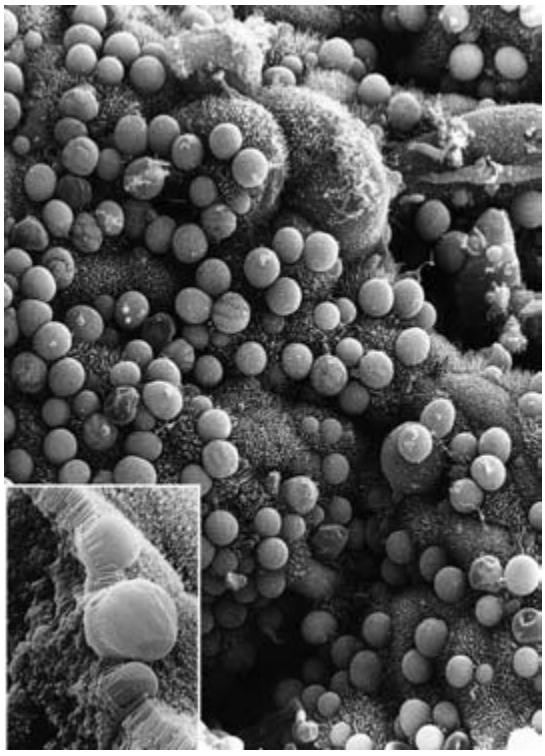


Figure 26.1: Endogenous stages of *Cryptosporidium parvum* on the surface of the ileum of an infected lamb. Original magnifications × 400 (main figure), × 15 000 (inset).

distension of the bowel, often with enteritis and colitis, although in some cases the mucosal surface may appear normal.

Histological examination reveals short, fused villi covered with the endogenous stages of *C. parvum* (Figure 26.1). The normal epithelium is replaced by a low columnar or cuboidal epithelium, resulting in villous atrophy and shortening of microvilli. Crypts are often elongated and the lamina propria may be infiltrated with mononuclear cells and neutrophils. The absorptive capacity is markedly reduced, leading to malabsorption and diarrhoea, although the exact mechanisms whereby *C. parvum* damages the intestine and induces clinical signs are not fully understood [3].

Diagnosis

Diagnosis of infection is usually by microscopic examination of stained faecal smears, commonly using the modified Ziehl–Neelsen method (MZN), the auramine fluorescent stains (AF) or fluorescein isothiocyanate-labelled monoclonal antibody (mAb) [3] to reveal the presence of oocysts. With the MZN technique, oocysts stain rose-pink–red against a blue–green counterstained background, while AF and mAb give a yellow-green or apple-green fluorescence, respectively, against an almost black or blue background.

If the number of oocysts in faeces is low, concentration is necessary using either flotation techniques (zinc sulfate, SG 1.18–1.20; saturated sodium chloride, SG 1.27; Sheather's sucrose) or sedimentation (formalin-ether, formalin-ethyl acetate) [4]. The oocysts should be examined as soon as possible or the medium diluted to avoid collapse and distortion in the flotation solutions.

Cryptosporidium antigen in faeces can be detected using enzyme-linked immunosorbent (ELISA) or immunofluorescent (IFA) assays, the latter technique exhibiting greater specificity and sensitivity than conventional staining methods. Assays based on the highly sensitive polymerase chain reaction (PCR) have been developed to detect oocysts in faeces [5, 6], but some techniques require purified oocysts and can be influenced by environmental contaminants. Such techniques reveal the presence of oocysts but do not indicate whether they are infective. This problem has recently been addressed by the development of tissue culture systems, sometimes coupled with the use of reverse transcription PCR to improve sensitivity, to determine the ability of the parasite to infect cells. However, only purified oocysts can be seeded into such systems.

In vivo viability tests in mice require relatively large numbers of oocysts, around 100 oocysts per mouse pup, therefore they are not suitable for the testing of environmental samples, but can be useful for determining disinfectant efficacy. Excystation *in vitro* is a good viability test but requires large numbers of oocysts to achieve accuracy and is not suitable for environmental samples that contain small numbers of oocysts. Dye inclusion techniques are most suitable for detecting viability in small samples, but variable staining quality can make interpretation difficult. DAP1 (4,6-diamidino-2-phenylindole) is most frequently used for environmental samples, often in conjunction with flow cytometer sorting to speed the counting process.

Post-mortem, ileal sections stained with haematoxylin and eosin will show the endogenous stages on

the luminal surface of epithelial cells as small round bodies (approximately 2–5 μm in diameter).

Epidemiology and transmission

C. parvum has a global distribution, and several features influence the epidemiology of disease. The oocyst shed in faeces is highly resistant to environmental stresses and, because it is excreted from the host fully sporulated, it is capable of direct transmission by the faecal–oral route from lamb to lamb or ewe to lamb, particularly in communal housing or via contaminated water. The infective dose is probably very low, fewer than five oocysts [2] but, once ingested, the parasite has the capacity to multiply rapidly in the host through asexual multiplication and re-infection of the intestine by mature merozoites and by autoinfection of the gut with sporozoites released from thin-walled oocysts, to reach large numbers ($>10^{10}$) of endogenous stages. Within 5–10 days of becoming infected, a single lamb can spread infection to a large proportion of the lamb crop. Synchronized lambing and intensification ensure that a large number of susceptible animals are available in confined areas, thus facilitating transmission.

Cryptosporidium is difficult to control within the environment, as it is ubiquitous and able to cross-infect a wide range of species of free-living animals in which infection is often asymptomatic. Rodents can be potential reservoirs of infection for livestock enterprises. Mechanical transmission via birds, insects and man may also be involved. Data on prevalence of *Cryptosporidium* in the UK are limited. The veterinary investigation service recorded 13 916 cases of cryptosporidiosis in calves in England and Wales between 1994 and 2003 [Veterinary Investigation Diagnosis Analysis (VIDA) data]. Data for sheep are scarcer, as faecal samples are not usually submitted for diagnosis from neonatal scouring lambs, but over the same period 1118 cases of cryptosporidiosis in lambs were recorded.

Treatment, prevention and control

Partial or demonstrable activity against *Cryptosporidium* has been reported for sulfaquinoxaline, halofuginone, lasalocid and paromomycin in experimentally infected neonatal ruminants, but little information is available for sheep [7]. Halofuginone lactate is licensed for therapeutic and prophylactic treatment of cryptosporidiosis in calves, but not for use in small ruminants. Treatment reduces clinical signs and diminishes oocyst output, but does not eradicate infection. Animals should be weighed accurately to achieve the recommended 0.1 mg/kg dose, since this drug has demonstrable toxicity at only twice the recommended therapeutic dose, and its use in severely dehydrated or inappetant animals is contraindicated. Under controlled experimental infections in lambs, clinical signs and oocyst excretion can be reduced by the ingestion of hyperimmune ovine or bovine colostrum [8]. Where bacterial co-pathogens are involved, appropriate antibiotic therapy should be given. Diarrhoeic lambs should be isolated and clean bedding provided to reduce the spread of infection and, if possible, newborn lambs should be kept separate from older lambs that may be infected. Disinfection of lambing pens and feeding troughs using an ammonia-based disinfectant or steam cleaning will reduce the number of oocysts in the environment.

It is not possible to control oocyst contamination on pasture but, as *C. parvum* is not host-specific, it is recommended that cattle slurries and manure are well fermented or composted prior to application on to pasture, as oocysts are killed by heat above 55–60°C and increasing ammonia concentration. Oocysts are also killed by desiccation.

COCCIDIOSIS

Coccidiosis is caused by protozoa of the genus *Eimeria*, which are intracellular parasites of the intestinal epithelial cells. They are highly species-specific, e.g. those which infect sheep will not infect goats or cattle, and vice versa. Other coccidial parasites such as *Cryptosporidium* and *Toxoplasma* can infect a wide range of host species. Clinical ovine coccidiosis is generally regarded as a disease of intensive animal husbandry.

Cause

Coccidial infection is initiated by the ingestion of sporulated oocysts shed in the faeces of previously

infected animals, which contaminate bedding, feed or the udders and teats of ewes. These oocysts excyst in the gastrointestinal tract, each oocyst releasing eight sporozoites that invade the epithelial cells and undergo cycles of asexual replication (schizogony). At each stage, an individual parasite enters a cell, grows and divides repeatedly to form a cluster of parasites. As the parasitized cells are damaged during this process, the intestinal epithelium gradually becomes eroded. After a number of asexual replications, sexual reproduction is initiated (gametogony) and results in the liberation of oocysts into the intestinal lumen, which are then shed in the faeces. The prepatent period is around 12–20 days. The oocysts are undeveloped (non-sporulated) and require time outwith the host to develop and become infective (sporulated). This may take 2–4 days under ideal conditions, but when the weather is cold, sporulation may take several weeks.

The life cycle of *Eimeria* spp lasts 2–3 weeks from ingestion of oocysts to the onset of oocyst shedding. Although 11 species of *Eimeria* infect sheep, only two are considered to be highly pathogenic: *E. crandallis* and *E. ovinoidalis*.

Clinical signs

Clinical coccidiosis is observed most commonly in lambs 4–6 weeks old. Clinical signs are acute diarrhoea, dullness, abdominal pain and anorexia, leading to dehydration and marked loss of weight and body condition. In outbreaks, most lambs usually will be affected. In severe cases, particularly in infections with *E. ovinoidalis*, there may be an acute, bloody diarrhoea as a result of extensive damage to the gut epithelium. Infection in adult animals is usually asymptomatic.

Pathology

The two pathogenic species, *E. crandallis* and *E. ovinoidalis*, are found in the ileum, but may also infect the caecum and colon. Both parasites have a similar effect on the intestinal tract. Damage to the epithelial cells, with subsequent reduction in their numbers, is reflected histologically by villous atrophy and crypt hyperplasia and cellular infiltration. Local oedema and haemorrhage may occur. Parasite numbers usually reach a peak at the start of the sexual multiplication phase, and this is when maximum tissue damage occurs. The damage to the gut may be sufficient to allow secondary bacterial infection to establish. The loss of absorptive surface area results in gut dysfunction, particularly impaired absorption of fluids and nutrients, leading to a profuse diarrhea and dehydration. This normally coincides with the onset of oocyst shedding, although in particularly heavy infections the damage caused by the endogenous stages may be sufficient to induce diarrhoea prior to oocyst shedding, which should be borne in mind during diagnosis.

Diagnosis

This should be based on the management history, age of lambs, clinical signs and outbreaks confirmed by post-mortem examination of lambs. Since only two of the 11 species of ovine coccidia are pathogenic, diagnosis cannot be based solely on the presence of clinical signs and a positive faecal oocyst count. Speciation of the oocysts will be required and involves allowing the oocysts to develop for several days to aid identification. Concentration of faecal oocysts by a simple saturated salt flotation and storage of oocysts in 2 per cent potassium dichromate will allow sporulation while preventing overgrowth of other contaminating organisms. Specific oocyst counts in faeces will support a diagnosis. Table 26.1 gives the characteristics of the 11 ovine species of coccidia.

The pathogenic species *E. crandallis* and *E. ovinoidalis* are of similar size (Figure 26.2). *E. crandallis* has a polar cap and broad sporocysts – on size alone it could be confused with *E. weybridgensis*, but this species has elongate sporocysts, demonstrating the value of sporulation to the identification process. *E. ovinoidalis* has no polar cap, a comparatively thin oocyst wall and elongate sporocysts. Table 26.1 gives mean sizes, around which there can be considerable variation. In severe infections, large numbers of the oocysts shed in faeces may be distorted owing to overcrowding in the gut, which can also make identification more difficult.

Severe diarrhoea in 4–6 week-old lambs associated with high oocyst counts where *E. crandallis* or *E. ovinoidalis* are the predominant species, or with high counts of deformed oocysts of these species, is indicative of clinical coccidiosis. Some husbandry practices may allow lambs to be exposed simultaneously to

Table 26.1: Characteristics of sporulated oocysts of *Eimeria* spp.

	Oocyst dimensions (μm)		
Species	Length	Width	Morphological characteristics
E. intricata	47	32	Polar cap, thick wall
E. ahsata	39	25	Prominent polar cap
E. bakuensis	31	20	Polar cap
E. granulosa	29	21	Egg-shaped, polar cap
E. faurei	29	21	Egg-shaped, no polar cap
E. parva	16	14	No polar cap
E. pallida	14	10	No polar cap
E. marsica	19	13	Small polar cap
E. weybridgensis	24	17	Shallow polar cap, elongate sporocytes
E. crandallis	24	17	Shallow polar cap, sometimes absent, broad sporocytes
E. ovinoidalis	23	18	No polar cap, thin wall

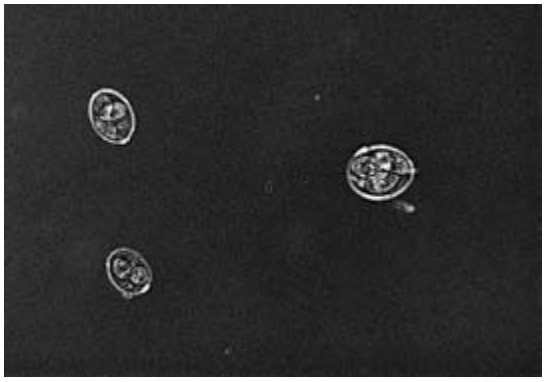


Figure 26.2: Oocysts of *Eimeria crandallis* (two on left with polar caps) and *E. ovinoidalis* (single on right).

coccidia oocysts and *Nematodirus battus* larvae and, under these conditions, the clinical syndrome will be more severe [9].

Epidemiology and transmission

The host specificity of *Eimeria* species means that the only source of infection for lambs is other ovines. All ages of sheep can be infected with *Eimeria,* and there is a balance between the rate of acquisition of immunity and the pathogenic effects of the parasite. Intensification often allows a massive challenge with oocysts which overwhelms the animal before its immune system can be effective. Adult sheep are usually resistant to disease but act as carriers, shedding low numbers of oocysts. The longevity of oocysts in faeces may allow some to survive over winter as residual environmental contamination, but the main source is the small numbers of oocysts shed by ewes, particularly around the periparturient period when the ewe's immune status is lowered. These may be insufficient to induce clinical coccidiosis, but subclinical infection in early lambs can multiply the parasite burden to very high levels so that later lambs introduced into the same pens or pasture receive a severe challenge from an increasingly contaminated environment. Thus, intensification tends to increase the likelihood of clinical coccidiosis. Clinical coccidiosis in grazing lambs is most common in those between 4 and 6 weeks old, particularly where stocking rates are high. Various factors can contribute to the severity of disease; a poor supply of colostrum and/or milk, cold wet weather, stress of transport and, in some circumstances, early weaning. The disease can occur also in lambs that have been housed and then turned out on to pasture, the signs occurring about 3 weeks after turnout. When young lambs are exposed to coccidial infection, they can usually be considered to be immune from around 6–8 weeks of age, although medication may delay the onset of immunity. Under extensive grazing, the challenge from oocysts is usually low and lambs are able to acquire a protective immunity without developing clinical disease.

The number of diagnoses of clinical coccidiosis made by the UK Veterinary Investigation Centres

fluctuates from year to year, but averaged 435 diagnoses per annum from 1994 to 2003 (source VIDA).

Host resistance

Ingestion of colostrum initially provides newborn animals with passive immunity to coccidiosis, but this wanes over time and susceptibility to infection increases. Animals then acquire resistance to infection as a result of exposure to the parasite – whether they show signs of disease depends on the size of the parasite challenge they face. Following exposure, animals develop a degree of immunity to the parasite, preventing recurrence of clinical signs, although asymptomatic re-infections can occur, as in mature ewes. This is an important feature when treatment is considered.

Occasionally, acute coccidiosis can occur in adult animals, most usually in those which are stressed, for example by environmental extremes, poor or unsuitable diet, prolonged travel or debilitation from concurrent infections.

Treatment and control

Treatment of clinical coccidiosis should be on a flock rather than on an individual basis, as lambs showing no obvious signs may contaminate the environment, and should include effective management as well as chemotherapy. Because 'trickle' infections with small numbers of oocysts tend to induce a strong immunity to clinical coccidiosis [10], prevention of excessive environmental contamination is desirable. Good hygiene and adequate clean dry bedding and drainage in lambing sheds is essential. Pens need to be cleaned regularly, and the raising of water and food troughs will reduce contamination with oocysts. Ideally, later-born lambs should be housed/grazed on different areas from early lambs, to prevent contact with heavily contaminated bedding/pasture. Overcrowding must be prevented. Regular movement of lambs to fresh grazing is also desirable to prevent excessive build-up of contamination. Rearing of lambs in batches of similar age will reduce the accumulation of oocysts and allow lambs most considered at risk to be targeted with treatment.

Coccidiostats may be incorporated into creep feed, although there is a possibility of clinical coccidiosis occurring once the medicated feed is removed. Immunity to the parasites is induced by contact with the developing stages in the gut and cannot occur to any significant extent if they are continuously cleared by blanket medication. Medication of feed for ewes will reduce the output of oocysts but may not prevent infection in lambs and is usually adopted in conjunction with lamb medication in creep feed.

Two effective anticoccidial drugs are licensed for use in sheep. Decoquinate may be incorporated in feed, while diclazuril may be administered therapeutically on an individual basis to lambs showing signs of infection. If possible, treated lambs should be moved to clean bedding or clean grazing (pasture which did not carry clinically affected lambs the previous year) to prevent re-infection before they have developed adequate immunity. Lambs that become dehydrated may require oral or intravenous rehydration.

Coccidial oocysts are quite resistant to disinfection. Both desiccation and elevated temperature (above 55–60°C) will kill oocysts, while some ammonia-based agricultural disinfectants also are effective, but cannot be used in the presence of livestock, only when the premises have been cleared. Steam cleaning of contaminated housing, water bowls and feeding troughs is advisable. Contamination on pasture will decline over time, although it will be more prolonged when mild, moist conditions prevail.

ZOONOTIC IMPLICATIONS

C. parvum is zoonotic and is transmitted to people via the faecal–oral route through close contact with infected livestock or companion animals, and many infections have been derived directly or indirectly from lambs or calves [11]. Contaminated drinking or recreational water is also a vehicle for transmission of oocysts to humans. *C. parvum* can infect susceptible adults, but the peak incidence is usually in children aged 1–5 years. It should be noted that human outbreaks may also occur as a result of infection with *Cryptosporidium hominis*, from human–human contact or environmental contamination with human wastes – this parasite is rarely found infecting animals.

In healthy children, *C. parvum* causes an acute enteritis, which is usually self-limiting within 1–2 weeks, but *Cryptosporidium* can be life-threatening in severely immunocompromised adults such as those

with AIDS or those receiving immunosuppressive treatments, in whom it causes chronic persistent enteritis and diarrhoea. Outbreaks in children have been associated with educational visits to urban farms and livestock markets. Infection has been reported as a result of occupational exposure in veterinarians and agricultural workers.

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27

Gastrointestinal helminthosis

F. Jackson and R.L. Coop

Gastrointestinal helminths are major contributors to reduced productivity and can lower the production of meat, milk and wool [1]. In intensive farming systems, control is achieved through regular treatment with anthelmintic medicines combined, where practicable, with grazing strategies [2].

Parasitic infection ranges from acute disease, frequently with high rates of mortality, chronic disease, resulting in various degrees of morbidity and premature culling, to subclinical infection, with sheep appearing relatively healthy but frequently performing below their full potential. The parasitic helminths of sheep can be subdivided into nematodes (roundworms), trematodes (flukes) and cestodes (tapeworms). This chapter will focus on the important nematodes that inhabit the abomasum and intestine. Although cestode

infections (*Moniezia*) are common in lambs, they are not considered generally to be a problem. Liver fluke is considered in Chapter 28.

CAUSE

The important nematode diseases of sheep in the UK are: (1) nematodirosis in young lambs; and (2) parasitic gastroenteritis (PGE) in lambs and occasionally older sheep.

Although several species of nematode may be present in the gastrointestinal tract of sheep (Table 27.1), the principal genera responsible for outbreaks of parasitic gastroenteritis (PGE) in the UK are *Ostertagia* (reclassified as *Teladorsagia*) and *Trichostrongylus*, although *Nematodirus battus* can cause outbreaks of diarrhoea when high numbers of larvae on pasture coincide with the presence of young susceptible lambs. Although formerly most common in the southern counties of England, the warmer prevailing climate in recent years has allowed sporadic infections of *Haemonchus contortus* throughout the UK. Haemonchus is, however, not a cold tolerant worm and thus transmission and persistence of larvae on pastures can be variable. Other nematodes are found sporadically in the gastrointestinal tract but are generally considered to be of low pathogenicity, and frequent treatments with modern broad-spectrum anthelmintics have considerably reduced the prevalence of those parasites with long pre-patency such as *Oesophagostomum* and *Bunostomum*.

Table 27.1: Gastrointestinal helminths of sheep in the UK

Abomasum	*Teladorsagia* (*Ostertagia*) *circumcincta* *Teladorsagia* (*Ostertagia*) *trifurcate* *Trichostrongylus axei* *Haemonchus contortus*
Small intestine	*Trichostrongylus vitrinus* *Trichostrongylus colubriformis* *Nematodirus battus* *Nematodirus fillicollis* *Cooperia curticei* *Strongyloides papillosus* *Bunostomum trigonocephalum* *Moniezia expansa*
Large intestine	*Chabertia ovina* *Oesophagostomum venulosum* *Trichuris ovis*

CLINICAL SIGNS AND PATHOLOGY

The severity of signs of parasitism and damage to the gastrointestinal tract will be influenced by host age, breed, immunological experience and nutritional status. Exposure of susceptible lambs to moderate-to-high numbers of infective larvae on pasture inevitably leads to clinical PGE unless the worm burdens are limited by anthelmintic treatment.

Teladorsagiosis

Clinical signs of acute *Teladorsagia* infection (type I) in lambs are watery diarrhoea, dehydration, loss of appetite and failure to gain weight. Pathological changes include a hyperplastic gastritis. The abomasal mucosa frequently is thickened, oedematous and numerous raised nodules are present on the surface of the abomasal folds. In severe infection, the hyperplastic nodules may coalesce to form the characteristic 'Morocco leather' appearance. Larvae developing within the gastric glands distend the lumen of the glands and stretch the cellular lining, which results in mature functional parietal and peptic cells being replaced by undifferentiated cells [3]. As infection progresses, adjacent non-parasitized glands become affected, parietal cells being replaced by non-functional undifferentiated cells and, as a consequence, the pH of the abomasal secretions increases. Leakage of macromolecules and protein across the damaged mucosa results in hypoproteinaemia and hypoalbuminaemia, and increased concentrations of pepsinogen in the plasma. A subacute or chronic form (type II teladorsagiosis) is seen occasionally in both housed and outwintered ewes and hoggs in late winter/early spring, caused by the mass emergence of large numbers of hypobiotic (arrested) larvae, which were acquired in the autumn and overwintered in the gastric glands. Affected sheep frequently show intermittent diarrhoea and progressive loss of condition and body weight.

Haemonchosis

Clinical features are anaemia, with pale mucous membranes, submandibular oedema, hyperpnoea and tachycardia. Pathogenesis is associated with the blood-feeding activities of developing larvae and adult worms. Diarrhoea normally does not occur. Acute disease results from large intakes of infective larvae, and young lambs rapidly become unthrifty, weak and lethargic. Hypoproteinaemia, hypoalbuminaemia and oedema occur, and morbidity is high. Chronic haemonchosis, due to a more gradual intake of infective larvae, results in a general wasting condition; sheep becoming unthrifty and emaciated, resembling a state of malnutrition. Growth rate declines and the fleece may be open and dull. Chronic haemonchosis with anaemia and hypoproteinaemia occasionally can occur in ewes in the spring, and results from maturation of hypobiotic larvae. At necropsy, the findings will depend on the severity and duration of the infection. The carcass is frequently pallid with ascites and fluid in the pericardium, the blood watery and the liver pale and friable. The abomasal contents are often dark brown and the mucous lining pale, oedematous, mucoid and covered with dark-red petechiae. Raised nodules may be present in the plicae. Microscopically, the main features relate to the local effects of individual adult worms or larvae, with the more diffuse effects resulting in hypertrophy of the mucosa. Adult *Haemonchus* usually lie between the surface epithelium and a thick layer of mucus, and local lacerations or erosions with haemorrhage from superficial capillaries are associated with their activity. Developing larvae in the gastric glands cause local distension, cytolysis and loss of parietal cells.

Intestinal trichostrongylosis

Although usually presenting as a chronic wasting disease in hoggs and ewes in early winter, intestinal *Trichostrongylus* infection can cause acute disease in lambs in late summer. Clinical features include anorexia, decrease in weight gain, with varying degrees of hypoalbuminaemia and hypophosphataemia. Dark coloured diarrhoea often is present in the more severely affected animals, and the fleece may be open and the wool brittle. At necropsy, large numbers (20 000–30 000) of *Trichostrongylus* spp. may be present. Gross lesions are enteritis with an increase in mucus, inflammation of the anterior small intestine and hypertrophy of the duodenal and jejunal mucosa. The villi are frequently short, distorted and, in severe infections, there may be total villous atrophy. Enzymic activity of the epithelial cell brush borders is frequently reduced. The intestinal crypts become dilated and elongated, and the lamina propria is thickened and heavily infiltrated with inflammatory cells. Developing larvae and adult worms burrow just beneath the surface epithelium (Figure 27.1), causing sloughing and disruption of cells and leakage of plasma protein into the lumen of the intestine. As resistance to *T. vitrinus* infection develops, the affected areas tend to be more localized, patches of villous atrophy ('finger-print' lesions) being surrounded by areas of relatively normal mucosa.

Figure 27.1: *Trichostrongylus colubriformis* burrowing through the mucosa of the small intestine.

Nematodirosis

Lambs infected with *N. battus* show acute enteritis with profuse watery diarrhoea, associated with emergence and development of the larval stages, frequently accompanied by lethargy and loss of appetite. The fleece is often dull and rough, and the lambs may show the typical 'tucked-up belly' appearance. Weight loss can be rapid with severe dehydration and, if the infection is untreated, mortality can be high. Lambs that survive the initial phase rapidly develop resistance to infection but it may take 2–3 months before they return to reasonable body condition. *N. filicollis* is less pathogenic as the infective larvae hatch over an extended period. At necropsy, there may be

masses of juvenile stages and adult worms coiled together in the intestine or very few parasites, the majority having been expelled during the diarrhoeic phase. Developing stages and adult worms are involved in the pathogenesis. Features include catarrhal enteritis, acute inflammation of the intestinal mucosa and dehydration of the carcass. Microscopically, the mucosa shows superficial lesions with local distortion and compression of the villi in contact with the parasites, leading to necrosis of the surface epithelium with the formation of local erosions. Villous atrophy may occur with the microvilli sparse and stunted, and the lamina propria infiltrated by inflammatory cells.

Other helminthoses

The abomasal parasite *Trichostrongylus axei* can cause catarrhal inflammation with erosion of the mucosa. *Cooperia curticei*, *Strongyloides papillosus* and *Bunostomum trigonocephalum* are found occasionally in the small intestine at necropsy, but generally are present in insufficient numbers to be pathogenic. Heavy infection can cause enteritis, erosion of the mucosa and local haemorrhage. *Moniezia expansa* is the commonest tapeworm in young lambs in the UK but, despite their large size (around 0.5–0.7 m), they are of low pathogenicity and are usually eliminated after a few months. The main nematodes in the large intestine are *Oesophagostomum venulosum*, *Chabertia ovina* and *Trichuris ovis*, which are normally present in small numbers and cause little damage. *O. venulosum* feeds on small plugs of tissue and may leave small ulcers on the intestinal mucosa, whereas the tropical/subtropical species *O. columbianum* is more pathogenic, the larvae migrating deeper into the mucosa and resulting in localized fibroblastic nodules. *C. ovina* may cause enteritis if present in sufficient numbers, producing oedema and small haemorrhages in the wall of the colon.

DIAGNOSIS

Parasitic gastroenteritis

Faecal egg counts are frequently used as a direct aid to diagnose nematode infections in lambs, but have several limitations. The presence of nematode eggs in faeces will indicate that adult worms are present but in some egg counts, particularly with *Teladorsagia*, these will not correlate with the size of the worm burden. The lack of correlation between egg count and worm burden may be due not only to reductions in *per capita* fecundity, but also to the presence of populations of inhibited larvae and/or developing immature stages. Interpretation of total faecal egg counts is further complicated by specific differences in fecundity, which, when several species are present, allows minor non-pathogenic species to make a disproportionate contribution.

Limited indirect diagnostic aids are available. Increase in plasma pepsinogen or gastrin concentrations can be a useful indication of damage to the abomasal mucosa in growing lambs as a result of infection with *Teladorsagia* or *Haemonchus* [3]. Values above about 0.7 IU pepsinogen suggest that the animals have been exposed to numbers of larvae that would affect their performance. However, pepsinogen concentrations can be elevated in older sheep that are not suffering obvious production losses as a result of a hypersensitivity reaction to incoming larvae.

No direct blood markers are available for intestinal parasitism. Increases in serum fructosamine concentrations have been investigated but appear to be variable. Similarly, intestinal *Trichostrongylus* infection lowers plasma phosphorus concentrations, but the reduction can be interpreted only in association with a knowledge of phosphorus levels of the forage or feed.

Anthelminitic resistance

The increasing prevalence of anthelmintic-resistant populations of gastrointestinal nematodes in the UK has led to the need to test for resistance and efficacy of the drugs that are used on farms. Practitioners and local veterinary investigation centres offer a range of assays, including the most commonly used on-farm *in vivo* test, the faecal egg count reduction test (FECRT) [4]. A simplified post-drenching efficacy (PDEC) test can also be conducted in which only post-treatment samples are examined. The timing of collection of samples in the PDEC is important; animals should be sampled 7 days post-treatment with the levamisole/morantel anthelmintics, 7–14 days for the benzimidazoles and 14–17 days post-treatment with the macrocyclic lactones [5]. *In vitro* assays tend to be conducted in more specialized laboratories and

include the egg hatch assay for benzimidazole resistance and the micro-agar larval development assay, which can detect resistance to both the levamisoles and benzimidazoles.

EPIDEMIOLOGY AND TRANSMISSION

The successful control of any helminth disease is based on a sound knowledge of its epidemiology, which often varies according to the helminth species. Thus, the control of each disease will be considered separately. Nematode parasites of sheep have a simple direct life cycle, involving only the host and the pasture environment.

Parasitic gastroenteritis

In the UK, PGE is primarily a disease of lambs and occasionally older sheep. The herbage numbers of third-stage larvae (L3) increase markedly from midsummer onwards, and this is when disease problems generally arise. Usually, *Teladorsagia* and *Haemonchus* larvae appear first, followed later in the summer and early autumn by *Trichostrongylus*. The warmer wetter springs and autumns recently experienced in temperate regions of Europe can influence patterns of PGE since these conditions favour parasite development and translation, and thus expose grazing stock to higher levels of challenge. Under these circumstances seasonal patterns of disease attributable to roundworm infections are less predictable than formerly. The two principal sources of these larval infections in temperate regions are:

- Strongylate eggs passed in the faeces of ewes during the periparturient relaxation of immunity (PPRI). The duration of the egg output during the PPRI is from approximately 2 weeks prior to lambing until about 6 weeks post-lambing.
- Strongylate eggs passed by lambs, resulting from the ingestion of overwintered L3; the latter can overwinter in fairly high numbers but decline in number rapidly during April and May, although a few will survive for up to 2 years in the soil and root matt, and remain as a reservoir of infection.

It is important to realize that it is the eggs deposited in the first half of the grazing season, i.e. April to June, that are responsible for the potentially dangerous populations of infective larvae that accumulate on pasture in the second half of the season, i.e. July to September. If ingested before October, most of these larvae mature in a few weeks; thereafter until the following spring, many of the larvae ingested become arrested in development for up to several months. Occasionally, the numbers of overwintered larvae are sufficient to cause a check in lamb growth in the spring.

Nematodirosis

Although several species of *Nematodirus* occur in sheep in the UK, *N. battus* is the species responsible for the severe outbreaks in lambs usually in late spring. Epidemiology of this disease is based on three main factors.

- The egg that contains the infective third-stage larva (L3) has a high resistance to freezing or desiccation and can survive on pasture for up to 2 years.
- Hatching, with release of the L3, requires specific stimuli and usually only occurs following a period of cold exposure followed by daily maximum temperatures of more than 10°C.
- Adult sheep are highly resistant to infection and only lambs in the first grazing season are susceptible.

Because of the ability of *N. battus* eggs to survive well in the environment, the infection is maintained by passing from one lamb crop to the next generation of lambs. Accumulation of infection on pasture therefore takes place over a period of years and not in a single season as in parasitic gastroenteritis. In the UK, disease never occurs on first-year grass, is rare on second-year pasture, but, by the third year of grazing by lambs, contamination may be sufficient to induce pathogenic infections. As a result of the critical hatching requirements, there can be an almost simultaneous appearance of large numbers of L3 on the pasture.

Although the mass hatch of eggs occurs each year, disease does not always follow even on heavily contaminated grazing for, if the hatch is very early (April), many young lambs are ingesting insufficient grass to take in large numbers of larvae, and if it is late (June), they are able to resist the larval challenge,

since age resistance appears by about 3 months of age and is high by 6 months. Clearly, due to the annual spring hatching of *N. battus* eggs, the disease can occur only in fields contaminated in the previous year. This contamination is usually derived from the previous year's lambs, but it has been demonstrated that young dairy calves can also harbour *N. battus* in high numbers and contribute to pasture contamination [6]. In recent years there have also been reports of nematodirosis occurring later in the year in lambs that have not been exposed earlier in the grazing season to sufficient *Nematodirus* challenge to induce adequate acquired immunity.

TREATMENT, PREVENTION AND CONTROL

The increasing prevalence of anthelmintic resistance has led to the realization that intensive chemoprophylaxis is not a sustainable option for the control of roundworms [5].

The object of any strategic control programme is to limit contact between the host and the infective stage of the parasite. This may be achieved by using anthelmintics to prevent or limit the contamination of pasture by ewes and lambs prior to June or by avoiding the grazing of pastures after June where contamination has occurred and larval populations are likely to be high. In some instances, a combination of both methods may be applied. The method of prophylaxis applied depends on availability of alternative grazing either on an annual basis or in midseason.

Farms with limited grazing management options

These often include upland and hill farms where most of the pasture is permanent. In these farms, control of nematodes may be achieved in two ways, namely: (1) chemoprophylaxis; and (2) by alternate grazing on an annual basis with cattle. Clearly, the former is the only method if the farm is stocked mainly with sheep, while the latter is to be recommended where both cattle and sheep are present in reasonable proportions.

Chemoprophylaxis

Control of *Nematodirus* disease usually can be achieved by avoiding the grazing of successive lamb crops on the same pasture. Where alternative grazing is not available, control can be achieved by anthelmintic prophylaxis, the timing of treatments being based on the knowledge that the peak time for the appearance of *N. battus* L3 on pasture is May to early June. Ideally, dosing should be timed at intervals of 3 weeks over May and June, and it is unwise to await the appearance of clinical signs of diarrhoea and dehydration before administering treatment. In years when the climatic conditions would predict severe disease, three treatments with anthelmintics such as benzimidazoles/probenzimidazoles, levamisole/morantel or avermectins/milbemycin are recommended during May and June; in other years, two treatments in May should suffice. It should be noted that the dose rate may need to be increased for some drugs and that others do not offer a persistent effect against *Nematodirus* spp.

The most important source of infection for the lamb crop is undoubtedly the periparturient increase in strongylate eggs in ewe faeces. Effective anthelmintic therapy of ewes during the fourth month of pregnancy should eliminate most of the worm burdens present at this time and, in the case of hill ewes, where nutritional status is frequently low, this treatment often results in improved general body condition. It is essential to use an anthelmintic that is effective against arrested larval stages and some of the new avermectins and milbemycins can prevent many larval species from establishing, giving a prolonged period of protection. Where ewes are housed over the winter or before lambing, this anthelmintic treatment may be given at the beginning of the housing period. However, during late pregnancy and early lactation, such treated ewes become re-infected from the ingestion of overwintered larvae on pasture. It is therefore recommended that, for optimal prophylaxis, a further treatment be given within 1 month of lambing.

Apart from specific treatment for *Nematodirus* infection, lambs should be treated at weaning and, if possible, moved to 'safe' or 'low-risk' pasture, i.e. pasture not grazed by sheep since the previous year (Table 27.2). Moving animals on to clean pasture carries an increased risk as far as anthelmintic resistance is concerned since it has the potential to rapidly select for resistance. If treated animals are moved on

Table 27.2: Strategies to provide minimal availability of infective larvae on pasture

'Safe' pastures	'Low-risk' pastures
Grassland for grazing in the spring and early summer	
New grass sown after an arable crop in the previous year	Pasture grazed only by dosed non-lactating ewes in the previous year
Pasture that has been grazed only by cattle in the previous year	Pasture grazed by cattle since the previous summer
Pasture used only for conservation in the previous year	Pastures grazed by dosed yearling sheep in the previous year should be 'safe' by early May
Grassland for grazing in mid-summer and autumn	
Aftermaths that have not been grazed by sheep in the spring	Grass grazed by non-lactating ewes in the spring/early summer
Grass that has not been grazed by sheep since the previous autumn and has carried cattle earlier in the spring	

to pasture where there are no free-living stages then eggs from resistant worms that have survived treatment will repopulate the pasture. The risk of having a population that contains only resistant worms is reduced if animals are moved on to pastures that are infected with predominantly susceptible worms. Where the only available grazing is heavily contaminated prophylactic treatments may need to be repeated until autumn or marketing. The frequency of dosing will be determined by the stocking rate, the level of pasture contamination and the type of anthelmintic. One treatment in September will generally suffice for hill lambs and two treatments under upland conditions. Rams should be treated in the spring and at pre-tupping in the autumn. The prophylactic programmes outlined are costly in terms of drug and labour handling, but currently are the only methods available in upland and hill farms where the enterprise is heavily dependent on one animal species.

Prophylaxis by alternate grazing

On farms where both sheep and cattle are present in significant numbers, good control of ovine PGE can be achieved by alternating the grazing of fields on an annual basis with the different host species. The basis for this control is twofold:

- The host specificity of the different nematode species. Theoretically, only *Haemonchus contortus* and *Trichostrongylus axei* can develop to maturity in both sheep and cattle although some of the *Ostertagia* spp, e.g. *O. leptospicularis*, are also adapted to both hosts.
- The annual mortality of overwintered L3, but evidence, particularly in cattle, suggests that it may require 2 years for the larval population on the herbage and upper soil layers to die out completely.

There is evidence also that *Nematodirus battus* can establish in calves and give rise to heavy larval populations on the pastures in the following year [6]. Although the main danger is to lambs grazed on these pastures, heavily infected calves may develop diarrhoea [6]. However, despite these qualifications about the role of dairy calves, good control is possible by simply exchanging, in the spring, the pastures grazed by sheep and cattle over the previous year, preferably combined with an anthelmintic treatment at the time of exchange, using an anthelmintic that is effective against arrested larvae.

Farms with plentiful alternative grazing

On lowland farms that rotate crops and grass, new leys and aftermaths are available each year. In such a situation, control should be based on a combination of anthelmintic prophylaxis and grazing management.

Chemoprophylaxis and grazing management

Good control can be obtained with only one anthelmintic treatment of ewes, carried out when the

ewes leave the lambing field, to terminate the periparturient increase in nematode eggs prior to moving the ewes and lambs to a safe pasture, such as a new ley. At weaning, the lambs should be moved to another 'safe' or low-risk pasture (Table 27.2). Although an anthelmintic treatment of the lambs at this time is good policy, it may be unnecessary if the new pasture is really 'safe', i.e. not grazed by sheep since the previous season and thus carrying only low levels of pasture contamination.

An excellent low-cost control system for farms with arable crops, sheep and cattle as equal components of the total enterprise can be adapted to suit farms where sheep and cattle dominate the livestock component. A 3-year rotation of sheep, cattle and conservation is recommended. Aftermath grazing after cropping, if available, can be used for weaned calves and sheep, but lambs or hoggs must not be allowed access to the cattle area, since this is intended as the next year's 'safe' grazing. It has been suggested that anthelmintic prophylaxis can be relinquished completely under this system, but clinical PGE sometimes has occurred when such a recommendation has been adopted. It is worth remembering that even the high-quality treatments currently available do not necessarily remove all the worms present, owing to development of resistant strains, that some cattle nematodes can infect sheep and vice versa, and that a few infective larvae on the pasture can survive for up to 2 years. So, it is advisable to use at least an annual spring treatment within the 3-year rotation outlined above. This treatment should be given around the time of moving to new pastures. Administering these treatments either before or shortly after the move should help to maintain susceptible genotypes within the worm population and hence reduce the selection pressure for the development of anthelmintic resistance.

Two other methods, namely strip grazing, in which sheep are confined to a narrow strip across the field by fences, which are moved every few days, and creep grazing, in which a single fence confines the ewes but allows the lambs to graze forward, can be effective in preventing PGE, but are costly in fencing and labour.

TAENIOSIS

Although tapeworms of several genera, namely, *Moniezia*, *Thysanosoma*, *Avitellina* and *Stilesia* have been reported as causing disease in sheep in various parts of the world, only *Moniezia* occurs in the UK. Mature tapeworm segments or eggs are passed in the faeces, and the eggs are ingested by free-living forage mites on pasture.

Development of the young tapeworms proceeds to a cyst stage in the mite, and infection occurs when the sheep ingests the infected mite during grazing. A seasonal fluctuation occurs in the incidence of *Moniezia* infection related to active periods of the forage mite vectors in the summer. The tapeworm cysts can overwinter in the mites. *Moniezia* is seen in lambs in the first grazing season but is less common in older animals. The pathogenicity of *Moniezia* spp. in lambs in the UK has yet to be conclusively demonstrated, and it is almost certainly the ease by which segments are recognized in the faeces that induces farmers to treat lambs for these tapeworms. Specific treatment is usually unnecessary, since several benzimidazoles routinely used to prevent nematodirosis and PGE simultaneously remove *Moniezia* spp. Tapeworm burdens are highest in lambs in spring and early summer and only if considered a problem is there need for routine prophylaxis at this time.

ANTHELMINTIC RESISTANCE

The gene(s) conferring resistance to a drug may be present initially at very low frequencies in a population of nematodes and resistance arises when an increased frequency of individuals within the population can survive treatments administered at the manufacturer's recommended dose rate. Resistance is heritable and therefore repeated anthelmintic treatment will select for an increasing proportion of resistant individuals. The mechanisms underpinning resistance involve either mutations at the target site of the drug and/or differences in how the parasites metabolize the drug.

Resistance to the three chemical classes of broad-spectrum anthelmintics, 1-BZ (benzimidazoles and probenzimidazoles), 2-LM (levamisole/morantel) and 3-AV (avermectins/milbemycins) has been recorded in nematode parasites of small ruminants throughout the world, initially mainly from the southern hemisphere. The reasons for the different rates of emergence of resistance in different agroclimatic zones

are complex but are thought to be due to the proportion of the total population left *in refugia* (i.e. unexposed to treatment), the number of generations and biotic potential of the parasite species involved. Resistance has been reported most frequently in *Haemonchus* spp. and *Trichostrongylus* spp. in tropical and subtropical regions, and in *Teladorsagia* in temperate regions [7, 8]. Nevertheless, although the rate of emergence of resistant isolates has been slower in temperate regions, there are increasing reports of resistant populations of nematodes in the UK [9] in sheep [10–14], goats [14, 15], cattle [16] and horses (17).

Surveys in the UK during the 1990s showed that nematodes resistant to the BZ anthelmintics were present on 24 per cent and over 40 per cent of sheep farms in Scotland and England, respectively. A more detailed study in south-west England showed that 44 per cent of farms harboured BZ-resistant nematodes. In 2000, a survey conducted in Scotland showed that over 60 per cent of the farms had BZ-resistant *Teladorsagia* and that on lowland farms the prevalence was over 80 per cent. Following the first reports of multiple anthelmintic resistance in sheep in the UK [12, 13] a small-scale survey in Scotland showed that over 30 per cent of the farms had evidence of ivermectin resistance [18]. Once resistant worms are present on a farm, for all practical purposes they can be considered as permanently established, as there is very little reversion to susceptibility in highly selected homozygous isolates [8]. Therefore, it is important to be able to detect the presence of emerging resistant isolates at an early stage. Unfortunately, the methods currently available – FECRT, egg hatch assay, larval development and larval inhibition assays – are relatively insensitive and are often only used to detect resistant nematodes when resistance genes are relatively common within the population.

Strategies to delay the development/ transmission of anthelmintic resistance

The realization that intensive chemoprophylaxis is not a sustainable option for the control of gastrointestinal nematodes has led to the development of a number of different approaches that can be used to reduce the rate of selection of resistance and its transmission through animal movement. Measures intended in the short term to reduce the selection and transmission of resistance have recently been described under the acronym SCOPS, Sustainable Control of Parasites in Sheep [5].

Three strategic approaches to reducing the rate of selection of resistance can be considered.

1. Reduce the frequency of treatment

Adopt a strategic dosing programme that minimizes the number of parasite generations exposed to a particular drug by treating at ecologically critical periods based on a sound knowledge of the epidemiology of the parasite species. On many farms, this may be feasible by adopting integrated grazing strategies to provide 'low-risk' pastures. Monitoring in its broadest sense, using egg counts in conjunction with information on previous grazing history, stocking densities, age and susceptibility of the stock, etc. can play a vital role in helping to reduce treatment frequency. Faecal egg counts can obviously provide information that enables treatments to be directed towards the key parasite(s) requiring chemoprophylaxis. In low-risk situations where susceptible stock are not subject to high challenge from pasture it may be possible to use faecal egg counts to indicate when to treat animals. Faecal egg counts also provide useful information on the contamination being laid down by grazing stock and hence an indication of the potential risk associated with the pasture. However, in high-risk situations where susceptible stock are exposed to high levels of challenge, such as *Nematodirus* infections in spring, it may not be possible to use faecal egg count monitoring to provide treatment timings since evident effects on performance may precede significant egg counts. Under these circumstances, treatment timings will be based on grazing history, previous occurrence of disease or drug resistance and all of the various factors that can be used to provide an assessment of the risk posed by the pastures being grazed.

2. Optimize the efficacy of anthelmintic treatment

It is important to calibrate the drenching equipment and also to weigh representative groups and classes of livestock and treat for the heaviest animals in the group. Slight overdosing is better than undertreatment. Anthelmintic resistance to a particular drug is more likely to develop if it is used consistently for a long period. Annually alternating the three main families of broad-spectrum anthelmintics, which have different modes of action, may delay the onset of

resistance, but recent modelling evidence predicts that changing the family each year may not confer any benefit over a change at less frequent intervals. The reason for using anthelmintics from different families is that resistance to one compound usually confers side- or cross-resistance to other drugs in the family. Recent research has shown that withholding feed for 24 hours prior to treatment will maximize the efficiency of orally administered benzimidazole or 3-AV [19], because a reduced rumen content slows the passage of the drug and prolongs bioavailability. Such practices should not be applied to ewes in late pregnancy where there is a risk of pregnancy toxaemia. Administering the anthelmintic over the tongue or using low-volume formulations of drugs will reduce the occurrence of closure of the oesophageal groove, assist deposition of drug in the rumen and maximize bioavailability.

3. Maintain biosecurity

Although on-farm selection is important, animal movement plays a key role in increasing the prevalence of resistant nematode populations. At present, most of the resistant populations in the UK and Europe are refractory to the 1-BZ group of drugs and therefore a sensible quarantine policy is to treat all sheep brought on to the farm with antelmintics from the other two families (2-LM and 3-AV). Since such combinations are not available at present in Europe the drugs should be administered sequentially and not mixed together prior to administration. Wherever possible, treated animals should be withheld from pasture for 24–48 hours to reduce contamination of grass with nematode eggs. Following quarantine-drenching animals should not be turned out on to 'clean' grazing since it will carry very few larvae and thus any 'resistant' larvae developing from eggs deposited by worms that have survived the treatment will not be 'diluted' by the resident population *in refugia*. Where fluke is endemic, additional quarantine treatments using an effective flukicide may be required.

FUTURE CONTROL OF NEMATODE INFECTIONS

Chemotherapy and grazing management will continue to form the major strategies for parasite control with a very limited number of new chemical compounds with novel modes of action becoming available in the next decade. Commercial emphasis will be directed towards formulation and delivery diversification. Control strategies will need to balance efficiency against the longer-term aim of conserving the effectiveness of the new anthelmintics. Current research is also focused on targeting treatments within a flock to those animals that most require them, thus helping to maintain an increased worm population *in refugia*. There is increasing research interest in non-chemotherapeutic sustainable approaches to parasite control such as vaccination [20], biological control of the free-living stages on pasture using nematophagous (predacious) fungi [21], improving the resilience of the host to infection through supplementation with rumen by-pass protein or urea/molasses feed blocks [1], genetic selection for resistant hosts [22] and the use of bioactive forages [23]. It is envisaged that, wherever appropriate, these novel approaches will eventually form components of integrated control strategies, minimizing reliance upon chemoprophyaxis and thus helping to conserve the efficacy of the currently available anthelmintics.

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28

Liver fluke

G.B.B. Mitchell

S*ynonyms*: ovine fasciolosis, 'pokey jaw', liver rot

Fasciola hepatica is the most important trematode of domestic ruminants in all countries where environments suitable for the intermediate snail host prevail. The parasite poses a major threat to animal welfare and causes substantial economic losses through mortality, ill-thrift, condemnation of livers at the abattoir, predisposition to other diseases, treatment and associated veterinary costs. In Britain, ovine fasciolosis has increased dramatically in recent years [1].

The risk of liver fluke disease is closely linked to summer rainfall which favours fluke development and provides an optimum habitat for the intermediate host, the dwarf pond snail *Lymnaea truncatula.* Acute disease has long been associated with high mortality

and sudden deaths in sheep flocks, while chronic disease causes anaemia, ill-thrift and poor production.

CAUSE

Fasciola hepatica, a member of the class Trematoda, sub-order Digenea, family Fasciolidae, is the most important digenetic trematode (flatworm or fluke) and occurs wherever climatic conditions favour the intermediate host, the dwarf pond snail *Lymnaea truncatula*. The parasite is of major veterinary importance in ruminants, but can infect most mammals, including humans. Adult flukes (see Figure 28.1 in the colour plate section) are leaf-shaped, hermaphrodite trematodes with distinct 'shoulders', measure 20–30 mm in length, and possess an oral and a ventral sucker for attachment in their predilection site, the hepatobiliary tree. Immature flukes from 2 mm in length are found in the liver parenchyma and the abdominal cavity of infected animals. *F. hepatica* eggs passed into the intestine via the bile ducts are distinctively large, golden brown/yellow when fresh, operculate and typically measure 150 × 90 μm, i.e. roughly twice the size of a roundworm (trichostrongylid) egg.

Life cycle

Fluke eggs passed in the faeces of a mammalian host develop and hatch to release motile ciliated miracidia (Figure 28.2). This process takes 9 days at optimal temperatures of 22–26°C. Development at lower temperatures takes longer and will not occur below 10°C. The miracidia, which usually hatch in large numbers over a few hours, have a short lifespan and must come into contact with their intermediate host, the dwarf pond snail, *L. truncatula*, within 3 hours if successful penetration of the snail hepatopancreas is to occur. In infected snails, miracidia develop through sporocyst and redial stages to the final stage, the cercaria. Under suitable conditions of temperature and moisture these are shed *en masse* from the snail as motile forms and attach themselves to firm surfaces, such as grass blades, where they encyst to form the infective metacercariae. It takes a minimum of 6–7 weeks to complete development from miracidia to metacercariae, but under unfavourable circumstances several months may be required. Within the snail one miracidium can produce over 600 metacarcariae.

Metacercariae ingested by the sheep excyst in the small intestine, migrate through the gut wall, cross the peritoneum and penetrate the liver capsule. The young flukes tunnel through the liver parenchyma for 6–8 weeks then enter the small bile ducts where they mature in about 4 weeks during which time they migrate to the larger ducts and, occasionally, to the gallbladder. The period from uptake of metacercariae to the presence of fluke eggs in the faeces is 10–12 weeks and therefore the minimum period for completion of one entire life cycle of *F. hepatica* is 17–19 weeks.

The disease

The number of outbreaks of ruminant fasciolosis in Britain has increased steadily in recent years. In Scotland, which enjoys a relatively mild, wet climate that creates a suitable environment for the intermediate host in many areas, the increase has been dramatic with unprecedented numbers of animals affected in 2002–3 (Figure 28.3). In sheep, as well as mortality and ill-thrift, fasciolosis has been associated with a variety of diseases including black disease due to *Clostridium novyi,* parasitic gastroenteritis and metabolic diseases.

CLINICAL SIGNS

Clinical fasciolosis in sheep is usually classified as acute, subacute or chronic, according to the number and stage of flukes present but there is considerable overlap between these categories. Critically, an outbreak of fasciolosis is a flock problem, even though only a few individuals may be showing typical clinical signs at any one time, and therapy must always be considered on this basis.

Acute fasciolosis

Outbreaks of acute fasciolosis are usually seen in late autumn and early winter, but in wet years may extend well into spring, and are associated with the presence of large numbers of immature flukes in the liver parenchyma. Acute disease results from simultaneous

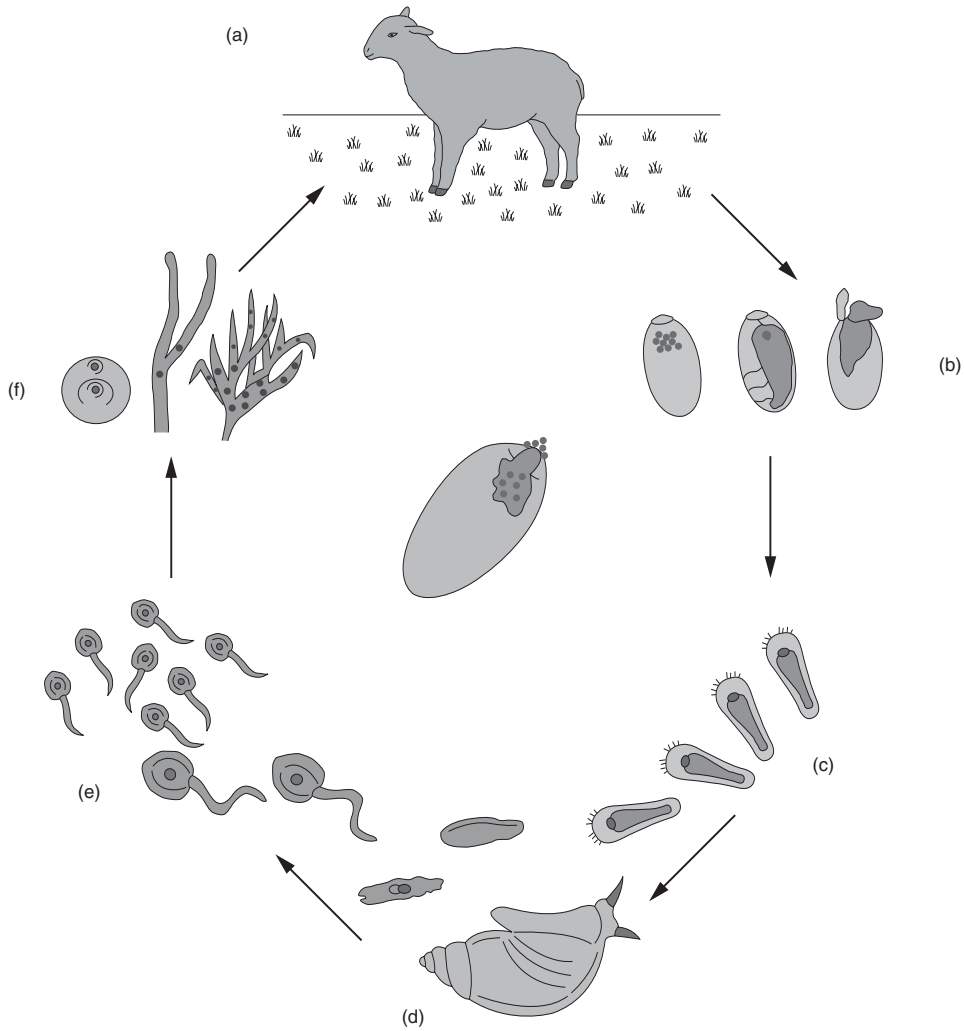

Figure 28.2: Life cycle of *Fasciola hepatica*: (a) sheep infected by metacerceriae on wet pasture, (b) eggs laid on pasture, (c) miracidia hatch, (d) miracidia find mud snail and multiply in it, (e) large numbers of cerceriae emerge from snail and (f) metacerceriae encyst on grass blades. The full cycle takes 4–5 months.

development of large numbers of immature flukes following ingestion of large numbers of metacercariae over a short period from very heavily infected pasture, or from prolonged migration of flukes in sheep previously exposed to the parasite. In both cases, acute haemorrhagic anaemia and hypoalbuminaemia will result, with sudden death occurring in animals in which sufficient numbers of flukes are present. On examination of the remainder of the flock, animals may be weak, with pale muscosae and, in some cases, exhibit abdominal pain with a palpably enlarged liver.

Subacute fasciolosis

Like the acute form of the disease, subacute fasciolosis occurs from late autumn to spring and also presents as an acute haemorrhagic anaemia with eosinophilia and hypoalbuminaemia. However, subacute fasciolosis

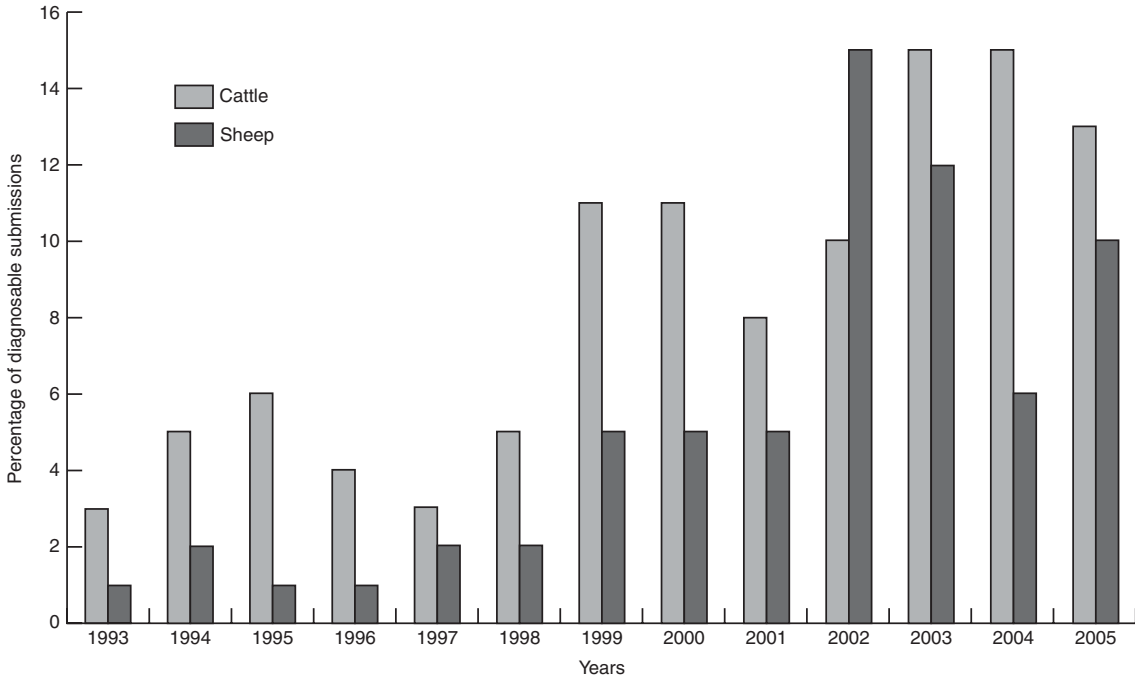

Figure 28.3: Outbreaks of fasciolosis in Scotland 1993–2005 as percentages of diagnosable submissions.

is not so rapidly fatal and affected sheep may show clinical signs for 1–2 weeks prior to death. Large numbers of immature flukes will be present in the liver parenchyma, although not in quite the numbers seen in acute fasciolosis. However, the parasites will have developed further and a substantial proportion of the population will be present as adults in the major bile ducts. This form of the disease may occur when sheep have ingested large numbers of metacercariae over a longer period of time or the number ingested at any one time has not been sufficient to cause the acute form of the disease.

Affected sheep lose condition rapidly, become markedly anaemic with obvious pallor, and may have a palpably enlarged liver and resent abdominal palpation. Submandibular oedema (bottle jaw) (Figure 28.4) may be present in some cases.

Figure 28.4: Subacute fasciolosis: bottle jaw [1]. (Courtesy of Novartis Animal Health.)

Chronic fasciolosis

Chronic liver fluke disease, seen most frequently clinically in the winter and spring, is characterized by weight loss, anaemia, eosinophilia and hypoalbuminaemia and is due to the blood-sucking activities of adult flukes in the biliary tree. In severe cases, submandibular oedema is prominent and terminal diarrhoea may occur. Concurrent infections with the

abomasal nematode *Teladorsagia* (*Ostertagia*) *circumcincta* may complicate the clinical picture. Liver fluke may also be responsible for subclinical disease, with only minor haematological and biochemical changes which, however, will result in lowered productivity reflected in inadequate food conversion rates, poor carcass formation and reduced milk production. Increasing evidence suggests that fasciolosis may predispose ewes to a variety of metabolic diseases around lambing time, including hypocalcaemia and pregnancy toxaemia.

PATHOLOGY

The pathogenic effects of *F. hepatica* on the host are exerted in various ways; during the migratory phase in the liver, parenchymal destruction is caused by the direct activity of the tunnelling fluke. The host inflammatory response results in cellular infiltration of the hepatic parenchyma which, in heavy infections, terminates in widespread fibrosis [2]. Should the migratory flukes enter any of the larger hepatic blood vessels, rupture with ensuing severe haemorrhage may occur. In the bile duct the adult fluke is an active blood-sucker and if sufficient numbers of flukes are present severe anaemia results.

Black disease (infectious necrotic hepatitis) may result from the activation and proliferation of the soil-borne toxigenic bacterium *Clostridium novyi*, hitherto dormant in the liver, in response to the anaerobic conditions produced by migrating flukes. Sudden or rapid death due to generalized toxaemia, may occur, with limited necrotic foci evident in the liver parenchyma and rapid autolysis of the carcass, despite very limited fluke damage to the liver in some cases (see Figure 28.5 in the colour plate section).

Necropsy of a case of acute fasciolosis reveals an enlarged haemorrhagic liver with a parenchyma severely damaged by the tracts of migrating flukes (see Figure 28.6 in the colour plate section). Large subcapsular haemorrhages may be present and a variable amount of sanguineous fluid containing immature flukes is evident in the abdominal cavity. The flukes recovered by expression from slices of liver tissue in cases of acute fasciolosis are generally only 5–8 mm long, indicating an infection of about 7–8 weeks' duration and there may be 800–2500 flukes in an affected liver.

In subacute fasciolosis fewer flukes are present in the liver and a substantial proportion present as adults in the major bile ducts.

The pathology of chronic fasciolosis is associated with adult flukes feeding in the biliary tree causing progressive anaemia and cachexia. In severe cases hepatic changes are characterized by extensive fibrosis and biliary thickening and may be accompanied by peritonitis. Parasitic abomasitis associated with *Teladorsagia circumcincta* may be a complicating factor.

DIAGNOSIS

Acute/subacute fasciolosis

A clinical history of sudden or acute deaths of sheep grazing wet pasture usually from September until December is suggestive of acute/subacute fasciolosis. In wet years outbreaks may continue from January until late spring. Confirmation is by post-mortem examination of fresh carcasses, gross pathology, and recovery and measurement of immature flukes. Histopathology may also be useful especially if black disease is suspected and hepatocellular damage is limited.

The salient features of the clinical pathology in cases of subacute fasciolosis are severe haemorrhagic anaemia with peripheral eosinophilia, and severe hypoalbuminaemia. Since the flukes are still immature no eggs are present in the faeces of affected sheep.

Chronic fasciolosis

This form of the disease normally occurs from late winter onwards with a clinical history of ill-thrift, progressive anaemia and diarrhoea in some cases. Confirmation of diagnosis is by post-mortem examination, clinical pathology and/or demonstration of *F. hepatica* eggs in faecal samples.

Liver enzymes

Elevation of aspartate aminotransferase (AST) and glutamate dehydrogenase (GLDH) can be useful for the diagnosis of acute disease as early as 2–3 weeks post-infection, while raised AST and gamma glutamyl transferase (GGT) levels can indicate chronic

disease once adult flukes are present in the biliary tree and may provide useful prognostic indicators.

Haematology

Haematology to confirm haemorrhagic anaemia can be useful in subacute disease and demonstration of peripheral eosinophilia has been used as an early indicator of fasciolosis.

Serology

Various serological techniques, including enzyme-linked immunosorbent assay (ELISA), can be used to detect antibodies to *F. hepatica* with a high level of specificity (>90 per cent). The test currently used for diagnosis by the UK Veterinary Laboratories Agency (VLA) and SAC Veterinary Services, based on detection of antibodies to excretory/secretory (ES) antigens of *F. hepatica* in serum, indicates only previous exposure to the parasite and does not provide information on current infection or the immune status of an animal.

EPIDEMIOLOGY

The extent of the habitat of the dwarf pond snail *L. truncatula*, which feeds mainly on algae, relates to climatic conditions and soil hydrology. Ideal conditions for survival and multiplication of snails include a slightly acidic environment and a slow-moving water medium to carry away waste products. Permanent habitats therefore include the banks of ditches or streams and the edges of small ponds. Following heavy rainfall or flooding, temporary habitats may be provided by hoofmarks, wheel ruts or rain ponds. Fields with clumps of rushes are common sites as these have a slightly acidic pH favoured by *L. truncatula.* The wet conditions required for snail breeding and also for *F. hepatica* development within the snails are achieved when rainfall exceeds transpiration. Such conditions also facilitate the development and hatching of *Fasciola* eggs, the search for snails by miracidia and the dispersal of cercariae after shedding from snails. A mean day/night temperature of 10°C or above is necessary for snail breeding, the development of *F. hepatica* within the snail, and the development and hatching of fluke eggs. As the mean day/night temperature increases during late spring and early summer, the development time for the stages of the liver fluke outside the final host (the suprapopulation) becomes shorter, with a minimum of 5 weeks in mid-summer.

The minimum temperature requirements for the development of a suprapopulation of *F. hepatica* normally prevail in the UK only from April to October, with relatively minor variations.

As a result, the main factor influencing the magnitude of the snail populations, and therefore the prevalence of fasciolosis, is summer rainfall. In favourable conditions one snail can produce several thousand descendants. Climatic factors dictate that, in the UK, most snails become infected in the summer by miracidia developed from eggs deposited in the spring and early summer which take a minimum of 5 weeks to develop to cercariae, resulting in increased pasture levels of metacercariae from late August onwards. Uptake of the infective stages results in clinical disease in sheep normally from September onwards. The higher prevalence of fasciolosis in recent years in sheep and cattle has been largely associated with milder, wetter weather. Increased rainfall raises the water table, thereby permitting *L. truncatula* to extend its habitat, while milder temperatures prolong the development period available. This has resulted in spread of the disease to previously unaffected regions. In Scotland, for example, this has been reflected in the spread of the disease from poorly drained pastures in the west to previously unaffected farms in the east of the country.

In climatically favourable years outbreaks of fasciolosis may continue into the spring and summer as the mild wet conditions permit overwintering of fluke eggs, metacercariae and snail survival.

While the foregoing account reflects UK seasonality, similar patterns occur in other regions.

TREATMENT, CONTROL AND PREVENTION

Control and prevention of fasciolosis using control measures integrated with a forecasting system is preferable to treatment of affected animals, when animal welfare may be compromised and economic

loss incurred. Control measures should be part of a flock health plan and have two main objectives:

- to improve animal welfare by eliminating flukes from animals
- to reduce the population of infected snails.

Control should limit the availability of *F. hepatica* eggs and, therefore, of miracidia to snail populations. Before snail control is undertaken an assessment of the control area for snail habitats should be made as this may be localized or extensive. Drainage is the best long-term method for permanent eradication of extensive snail habitats but may prove prohibitively expensive. Where snail habitat is localized, pasture management, e.g. fencing off wet areas or avoiding grazing during periods of high risk, should reduce infection. On many affected farms this is not practical and control measures rely heavily on the use of flukicides.

Flukicides

In recent years, the choice of licensed flukicides has been reduced. Most are effective against adult flukes but activity is variable against the immature stages. It is therefore extremely important to consult individual datasheets for product efficacy before treating animals, particularly in the autumn when immature flukes may predominate. Combined fluke and worm products may not be suitable if immature flukes are present, and there is evidence that acute and subacute disease due to immature flukes may occur from August until May of the following year. Specific control measures for fasciolosis are required, tailored to individual farm needs, in addition to a strategic programme for the control of gastrointestinal helminths, an important part of a health plan drawn up with veterinary consultation. Advice for farmers on flukicide usage, particularly with regard to product and dosage frequency, should take account of published forecasts for the year, previous farm history, abattoir returns if available and faecal monitoring. Advice must also consider the possibility of flukicide resistance.

Flukicide resistance

Since 1995, triclabendazole (TCB)-resistant flukes have been recorded in sheep in Ireland, Scotland, Wales and England [3]. If signs persist following treatment, faeces samples should be checked for fluke eggs 3 weeks after treatment. If fluke eggs are still present, affected animals should be treated with an alternative flukicide. Casualty animals should be examined at post-mortem and investigations conducted on ill-thrifty animals.

Quarantine treatment for fasciolosis

Quarantine treatments are vital to avoid the introduction of flukes to uninfected farms having habitats that could support the intermediate snail host. Such treatments should also reduce the spread of TCB-resistant flukes. Animals (including cattle) brought on to farms where fluke transmission is possible, should be treated with a product effective against immature flukes and kept off snail-contaminated pastures for 4 weeks. If TCB resistance is suspected sequential treatment with two products effective against immature flukes should be carried out. Follow-up treatment may be required 6–8 weeks after the initial treatment.

Control and prevention

Recently published UK government guidelines on fluke control expressed concern about the widespread use of combination 'fluke and worm' products for the control of liver fluke [4]. The guidelines suggested that this often resulted in mistimed fluke treatments and could lead to extensive, inadvertent use of anthelmintics and unnecessary additional selection pressure on nematode populations likely to increase the risk of anthelmintic resistance. Consequently, use of these products should be discouraged and flukicides used as part of a specific fluke control strategy. On fluke-infested farms all animals should be vaccinated against black disease using a multivalent clostridial vaccine as unvaccinated animals are at risk even when fluke challenge is minimal.

Monitoring

Monitoring is a key feature of control and flocks should be checked for the presence of flukes before flukicides are used, unless they are known to be

infested and monitoring is conducted at regular intervals. This should include post-mortem examination of casualty animals, essential for diagnosis of acute/subacute disease, and investigation of ill-thrifty animals by blood and/or faecal sampling and regular checks on fluke egg counts.

Treatment guidelines

Fasciolosis should always be approached as a flock problem as, unlike cattle, sheep do not develop a functional immunity to the disease and reinfection on wet pasture is likely. Therefore, all animals at risk should be treated. The following gives a guide to treatment frequency, but the risk of flukicide resistance and the importance of monitoring and quarantine treatments should be borne in mind.

Where fluke is present on a farm, a flukicide active against immature forms should be used in October, or earlier if a high prevalence of disease is forecast following a wet summer, and again in January if faecal egg counts indicate a need. A flukicide with activity against adults only should be given, if indicated by monitoring, to all animals at risk in May to June. Flukicides effective against immature flukes should be rotated annually for autumn/winter treatments. Additional treatments between October and January may well be necessary in wet years. As deer and rabbits act as fluke reservoirs, fluke eradication is not possible where the snail habitat exists.

Checklist to investigate and control fluke disease

1. Incorporate a dedicated fluke control programme in the flock health plan based on veterinary advice and review its effectiveness annually.
2. Investigate all cases of ill-thrift by post-mortem examinations, fluke egg counts and/or blood tests.
3. Monitor fluke levels regularly by having fluke egg counts carried out on sheep and cattle.
4. Ask abattoir for details of fluke infection in animals going for slaughter.
5. Check data sheet of flukicides, especially for effectiveness against immature flukes.
6. Treat all cattle and sheep at risk with a flukicide capable of removing immature and adult flukes in October and January.
7. In some years cattle and sheep may need additional treatments between October and January (see 'Forecasting' below), seek veterinary advice if unsure.
8. Depending on monitoring results animals at risk may require treatment in May to June with a product capable of removing adult flukes.
9. Ensure all animals at risk are vaccinated against black disease.
10. Give quarantine treatments to all purchased or away-wintered animals.
11. If TCB resistance is suspected check faeces for fluke eggs 3 weeks after treatment.

Forecasting

The risk of severe outbreaks of fasciolosis increases following wet springs and summers. Forecasting systems, such as the Stormont 'wet day' forecast [5] and the 'Mt' systems [6], based on rainfall and evaporation have been developed. In addition, geographic information systems (GIS) using sophisticated computer models which simulate the life cycle of *F. hepatica* and incorporate climatic, geographic and soil hydrology data are being introduced to predict the likely incidence and severity of fasciolosis. Accurate forecasts can normally be made by the end of the summer, but 'early warnings' may also be publicized in the press (e.g. if May and June have been exceptionally wet) to enable control measures to be instituted. Monthly disease surveillance reports for Scotland, England and Wales, published in the *Veterinary Record* incorporate information on fluke disease in the UK.

VACCINE DEVELOPMENT AND OTHER AREAS OF RESEARCH

Research in recent years has focused on the pathophysiological changes accompanying fasciolosis, diagnostic techniques and vaccine development. Recombinant technology has been used to produce a cathepsin L-like protease, an excretory product of *F. hepatica* believed to play an important role in tissue degradation and so facilitate parasite migration. Cathepsin L has been used to develop a highly specific ELISA which enables *F. hepatica* infection to be

detected 5 weeks after infection in sheep and cattle. The same product has been used with various adjuvants in vaccine trials in which a protective efficacy of 79 per cent was achieved in sheep [7, 8]. The vaccine also exhibited effects on the fecundity of parasites remaining in vaccinated animals and could therefore also affect transmission to the intermediate host.

OTHER TREMATODES OF SHEEP

Whilst *F. hepatica i*s the most important trematode of sheep, in some areas of the world two other species can assume importance, and it is pertinent to include a brief account of them.

Fasciola gigantica

This fluke is also a member of the sub-class Digenea, family Fasciolidae, genus *Fascioloides*. It has a morphology similar to *F. hepatica* but is larger: 5–7 cm long by approximately 1.2 cm wide. Characteristically, it has less well-defined shoulders and long straight sides in comparison with *F. hepatica.* Its distribution includes Africa, Asia, southern USA, Spain, southern Russia and the Middle East. The egg is larger than that of *F. hepatica*. The most important intermediate host is *Lymnaea auricula*. The biology of this snail and of the others that can act as intermediate hosts of *F. gigantica* is similar to that of snails associated with *F. hepatica*.

The life cycle of *F. gigantica* is similar to that of *F. hepatica*, although the different phases are longer, and it has a closer association with water. The prepatent period is 13–16 weeks. The pathogenesis of this fluke is similar to that of *F. hepatica*. In sheep, both the acute and chronic forms occur. Control measures based on those applicable to *F. hepatica* infection are recommended.

Dicrocoelium dendriticum

This trematode is a member of the sub-class Digenea, family Dicrocoeliidae, genus *Dicrocoelium*. It has a global distribution [9] and, in the UK, is important only in the islands off the west coast of Scotland. It is a small lanceolate fluke approximately 1.2 cm long by 0.25 cm wide. The egg is small ($45 \times 30\,\mu m$), dark brown, with an operculum and, characteristically, the egg has one flat side. When passed in the faeces, it already contains miracidia. This fluke does not require water. The egg is ingested by various species of land snail, then the miracidium emerges to reach the hepatopancreas of the snail. Two generations of sporocysts are produced from which cercariae, in masses held in a form known as slime-balls, are expelled by the snail. This phase takes approximately 3 months. Thereafter, further development is achieved when the second intermediate host, an ant of the genus *Formica*, ingests the slime-ball. Whole ant colonies can become infected in endemic areas. In the ant, the cercariae migrate to the abdominal cavity to become metacercariae. Some cercariae migrate to the central nervous system of the ant, and this aberrant form makes the ant climb up herbage, increasing the availability to grazing animals. Ingestion of the ant releases the metacercariae, which then migrate to the liver via the bile duct where, spreading through the biliary system, they pass throughout the liver.

Very large numbers of these flukes can be acquired by individual sheep. Diagnosis requires post-mortem examination or demonstration of the typical eggs in faeces samples. Control cannot be achieved in a manner similar to *F. hepatica* because snail sites cannot be adequately defined, and anthelmintics used for *F. hepatica* are not usually effective unless at dosage rates considerably larger than those employed with *F. hepatica*. It has been reported that albendazole, at dosage rates of 20 mg and 15 mg/kg, is safe and efficacious against *D. dendriticum* in sheep whilst, in separate work, two doses of 10–12 mg/kg given a week apart reduced faecal egg counts by about 90 per cent.

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Part VI
Diseases of the respiratory system

29

Acute respiratory virus infections

J.M. Sharp and P.F. Nettleton

The acute respiratory disease complex of sheep is dominated by pneumonic pasteurellosis caused by *Mannheimia haemolytica*. Although several viruses have been isolated from sheep with acute respiratory illness, it is unlikely that a syndrome occurs in the field that can be attributed solely to virus infection. Nevertheless, available evidence indicates that some viruses may be involved in the aetiology of acute ovine respiratory disease. For example, viruses have been isolated from a high proportion of outbreaks of acute illness and also have been closely related to high levels of pneumonia in slaughtered lambs. Several studies have suggested that mainly parainfluenza virus type 3 and adenoviruses are involved, whereas the roles of other viruses, such as respiratory syncytial virus and reovirus, are less clear.

PARAINFLUENZA VIRUS TYPE 3 (PI3)

PI3 is an enveloped single-stranded negative sense RNA virus that matures by budding from the surface of infected cells. It contains six polypeptides ranging in mass from 34 to 88 kilodaltons (kDa). The two major glycoproteins, which are present as short spikes on the envelope, are HN (73 kDa), responsible for haemagglutination and neuraminidase activity, and F (51 kDa), responsible for cell fusion and haemolysis. These two glycoproteins comprise about 15 per cent of the virus and appear to be necessary for stimulation of immunity. There is only one serotype of ovine PI3, which is antigenically related to, but distinct from, bovine and human strains of PI3. The distinctness of ovine PI3 virus has been further demonstrated by genomic sequencing of the F gene [1].

Clinical signs

PI3 virus is associated with a range of illnesses in sheep. Most infections are inapparent or mild, but outbreaks of acute illness associated with the presence of PI3 virus have been recorded, in which there is a high morbidity. Affected animals usually are afebrile, may cough frequently, and have a copious serous nasal and sometimes ocular discharge. The illness that follows experimental inoculation largely depends on the route of inoculation. Intranasal instillation or aerosol exposure of lambs with PI3 virus results in viral replication in the upper respiratory tract without clinical signs, whereas combined intranasal and intratracheal inoculation causes a severe respiratory illness 3–7 days after inoculation, characterized by pyrexia, tachypnoea, dyspnoea and dullness lasting 3–5 days.

Pathology

Experimental inoculation with PI3 virus can result in linear or patchy areas of dull, red consolidation in the apical lobes of the lungs (Figure 29.1). These lesions, which are most extensive 6–8 days after inoculation, show histological features of hyperplasia of the bronchiolar epithelium, infiltration of interalveolar septa by mononuclear cells and cellular exudate in the bronchiolar lumen. Acidophilic intracytoplasmic inclusion bodies may be detected in the bronchiolar epithelium up to 6 days after infection. These lesions resolve fairly quickly, although a residual interstitial pneumonia

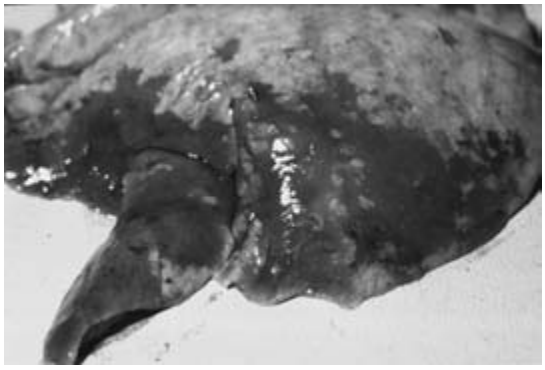

Figure 29.1: Lungs of specific pathogen-free lamb infected with ovine PI3 virus and killed 5 days later. Extensive consolidation is evident in apical, cardiac and diaphragmatic lobes.

and focal alveolar epithelialization persists for at least 28 days after inoculation. Resolution of acute lesions in the lungs is complete by 75 days post-infection.

Diagnosis

PI3 virus infection can be confirmed by isolation of the virus from swabs or aspirates taken from the upper respiratory tract during the first 6 days of infection, which usually coincides with the presence of a copious serous nasal discharge, or from pieces of tissue from the respiratory tract. Immunohistochemical fluorescent antibody tests and reverse-transcriptase-polymerase chain reaction (PCR) also can be used to demonstrate the presence of virus [1, 2]. A rise in serum antibody titre may be detected in paired sera by either the enzyme-linked immunosorbent assay (ELISA) or haemagglutination inhibition (HAI) test. However, there are limitations to this approach, as infections by PI3 virus can occur in the absence of an apparent rise in serum antibody titre.

Epidemiology

PI3 infections of sheep occur globally, and researchers in many countries have reported the presence of antibodies in the sera of both pneumonic and healthy sheep [3]. It appears that such infections are common, as the proportion of sheep with antibody is rarely less than 70–80 per cent. Most lambs acquire colostral antibodies to PI3 virus, and infections are rarely detected while these are present. However, these antibodies wane quickly and the lambs become susceptible to infection with PI3 virus, so that most become infected within the first 12 months, although outbreaks have been detected in adult sheep up to 5 years of age. The method by which the virus is maintained in the flock is uncertain. It seems likely that virus may continue to circulate even among immune animals and some become persistently infected, even in the presence of an immune response. Although most infections with PI3 virus pass unnoticed, field observations indicate that the virus may predispose sheep to infection by bacteria, notably *M. haemolytica*. These observations have been borne out by experimental findings, which have shown that prior infection of lambs with PI3 virus exacerbates disease induced by *M. haemolytica*. The clinical illness and lesions produced by combined infection with PI3 virus and *M. haemolytica* are identical with those observed in naturally occurring pneumonic pasteurellosis, and lesions in surviving lambs persist for at least 8 weeks after infection.

Control

PI3 virus is probably involved in a proportion of outbreaks of acute respiratory disease, many of which also involve *M. haemolytica*. Some means of control would be desirable and this might be best achieved by incorporating PI3 virus in any vaccines that are designed for the prophylaxis of pneumonic pasteurellosis.

Immunity to PI3 virus can be stimulated experimentally by local or parenteral administration of PI3 antigens, which will prevent virus replication, clinical illness and pneumonic lesions. Such immunity has the advantage of being able to reduce the extent of the pneumonic lesions produced by combined infection with PI3 virus and *M. haemolytica*. This effect could supplement the effect of other components in any vaccine directed against *M. haemolytica*. Field trials in New Zealand and the UK of live attenuated PI3 virus vaccines, administered intranasally, have indicated that such procedures may reduce the prevalence of pneumonia [3].

ADENOVIRUSES

Adenoviruses are unenveloped icosahedral viruses comprising 252 capsomeres. Each capsomere at the 12 vertices has a filamentous projection, which is

involved in the haemagglutination exhibited by some serotypes. The isolates of ovine adenovirus possess the group-specific antigen that is common to all mammalian adenoviruses. In addition, seven distinct serotypes of ovine adenovirus (OAdV) have been determined besides many untyped isolates. Recent sequencing and phylogenetic analysis have indicated that ovine adenoviruses belong to two different genera. Five, plus one tentative species, are classified in the genus mastadenovirus but ovine adenovirus 7 and an Australian isolate (OAV287) are included in the new genus atadenovirus [4, 5].

Clinical signs

Adenoviruses have been isolated from sheep with a variety of clinical conditions ranging from apparently healthy to severe pneumoenteritis and acute illness [6], although most isolates have come from apparently healthy lambs. The clinical illness following experimental inoculation of lambs with adenoviruses appears to be related to the individual serotypes and, in some cases, strains within serotypes. OAdV types 1–4 appear to cause little illness, although the Hungarian isolate of OAdV-1 induces anorexia, sneezing and pneumonia. Infection with OAdV-5 or bovine adenovirus 7 (BAdV-7) results in nasal discharge and pyrexia, and OAdV-6 causes mild upper respiratory tract illness with possible central nervous system involvement.

Pathology

Lesions have been found in lambs that have been experimentally infected with serotypes that cause clinical illness, that is OAdV-1, OAdV-5, OAdV-6, BAdV-2 and BAdV-7. The apical and cardiac lobes of the lungs from such lambs show atelectasis and dull, red consolidation of varying extent, which may last for longer than 14 days. The bronchial and mediastinal lymph nodes can be enlarged. The principal histological change appears to be a proliferative bronchiolitis with an associated exudate of desquamated epithelial cells, macrophages and lymphoid cells. The bronchiolitis induced by some serotypes may extend into alveoli to form a bronchopneumonia. These lesions appear to be most extensive about 7 days after inoculation, and regress by day 14–21. A particular feature of experimental infection by OAdV-6 is cytomegaly and karyomegaly of bronchiolar epithelial cells, which also has been reported in lambs with a naturally occurring pneumonia attributed to adenovirus. Some serotypes induce lesions in organs other than the respiratory tract, such as focal hepatic necrosis and lymphangitis by OAdV-4, and nephritis by OAdV-5. Adenovirus inclusions have been reported in the intestine of lambs with a necrotizing enteritis [7] and OAdV-7 was demonstrated in multiple organs of lambs that succumbed to an acute illness [6].

Diagnosis

As all ovine adenoviruses possess the mammalian group-specific antigen, serological tests can be used to detect antibodies to this antigen. The complement fixation (CF) or agar gel immunodiffusion (AGID) tests are convenient and rapid to perform but have disadvantages. The immune response on which they depend takes at least 4–5 weeks to develop, and they are less sensitive and less specific than microneutralization or HAI tests. However, these latter tests, although more sensitive, are not used routinely because they are specific for each serotype. Isolation of the virus is an important adjunct to serology as a means of diagnosis. It is unlikely that the number of ovine adenoviruses identified is complete, and in any unisolated serotype, as with many bovine serotypes, the common group antigen or a minor component may be absent. Infection by such serotypes would therefore go undetected if serology were the sole means of diagnosis.

Epidemiology

Antibodies to the adenovirus group antigen have been detected in sheep sera in many countries, indicating the wide distribution of this virus group. Serological and virological studies in a few countries have indicated that such infections are common, particularly in young lambs. The prevalence of antibodies to individual serotypes varies between 20 and 70 per cent, most infections occurring before the lambs are 1 year old. Surveys of flocks have shown that few viruses could be isolated whilst colostrum-derived antibodies were high but, when the majority of the flock became susceptible, adenoviruses could be isolated frequently from the faeces and sometimes the upper respiratory tract. The widespread occurrence of adenovirus infections at an

age when respiratory illnesses are commonplace in lambs has compounded the difficulty in determining the role of such viruses.

Ovine adenoviruses have been shown experimentally to form persistent infections, and virus may be excreted for at least 80 days after infection. Such persistently infected sheep probably form an important role in the maintenance of infection within a flock. The interaction of ovine adenoviruses with *M. haemolytica* has been studied experimentally and OAdV-6 at least can enhance the pneumonia caused by *M. haemolytica*. Although the detection of neutralizing antibodies is insufficient to confirm interspecies infections by adenoviruses [8], the isolation from sheep of two serotypes of bovine adenovirus, and the isolation of OAdV-5 from goats, highlight the opportunities for interspecies transmission by these ruminant adenoviruses.

Control

As adenoviruses commonly cause inapparent infections of young sheep, there seems little need for their control in most countries. This does not seem to apply in Hungary, where the collection of young lambs from many sources into large fattening units is thought to alter the situation, and adenoviruses, particularly OAdV-1 and BAdV-2, become major pathogens. In that country, vaccines have been developed that protect lambs both by ingestion of colostrum from vaccinated ewes and by active immunization. In Australia, OAV287 has been studied for its potential as a gene delivery vector [5].

MISCELLANEOUS VIRUSES

Respiratory syncytial virus (RSV) causes serious respiratory illness in human infants and cattle but appears to be less important in sheep. Ruminant RSVs have been tentatively classified into two subgroups based on antigenic and genetic differences that have been exploited to develop new diagnostic assays [9–11], but caprine and ovine strains of RSV might represent, with bovine RSV, a subgroup of ruminant strains rather than different species. Antibodies to RSV are widespread in sheep in North America, and the virus has been isolated from sheep with rhinitis. Experimental inoculation of lambs with either the bovine or the ovine isolate of RSV causes only a mild respiratory illness and pneumonia, characterized by bronchiolitis. Thus, the available epidemiological and experimental studies have, so far, failed to demonstrate a major role for RSV in respiratory diseases of sheep. However, RSV infection of lambs presents a useful model in which to develop effective vaccines to human RSV and to study the potential role of this virus in asthma [12].

Reovirus types 1–3 have been isolated from sheep but, as in other species, their role in disease appears equivocal. One exception to this may be reovirus type 1 which is regarded in Hungary as one part of the pneumo-enteritis complex.

Bovine herpesvirus 4 has been isolated from the lungs of a sheep with pneumonia, but was not thought to be the causal agent [13].

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30

Contagious respiratory tumours

J.M. Sharp and M. De las Heras

Although tumours are generally uncommon in domestic livestock, sheep are affected by two neoplastic diseases, ovine pulmonary adenocarcinoma (OPA) and enzootic nasal adenocarcinoma (ENA), which arise from secretory epithelial cells that retain their secretory function after transformation. Both diseases occur throughout the world and are endemic in some countries, such as the UK, Spain, South Africa. These diseases may be present in the same country, although the nasal tumour has not been recognized in some. Each disease is associated exclusively with betaretroviruses that are highly related but distinct.

OVINE PULMONARY ADENOCARCINOMA

Synonyms: Jaagsiekte (Afrikaans), sheep pulmonary adenomatosis or SPA (UK), pulmonary carcinoma (USA)

OPA is a contagious disease produced by a tumour in the lungs of sheep. The tumour also has been recognized in goats but more rarely. It is experimentally transmissible in these two species but does not affect cattle or other animals. The disease has been recognized in over 20 countries on the continents of Europe, Africa, America and Asia, and in a wide variety of breeds. Studies in Britain and South Africa show that OPA accounts for almost 70 per cent of all sheep tumours.

Cause

The role of viruses in the aetiology of OPA has been speculated for many years. Intensive research over the past 10 years has now demonstrated conclusively that OPA is caused by an exogenous betaretrovirus known as Jaagsiekte sheep retrovirus (JSRV).

OPA can be transmitted only with material that contains JSRV. Although all sheep contain JSRV-related endogenous viruses (SERVs), it has been

shown that JSRV is an exogenous virus that is associated exclusively with OPA. JSRV is detected constantly in the lung fluid, tumour and lymphoid tissues of sheep affected by both natural and experimental OPA [1]. The virus also can be detected in unaffected in-contact flockmates but never in sheep from unaffected flocks with no history of the tumour [2]. Definitive evidence that JSRV causes OPA was provided by induction of OPA in experimentally inoculated lambs with JSRV virions obtained by transient transfection of a cell line with a full-length molecular clone of JSRV [3, 4].

It is not fully understood how JSRV transforms the epithelial target cells, the alveolar type 2 cells and bronchiolar Clara cells. However, the JSRV envelope can cause proliferation of respiratory epithelial cells [5, 6].

Clinical signs

The incubation period in naturally infected sheep appears to be long so that the disease is not usually seen until sheep are about 2–4 years old. Tumours, however, have been seen exceptionally in lambs as young as 2 months old and in sheep 11 years of age. Recent experimental studies suggest that the age at infection has an effect on the development of OPA and that resistance increases with age [7].

Clinical signs are those of a progressive respiratory illness associated with loss of weight. Although small areas of adenomatous tissue may be present in the lungs without producing obvious clinical signs, tumours that are sufficiently large to interfere with normal lung function result in respiratory embarrassment, which is most obvious following exercise. The degree of rapid or exaggerated breathing, often associated with noticeable movement of the abdominal wall (abdominal lift), depends on the extent of the tumour and the loss of normal functional lung. A unique feature of OPA is the accumulation of fluid within the respiratory tract, which may flow from the nostrils when the head is lowered (Figure 30.1). In advanced cases, high-pitched and moist sounds may be heard on auscultation or even by the unaided ear.

Appetite is maintained, although loss of weight is obvious. Death inevitably occurs, often suddenly, from a complicating *Mannheimia haemolytica* pneumonia.

Figure 30.1: Sheep affected with OPA. When the head is lowered fluid flows from the nostrils into the container (arrows). As much as 300 ml may be collected, although 30–50 ml is more usual.

In some countries, another form of the tumour (atypical OPA) has been reported, which is not associated with excess accumulation of fluid in the lung (see later) and, therefore, generally presents as an incidental finding at necropsy or the abattoir [8].

Pathology

OPA lesions are confined to the lungs but occasionally metastases have been observed in the associated lymph nodes and, rarely, in extrathoracic sites [9]. Although JSRV establishes a chronic infection of the lymphoreticular system, no pathological changes in these tissues have been recorded.

Affected lungs are considerably enlarged and heavier than normal due to infiltration by areas of tumour that may vary from small discrete nodules, measuring only 5–20 mm, to extensive tumours involving the entire ventral half of the diaphragmatic and other lobes. Usually tumour tissue is present in both lungs, although the extent on either side does vary. The lesions can be segregated into two general types, classical and atypical:

- *Classical OPA*: tumours are solid, grey or light purple with a shiny translucent sheen and often separated from adjacent normal lung by a narrow emphysematous zone. The presence of frothy white fluid in the respiratory passages is a prominent feature and is obvious even in lesions as small as a few millimetres (Figure 30.2a). In advanced cases, this fluid flows out of the trachea when it is cut or pendant. Histological examination of OPA lesions shows areas of lung where cuboidal or columnar cells replace the normal thin alveolar cells. Sometimes these abnormal cells form papilliform growths that project into the alveoli. Intrabronchiolar proliferation also may be present. A third type of change consists of nodules of loose connective tissue in a mucopolysaccharide substance. Accumulations of large macrophage cells occur in the lung tissue around the tumours [10].
- *Atypical OPA*: tumours comprise solitary or aggregated hard white nodules, which have a dry cut surface and show clear demarcation from surrounding tissues. The presence of excess fluid is not a prominent feature (Figure 30.2b). The histological appearance of these tumours is essentially the same as classical OPA but with an exaggerated inflammatory response (mostly lymphocytes and plasma cells) and fibrosis [8].

Pleurisy may be evident over the surface of the tumour and in some cases abscesses are present in the adenomatous tissue. Adult sheep, which on postmortem examination appear to have died from acute

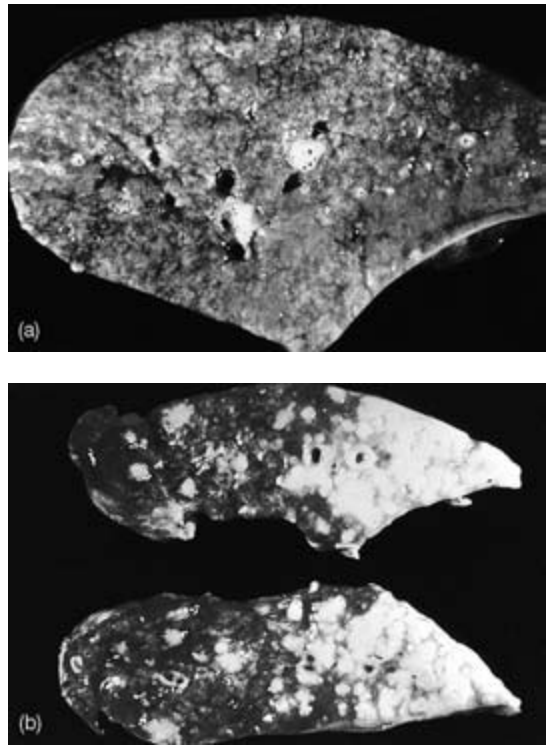

Figure 30.2: (a) Classical OPA. Cross-section of affected lung. The tumour is diffuse with poorly demarcated margins and a granular cut surface. Characteristic frothy fluid is exuding from bronchioles. (b) Atypical OPA. Cross-section of affected lung showing smooth, white cut surface of well-demarcated tumour nodules and absence of excess fluid.

mannheimiosis, should have their lungs examined carefully as lesions of OPA may be masked by the acute reaction to the *M. haemolytica*.

Ultrastructural and immunohistochemical studies have demonstrated that alveolar type 2 cells and Clara cells are the predominant neoplastic cell types in OPA and that the tumour cells exhibit variable degrees of differentiation [8, 11].

Diagnosis

Clinical detection of a case of classical OPA should be based on the presence, in a single mature sheep of an afebrile, wasting disease, with marked respiratory signs. A useful aid to diagnosis is to raise the hindquarters and lower the head of the sheep, which causes

mucoid fluid, sometimes copious in amount, to run from the nostrils. Atypical OPA is generally subclinical and detected only at necropsy. Large tumours are unmistakable in appearance at necropsy but confirmatory histology may be necessary where the amount of adenomatous tissue is small or secondary bacterial pneumonia has developed. Where tumours are not obvious, at least one or two samples for histological examination should be taken from each lobe. The transformed epithelial cells are the major sites of virus replication and JSRV antigens can be revealed by immunohistochemical techniques [8].

At present, there are no immunological tests to support a clinical diagnosis of OPA in the live animal. JSRV has been associated exclusively with both classical and atypical forms of OPA but antibodies to this virus have not been detected in the sera of affected sheep, even with highly sensitive assays [10, 12].

Sequencing of JSRV and related endogenous retroviruses has led to the development of polymerase chain reaction assays (PCRs) that can detect the exogenous virus in a background of genomic DNA and endogenous sequences. Using this sensitive procedure, JSRV has been detected in the blood of experimentally-infected lambs and, more recently, in sheep with OPA and in-contact sheep from flocks with OPA [2, 3, 13, 14]. More extensive investigations demonstrated that sheep with clinically recognizable OPA were consistently positive but infected sheep with no lesions or very early lesions were intermittently positive. This result suggests that this PCR assay may not be appropriate as a screening test for individual animals, although it could be useful for flock testing [13].

Epidemiology

OPA has been recognized for many years to be experimentally transmissible by the respiratory route. It is probable that an infected sheep, even before it develops obvious respiratory signs, excretes virus-containing droplets as it breathes. Later, as the disease progresses, it will discharge quantities of infective respiratory fluid, especially during feeding when the head is lowered. This causes the sheep to snuffle and doubtless creates an aerosol of infected droplets. Close confinement of a flock obviously increases the probability of transmission so that housing, with trough feeding and watering, may allow the infection to spread.

OPA is generally introduced into flocks and even countries by the acquisition of infected sheep. Losses from classical OPA can be high when the disease first appears as occurred in Iceland, where in some flocks the annual loss for OPA was recorded as reaching 50–80 per cent. In countries where the disease is endemic, OPA may be more common in certain areas or breeds. This appears to be the case in Scotland where losses in infected flocks are generally between 2 and 10 per cent annually. During the commercial life of an infected flock, classical OPA is associated with 50 per cent of the on-farm mortality and is present in up to 20 per cent of culled sheep. Similar information on the prevalence of atypical OPA is not available at present.

Although an association of the disease with cold conditions has been suggested this has not been proven and close contact between sheep is probably the vital factor in transmission, as when sheep are housed together.

Until recently, all existing epidemiological information has been based on clinical diagnosis and pathology and the extent of infection by JSRV in affected flocks has been unknown. However, recent longitudinal studies using PCR to investigate natural transmission of JSRV have shown that lambs become infected at a very early age and a high proportion of animals in an OPA-affected flock become infected; only a minority develop OPA [14, 15]. Moreover, JSRV has been found in colostrum and milk obtained from sheep in OPA-affected flocks and JSRV can be detected within a few months in the blood of lambs fed artificially with colostrum and milk (De las Heras *et al.*, unpublished observations). Colostrum and milk, therefore, may be important in the transmission of JSRV under natural conditions.

Control and treatment

No method of treatment is recognized or advised. For many years, control has been based on regular inspection of adult sheep in affected flocks, with prompt culling of any suspicious animal, as well as the offspring of affected ewes, which frequently develop OPA also. While these methods are unlikely to eradicate OPA from a flock in which the disease is endemic, reduction in the prevalence of infection may be obtained.

Embryo transfer has been reported as one way to obtain animals free of OPA, based on clinical and

pathological evidence [16]. It would worth re-evaluating this approach with the more sensitive PCR techniques to confirm that the offspring were free of JSRV infection. More recently, Voigt *et al.* (personal communication) appear to have eradicated OPA and JSRV from two OPA-affected flocks by removing the lambs at birth, depriving them of maternal colostrum and rearing them in isolation. No clinical cases have been observed after 3 years and no JSRV infection has been detected by PCR examination of brochoalveolar lavage samples. These results and the new epidemiological data described above suggest that removing the lambs at birth and rearing with artificial colostrum and milk may be a control measure to consider in the control and treatment of OPA.

ENZOOTIC NASAL ADENOCARCINOMA (ENA)

Synonyms: Infectious nasal adenopapillomatosis, infectious nasal adenocarcinoma, tumor intranasal enzootico, enzootic nasal tumour

ENA is a contagious disease of the respiratory system characterized by neoplastic changes of the glands of the nasal mucosa of sheep. ENA occurs in many countries throughout the world, although it appears to be absent in some major sheep-rearing countries, such as Britain, Australia and New Zealand.

Cause

The contagious nature of ENA has been known for many years as a result of observations such as its repeated occurrence in the same flocks and its introduction to previously unaffected flocks. Tumour homogenates can transmit the disease experimentally and recent studies have associated an exogenous retrovirus, known as ENTV, with the disease. ENTV has been demonstrated constantly in tumour tissue and nasal fluid of affected sheep and never in unaffected animals. ENTV is highly homologous to JRSV but is a distinct virus, with clear differences located in the LTR and env regions of the genome. Confirmation of ENTV as the aetiological agent of ENA will require a similar approach to that which demonstrated JSRV to be the cause of OPA.

Clinical signs

Young adult sheep (2–4 years old) are most often affected. Clinical signs include intense seromucous nasal discharge (Figure 30.3), accompanied by stertorous breathing, coughing and also dyspnoea. The continuous flow of the nasal secretion causes a characteristic discoloration and hair loss around the nostrils. Skull deformation, softening of the cranial bones and exophthalmus can arise as a result of the tumour mass. Pyrexia is not a feature of the disease. Gradually, during the course of several months, the animal loses weight and finally dies due to complications.

Pathology

At necropsy, ENA presents as a tumour arising from the ethmoid turbinates, either unilaterally or bilaterally, although bilateral cases are more common [17]. The tumours are soft, grey or reddish-white with a granular surface covered by mucus. Growth of the tumour, which can fully occupy the nasal chambers, results in compression of the surrounding structures and may even invade maxillary or frontal areas (Figure 30.4).

Histologically, ENA is classified as a low-grade adenocarcinoma of the nasal glands. Ultrastructural examination of the tumours indicates that the transformed cells arise from mucosal secretory glands. The stroma of the tumour is infiltrated by lymphoplasmocytic cells. No metastases to regional lymph nodes or other organs have been found.

Figure 30.3: Sheep affected by ENA showing chronic seromucous nasal discharge.

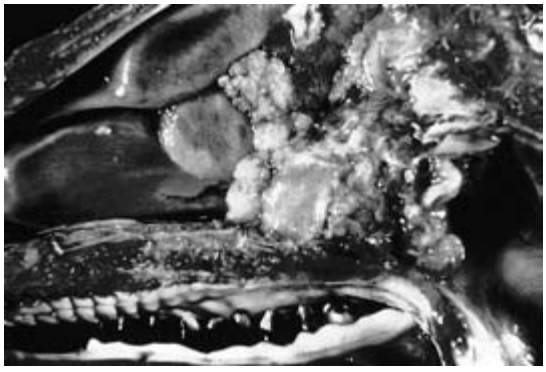

Figure 30.4: Sagittal section of head of sheep affected by ENA. The cauliflower-like tumour occupies the caudal part of the nasal cavity and arises from the ethmoid turbinates.

Diagnosis

Clinical detection of a case of ENA should be based on the presence of the clinical signs described above. Tumours are unmistakable in appearance at necropsy but confirmatory histology may be necessary. As in OPA, the transformed epithelial cells are the major sites of virus replication and ENTV antigens can be revealed by immunohistochemical techniques [17].

There are no laboratory tests to support a clinical diagnosis of ENA in the live animal and antibodies to ENTV have not been detected in the sera of affected sheep [12]. A specific PCR for ENTV consistently detects the virus in tumour and nasal fluid but, unlike OPA, virus has been detected infrequently in the blood.

Epidemiology

ENA is probably spread by the respiratory route but may be less contagious than OPA because it is more difficult to transmit experimentally. In affected flocks the prevalence of disease is usually 0.5–2 per cent, although it can be as high as 15 per cent. The disease can be introduced into a flock by purchasing infected animals. There is no information at present on the prevalence of ENTV infection.

The coexistence of OPA and ENA lesions in the same animal has been described, and virological data suggest a synergistic relationship between ENTV and JSRV [18].

Control

No treatment or vaccine is available. In general, the same procedures recommended for OPA can be applied to control of ENA.

ENZOOTIC NASAL TUMOUR IN GOATS

Goats are affected by a tumour of the ethmoid turbinates that shows all of the features just described [19]. It is associated with a retrovirus that is highly homologous to both JSRV and ENTV, but can be distinguished by unique genomic sequences. In mixed flocks of goats and sheep, both diseases have been observed, but it is not known whether the goat ENTV can infect sheep and induce ENA, or the reverse.

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31

Maedi-visna

G.C. Pritchard and I. McConnell

Maedi-visna (MV) is the composite name for an important multisystemic disease of adult sheep caused by infection with a retrovirus of the lentivirus subfamily. Maedi and visna are Icelandic words describing two apparently different clinical syndromes that affected Icelandic sheep in the1940s and 1950s. Maedi, or 'laboured breathing', was a fatal, progressive pneumonia of mature sheep; visna, or 'wasting', was a meningo-encephalitis which caused progressive paralysis and death. The disease appeared

in Iceland in the years following the importation of Karakul sheep from Germany in 1933. Its viral aetiology was established in the late 1950s, when it also became clear that maedi and visna were caused by essentially the same virus. The disease was eventually eliminated from Iceland in 1965 following phased de-stocking of the sheep-rearing areas and repopulation with sheep from unaffected parts of the country. Iceland is the only country that has eradicated maedi-visna successfully. In North America, where MV infection is widespread, the associated disease is termed ovine progressive pneumonia, or Montana sheep disease. In the Netherlands, the disease is known historically as *zwoergerziekte* and in France as *la bouhite*. Although MV has been reported from most of the major sheep-producing countries of the world, it has not been seen in Australia and New Zealand. However, the very closely related caprine arthritis encephalitis (CAE) virus infection occurs in both countries. Comprehensive up-to-date information about the prevalence of MV and CAE in many European countries is lacking but they appear to occur widely in the Mediterranean region, particularly Spain, Greece and Italy [1]. The term 'small ruminant lentivirus' (SRLV) is increasingly used to describe both MV in sheep and CAE in goats [1, 2].

In Britain, MV virus infection was first detected in the 1970s in exotic sheep (or their progeny) imported from continental Europe, or in indigenous breeds that had been in contact with exotic sheep. By 1983, it was demonstrated in commercial flocks of indigenous sheep [3] and serological surveys in the 1990s revealed infection in about 1.5 per cent of flocks. Despite concerns, confirmed clinical disease (mainly maedi), as opposed to serological evidence of subclinical infection, was recorded in only about 20–25 different indigenous flocks in Britain from on-going national surveillance activities up to the end of 2005.

CAUSE

MV virus is a non-oncogenic, exogenous, retrovirus belonging to the lentivirus subfamily, which includes human, simian, feline and bovine immunodeficiency viruses, equine infectious anaemia (EIA) virus and CAE virus. Retroviruses are RNA viruses that replicate via a DNA provirus that becomes integrated or closely linked to genomic DNA in the virus target cells. There is a very close relationship between MV and CAE viruses and cross-species transmission is possible, especially after feeding infected milk from one species to the other; both viruses are now commonly classified together as small ruminant lentiviruses (SRLV).

Lentiviruses are so-called because they are slow viral infections that persist for the lifetime of the individual and are progressively fatal with no immune recovery [apart from the vector-borne lentiviral infection of horses (EIA) in which some animals recover and become immune]. Antigenic variation in MV virus is well documented and in all lentiviral infections there is extensive mutation of the gene encoding envelope glycoproteins that allows mutated viruses to escape immune elimination. There has been intense interest in this group of viruses since the discovery that human AIDS was caused by infection with the lentiviruses of HIV-1 and HIV-2. The hallmark of lentiviral disease is a progressive multisystem chronic active inflammatory process occurring predominantly, but not exclusively, in the lungs and mammary gland, due to the action of the virus on cells of the macrophage monocyte series. Arthritis and degenerative joint disease also occur due to virus-induced pathology. The immunodeficiency state, which arises in some lentiviral diseases (such as HIV), is due to selective targeting and destruction of CD4 T cells. Additional lymphocyte subsets are targeted in feline immunodeficiency (FIV) and thus in cats the immunodeficiency state is severe. The primate lentiviruses of simian immunodeficiency virus (SIV) also resemble AIDS-like diseases in a range of primate species. Importantly, however, selective destruction of lymphocyte subsets is not a feature of MV infection since T cells are not targeted. Therefore, severe immunodeficiency does not occur and effects are restricted to cells of the monocyte macrophage series. Lentiviruses are an important group of viruses where there is no suitable laboratory animal model. Studies of naturally occurring lentiviruses, such as MV virus, are therefore of considerable importance to comparative medicine, with MV providing a unique insight into the chronic inflammatory process characteristic of all lentiviruses, uncoupled from T cell immunodeficiency. Although MV virus is commonly recognized as a prototype AIDS virus, it is important to stress that it does not infect other species such as humans.

CLINICAL SIGNS

The incubation period is protracted and most infection is subclinical. Clinical signs develop insidiously, epitomizing slow virus infection. The interval between the first introduction of infection and its overt manifestation can be as long as 10 years, by which time a high proportion of the flock is likely to be seropositive. Disease is not evident until at least 2 years of age, or more usually, 4–5 years. Maedi is the most common clinical manifestation: affected animals remain bright, alert and continue to eat but exhibit gradual loss of condition; tachypnoea and respiratory distress develop slowly and may initially only be evident on exertion. Pyrexia is not a feature unless secondary bacterial infection is present; auscultation of the thorax is generally unrewarding. Coughing and nasal discharge are occasional features; flared nostrils, neck extension and open-mouth breathing occur in more advanced cases; wool loss may be seen as debilitation progresses. Maedi is clinically much less obvious in large sheltered lowland flocks on a high plane of nutrition (as in parts of Britain), where mildly affected sheep may pass unnoticed for several months and can survive for a year or more until culled or destroyed on humane grounds. In some flocks the disease appears to persist virtually unrecognized almost indefinitely or its features are attributed to other causes.

Although maedi is untreatable and ultimately fatal, mortality rates due to uncomplicated disease are generally very low – less than 2 per cent in a heavily infected flock studied in the East Anglia region of England. Deaths are commonly associated with secondary bacterial infection, particularly mannheimiosis (formerly pasteurellosis), and there may also be an increased predisposition to other conditions such as pregnancy toxaemia. Mortality rates can be higher under harsher climatic and environmental conditions, such as those encountered in an infected flock in the north-east of Scotland [4], in which ewe mortality was 14 per cent, with associated losses in lambs due to poor viability and insufficient colostrum. However, the extent to which losses are due directly to MV virus infection or to secondary and opportunistic infections is not clear.

Visna, the nervous form of the disease, is also characterized by weight loss. It occurs much less commonly and is usually seen only sporadically in heavily infected flocks, often several years after the initial recognition of maedi. Both conditions may be present to varying degrees in the same animal or they can occur independently. Somewhat unusually, visna (rather than maedi) was the first clinical presentation of MV virus infection in two unrelated commercial flocks in Britain in 2003 [5]. Clinically, this suggested the possible emergence of an apparently neurotropic strain of virus, as was seen in Iceland during the last century. Clinical signs of visna relate to lesions localized in either the brain stem or spinal cord. Gait abnormalities of the hind limbs are the main initial feature, particularly unilateral dragging of the foot due to paresis of the affected limb, resulting in scraping of the hoof. Knuckling over and stumbling soon follow, leading to ataxia, general incoordination, hindlimb paralysis, recumbency and death. Other features include circling, progressive head tilt, fine tremor of the lips, dazed appearance, hyperaesthesia, separation from the rest of the flock and aimless wandering. Visual impairment and blindness, features not recognized until fairly recently, can also occur. Unlike maedi, visna cases usually progress relatively rapidly, resulting in death or euthanasia within a few weeks.

A slowly progressive, indurative mastitis [6] is a common feature in some heavily infected flocks but this has not been widely reported in Britain. This can be detected only by careful palpation of the udder; there are no gross changes to the character of the milk. The main consequence seems to be delayed weight gains in unweaned lambs, particularly twins and triplets. Arthritis is a rare manifestation of MV virus infection. Carpal joints are mainly affected, and joint enlargement is not usually associated with acute lameness.

PATHOLOGY

Maedi is the manifestation of MV virus infection most likely to be suspected from gross pathology. In cases uncomplicated by secondary infections, the lungs have a uniform dense rubbery texture and a swollen voluminous uncollapsed appearance; chest wall/rib impressions are sometimes evident. Pulmonary oedema, consolidation and abscessation are absent in uncomplicated cases. Despite usually weighing at least 1–2 kg, which is two to four times the normal weight (see Figure 31.1 in the colour plate section), the lungs remain aerated and invariably float in water until cases are very well advanced. Lung colour varies

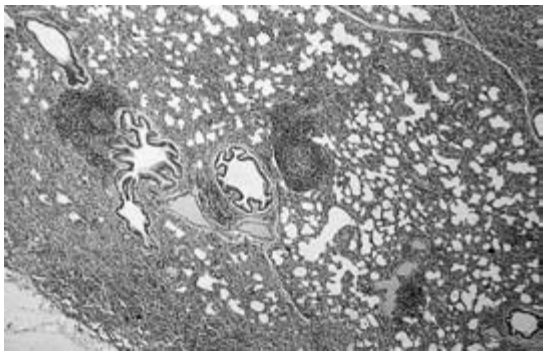

Figure 31.3: Histology of maedi lung, showing lymphoid infiltration in the septa and lymphoid nodules.

from uniform shades of dull red or pink (which can appear superficially similar to non-specific congestion) to a mottled pinkish-brown, variegated with firm greyish granular focal spots up to 5 mm in diameter. These lesions, which are visible on the surface and on cut section, are distributed throughout all lung lobes where they can extend to hard, grey confluent areas (see Figure 31.2 in the colour plate section), depending on the age of the lesions and degree of lymphoproliferative change. Associated lymph nodes are invariably grossly enlarged; the caudal mediastinal may reach up to 10 times normal size. In some affected flocks, uncomplicated maedi is the norm, whereas, in other flocks, cases usually present as mixed infection with lungworm, *Mannheimia haemolytica* pneumonia (pasteurellosis), purulent bronchopneumonia or, more notably [7], ovine pulmonary adenocarcinoma (OPA) – formerly sheep pulmonary adenomatosis (SPA). In such cases underlying maedi lesions can easily be masked and may not be detectable at routine necropsy unless suspected from the flock history. Visna lesions are located in either the spinal cord or brain and are not usually grossly apparent. When the udder is affected, the mammary glands are grossly small and firm to cut. Arthritis associated with MV virus infection produces gross thickening and sometimes a red/tan discoloration of the synovial membrane with joint enlargement, particularly of the carpus. Histological lesions [8] comprise immunologically mediated chronic active inflammatory changes, with lymphoid infiltration (Figure 31.3) and proliferation in one or more target organs including lung, central nervous system, mammary glands and joints. Main organ-specific changes consist of smooth muscle hyperplasia (lungs), demyelination (central nervous system), fibrosis (mammary gland) and proliferation of synovial membrane, and degenerative changes to the articular cartilage (joints). In advanced mixed pulmonary infections, particularly involving OPA, attribution of specific histological lesions can be problematical [3, 7].

DIAGNOSIS

Most infected sheep within a flock show few overt signs of disease but remain clinically silent virus carriers. For control purposes, it is essential that such animals are identified as soon as possible after infection. Antibody detection tests are routinely used for diagnosis and, since virus infection persists, the presence of antibody signifies current infection. The time period from infection to seroconversion is variously estimated at about 6 months [1] or from 3 weeks to several months [2] but it may take several years and some infected animals never seroconvert. It is shorter in more heavily infected flocks than those with low seroprevalence.

The agar gel immunodiffusion test (AGIDT) has been the classical gold standard test for the past 25 years but it is gradually being replaced by a range of enzyme-linked immunosorbent assay (ELISA) methods, which employ purified virus, recombinant proteins or synthetic peptides as antigens. ELISA shows good concordance with AGIDT [9]. It is increasingly recognized through flock testing regimes to be more sensitive than AGIDT and the Office International des Epizooties (OIE) has now accepted it as an alternative test for MV (AGIDT remains the prescribed test for international trade). It can detect seroconversion earlier and is particularly suitable for screening large numbers of serum samples in eradication and accreditation schemes [10]. The sensitivity of commercially available ELISAs appears to vary, however (this may be due to viral genomic heterogeneity), and ideally the antigen used for ELISA should contain epitopes present in the virus strains circulating in the population being investigated, if known [2]. In the MV accreditation scheme (Sheep and Goat Health Scheme) in Britain operated by the Scottish Agricultural College (SAC) (see 'Control and prevention' later), it is their policy to use the AGIDT for preliminary flock screening and monitoring of status, while ELISA is preferred

for within-flock eradication purposes (B.A. Synge, personal communication). ELISA has been used successfully for detecting MV antibodies in milk, which offers the potential for low-cost screening of milking sheep flocks (and goat herds for CAEV) and as a useful tool in national eradication schemes. Western blot, where available, is becoming established as a routine standard for confirming suspect ELISA results. Polymerase chain reaction (PCR) technology can be used for cases of special interest, such as when infection is suspected in a seronegative animal or in very young lambs which have received colostrum from infected ewes.

MV virus infection should always be suspected in flocks with a history of progressive weight loss, emaciation and chronic pneumonia in older ewes, or unusual nervous signs, especially where breeding replacements are purchased from sources not monitored for MV virus infection. However, maedi (although less likely with visna) can easily remain unrecognized in a flock for several years, particularly if mildly affected ewes are culled for other reasons before overt signs develop. Also, unless there is a serious flock problem, veterinary surgeons may not be consulted and, for economic reasons, necropsies are rarely undertaken routinely on older ewes. Clinically, maedi needs to be differentiated primarily from OPA, but atypical pneumonia, lungworm infection and chronic suppurative pneumonias also need to be considered. Histological examinations are essential to confirm the diagnosis. However, it should be emphasized that there can be difficulties in reaching a definitive diagnosis in the presence of secondary infection. Furthermore, the isolation, for example, of *M. haemolytica* from the lungs does not exclude the possibility of underlying MV virus infection and associated pathology. Gross examination of the lungs can provide a useful guide, particularly checking whether the weight (excluding visible trachea) exceeds 1 kg. Although clearly non-specific, this approach offers a simple preliminary screening test, particularly for lungs not exhibiting obvious consolidation or gross pathology. If maedi is suspected in home-bred sheep, the overall within-flock seroprevalence is likely to be at least about 60 per cent, with a higher prevalence in the older sheep. Hence, in most infected flocks, blood sampling just a few aged poorer ewes should be sufficient to detect at least one seropositive animal.

Visna is unlikely to be the main initial presentation of MV virus infection in a flock with no previous history, although primary visna was seen in two flocks in Britain referred to earlier [5] where the emergence of an apparently neurotropic strain of virus was suspected. Scrapie is a major differential diagnosis for visna and, significantly, some recent cases of visna seen in the UK were initially reported to the authorities as suspected scrapie. Cases also need to be differentiated from space-occupying brain and vestibular lesions. It may be necessary to differentiate visna from periparturient metabolic conditions, cerebrocortical necrosis and cerebral listeriosis, particularly if the flock has a previous history of these diseases. Definitive confirmation requires brain and spinal cord histopathological examinations, supported by serology.

EPIDEMIOLOGY AND TRANSMISSION

Following primary exposure, the virus establishes a persistent infection (by latent carriage in monocytes and macrophages) in peripheral blood. Viral expression is closely dependent on maturation of monocyte to macrophage. Variations in immune response, genetic susceptibility and virus phenotype appear to dictate what target organ system is involved and the progress of lesion development. Lesions develop over a protracted period and sheep may be relatively old in relation to their productive lifespan before overt signs appear. Despite mounting virus-specific humoral and cellular immune responses, infected sheep do not develop a protective immunity.

The main route of transmission is from an infected ewe to its offspring via colostrum (including pooled colostrum banks) and milk. Aerosol transmission between animals of all ages in close contact and over distances of several metres appears to be an almost equally significant route of spread both within and between flocks, particularly under intensive housing or grazing conditions or where concurrent OPA, which has a synergistic effect on transmission [7], is also present. Breed susceptibility and management practices also play significant roles. If naïve animals are introduced into a heavily infected flock, up to 50 per cent may seroconvert within 9 months. In the presence of OPA, seroconversion rates of 90 per cent within 18 months have been recorded: one such infected flock in England, for example, showed an

increase in seroprevalence from 4 to 93 per cent within 4 years [7]. In all endemically infected flocks, there is an increase in seroprevalence with age, from about 25 per cent seropositive at 2 years to over 90 per cent at 5 years or more.

Placentas contaminated with maternal blood, contaminated milking equipment and poor personal biosecurity practices by operatives may also contribute to the spread of infection [2]. Semen has been demonstrated to contain virus but its role in viral transmission is not known. The significance of intrauterine viral transmission remains unclear but it is generally thought that it is of minor importance compared with other routes [2].

ECONOMIC ASPECTS

The economic effects of clinical and subclinical MV virus infection are poorly understood and difficult to quantify because of the complex interaction between the virus, its host and the environment [2]. The disease develops very slowly and only about 30 per cent of infected animals ever develop clinical signs. Flock seroprevalence, genetic susceptibility, husbandry and management practices, environmental influences, intercurrent disease, culling policy and the relative value of cull ewes compared with breeding replacements are all important factors. The potential cost is inevitably much greater in pedigree flocks selling breeding stock than in commercial flocks engaged in finished-lamb production. The effects on ewe mortality, milk production and quality, lambing per cent, lamb birth weight, viability and weaning weight are all very variable. Some studies have described a weight shortfall of up to 5 kg per lamb at weaning due to decreased milk production from indurative mastitis [6], but this has not been confirmed by other workers. The effect of age is a major confounding factor when comparing the productive performance of MV-positive and MV-negative ewes because older ewes generally have a higher lambing percentage and heavier lamb birth weights regardless of MV status. Under extreme conditions, losses of 10–20 per cent of gross margin per ewe have been described in some affected flocks [4], whereas, under sheltered lowland conditions [7], the only measurable economic effect of MV virus infection is the need to cull ewes a year earlier than usual.

CONTROL AND PREVENTION

In common with other viruses of the Lentivirus subfamily, clearance of virus is never seen following either natural or experimental infection. The reasons for this include: rapid mutation of the virus envelope glycoproteins to give antigenically different subgroups of the virus; a failure to make IgG2 antibody essential for cell-mediated immunity and an inherent capacity of the virus to 'go-to-ground' as a latent proviral infection in cells of the monocyte macrophage lineage [11]. Latently infected cells do not express any viral antigens for recognition by the immune system and hence cannot be eradicated. However, they act as a reservoir of infected cells which can switch on production of fully infectious virus when they differentiate in response to inflammation. This may be why maedi is more evident in situations of poor husbandry and in association with secondary infections.

Several research groups are pursuing the possibility of controlling MV virus infection by vaccination. However, vaccination against MV can be a 'double-edged sword' and there are good experimental data to show that using whole inactivated virus will make the disease worse through the development of immunopathological lesions. To overcome this problem, novel vaccines based on recombinant viral proteins are being developed. Preliminary results show that the choice of viral antigen is critical in determining the outcome of vaccination since immunization with certain recombinant viral antigens enhances immunopathology rather than immunity.

Control programmes adopted for SRLV infection by different countries need to take into account national or regional characteristics and achievable objectives: merely reducing seroprevalence will lead to a useful decline in the incidence of clinical disease but reducing low seroprevalence to serologically negative is required to eradicate infection [2]. The potential for virus transmission between sheep and goats proved to be a significant risk factor which hindered final progress in the otherwise highly successful CAEV eradication programme in Switzerland [12]. For uninfected flocks, the first priority is to prevent the introduction of infection. This can be achieved by applying good flock biosecurity practices, including not sharing rams or mixing sheep with other flocks and maintaining appropriate segregation at

shows and sales. Quarantining and blood sampling of breeding replacements, or purchasing only from established MV virus-free sources is desirable, but may be economically viable only for pedigree flocks. In Britain, where the infection appears to be maintained within commercial flocks, strict segregation from pedigree flocks is essential. Several countries operate compulsory or voluntary monitoring, eradication and control schemes based on systematic flock serological examinations. If flock infection is detected sufficiently early, when seroprevalence is low, a simple test-and-cull programme at 3–6-monthly intervals may be successful at eliminating infection, but it is only likely to be economically feasible in valuable pedigree flocks. It is essential that lambs born to seropositive ewes are also removed. In Britain, a MV accreditation scheme is currently operated by the SAC veterinary services in partnership with practising veterinary surgeons.

Once infection is firmly established in a flock, the options are limited. Attempts to produce MV-free flocks from heavily infected flocks by snatching lambs at birth for artificial rearing or by adopting segregation and serological testing approaches have generally been unsuccessful or impractical under commercial conditions. Underestimating the significance of horizontal transmission and having inadequate separation between infected and uninfected sheep are important issues. However, a recent report [13] described the successful containment of an infected cohort of purchased sheep for two breeding seasons using rigorously enforced biosecurity practices. In many endemically infected commercial flocks operating under favourable environmental conditions, reasonably effective control can be achieved by increasing the flock replacement rate and adopting a strict culling policy for suspect clinical cases and not keeping ewes beyond 4–5 years of age. This has the effect of keeping flock seroprevalence down, thereby reducing the incidence of clinical disease. Complete flock replacement with assured MV virus-free stock, if available, is the other main option but is unlikely to be economically viable for most commercial flocks.

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32

Pasteurellosis

W. Donachie

Synonym: enzootic pneumonia (pneumonic forms only)

There are two clinical forms of pasteurellosis: pneumonic and systemic. The pneumonic form is caused by *Mannheimia haemolytica* (formerly designated *Pasteurella haemolytica* [1]) (see below for more details), and the systemic disease by *Pasteurella trehalosi.* In sheep in temperate climates *P. multocida* rarely causes pneumonia and little is known of the epidemiology of that infection.

Pasteurellosis caused by *M. haemolytica* is one of the most common bacterial infections of sheep, and by far the most important respiratory one, with a widespread distribution, occurring in temperate, subtropical and tropical climates. Pneumonic pasteurellosis in sheep was first described in 1931, but not until the 1960s did serotyping and biotyping help to define the epidemiology of the disease. In sheep, two '*Pasteurella*' species, *M. haemolytica* and *P. trehalosi,* share a common serotyping system comprising a total of 17 serotypes [1], with approximately 90 per cent of all isolates serotypable. Each of the two species, originally biotypes of *M. haemolytica*, is associated with a distinct clinical syndrome. *M. haemolytica* (formerly biotype A) strains are responsible for pneumonic pasteurellosis in sheep of all ages while *P. trehalosi* strains (formerly biotype T) cause a systemic disease in 6–10-month-old lambs.

In 1999 the taxonomy of the family *Pasteurellaceae* changed in response to new information on the relatedness of strains following studies on DNA and ribosomal RNA homology [1]. The revised taxonomy introduced a new genus, *Mannheimia,* to replace *P. haemolytica* and *M. haemolytica*-like strains. The prototype species of the new genus is *M. haemolytica*, which includes all the former *P. haemolytica* A serotype strains apart from A11. Strains of this latter serotype are now placed in another *Mannheimia* species*, M. glucosida*.

Together, *M. haemolytica, M. glucosida* and *P. trehalosi* are divided into 17 serotypes on the basis of an indirect haemagglutination test, which depends on the serotypes having specific polysaccharide capsules. Serotypes 1, 2, 5, 6, 7, 8, 9, 12, 13, 14, 16 and 17 belong to *M. haemolytica,* A11 is now *M. glucosida* and serotypes 3, 4, 10 and 15 are *P. trehalosi.* The taxonomy of *P. trehalosi* remains unchanged. The main differences between *Mannheimia* and *Pasteurella* spp. are shown in Table 32.1.

Table 32.1: Characteristics used to differentiate *Mannheimia* and *Pasteurella* species (from [1])

	Mannheimia	*Pasteurella*
β-Haemolysis	−	−
α-Haemolysis	+	−
V-factor dependency	−	d
X-factor dependency	−	−
VP, 37°C	−	−
Urease	−	d
ODC	d	d
Indole	−*	+†
L-arabinose	d	−
Glucosides	d	−
Mannitol	+	d
D-mannose	−	+
D-melibiose	−*	−
meso-Inositol	d	−
D-sorbitol	d	d
Trehalose	−	d
ONPG	d	−

+, positive; −, negative; d, positive or negative; *, deviating strains occur; †, *P. avium* indole −ve.
VP, Voges Proskauer;
ODC, ornithine decarboxylase;
ONPG, ortho-nitrophenol test for beta-glactosidase production.

CAUSE

M. haemolytica and *P. trehalosi* are identical in their morphology, both being encapsulated, small (1–2 μm × 0.3–0.6 μm), Gram-negative, aerobic coccobacilli. In carbohydrate fermentation tests, most strains of *M. haemolytica* ferment arabinose (hence the former biotype A) but not trehalose, whereas all *P. trehalosi* strains ferment trehalose. Colonies of *M. haemolytica* are small and grey with a narrow zone of haemolysis after 24 hours of incubation. The colonies of *P. trehalosi* strains are darker, larger (up to 2 mm in diameter) and have brownish centres (Figure 32.1). *P. trehalosi* strains are more resistant than *M. haemolytica* strains to penicillin, ampicillin, chloramphenicol, oxytetracycline, erythromycin and nitrofurantoin. Basic fuchsin (0.2 μg/ml) in brain–heart infusion broth inhibits *M. haemolytica*. Both species produce an exotoxin (leukotoxin), which acts specifically on ruminant macrophages and plays an important part in pathogenesis. The prevalence of the serotypes varies (Figure 32.2). In the UK, between 1982 and 1998, 33 per cent of all (then) *Pasteurella* isolates were serotyped as A2, while A1, A6, A7 and A9 represented about 22 per cent of the total. T10 is the commonest *P. trehalosi* serotype (11 per cent), followed by T15 (9 per cent) and T4 (7 per cent).

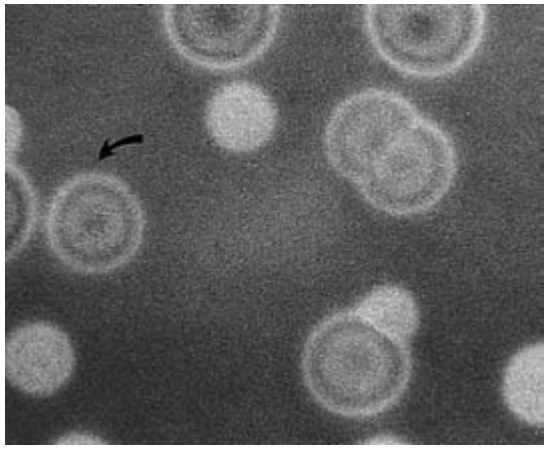

Figure 32.1: Bacterial colonies on blood agar. The smaller lighter colonies are *Mannheimia haemolytica* and the larger darker colonies (arrow) are *Pasteurella trehalosi*.

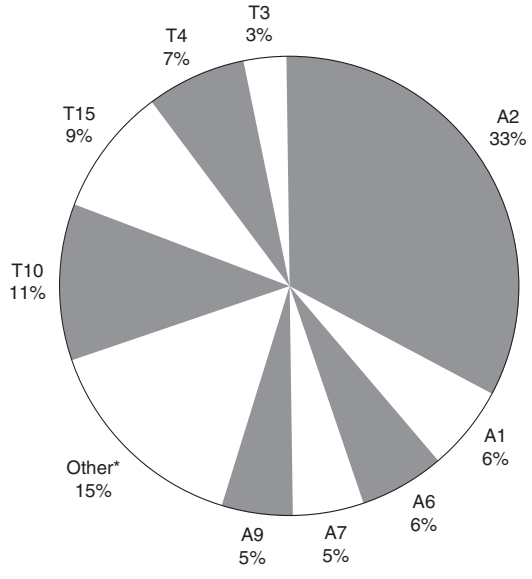

Figure 32.2: The prevalence of *M. haemolytica* and *P. trehalosi* from 1982 to 1998.

Strains that are untypable with existing typing sera are isolated from both healthy and diseased sheep. The majority of such strains show similarities to *M. haemolytica*. Clinical cases associated with each species have a seasonal distribution with *M. haemolytica* more prevalent in the spring and summer and *P. trehalosi* predominant in the autumn. *M. haemolytica* and *P. trehalosi* occur in the nasopharynges and tonsils of apparently normal sheep. *M. haemolytica* predominates in the nasopharynx whereas *P. trehalosi* is the major species in the tonsils.

DISEASE ASSOCIATED WITH *M. HAEMOLYTICA*

The predominant disease caused by this species is pneumonia.

Clinical signs

In a proportion of cases, clinical signs are not noticed and the animal is found dead. The clinical signs of acute, pneumonic pasteurellosis are dullness, anorexia, pyrexia of greater than 40.6°C and varying degrees of hyperpnoea or dyspnoea. On auscultation, adventitious

sounds are not prominent and the respiratory sounds are loud and prolonged. There are often serous nasal and ocular discharges. A frothy fluid drooling from the mouth is usually present in the terminal stages. In subacute or chronic cases, the clinical signs may be transient and less obvious than in the acute disease.

Pathology

The initial observation at necropsy of most adult sheep with pneumonic pasteurellosis is extensive ecchymotic haemorrhage in the throat region and over the ribs. On opening the thorax, subpleural and subepicardial petechiation and varying amounts of clear, yellow pleural and pericardial exudate are found.

In hyperacute cases, the lungs are swollen, heavy and cyanotic, with bright purplish-red solid areas, which exude a frothy haemorrhagic fluid when incised (Figure 32.3). Cases of slightly longer duration develop consolidation, particularly in the cranial and ventral parts of the lungs and may be covered by a greenish, gelatinous pleural exudate. The consolidated portions of such lungs contain irregular greenish-brown areas of necrosis, each with a dark, haemorrhagic margin. The tracheobronchial linings in hyperacute and acute pneumonic pasteurellosis are red to dark purple, and the airways contain pink-stained froth. In less acute cases a greyish-pink, raised consolidation of cranial lobes predominates which, when cut, resembles liver in appearance and consistency, with red or grey solid lobular tissue prominently separated by thickened septa. In such tissue, dark necrotic foci and pleurisy characterized by organizing adhesions may be present. Very occasionally the only lesions are solid, raised nodular masses, scattered throughout the lungs. These may be mistaken for lung tumours, but histology reveals the characteristic pathology of pasteurellosis. The bronchial lymph nodes in pneumonic pasteurellosis are enlarged, soft and pale or petechiated.

In lambs up to 12 weeks of age, *M. haemolytica* is associated with septicaemia rather than a primary pneumonia. There may be either no lung involvement, or pleurisy and pericarditis with only focal lung lesions. Petechiae, however, are usually present in the myocardium, spleen, kidney and liver, the carcass lymph nodes are enlarged and haemorrhagic, and hepatic fatty degeneration is present.

Histologically, the hyperacute disease exhibits intense hyperaemia with haemorrhages, and a pale pink-staining fluid rich in protein, mingled with masses of Gram-negative coccobacilli, fills the alveoli. The acute consolidated lesion shows a widespread cellular exudate accompanying congestion and capillary haemorrhage. Clusters of neutrophils may be present but the predominant cell filling the alveoli is elongated with an intensely basophilic spindle-shaped nucleus and is called an 'oat cell', masses of which form whorls and appear to stream between adjacent alveoli (Figure 32.4). When present, the characteristic

Figure 32.3: Pneumonic pasteurellosis. Lungs of a lamb showing congestion and oedema (note frothy fluid in the trachea) and an area of pneumonia and fibrinous pleurisy affecting the apical and cardiac lobes of the right lung. (Courtesy of Dr J.A. Garcia de Jalon, Faculdad de Veterinaria, Zaragoza, Spain.)

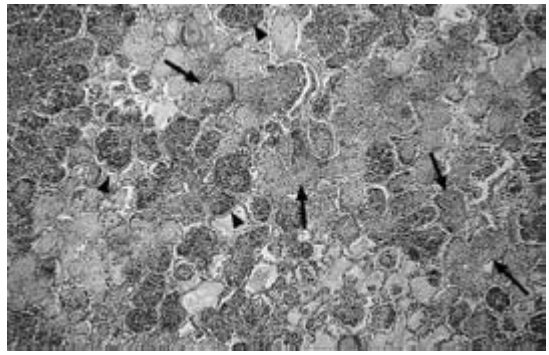

Figure 32.4: Fibrinous pneumonia due to *M. haemolytica*. Generalized congestion and oedema. Some alveoli are filled with a fibrino-cellular exudate (arrows) and in others accumulations of 'oat' cells are present (arrow heads). (Courtesy of Dr L. Gonzalez, VLA, Lasswade.)

necrotic lesions have a zonal structure with necrosis of all elements centrally surrounded by a dense layer of oat cells, macrophage-like cells and the products of cell death. Progressive stages of pleurisy are observed: the most acute type displays oedema and a diffuse light mononuclear cell exudate with masses of coccobacilli, while a thick layer of fibrino-cellular exudate characterizes the less acute form.

Diagnosis

Pasteurellosis is the commonest cause of acute pneumonia in sheep, but only a provisional diagnosis should be made on clinical signs and history [2]. The presence of *M. haemolytica* in nasal swabs is of no diagnostic significance and serology is not useful on either an individual or a flock basis. A diagnosis may be confirmed at necropsy by finding the acute inflammatory changes in the thorax and the characteristic hepatized and/or necrotic lung lesions. Further confirmation may be achieved by histological demonstration of lesions containing oat cells. However, hyperacute pneumonic pasteurellosis requires bacteriological confirmation, since other conditions, e.g. clostridial disease and even post-mortem autolysis, may cause similarly congested, heavy lungs.

In smears from exudates and from the cut surfaces of lung lesions, Gram-negative bacteria may be seen, but only the isolation in culture of large numbers [10^6 or more colony-forming units (CFU) per g of lung] confirms acute pasteurellosis. In subacute and chronic cases, 10^3–10^5 CFU/g would be expected. Samples should be collected from untreated cases and include a range of sites, e.g. thoracic fluids, heart blood, lung lesions, kidney, spleen and liver. Routine serotyping of isolates of *M. haemolytica* is of little value, but can be useful in epidemiological studies and investigations into putative vaccine breakdowns.

Epidemiology

Most outbreaks of pneumonic pasteurellosis in Europe occur in May, June and July, and many involve both ewes and lambs. Flock outbreaks usually start suddenly with deaths, often in young lambs in which the disease is hyperacute and septicaemic rather than pneumonic. As lambs get older, the disease becomes more confined to the thorax, with prominent lesions of pleurisy and pericarditis. Beyond 3 months of age most cases are frankly pneumonic, although sudden deaths with septicaemia rather than pneumonia may still occur. As the outbreak progresses over the next few days, a number of sheep will be noticed with clinical signs of pneumonia. Observations of the flock show that some sheep have an occasional cough and slight oculo-nasal discharges. Morbidity and mortality vary. No figures for morbidity rates are available but a total mortality of almost 2 per cent was derived from 450 outbreaks in which 125 731 sheep were at risk. Mortality was highest (2.5 per cent) in hill and upland sheep between weaning and entering the breeding flock. Pneumonic pasteurellosis also occurs in individual sheep sporadically rather than as part of a clearly defined flock outbreak.

It is generally assumed that environmental factors are important predisposing causes of pneumonic pasteurellosis, but precise epidemiological data are lacking. Some outbreaks can be linked to previous stressful situations such as warm or cold, wet weather, and dipping, castration or dosing [3]. There is evidence from experimental investigations and epidemiological studies that infection with parainfluenza virus type 3 (PI3) and ovine pulmonary adenocarcinoma are factors predisposing to pneumonic pasteurellosis. Infection with PI3 virus generally produces a mild illness (see Chapter 29), and cases of pneumonia in a flock can be attributed to a superimposed infection of the lungs with *M. haemolytica* strains. Viral infection is thought to create an ideal micro-environment consisting of necrotic cells and proteinaceous fluid in the lung, favouring bacterial growth by interfering with the mucocilliary clearance mechanisms of the respiratory tract, and by depressing the capacity of resident lung macrophages to take up and kill bacteria. The subsequent *M. haemolytica* infection is exacerbated by these events. In most cases, flock outbreaks on individual farms are sporadic and do not occur every year, although on some farms small numbers of sheep may succumb annually. There is a tendency for the prevalence of the disease to be higher overall in some years than in others for which there are two possible explanations. Either environmental factors, e.g. climate, are particularly favourable for the disease over a wide area in some years or immunity to the predisposing virus infection is cyclic.

Like viruses, some respiratory bacterial infections also increase the susceptibility of sheep to secondary *M. haemolytica* infections. *Mycoplasma* spp. are

common in the respiratory tract of sheep and the combination of *M. ovipneumoniae* and *M. haemolytica* A2 induces a proliferative (atypical) pneumonia in lambs. *Bordetella parapertussis* has been isolated from ovine lungs in Scotland and, under experimental conditions, can predispose to secondary *M. haemolytica* infections.

Treatment, control and prophylaxis

Since pasteurellosis is sporadic, optimum control is achieved by vaccination. However, there are circumstances in which the use of antibiotics is useful, for instance in outbreaks of pasteurellosis in lambs during the period when passive immunity from colostrum has waned and active immunity is being generated by vaccination. Long-acting oxytetracycline has been shown to be effective therapeutically and prophylactically in experimentally infected lambs and under field conditions.

Early commercial vaccines against pasteurellosis were bacterins and contained a very limited range of serotypes. There was no experimental evidence of their efficacy. The development of a method for producing pneumonic pasteurellosis in specific pathogen-free (SPF) lambs allowed the challenge of novel vaccines based on cell extracts and a range of serotypes. It was shown that these extracts were more immunogenic than bacterins, and the protection was serotype-specific.

New vaccines based on protein antigens that are involved in iron uptake have now been developed and became available in 1997. This development was based on the findings that SPF lambs that had recovered from an antibiotic-terminated episode of pasteurellosis induced by exposure to an aerosol of A2 were solidly immune to challenge with this serotype. This led to the discovery that *Pasteurellae* collected from the pleural fluid (i.e. bacteria grown *in vivo*) contained proteins not present in *Pasteurellae* grown *in vitro*, and that sheep produced an immune response to those proteins. Identical iron-regulated proteins (IRPs) were produced by the *Pasteurellae* when grown *in vitro* under conditions of iron limitation, and vaccines made from such bacterial cells were highly protective [4]. Since IRPs are antigenically similar in all serotypes, they offer cross-protection against serotypes not in the vaccine [5]. Other antigens are also involved in immunity and leukotoxin-containing vaccines have been found to be immunogenic. Serological responses in SPF lambs have shown that the leukotoxin-neutralizing titre and the ability of specific antibody and complement to kill *M. haemolytica in vitro* correlated with immunity to challenge.

Studies on the humoral and cell-mediated immune responses in the sera and lung washings of vaccinated and recovered lambs suggested that humoral immunity was the more important. This was confirmed by the finding that sera or immunoglobulins from infected or hyperimmunized sheep passively protected SPF lambs against aerosol challenge. The unpredictable, sporadic nature of pasteurellosis necessitates a vaccination pattern that will ensure year-round immunity for all ages of sheep. Breeding sheep should be vaccinated twice at an interval of 3–4 weeks, around tupping time and given a third, booster dose 4–6 weeks before lambing to ensure high concentrations of antibodies in the colostrum. Epidemiological evidence suggests that this schedule gives immunity to the lambs for up to 5 weeks. Active immunity in lambs should be induced by two doses of vaccine, the first being given as early as 10 days after birth, as colostrally acquired antibodies do not interfere with the response to vaccination. Weaned lambs brought in for autumn and winter fattening should be given two doses of vaccine, ideally before arrival on the farm. Annual boosters of breeding stock should be given before lambing, but more frequent vaccination can be used to cover periods of high risk as indicated by the pattern of disease on individual farms.

DISEASE ASSOCIATED WITH *P. TREHALOSI*

Systemic pasteurellosis

This is epidemiologically and pathogenetically distinct from the pneumonic form of pasteurellosis.

Clinical signs

The main feature is sudden death, so that affected sheep are seldom seen alive. Those that are, usually are recumbent, extremely depressed, dyspnoeic and froth-

ing at the mouth. This clinical description is consistent with that of endotoxic shock, and experimental studies in SPF lambs have demonstrated the typical biochemical characteristics of this condition [6].

Pathology

The carcass is usually that of a young sheep in good condition. Subcutaneous haemorrhages are found over the neck and thorax, and ecchymoses are also frequently seen on the pleura and diaphragm, or under the epicardium. The lungs are swollen and oedematous with widespread focal haemorrhages, and frothy blood-tinged fluid exudes from the bronchioles. Consolidation is not a feature. Lesions also occur in the pharynx and upper alimentary tract. In the former site, they take the form of necrotic erosions, which are especially prominent around the tonsillar crypts (see Figure 32.5 in the colour plate section). Similar erosions may be found also in the nasal mucosa, tongue or soft palate. Necrosis of the oesophageal lining, with extensive sloughing of mucosa, may be present and similar necrotic lesions are found variably in the omasum and rumen. The abomasum may contain considerable areas of haemorrhagic inflammation, or shallow haemorrhagic ulcers, most numerous at the pyloric end. Rarely, similar lesions may be seen in the duodenum.

The liver is usually swollen and congested and may contain numerous small (0.5–5 mm), grey, necrotic foci scattered throughout its substance. Small infarcts may sometimes be seen in the spleen, and the kidneys appear blotchy. The tonsils and retropharyngeal lymph nodes are usually enlarged and oedematous. Microscopically, the necrotic lesions in the pharynx and alimentary tract show necrosis of the mucosa with extensive sloughing. Underlying tissues are hyperaemic but exhibit surprisingly little cellular reaction. Masses of Gram-negative coccobacilli and Gram-positive cocci can be seen adhering to the luminal surface of many ulcers or eroded areas, and similar masses occlude local vessels and lymphatics. Lesions in the lungs, liver, spleen, adrenals and, less frequently, kidneys can be attributed to the dissemination of bacterial emboli in the terminal arterial system. The lesions are focal and consist of masses of Gram-negative coccobacilli, usually surrounded by zones of necrosis enclosed by basophilic spindle-shaped leucocytes (oat cells). In the brain, serum protein leakage in the cerebrocortical leptomeninges, with mononuclear cell infiltrates in the choroid plexuses of the lateral and fourth ventricles have been reported [2].

From the evidence of these findings and the results of experimental work with *P. trehalosi* serotypes in sheep, possible pathogenetic mechanisms can be postulated. It is thought that multiplication of *P. trehalosi* serotypes resident in the tonsils occurs under the influence of poorly understood environmental factors, e.g. change in pasture, with the development of necrotizing lesions in the pharynx and upper alimentary tract. Bacterial emboli from these sites pass by way of the general circulation to the lungs and other organs, where further multiplication and toxin release cause the death of the animal.

An alternative hypothesis sites the primary lesions in the forestomachs and intestines, emboli passing to the lungs via the liver and portal system.

Diagnosis

The diagnosis of systemic pasteurellosis depends on the isolation in culture of large numbers ($>10^6$ CFU/g of tissue) of *P. trehalosi* from the lungs, livers and spleens of sheep with the gross pathological changes described. *P. trehalosi* can be isolated from the nasopharynges and tonsils of apparently normal sheep and in small numbers from other sites, including the lungs, but their presence in these sites can be ignored. It should also be remembered that antibiotic therapy may reduce the numbers of bacteria in the lesions.

Epidemiology

Most outbreaks of systemic pasteurellosis conform to a well-defined pattern. In the UK, the disease affects 6–9-month-old sheep during October, November and December. The onset of the disease frequently coincides with folding on rape or turnips or a change to improved pastures, and both circumstances have been postulated as predisposing causes. However, there is as yet no proof that they are so. A change to wet, cold weather has also been noted as a possible contributing factor. A typical episode usually starts with a number of sudden deaths, but the number of deaths then

quickly drops over the next few days. Mortality is quite variable but seldom exceeds 10 per cent of the flock. Overall mortality rate for 116 outbreaks involving 24 040 sheep at risk was 2.5 per cent. Sporadic deaths due to systemic pasteurellosis do occur at other times of the year and in all ages of sheep, but even less is known about the factors that predispose to these cases.

Successful experimental production of systemic pasteurellosis has been reported. Feedlot lambs given hydrocortisone and a changed diet from 100 per cent roughage (alfalfa) to 90 per cent high protein concentrates succumbed to systemic pasteurellosis [7]. Dosing with *P. trehalosi* was not necessary. It was concluded that the dietary changes induced erosions and ulcers of the gastrointestinal mucosa, which were the portals of entry of the infection leading to the systemic disease. Thus, both epidemiological and experimental evidence points to the same pathogenesis. Pathology similar to that seen in natural causes has also been produced in SPF lambs challenged subcutaneously with *P. trehalosi*. However, these were young lambs (10 weeks of age) compared with the naturally affected animals [6].

Control, prevention and treatment

Because of the epidemiology of the disease, control is best achieved by vaccination. As with pneumonic pasteurellosis, field trials are difficult owing to the sporadic nature of the disease on individual farms from year to year. Experimental studies in SPF lambs have demonstrated that vaccines containing iron-regulated proteins, such as those used in *M. haemolytica,* conferred significant protection against T10 and T15 challenges [7].

Most isolates of *P. trehalosi* are sensitive to oxytetracycline (25 μg discs), but as sheep are rarely seen in the early stages of the disease therapy is seldom possible, and there are no reports on the value of prophylactic therapy.

Since stress may play some part in predisposing to this disease, flock management should be designed to minimize the stress involved in changes of environment and nutrition during the period from October to December.

OTHER *M. HAEMOLYTICA* AND *P. TREHALOSI* INFECTIONS

Occasionally *M. haemolytica* does cause other pathological conditions apart from than pneumonic and systemic pasteurellosis but these occur much less frequently. For example, strains of *M. haemolytica* and *P. trehalosi* have been isolated from cases of mastitis in ewes where the condition is occasionally fatal. Arthritis is a common sequel to experimental intravenous inoculation of *P. trehalosi* and is also occasionally diagnosed in the field. Sporadic *Mannheimia*/*Pasteurella* meningitis affects ewes and lambs. The isolation of large numbers of *Mannheimia*/*Pasteurella* cells from lesions is essential for diagnosis of all these conditions.

BORDETELLA PARAPERTUSSIS INFECTION

Bordetella parapertussis, the cause of a mild form of whooping cough in humans, has been isolated from sheep lungs in New Zealand and in the UK [8, 9] and is believed to be involved in ovine pneumonia. *B. parapertussis* predisposes lambs to secondary *M. haemolytica* pneumonia in an experimental model [10]. In common with other pathogenic *Bordetella* species, *B. parapertussis* has a large array of virulence factors that contribute to tissue damage in the host. The bactericidal mechanisms of the lung can be detrimentally affected by the destruction of ciliated cells and the presence of alveolar oedema fluid and alveolar exudate. *B. parapertussis* infection results in the reduction of the phagocytic capacity of alveolar macrophages and killing of *M. haemolytica* [11].

B. parapertussis is a small ($1 \times 2\,\mu m$) Gram-negative aerobic coccobacillus. The colonies are slow-growing (up to 48 hours) on Brodet–Gengou medium showing a β-haemolysis. Isolation from lungs and upper respiratory tract samples can be difficult, but specialized isolation media have been developed [11].

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33

Mycoplasma respiratory infections

R.D. Ayling and R.A.J. Nicholas

Synonyms: atypical, non-progressive, prolific interstitial pneumonia, coughing syndrome, enzootic pneumonia, mycoplasma pneumonia, summer pneumonia

Respiratory disease in sheep may result in sudden death or in protracted illness causing suffering to affected animals as well as high financial losses to owners. In addition to death and sickness, reduced feed efficiency, slaughter condemnations, prevention and treatment measures contribute substantially to losses. Some consider that this condition is dominated by pneumonic pasteurellosis caused by *Mannheimia*

haemolytica, particularly in newborn and feedlot lambs as well as in mature ewes, but the role of mycoplasmas, in particular *Mycoplasma ovipneumoniae*, is often overlooked. As well as causing disease in its own right *M. ovipneumoniae* may also predispose animals to pasteurellosis and viral infections. Sheep are generally less susceptible to mycoplasmas than goats but a reassessment of the mycoplasma flora of sheep is necessary, particularly in regions where mixed flocks of small ruminants are kept.

CAUSE

M. ovipneumoniae, first isolated in New Zealand in 1974, is the cause of atypical or ovine non-progressive pneumonia. Mycoplasma pneumonia is well recognized in both Australia and New Zealand where it is known as 'summer pneumonia' because of a seasonal increase in the prevalence of the disease. A coughing syndrome identified in the USA with persistent and long-term coughing, as well as rectal prolapse, was attributed to a combination of *M. ovipneumoniae* and *M. arginini* [1]. It is believed that a primary infection with *M. ovipneumoniae* may predispose sheep to invasion of the lower respiratory tract by other organisms such as parainfluenza 3 virus, a frequent pathogen of housed lambs, and *Mannheimia haemolytica* which may enhance the pathological process.

Confirmation of the role of *M. ovipneumoniae* in disease has been shown experimentally following endobronchial inoculation of infected lung lesion homogenates and mixtures of isolates [2]; inoculation of pure cultures is much less successful and leads to mild disease suggesting differences in virulence among isolates [3]. In the UK, an apparent increase in isolation of *M. ovipneumoniae* from pneumonic flocks since 2001 may be related to overcrowding following restrictions of movement imposed during the foot-and-mouth disease outbreaks [4]; many of these outbreaks were in flocks fully vaccinated with pasteurella vaccines.

Low numbers of *M. ovipneumoniae* are often isolated from the lungs of healthy sheep but during times of stress or inclement weather, subclinical infection may predispose sheep to acute fibrinous pneumonia, pulmonary abscessation or pleurisy. In one study, over 70 per cent of lambs with lungs affected by chronic non-progressive pneumonia had titres greater than 10^6 organisms per gram of lung, whereas only 25 per cent of non-pneumonic lungs contained mycoplasmas with under 3 per cent having a titre of 10^6 organisms per gram of lung [5].

M. ovipneumoniae has several mechanisms that may help cause disease or evade the host's immune system. It has a polysaccharide capsule which is thought to have a role in pathogenicity and may facilitate adherence of the organism to the ciliated epithelium. The capsule may also interfere with macrophage activity and thus contribute to disease [1, 6]. The production of autoantibodies to the ciliary antigen of the respiratory tract is thought to be a mechanism involved in the coughing syndrome of lambs. Colonization of the respiratory tract by *M. ovipneumoniae* precedes the production of these antibodies [1].

Extensive heterogeneity has been shown among *M. ovipneumoniae* strains by DNA and protein analysis revealing 58 different profiles in 60 isolates [7, 8]. Consequently, it is unlikely that immunity to any one strain will confer total protection against all other strains [5].

CLINICAL SIGNS

Reports from Australia describe outbreaks of pneumonia in housed lambs in the first year of life with high morbidity and low mortality, poor growth rates and exercise intolerance [9–11]. Clinical signs may be mild with dull lambs showing increased respiratory rates; in contrast, infections may also result in mortality, acute fibrinous pneumonia, consolidated lesions, pulmonary abscesses and pleurisy, depending on exacerbating circumstances. The signs initially present as coughing, temperature rise, depression of appetite and growth rate, with a drop in milk yield. A variable percentage of lambs will be affected but, over a period of weeks, the problem may involve most of the lambs. Growth rates may be slowed and carcass weights reduced. The main clinical signs are chronic, persistent and irregular cough which may lead to rectal prolapses, and a mucopurulent nasal discharge; other bacterial infections may also be

involved that cause a more severe inflammatory response and clinical signs [1].

M. ovipneumoniae has also been isolated from cases of mastitis and keratoconjunctivitis but its role in these diseases is unknown [12].

PATHOLOGY

Typically, lesions begin with dull red ventral areas of collapse which are accompanied by bronchiolitis in associated airways (see Figures 33.1 and 33.2 in the colour plate section). They progress to firm red–grey areas of consolidation over 2–3 weeks but may continue as grey areas of consolidation, often with attached localized pleural adhesions [5].

Histologically, a wide range of lung changes are seen, including hyperplasia of the lymphoid nodules and bronchiolar epithelium and a mononuclear cell reaction in the alveolar septa. Evident also are lymphocytic cuffs around bronchioles and vessels, collapsed alveoli and others with exudates containing mononuclear cells. Lymphoid hyperplasia may result in extensive cuffs around airways with consequent compression of the passages. Ultrastructurally, *M. ovipneumoniae* has been reported to attach to cilia in airways of lambs and to colonize the upper respiratory tract of sheep [1].

DIAGNOSIS

Gross lung pathology is not pathognomic so laboratory diagnosis is essential to confirm mycoplasma involvement in disease. Good serological responses have been detected in experimentally infected lambs but, in the field, titres can be much lower and clearly reflect the immunogenic heterogeneity of strains. Because of its wide prevalence, examining paired sera for a rising titre gives a better indication of an active *M. ovipneumoniae* infection. The isolation or detection of *M. ovipneumoniae* is only an additional aid to diagnosis of the disease, as many healthy sheep carry low levels of *M. ovipneumoniae*; however, quantification of the mycoplasma concentration in the lungs may be useful. Unlike most mycoplasmas, *M. ovipneumoniae* produces small centreless colonies but the identity must be confirmed by serological tests like growth inhibition or, increasingly, polymerase chain reaction (PCR). Newer molecular methods such as PCR [4] and the 16S rDNA PCR and denaturing gradient gel electrophoresis [13, 14] offer rapid methods for detecting mycoplasma and can detect multiple mycoplasma infections.

Immunohistochemistry, using a specific polyclonal or monoclonal antibody, confirms the presence of the organism in the diseased lungs and provides an indication of the involvement of *M. ovipneumoniae* in lung damage [15].

Suitable samples for diagnosis from live animals include sera, nasal swabs, ear swabs or bronchoalveolar lavage. A transtracheal bronchoalveolar lavage technique that successfully recovered *M. ovipneumoniae* and *Mannheimia haemolytica* has been described [16], but its general use in the field may not be widely accepted. With dead animals, lung taken from the interface between healthy and diseased tissue and pleural fluid is desirable.

It is important to differentiate atypical pneumonia from the progressive pneumonias maedi and pulmonary adenocarcinoma. The age, pattern of disease, clinical signs and histological changes are useful distinguishing features. Furthermore, chlamydial invasion of the lower respiratory tract can also produce lung damage similar to that seen in atypical pneumonia.

EPIDEMIOLOGY AND TRANSMISSION

Transmission of the causal organism is mainly by the respiratory route. Mycoplasmas frequently occur in the upper respiratory tract of healthy sheep, which may act as a major source of infection to lambs. Lambs are thought to become infected within a few days of birth but disease progresses slowly and occurs often with secondary bacterial infections from 5 to 10 weeks of age. The pneumonia may be severe, but some lambs recover within a few weeks while it persists in other lambs for much longer. Outbreaks can occur when groups of lambs from different sources are housed together and may be a result of mixing uninfected with infected lambs or the effect of encountering different strains of *M. ovipneumoniae.* In Spain,

high summer temperatures were a precipitating factor in exacerbating a mild condition and principally involved *M. ovipneumoniae* with *Actinobacillus pleuropneumoniae* biovar A [17].

TREATMENT AND CONTROL

Improved husbandry practices, such as lower stocking densities and improved ventilation, are important in preventing and reducing the spread of respiratory disease. Contact, even through shared airspace, with older sheep is best avoided and bought-in sheep or lambs should be isolated before mixing with the home flock.

Treatment with antimicrobials effective against mycoplasmas often produces immediate respite, which may be sufficient for the animal to recover and may be the result of elimination of secondary bacteria. However, where *M. ovipneumoniae* is involved, the animals may quickly relapse and require further treatments. Antimicrobials likely to be effective include the newer fluoroquinolones, oxytetracycline or a macrolide. However, recent *in vitro* antibiotic trials on UK isolates of *M. ovipneumoniae* indicated variation in antimicrobial susceptibility between strains, particularly to the macrolides where antimicrobial resistance seems to have developed [15].

The outbreaks of pneumonia recently seen in *Pasteurella*-vaccinated flocks in the UK suggest that consideration should be given to incorporating a mixed selection of *M. ovipneumoniae* strains into these vaccines to provide greater protection against respiratory disease [4].

OTHER MYCOPLASMAS

Other *Mycoplasma* species are occasionally associated with respiratory disease in sheep. *M. arginini*, a ubiquitous mycoplasma of many animal species, is frequently isolated from the respiratory tracts of lambs with disease, sometimes mixed with *M. ovipneumoniae*. While it is not thought to be a major pathogen it may increase pathological damage.

M. agalactiae, a major cause of contagious agalactia in sheep and goats, has also been isolated from pneumonic lungs [18], although its role in respiratory disease is not clear. The cattle pathogen, *M. bovis* has been isolated infrequently from the respiratory tracts of sheep and goats [12]. In a recent study in the Middle East the goat pathogens *M. putrefaciens*, *M. mycoides* subsp. *mycoides* large colony variant and *M. capricolum* subsp. *capricolum* were isolated from both nasal swabs and milk samples of pneumonic sheep and goats in mixed herds [19]. In North Africa, *M. c. capricolum* appears to be the major cause of pneumonia and contagious agalactia in sheep [20]. Similarly, *M. capricolum* subsp. *capripneumoniae*, the cause of contagious caprine pleuropneumoniae, has been isolated from respiratory tracts of sheep in contact with affected goats [21].

While only a few *Mycoplasma* species have been reported to cause respiratory disease in sheep, it is apparent that sheep may be equally susceptible to infection by a range of *Mycoplasma* species if the opportunity presents itself. Sheep may also act as carriers of *Mycoplasma* species for other susceptible host species.

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34

Parasitic bronchitis and pneumonia

F.E. Malone

Synonyms: lungworm, hoose, husk, verminous pneumonia

Parasitic bronchitis and pneumonia in sheep in Western Europe is not as economically important as the equivalent disease in cattle. The Veterinary Investigation Surveillance Report in Britain stated that lungworms were associated with 7.3 per cent (range 3.3–10.3 per cent) of ovine respiratory diseases diagnosed at post-mortem between 1997 and 2004 [1]. The disease is particularly important in countries where sheep are grazed intensively in warm moist conditions [2]. Parasitic bronchitis primarily affects young sheep. The disease is characterized by coughing and is seen more commonly in autumn and early winter [3].

CAUSE

A number of parasitic nematodes in the families Dictyocaulidae and Protostrongylidae may infect sheep lungs [4]. The most important of these are *Dictyocaulus filaria*, *Muellerius capillaris* and *Protostrongylus rufescens*. Other sheep lungworms reported include *Cystocaulus ocreatus* and *Neostrongylus linearis*.

CLINICAL SIGNS

The severity of clinical signs depends on the level of infection and the lungworm involved. *D. filaria* may cause coughing, increased respiratory rate and bilateral nasal discharge. Severe infestations are associated with reduced appetite and weight loss. However, deaths due to *D. filaria* infection are uncommon unless there is secondary bacterial infection. *M. capillaris* is more common than *D. filaria* or *P. rufescens*, but it causes minimal clinical signs in sheep. It is considered more pathogenic in goats [2]. *P. rufescens* is not as pathogenic as *D. filaria*, but heavy infestations can be associated with respiratory distress and weight loss.

LIFE CYCLE AND PATHOGENESIS

D. filaria is the most pathogenic lungworm of sheep. The adult worms are 30–100 mm long and live in the bronchi, where the females lay embryonated eggs, some of which may hatch in the lungs. Both embryonated eggs and first stage larvae are expelled from the lungs by coughing and are then swallowed. The remaining embryonated eggs hatch as they pass through the sheep's intestine and first-stage larvae are expelled in the faeces, where they develop to the infective third stage and are ingested with herbage. Ingested larvae penetrate the small intestinal wall and pass via the lymphatic vessels to the mediastinal lymph glands, where they develop to fourth-stage larvae. These migrate via the thoracic duct, anterior vena cava and heart to reach the pulmonary alveoli 7 days after ingestion. The larvae then migrate to the bronchi, where they develop to fifth-stage larvae and finally to adults in about 4 weeks post-infection [4].

The migrating larvae induce an inflammatory reaction in the alveoli and stimulate a cellular infiltrate consisting of neutrophils, eosinophils, macrophages and macrophage giant cells. As the larvae progress to the bronchioles and bronchi, both fifth-stage larvae and adult worms cause a severe bronchiolitis and bronchitis characterized by hyperplasia of respiratory epithelium, which becomes infiltrated with neutrophils and eosinophils. There is peribronchial and peribronchiolar lymphoid hyperplasia, sometimes forming lymphoid nodules. Some of the embryonated eggs and hatched first-stage larvae are aspirated into the bronchioles and alveoli, causing an inflammatory reaction and resultant pneumonia. Alveolar epithelial hyperplasia, which may be widespread, develops

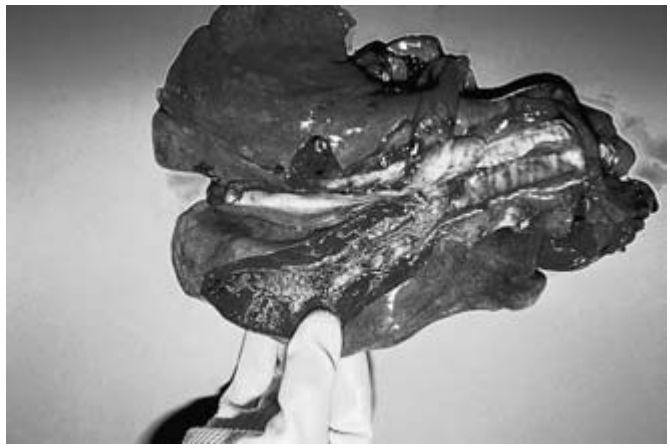

Figure 34.1: Severe adult *Dictyocaulus filaria* infection in the trachea and bronchi of a 9-month-old lamb.

during this phase of the infection. Bacterial pneumonia may develop as a secondary complication.

On post-mortem examination, adult *D. filaria* may be seen in the bronchi (Figure 34.1). Coalescing foci of consolidation may be present in the lungs, predominantly in the posterior regions of the caudal lobes. Atelectasis or emphysema may be seen at the edges of affected areas, but the severe interstitial emphysema characteristic of bovine *D. viviparus* infection is not a feature of the ovine disease.

M. capillaris has a similar life cycle to *D. filaria* but, after expulsion in the faeces, requires a suitable mollusc (slugs or land snails) as an intermediate host [4]. Sheep become infected when they consume the intermediate host. The adult worms live in the alveoli or alveolar ducts and produce multifocal, subpleural nodules about 2 mm in diameter throughout the lungs, but predominantly in the caudal lobes. Initially, the nodules are red-purple and soft, later becoming greyish, firm and occasionally mineralized. Such nodules may also be found deep in the lung parenchyma. Histologically, the reaction around the worms varies from mild interstitial pneumonia to a marked granulomatous reaction, with infiltration of eosinophils, lymphocytes, macrophages and macrophage giant cells [5].

P. rufescens also has an indirect life cycle and requires a land snail as intermediate host to develop to the infective stage, when it is consumed by the sheep. The adult worms are slender, red, 16–35 mm long and live in the small bronchioles. On post-mortem examination, small, yellowish raised lesions are seen in the caudal lobes; older lesions are grey and occasionally have mineralized necrotic centres. The worms cause a granulomatous reaction, containing occasional mineralized foci, with associated inflammatory changes in the surrounding alveoli [5].

EPIDEMIOLOGY AND TRANSMISSION

Important sources of infection for lambs are increased numbers of *D. filaria* first-stage larvae in the faeces of infected ewes or yearlings in the spring, or infective third-stage larvae surviving over the winter on pasture. More suitable conditions for larval survival occur in autumn/winter than in spring/summer, so the number of infective larvae on pasture is minimal during the summer and greatest in the autumn [3]. The level of excretion of *D. filaria* larvae in the faeces of infected sheep shows considerable individual variation, both weekly and from year to year [6]. During the grazing season at least three generations of the parasite may occur, so autoinfection is possible [3].

In the UK, prevalence of *D. filaria* infection is low in spring and summer, increasing in late autumn and winter and falling to a low level by May. Moist summer conditions give rise to a higher level of infection in autumn and winter than a dry summer [3]. Clinical disease is reported more frequently in hill lambs in autumn, occasionally in association with parasitic gastro-enteritis. Most lambs become infected at some time during their first year of life, but there is a

wide range in susceptibility of individual lambs to *D. filaria*, with most lambs developing a light, but prolonged infection [3, 6]. Most infections are in lambs, with only a few light, short-lived patent infections in yearlings or adult ewes [6]. Host ranges for *D. filaria* (sheep), *D. viviparus* (cattle) and *D. capreolus* (moose and roe deer) are considered to be mainly restricted to these species [7].

Both *M. capillaris* and *P. rufescens* require intermediate hosts for their development. The latter are the sole source of infection for sheep and the infective larvae are reported to persist in the intermediate host for its lifetime [4].

DIAGNOSIS

A presumptive diagnosis of *D. filaria* infection may be made on the basis of mild-to-moderate respiratory disease in lambs that have been grazing in late summer or early autumn. The characteristic first-stage larvae may be demonstrated in the faeces by use of the Baermann method or flotation procedures [8]. First-stage larvae of *D. filaria* are 0.55–0.58 mm long, contain numerous brownish food granules in the intestinal cells and have a small button-like structure at the anterior end [4]. On post-mortem examination the adult worms are demonstrable on opening the trachea and bronchi. The presence of *D. filaria* larvae in bronchioles and alveoli may also be detected by the Baermann method [8].

M. capillaris lungworms may be extracted from non-calcified nodules by compressing the nodule between two pieces of glass and teasing the worm from the tissues with dissection needles [8].

TREATMENT, PREVENTION AND CONTROL

As clinical lungworm infections in sheep are uncommon in Western Europe, specific strategies for control of parasitic bronchitis have not been reported. Control strategies for gastrointestinal worms, such as anthelmintic prophylaxis, will also be effective in controlling parasitic bronchitis. Levamisole, the benzimidazoles, ivermectins, doramectin and moxidectin are effective in treating *D. filaria* infection. However, after anthelmintic treatment re-infection may occur until late autumn or winter [6]. The ivermectins, doramectin and moxidectin all have persistent activity against *D. filaria*.

While an irradiated larval vaccine is available for *D. viviparus* in cattle, a similar vaccine for *D. filaria* in sheep is not developed commercially in Western Europe.

Control of *M. capillaris* and *P. rufescens* is more difficult, as intermediate hosts that are present on poorly drained pastures are involved. The benzimidazoles, avermectins and moxidectin are reported to be effective for treatment of these infections.

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Part VII
Diseases of the nervous system

35

Scrapie

M. Jeffrey and L. González

Synonyms: la tremblante (French: trembling), traberkrankheit (German: trotting disease), gnubberkrankheit (German: nibbling disease), rida (Iclandic: ataxia or tremor), prurigo lumbar (Spanish: lumbar itchy skin eruption)

Scrapie is a chronic, progressive and invariably fatal neurodegenerative disorder naturally affecting sheep and goats. This infectious disease has been present in Britain and parts of Europe since at least the eighteenth century, and is currently identified in several countries of the Americas, Africa and Asia. It is notably absent from Australasia, which has remained free of disease due to rigorous quarantine measures and to eradication programmes taken following importations of infected sheep.

Scrapie belongs to a group of disorders commonly referred to as prion protein disorders or transmissible spongiform encephalopathies (TSEs) (Table 35.1). Previously little known outside the sheep farming, veterinary and medical communities, the epidemic of bovine spongiform encephalopathy (BSE) and the occurrence of new variant Creutzfeldt–Jakob disease (vCJD) have served to increase public and scientific awareness of these peculiar diseases of man and animals.

In the absence of a live animal diagnostic test, the reporting of scrapie has historically depended on submissions of sick sheep for laboratory investigation. However, sheep farmers have been reluctant to acknowledge the occurrence of the disease in their flocks because of its impact on the value of the breeding stock. For this reason, and because of the difficulty of clinical diagnosis, an accurate estimation of the incidence of scrapie has been hard to establish. In fact, when scrapie became a notifiable disease in the UK, and in other European Union (EU) states, in 1994, a reduction in the reported incidence of the disease in Britain was observed. Since the BSE epidemic and its proven transmission to other species, the EU has initiated widespread active surveillance for scrapie throughout Europe, leading to a greatly heightened awareness of this group of diseases. This, coupled with an explosion in research activity, is resulting in a greatly increased number of scientific publications in the field. This chapter is written, therefore, against a background of rapidly emerging data and a consequent shifting of emphasis between ideas and priorities.

Scrapie can be transmitted experimentally to a number of different experimental host species, including livestock and laboratory animals. Moreover, there are several naturally occurring disorders related to scrapie currently recognized in various species (Table 35.1). Both transmissible mink encephalopathy (TME) and BSE have been linked to scrapie of sheep. Although in neither of these instances is there conclusive proof of a causal relationship, there is a common acceptance of epidemiological data suggesting that sheep scrapie fed to cattle in the form of meat and bone meal initiated the BSE epidemic in the UK. Subsequent transmission and biochemical studies have established that BSE, vCJD, feline spongiform encephalopathy (FSE) and other TSEs in captive felids, ungulates and primates are caused by the same agent strain. On the other hand, there is no convincing evidence to relate chronic wasting disease (CWD) and other human prion diseases to either sheep scrapie or BSE. A group of recently emerged conditions generically called ‘atypical scrapie’ (including ‘Nor 98’) are considered by some to be prion protein disorders of sheep, but its actual relationship to scrapie is unclear.

CAUSE

It has long been evident that, unlike many other diseases of humans and animals, scrapie was not caused by a conventional infectious agent. Although controversy still remains concerning the precise molecular

Table 35.1: Naturally occurring scrapie-like diseases

Name of disease	Species affected
Scrapie	Sheep Goat Moufflon
Chronic wasting disease (CWD)	Mule deer White tailed deer Elk
Transmissible mink encephalopathy (TME)	Farmed mink
Bovine spongiform encephalopathy (BSE)	Cattle
Spongiform encephalopathy in primates	Nyala, gemsbock, oryx, eland, kudu, ankole, bison Lemur
Feline spongiform encephalopathy (FSE)	Domestic cat Puma, cheetah, ocelot, lion, panther
Kuru	Man
Creutzfeldt–Jakob disease (CJD)	Man
Variant CJD	Man
Gerstmann–Staussler syndrome	Man
Fatal familial insomnia	Man

and biochemical nature of the causal agent of TSEs, most researchers adhere to the prion or protein only hypothesis [1].

During TSE infections, an abnormal isoform of a host-encoded membrane glycoprotein, called prion protein (PrP), accumulates in certain tissues, notably of the central nervous system (CNS), peripheral nervous system (PNS) and, in most cases, lymphoreticular system (LRS). This abnormal isoform is referred to as PrP^{sc} (where 'sc' stands for scrapie), PrP^{d} (disease-associated) or PrP^{res} (protease resistant), and its detection in tissue extracts or sections is commonly used as a diagnostic criterion. Transmission studies demonstrate that those tissues also contain the scrapie agent (or any other TSE agent, depending on the specific disease), which is unusually resistant to denaturation and sterilization processes. These and other data led to the formulation of the prion hypothesis, which proposed that PrP^{sc} was the causative agent or prion. As originally defined, the prion was a 'proteinaceous infectious particle that lacks nucleic acid; composed largely, if not entirely, of PrP^{sc} molecules' [1], which was capable of inducing conversion of the normal or 'cellular' form of PrP (PrP^{c}). Within the same context, PrP^{sc} was defined as an 'abnormal, pathogenic isoform of the prion protein that causes sickness; the only identifiable macromolecule in purified preparations of prions' [1].

In many studies, PrP^{sc} has been shown to possess a number of qualities that differentiate it from normal PrP^{c}, including partial protease resistance, insolubility and high beta-pleated sheet content [1]. An impressive amount of data in support of the prion hypothesis has been garnered from a wide range of experiments and includes: (1) the failure to transmit disease to mice engineered to lack PrP; (2) the apparent transmissibility of a spontaneous disease in transgenic mice expressing human inherited forms of prion disease; (3) the transmission of infection from contaminated instruments and wires; and, most recently, (4) the transmission of infection with abnormal PrP generated *in vitro* from synthetic peptides. Proof of principle of the prion hypothesis has unequivocally been established in yeast, but remains unproven in mammals, as some of the key experiments have not yet been successfully reproduced by different research groups. Furthermore, the prion hypothesis is still unable to provide satisfactory explanations for the transmission of the more than 20 laboratory strains of

scrapie that have been characterized in mice [2] and are increasingly being recognized in sheep [3, 4]. It has been proposed that numerous conformationally stable forms of abnormal PrP would exist and be capable of producing strain-specific patterns of pathology and incubation period. At present, however, there are no data to identify PrP^{sc} conformational variants with such properties, which could potentially carry strain-specific information and still be naturally transmitted between animals. A minority view therefore still maintains that a conventional nucleic acid is more likely to contain the specific information necessary to convey strain diversity [5].

The scrapie and other TSE agents are exceptionally resistant to inactivation. They remain infectious after heat treatment in excess of 100°C for 1 hour, ultraviolet and ionizing radiation, and most chemical disinfectants. Wet heat (autoclaving) is far more effective than dry heat, with one report even suggesting that the scrapie agent resists heating to over 300°C in a desiccated state. Some chemicals, such as formalin, seem to protect the agents from heat inactivation, while others, such as sodium hydroxide, may increase the effect of heat. Thus, autoclaving for 30 minutes at 121°C in 1 M sodium hydroxide is completely effective. Sodium hypoclorite (21 000 ppm active chlorine) appears to be one of the best disinfectants for routine use on equipment and tools.

ANTE-MORTEM DIAGNOSIS OF CLINICAL SCRAPIE

Most cases of clinical scrapie occur in sheep 2–4 years old, but where infection pressure is low, individual cases can occur in much older animals. Conversely, exceptional cases have been reported in sheep younger than 1 year. The incubation period is probably modulated by the infectious dose, the age at infection and the prion protein (PrP) genotype of the host [6], and possibly by the infecting strain.

Clinical diagnosis of individual scrapie cases may be difficult, as signs are insidious and variable in different sheep populations and in flocks with different levels of infection, and can resemble, especially in the early stages, those of some other conditions of adult sheep. The first signs are usually behavioural changes that are most readily evident to shepherds. Affected sheep may lead or trail the rest of the flock when driven, or disengage from it; they may show abnormal reactions to the sheep dog or appear momentarily confused or anxious. These early signs progress to a more definite neurological illness frequently characterized by signs of pruritus and ataxia, one of which usually dominates the clinical course. Intense itch is recognized principally by compulsive rubbing or scraping against fixed objects (Figure 35.1), nibbling

Figure 35.1: Sheep showing clinical signs of scrapie including emaciation, skin lesions over ischial tuberosity due to scratching and biting due to pruritus. (Courtesy of Dr J.A. Garciá de Jalón, University of Zaragoza, Spain.)

at the skin and scratching with a hind foot. This may result in extensive loss of wool and self-inflicted skin lesions, particularly over the lateral thorax, flanks and hindquarters. A characteristic 'nibbling reflex' can often be elicited by palpation of the lumbar region, and may also be evoked by the sheep's own scraping movements. Locomotor incoordination is first apparent as an awkwardness of turning, difficulty in positioning or swaying of the hind limbs, and a high stepping or trotting gait of the forelimbs. Stumbling and falling occur, but the sheep is generally able quickly to regain a standing posture, until ataxia progresses to quadriplegia and recumbency. Other neurological signs may include teeth grinding (bruxism), cud-dropping (dysphagia), excess salivation, abnormally low carriage of the head and ears, fine tremor, seizures and amaurosis (blindness). There may also be apparent hyperaesthesia to sound, movement or touch, as well as polydipsia and polyuria. In most cases, there is also a loss of body condition; although in some cases this can be the earliest detectable change, significant weight loss usually occurs in the late clinical stages. This pre-terminal decline may be associated with the reduction in rumination time reported for sheep with scrapie.

The clinical phase of the disease can last for weeks or even longer, but much more abrupt cases can also be seen in which sheep collapse within days of onset, or even die without premonitory signs.

POST-MORTEM CONFIRMATION OF CLINICAL DISEASE

There are no specific gross pathological changes seen in scrapie. Histological lesions are confined to the CNS where the most striking change is vacuolation of the neuroparenchyma, without primary degenerative changes of the white matter. There are no specific inflammatory changes, but there may be neuronal loss, rare neuronophagia and gliosis. Both astrocytosis and microgliosis occur but these are highly variable tending to parallel the magnitude of vacuolation.

Vacuolated neuronal perikarya are distended by characteristic single or multiple vacuoles often containing intra-vacuolar membranous debris. Vacuolation in neuronal processes produces the distinctive appearance of spongiform change in the grey matter neuropil. Vacuolar changes are sometimes accompanied by less conspicuous microscopic features such as cerebrovascular amyloidosis. Typically, vacuolar lesions have a bilaterally symmetrical distribution and are usually most apparent in the brain stem and thalamus. The magnitude and distribution pattern of vacuolation appears to be influenced by both agent strain and host genotype, although other factors may also contribute to the overall magnitude, so that there is not always a correlation between the severity of vacuolation and that of clinical signs or the duration of the clinical phase.

Careful examination and experience is necessary to differentiate scrapie-associated vacuolar changes from those occurring as a result of other infectious and non-infectious ovine conditions. Confirmation of histopathological examination-based diagnosis currently relies on the detection of accumulation of abnormal PrP in the CNS. This can be achieved by Western immunoblotting, of which there are several different protocols, whereby abnormal PrP is distinguished from normal PrP by its increased resistance to protease digestion (PrP^{res}). Once subjected to detergent extraction and enzyme digestion, samples of brain containing abnormal PrP result in a characteristic three-band pattern, which corresponds to the unglycoslyated, monoglycosylated and diglycoslyated fractions of the protein. Another confirmatory method is immunohistochemistry, in which disease-associated PrP (PrP^{d}) is specifically identified by a combination of pre-treatment of tissue samples, appropriate antibodies and cell-based morphological features. The most intense and consistent accumulations of PrP^{d} are found in the hindbrain and spinal cord, while the variation in intensity of PrP^{d} deposition in forebrain areas is considerable. A further confirmatory method is the detection of scrapie-associated fibrils in CNS tissue extracts by electron microscopy.

Histopathological examination, Western immunoblotting and immunohistochemistry are the three tests used for the statutory diagnosis of scrapie in the UK. Results from a blind test correlation study have shown that the results for the three tests give a high degree of correlation in clinically suspect scrapie cases.

Transmission from infected tissues, usually to laboratory rodents by injection, is the only available means of detection of infectivity. Although mice have proved the most useful species for this purpose, attempts to transmit natural scrapie to mice are not always successful, although it is likely that transgenic mice expressing sheep PrP genes will be generally more efficient. Because of the long incubation periods (1–2 years), it is impractical to use the criterion of transmissibility for diagnosis.

POST-MORTEM DETECTION OF PRE-CLINICAL SCRAPIE

Pathogenesis studies and surveillance schemes have shown that some asymptomatic sheep may accumulate abnormal PrP in brain at relatively early stages of the disease process, always preceding the onset of vacuolar changes. Early PrP^d deposits can be found more or less simultaneously in the intermedio-lateral columns of the thoracic spinal cord and in the dorsal motor nucleus of the vagus nerve in the hindbrain (see Figure 35.2 in the colour plate section), pointing towards the enteric nervous system as the common source of these, respectively, sympathetic and parasympathetically innervated sites. In some heavily infected flocks, early CNS accumulation of PrP^d can be detected at 10 months of age (i.e. at around 40 per cent of the incubation period, assuming infection at lambing time), while in other studies of natural and experimental disease by the oral route, initial accumulations are not detected until approximately 14–16 months of age (approximately 60 per cent of the incubation period).

Several enzyme-linked immunosorbent assay (ELISA)-like immunochemical methods can be applied to the early detection of abnormal PrP in CNS samples. In fact, these so-called 'rapid tests', because of their high sensitivity, are currently used for the screening of subclinical scrapie in active surveillance programmes. As with immunoblotting, these methods generally rely on enzymatic pre-treatment of tissue samples to differentiate between normal and abnormal PrP, though one assay based on abnormal PrP-specific antibodies is currently available.

Outside the CNS, accumulation of PrP^d can be found in the sensory retina, major nerve trunks, the enteric nervous system, peripheral and cranial nerve ganglia, and other sites including the adrenal medulla. Accumulations of abnormal PrP have recently been demonstrated in muscle, either in association with nerves or with the peripheral nervous component of muscle spindles. However, little is known about whether these accumulations, which are mostly associated with the peripheral nervous system, precede or succeed CNS involvement, as most of those findings refer to clinical disease.

One important feature of sheep scrapie is the consistent and significant aggregation of abnormal PrP, both PrP^{res} and PrP^d, in LRS tissues [7, 8]. Direct neuroinvasion without accumulation of abnormal PrP in LRS had been reported to be a feature of infection of sheep of a particular PrP genotype (VRQ/ARR;). However, recent studies demonstrate that the frequency of scrapie cases, clinical and pre-clinical combined, without LRS accumulation of PrP^d is very low (less than 3 per cent), and there is no clear association between LRS involvement and PrP genotype [9]. All LRS tissues are equally affected in terminal disease except for thymus, which does not show significant PrP^d accumulation. Accumulation of PrP^d in LRS tissues is mainly restricted to secondary follicles where it is associated with tingible body macrophages (see Figure 35.3 in the colour plate section) and follicular dendritic cells. Some macrophage-like cells within adjacent paracortical T cell areas and in the marginal zone of the spleen also show PrP^d accumulation [10].

The onset of accumulation of abnormal PrP in LRS tissues is similarly variable as in the CNS. Some reports describe PrP^d positivity in retropharyngeal and/or mesenteric lymph nodes, ileal Peyer's patches and palatine tonsil in naturally infected sheep as young as 2 months, while in other studies such accumulations are not detected until the second year of life. The time dynamics of accumulation of PrP^d in the LRS appear to be influenced by age at exposure, dose, genotype and strain of the infectious agent, but in most sheep scrapie cases, either natural or experimental, accumulation of PrP^d in LRS tissues precedes that in the CNS, and is often detectable in apparently healthy sheep in the absence of PrP^d in the brain [9].

ANTE-MORTEM DETECTION OF PRE-CLINICAL SCRAPIE

Although infectivity of blood during the clinical and pre-clinical stages of sheep scrapie has been demonstrated by blood transfusion [11], there is currently no suitable blood test for the diagnosis of the disease. Therefore, the consistent and early accumulation of PrP^d in LRS tissues has prompted the use of biopsies for the identification of pre-clinically infected animals. Most efforts have concentrated on palatine tonsil and third eyelid samples which have provided successful results in experimental situations. These procedures

have, however, technical drawbacks and handling requirements that prevent their use for the screening of large populations of sheep in the field. More recently, detection of PrP^d in biopsies of rectal mucosa, a site containing abundant lymphoid follicles, has been reported to have a similar diagnostic efficiency to that of tonsil biopsy [12]. Owing to its practicality and lack of adverse effects on the sheep, this procedure offers good prospects for field diagnosis of pre-clinical scrapie.

STRAIN DIVERSITY IN SHEEP SCRAPIE

Evidence for strain diversity originally came from transmission experiments of individual and pooled sheep scrapie brains to mice. After serial passage at limiting dilution, different mouse-adapted strains have been isolated and characterized on the basis of the incubation period and the lesion profile in the recipient mice. However, it is unclear at present how these murine strains reflect sheep scrapie strain diversity in the field, or result from adaptation due to serial passage. Two experimental sheep scrapie strains are currently recognized, namely CH1641 and SSBP/1, which target sheep of different PrP genotypes or result in very different incubation periods in the same genotype [13]. TSE strain recognition in sheep has assumed more importance in recent years with the concern that the BSE agent may have entered the sheep population. Although there is no evidence to date from transmission studies of natural sheep TSEs to mice [3] that BSE can be present in sheep, the numbers of experiments completed so far have been too few to draw meaningful data. However, recent large surveys of clinical scrapie cases in the UK have not detected the BSE-characteristic Western blot signature in any of the sheep examined [14].

Characterization of sheep TSE strains in the natural host can be attempted through detailed examination of the pathological phenotype in brain. Studies done on vacuolar lesion profiles show significant individual variation between animals challenged with a single sheep scrapie source, while patterns of PrP^d accumulation are more consistent. Several different morphological types of PrP^d accumulation may be detected by immunohistochemistry and these can be grouped into four different cell-specific, extracellular PrP^d patterns and two intracellular, truncated forms of PrP^d. The PrP^d profiles created on the basis of these morphological types [4] appear to be consistent within, but different between, groups of sheep experimentally challenged with particular sheep TSE sources. Thus, the PrP^d profiles of the diseases resulting from CH1641 and SSBP/1 infections are distinct, regardless of differences in breed or PrP genotype of the inoculated sheep or in routes of challenge. Different phenotypes of PrP^d accumulation in the brain are observed when dealing with natural disease, although in these circumstances it is more difficult to establish whether the source or strain effects are linked with genotype.

Following the discovery that vCJD was linked to BSE of cattle, extensive research has been undertaken to determine the transmissibility of cattle BSE to sheep and to determine means by which sheep BSE may be recognized against a background of natural sheep scrapie. The PrP^d profile approach applied to sheep experimentally infected with BSE shows that the pathological phenotype of BSE is very consistent, is not affected by host factors or by the experimental design, and is different from all natural and experimental scrapie sources examined [15]. Differentiation between sheep scrapie and experimental ovine BSE can also be achieved by another approach called 'PrP^d epitope mapping' [16]. This is based on the fact that PrP^d accumulating in neurones and microglial cells (and in LRS tissue macrophages) shows a different truncation pattern depending on the source of infection. Thus, antibodies which recognize epitopes in the 93–99 amino acid sequence of the N terminus of PrP provide specific immunolabelling for intracellular PrP^d in tissues from several sheep scrapie sources but fail to do so in BSE-infected sheep tissues. Intracellular PrP^d accumulations are however marked and consistent in BSE-infected sheep, and can be revealed with antibodies to the globular domain or C terminus of PrP. It has been suggested that these truncation properties and consequently distinct immunolabelling patterns represent differences in the conformation of the abnormal PrP produced as a result of BSE and scrapie agents infection. These differences can also be revealed by Western immunoblotting, combining the use of two PrP antibodies, one to the N terminus and another to the globular domain of the protein. The hypothesis of a differential truncation also agrees with the lower molecular weight of the unglycosylated fraction of BSE-derived PrP^{res} (19 kDa) compared with that of scrapie (21 kDa).

EPIDEMIOLOGY AND TRANSMISSION

Scrapie has a widespread geographical distribution, with the notable exceptions of Australia and New Zealand, but its real incidence is difficult to estimate due to biases in the surveys conducted. Historically, there have been fluctuations in incidence of scrapie within and between breeds of sheep and regions. In Britain, since scrapie became notifiable in 1994, between 235 and 454 cases have been reported annually, on 82–261 flock-owning premises; that is much less than previous estimates.

Several studies have shown that scrapie can be transmitted by contact from affected, or infected healthy, sheep to uninfected sheep and goats, and more prolonged or higher levels of exposure lead to higher incidences of contagious transmission, as occurred with over-winter housing of sheep in Iceland. The duration of exposure after lambing affects transmission to offspring; lambs removed at birth from their scrapie-infected dams and flock had a lower incidence (10 per cent) of scrapie in adulthood than those separated later (16 per cent) or those not segregated at all (41 per cent). Sheep placenta may be one source of direct infection and pasture contamination, and recent studies have shown that farmers who do not dispose of placenta by incineration or burial, have a higher risk of scrapie on their farms [17]. However, it is plausible that faeces contribute to pasture contamination more continuously or consistently than placenta and the frequently quoted role of the sheep placenta as a major source of infection needs careful re-assessment. Infection is probably transferred both by direct contagion between animals and through pasture contamination.

Progeny of sheep that develop scrapie are more likely to get the disease than those from apparently scrapie-free sheep, owing both to genetic influences and to transmission of infection. It is exceptional for those born of two scrapie-infected parents not to develop the disease. When only one parent is infected, the risk to the offspring is reduced, more so if only the sire is infected. Thus, the conclusion has been reached that maternal transmission is far more important than sire transmission, but this difference is less if the progeny are separated and hence protected from later horizontal infection (Table 35.2).

In practical sheep farming these parental differences on risk of transmission are very important to understand, because they demonstrate clearly the role of the infected ewe as the source of scrapie infection not only to her offspring but to the entire flock; the sire, on the other hand, has an enormous effect on the flock's genotype.

Maternal transmission is generally acknowledged as important in natural sheep scrapie but is hard to evaluate in view of potential lateral contagion between sheep of all ages. There is uncertainty concerning the route of infection from a scrapie-infected ewe to her offspring and whether infection is passed *in utero*, postnatally or both. *In utero* transmission was achieved by subcutaneous infection of pregnant dams, and is regarded as the most likely route of infection for those sheep which developed scrapie despite having been separated from their dams at birth. Concerning embryo transfer (ET), the present situation is that UK sheep derived from unwashed embryos apparently developed scrapie; these embryos came from scrapie-susceptible ewes inseminated by a susceptible ram and were transferred into genetically resistant ewes. Had they remained scrapie-free, this would have confidently secured ET as a reliable means of obtaining scrapie-free stock. However, the source of scrapie in these ET-derived sheep may have been natural post-natal infection rather than the embryos themselves being infected. Similar studies in the USA, which suggested that ET was useful for obtaining scrapie-free stock, are also difficult to interpret because of problems in

Table 35.2: Parental scrapie status and the infection risk (%) to progeny

	Both parents unaffected	Both parents affected	Sire only affected	Dam only affected
Progeny groups not separated	18	94	18	81
Progeny groups separated	15	88	36	46

Data accumulated by Parry, Dickinson and Hourrigan and reviewed in reference [6].

experimental design. More recently, in extensive controlled studies, sheep derived from washed or unwashed embryos obtained from scrapie-infected and uninfected susceptible donors have survived for extended periods without getting scrapie. In summary, although data are incomplete, the aggregate evidence does not now favour pre-natal transmission, and the increased risk of scrapie for lambs born to clinical and pre-clinical scrapie dams is most likely to be the result of post-natal contact. Whether placentas are the main, or even the sole, infectious product, or transmission can occur through other contaminated birth products or through milk and colostrum remains to be elucidated.

CONTROL OF SCRAPIE

In scrapie-affected flocks, controlling the disease and reducing its incidence depends on three approaches: identifying infected animals, reducing infectious load and selecting for resistance. The first approach has been dealt with under ante-mortem diagnosis of pre-clinical disease. One key point in this respect refers to the sensitivity of the methods, which should be able to identify as many infected sheep as early as possible, hopefully before they become contagious. Applicability of sampling procedures in the field and high throughput of laboratory tests are factors that also need to be considered.

The early detection and elimination of infected sheep will result in a reduction of the level of infection in a flock. Good husbandry can also help in lowering the risk of transmission between infected and susceptible sheep. The practice of indoor and in-pen lambing in a flock with endemic scrapie is a potent means of exposing the whole flock to infection and of amplifying it. The use of straw bales to construct lambing pens and their destruction after each lambing, the burial or incineration of placentas, and the removal of ewes and lambs to clean paddocks in small groups to limit cross-contamination will reduce the spread of infection at vulnerable times. Where scrapie is a known problem, the use of several lambing areas at a distance from each other reduces the risk of cross-infection. In general terms, intensive production methods increase the opportunities for spread from scrapie-infected to uninfected sheep of any age.

Attempts are currently underway to control and eradicate scrapie based on genetic selection for resistance. Three common polymorphisms have been identified within the protein-coding region of the highly polymorphic sheep *PrP* gene at codons 136 (alanine/valine, A/V), 154 (arginine/histidine, R/H) and 171 (arginine/glutamine/histidine, R/Q/H), which strongly influence susceptibility to disease and/or incubation period. Although the permutations of these alleles would provide theoretically 36 PrP genotypes, only 15 are commonly found in nature, and they likely arose from mutations of the 'wild type' ARQ/ARQ genotype. The ARR/ARR genotype is considered to provide full resistance to natural scrapie, while the VRQ/VRQ is the most susceptible. In some breeds, like the Suffolk, in which valine at codon 136 is generally absent, sheep of the ARQ/ARQ genotype may show high incidence of scrapie. *PrP* genotyping can therefore be used as an aid to the control of scrapie: breeding stock, particularly rams, of appropriate PrP genotype can be selected to produce progeny with reduced risk of developing disease. Such genotyping services are available on a commercial basis in North America and Europe and is the basis of the National Scrapie Plan in the UK and in several countries in Europe. Some individual farmers and breed societies are already basing selection of breeding stock on the most scrapie-resistant animals, but these are not common in many flocks, and in some breeds the ARR/ARR genotype is actually absent. Other considerations to be taken into account while selecting for increased genetic resistance to scrapie are the possible adverse effects on the susceptibility to other diseases or on production traits. These aspects are currently being investigated, but the results so far do not show detrimental effects that could rival those of scrapie. Thus, it has been reported that, because of their shorter lifespan, scrapie-affected sheep produce fewer lambs than non-infected sheep, and that lambs of resistant genotypes have higher chances of survival than those of susceptible genotypes, possibly due to the effects of sub-clinical disease in the latter.

NOR-98 AND ATYPICAL SCRAPIE

Cases of a scrapie-like neurological condition with unusual pathological features were initially recognized in 1998 in Norway and called 'Nor-98' [18]. The PrP protein found in those brains, while abnormal,

also showed different biochemical and immunochemical properties than abnormal forms of PrP commonly found in scrapie-affected sheep. Further similar cases have been identified in several European countries from surveys of fallen stock and in routine abattoir samples. These cases are almost invariably found in asymptomatic sheep and are variously referred to as atypical scrapie or Nor-98. While it is clear from the accumulating data that these reports describe prion protein disorders, the incomplete nature of the samples collected in active surveillance programmes and subtle differences in the biochemical profiles make it uncertain whether they represent a single entity or a heterogeneous group. We will therefore refer to these cases collectively as 'Nor-98-like'.

Nor-98-like cases have been identified throughout Europe including countries where scrapie is endemic and others, such as Portugal and Sweden, where scrapie has not previously been reported or not for a long time. These cases generally come from flocks without recorded occurrence of scrapie. Most cases are detected in sheep older than 3½ years and occur sporadically, although in one or two instances there is an association with an additional Nor-98-like case in the same flock. Nor-98-like cases generally target combinations of ARR, ARQ, AHQ and ARH alleles, that is, those considered more resistant to classical scrapie. Sheep with the ARQ allele may have an additional phenylalanine polymorphism at codon 141 of the PrP protein but not all sheep with a Nor-98-like prion protein disorder have this polymorphism.

Few complete data are available on brain pathology of Nor-98-like cases, as available tissue samples are usually restricted to caudal brainstem. However, some cases lack histological or immunohistochemical changes and pathological investigations may not provide supportive diagnostic data. When present, Nor-98-like cases have small, often confluent vacuoles of the cerebellar and cerebral cortices but not in the brainstem. Where present, abnormal PrP may be seen as diffuse accumulations in the cerebellar cortex and in the spinal tract nucleus of the trigeminus nerve, but not in the dorsal motor nucleus of the vagus.

Most Nor-98-like cases have been identified by 'rapid' ELISA-like immunochemical tests, but are negative in the confirmatory Western immunoblotting using conventional proteinase K digestion. However, these samples are positive with Western blotting methods that use low concentrations of proteinase K and milder conditions of temperature, detergents, chaotropic agents and pH. In such conditions, a variable number of bands of different molecular weights is obtained, depending on the precise conditions and the antibodies used, but all appear to produce a band at less than 15 kDa. The signal is generally stronger if cerebellar tissue is used, but the same pattern is recognized with testing of brainstem.

Data for Nor-98-like cases are rapidly accumulating. It is an area of research keenly observed by those in the field as it is likely that the studies of Nor-98-like cases will lead to a significant increase in the scope and understanding of prion protein disorders.

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36

Louping-ill

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Synonym: trembling

Louping-ill is an acute virus disease of the central nervous system (CNS) affecting most species of domestic animal as well as man. Although a disease primarily associated with sheep, clinical signs may occur following infection of cattle, goats, pigs, horses, farmed red deer, dogs and humans as well as wild red grouse. As a disease of sheep, with ataxia and incoordinated, louping-ill has been recognized in the upland grazings of Scotland for many years. However, despite numerous investigations, the identity of the causal agent remained elusive until 1930, when workers in Edinburgh isolated the virus from the brains of affected sheep and demonstrated its transmission by the sheep tick (*Ixodes ricinus*) shortly thereafter. By 1934, a vaccine prepared from formalinized infected sheep brains had been developed and further research was minimal until the late 1960s.

CAUSE

Louping-ill virus is a member of a diverse group of viruses that are transmitted between vertebrates by haematophagous arthropods. Initially, on the basis of this biological characteristic, they were termed the arboviruses (arthropod-borne) and on

antigenic criteria subdivided into groups A, B and C. On morphological grounds, groups A and B were allocated, respectively, to the genera *Alphavirus* and *Flavivirus* of the family Togaviridae. Finally, on molecular criteria, the *Flavivirus*es were given family status and are now known as the Flaviviridae. Within this family is an antigenically closely related complex of viruses transmitted by Ixodid ticks distributed throughout northern temperate latitudes known as the tick-borne encephalitides. Disease caused by these viruses is primarily a problem of infections in humans and it is only louping-ill virus that predominantly produces disease in domestic animals and native fauna. Initially, louping-ill was recognized as a disease of sheep restricted to the British Isles but indistinguishable disease has since been reported in Bulgaria, Turkey, Norway and Spain. Thus, louping-ill can no longer be regarded as exclusively a problem in the British Isles, but may have a wider distribution on continental Europe. Although the viruses involved are very similar and cannot be distinguished by conventional techniques, molecular analysis indicates that the Turkish and Spanish viruses are distinct, while the Norwegian viruses are identical to louping-ill virus. Molecular analysis of isolates of virus from the British Isles indicates that louping-ill virus has evolved from an ancestral European origin only over the past 300–500 years.

Like all *Flavivirus*es, louping-ill virus is readily destroyed by heat, disinfectants and acidic conditions. The capacity of the virus to agglutinate goose red blood cells is exploited in establishing the identity of virus isolates and in a haemagglutination-inhibition (HI) test for detecting antibody. In the laboratory, virus can be propagated either in mice by intracerebral inoculation or in cell cultures including baby hamster kidney (BHK-21), pig kidney, chick embryo and sheep kidney cells.

CLINICAL SIGNS

All ages of sheep appear to be equally susceptible although, in endemic areas, louping-ill is most frequently diagnosed in lambs and yearlings. Following infection, 5–60 per cent of animals develop clinical signs, which vary from slight transient ataxia to sudden death. In affected animals, incoordination progressing to paralysis, convulsions, coma and death within 24–48 hours is the general course. In a proportion of non-fatal cases, residual torticollis or posterior paralysis may remain for weeks or months. Fever is not consistently present during the clinical phase of infection.

Following subcutaneous injection of virus, initial replication occurs in the draining lymph node. Thereafter, virus is disseminated by the circulation, attaining high titres in blood and lymphatic tissues. During this viraemic phase, there are few clinical signs apart from an elevated rectal temperature. After 3–5 days, virus can no longer be detected in the blood or lymphatic tissues but persists in the CNS for up to a further 5 days during which clinical signs may develop. Serum antibodies may be detected 5–6 days after infection, which at first are largely of the IgM class and are progressively replaced by IgG antibody over the following 7–10 days. At one time, it was considered that clinical signs developed only in those animals in which virus gained access to the brain, and great emphasis was placed on factors that might predispose to this. However, it is now recognized that, following infection, virus invariably replicates in the CNS. In subclinical infections, only limited neuronal damage occurs before viral replication is interrupted by the immune response [1, 2].

When sheep are infected with louping-ill virus a few days after infection with another tick-borne pathogen, *Anaplasma phagocytophilum* (Chapter 51), the reaction is very much more severe than that of animals infected with either organism alone. In addition to the anticipated neurological signs, dysentery frequently develops and infection by normal commensal fungal agents may result in extensive pathological changes with mortality approaching 100 per cent. This effect has been attributed to a profound immunological dysfunction resulting from the dual infection and may contribute to the high mortality that can occur when unacclimatized animals are introduced to tick-infested pasture.

PATHOLOGY

Pathological changes directly attributable to louping-ill virus are restricted to the CNS, although terminally, secondary pneumonic lesions may develop. Hyperaemia of the meningeal blood vessels can be observed

at post-mortem, but does not represent a specific gross finding. The principal histological lesion is widespread non-suppurative meningo-encephalomyelitis. Neurone necrosis and neuronophagia are most prominent in the motor neurones, cord, medulla, pons, midbrain and the Purkinje cells of the cerebellum with the distribution of cytoplasmic viral antigen following a similar pattern. Focal gliosis and lymphoid perivascular cuffs are most prominent in the hindbrain, brainstem and meninges. These inflammatory cells appear to be predominantly B-lymphocytes specific for louping-ill virus antigens and the intrathecal synthesis of antibody to louping-ill virus by these cells is considered responsible for limiting viral replication in the CNS of the non-fatal cases [1].

DIAGNOSIS

A diagnosis of louping-ill should be considered in animals exhibiting signs of neurological dysfunction or that have died suddenly in areas where ticks are active. In areas where ticks are absent, louping-ill may also affect sheep with a history of recent transportation. When louping-ill is suspected, laboratory confirmation should be sought, as the clinical signs are similar to those of other diseases of the CNS.

By the time clinical signs are apparent, virus can be detected only in the CNS. In dead or moribund animals, the brains should be removed carefully and small pieces (approximately 1–2 cm^3) of brainstem placed in 50 per cent glycerol saline, while the rest of the brain is placed in 10 per cent formal saline. Details of the method employed and the precautions that must be taken can be found in Chapter 75. Histological examination of the brain provides evidence of a non-suppurative encephalomyelitis but a definitive diagnosis relies on detection of the virus either by immunohistochemistry (IHC), polymerase chain reaction (PCR) or isolation of virus. IHC is performed on formalin-fixed paraffin-wax embedded brain sections, mainly from the hindbrain where viral antigen is most frequently found (see Figure 36.1 in the colour plate section).

The pieces of brain collected in glycerol saline are washed and homogenized for virus isolation in cell culture or detection of viral RNA by reverse transcription PCR (RT-PCR). Identification of virus isolated in culture may be made by preparing duplicate cultures incorporating specific antiserum in the inoculum or by RT-PCR. The RT-PCR employed amplifies a base sequence that appears to be present in all strains of louping-ill virus and distinct from other Flaviviruses.

Where it is not feasible to obtain brain material, serological confirmation may be sought. Various tests have been described for detecting antibody, including complement fixation, gel precipitation, neutralization, enzyme-linked immunosorbent assay (ELISA) and HI. In practice, however, the HI test has proved of greatest value. Serum that is collected from a clinically affected animal is tested for HI antibody both as unheated serum and after heating to 64°C for 30 min. A fourfold or greater reduction in titre in the heated sample indicates that much of the antibody activity is due to IgM, from which it may be inferred that the serum has been collected from a recently infected animal. However, as IgG may largely replace IgM before clinical signs are manifest, the absence of readily detectable IgM antibody in positive sera does not preclude the possibility of recent louping-ill virus infection [2]. Following infection, moderately high titres of IgG antibody are maintained for the life of the animal.

EPIDEMIOLOGY AND TRANSMISSION

Transmission of louping-ill is dependent on the sheep tick *Ixodes ricinus* and thus the epidemiology of the infection is intimately linked to the vector. The two fundamental requirements for the tick are a moist microclimate for its survival when the tick is not feeding and the availability of large mammals such as sheep, cattle, hares, deer, etc. on which adult ticks rely for their blood meals [3].

During the 3-year life cycle of the tick, feeding occurs for only approximately 17 days, the remaining time being spent on the ground, where it is essential that the relative humidity remains close to saturation. Such conditions are present in the vegetational mat of the upland grazings of the UK, but are absent in the relatively well-drained, intensively farmed lowland areas. The distribution of the tick is therefore largely restricted to the upland sheep grazings, sheep being the principal vertebrate host. Thus, louping-ill may occur in the majority of hill sheep flocks, although the extent of challenge varies widely. The

geographical distribution of louping-ill is determined by that of the tick and similarly the annual periodicity of tick activity determines the seasonality and incidence of infection.

Ticks are generally inactive during the winter months and become active in the spring, when the mean maximum weekly temperature exceeds 7°C. The period during which a tick quests before its final exhaustion and desiccation is governed by the temperature and relative humidity but seldom exceeds 4–8 weeks. Thus, the time of tick activity varies throughout the country, depending on the prevailing local climatic conditions. In all locations, exhausted ticks that have failed to feed will die, which explains the relative absence of ticks during the summer months. Spring-fed ticks will not have moulted and be ready to feed until the following spring. However, in certain regions, particularly in the west of the UK, there is a second period of tick activity in the autumn. However, with recent milder winters tick activity may extend into the winter months. Ticks that feed in the autumn or winter do not moult until the following summer, and are therefore not ready to feed until the autumn. Such autumn-feeding ticks are considered to be a distinct population from the spring-feeding ticks. In certain upland areas where temperatures remain low until May, the life cycle of the tick may be extended by several years. Louping-ill virus is transmitted transtadially and there is no evidence that transovarial transmission can occur. Thus, transmission of virus is only through the bite of nymphal or adult ticks that have ingested infected blood meals as larvae or nymphs, respectively. It is self-evident that the incidence of louping-ill virus infection follows closely the periodicity of the tick, losses occurring in two peaks: one in the spring followed in some areas by a second peak in the autumn.

Two patterns of mortality are seen. The disease may affect all ages of animals when either infection is present at a low incidence or where infection has only recently been introduced to a farm. All ages of bought-in sheep also may be susceptible. On farms where louping-ill is endemic, losses occur primarily in lambs and replacement breeding stock, older animals being immune. Colostral antibody of immune ewes provides highly efficient protection to their lambs, which are therefore unlikely to become infected during their first spring. Thus, on endemic farms, only lambs acquiring insufficient colostral antibody are likely to die from louping-ill. Lambs protected by colostrum in their first year of life are fully susceptible to infection the following spring, and it is therefore in the ewe lambs retained for breeding that the heaviest mortality frequently is seen.

The sheep tick is not a fastidious feeder and, while adult ticks are restricted to larger mammals, the larval and nymphal stages feed on any category of vertebrate. It is, therefore, not surprising that infection with louping-ill virus has been reported in a wide variety of domestic and wild species of vertebrate. Most illuminating, however, have been the experimental studies of infection in red grouse (*Lagopus scoticus*), which generally proved fatal, and field studies which indicated that, in some areas where louping-ill was endemic, a high mortality due to virus infection occurred in the grouse. Studies of infection in other vertebrates indicated that, apart from sheep, the intensity of viraemia that developed was insufficient to transmit virus to the tick. Thus, contrary to the previous view that louping-ill virus is maintained primarily in a wild vertebrate–tick cycle, the converse appears to be true and present evidence indicates that the maintenance of louping-ill in nature is essentially a sheep–tick cycle. Grouse contribute only occasionally to maintaining infection, as they die out in areas where louping-ill virus becomes established and infection of other vertebrates is of limited importance [4, 5]. However, there is some evidence to suggest that, where mountain hare (*Lepus timidus*) populations are large, transmission of virus by ticks feeding on hares may occur in the absence of a viraemia. Although transmission is normally through tick bites, animals are also susceptible to infection by the oral route. This does not usually occur in sheep, but an outbreak of louping-ill in pigs was attributed to the ingestion of infected lamb carcasses. In addition, drinking unpasteurized goat milk has given rise to human disease caused by the closely related European tick-borne encephalitis virus. As high titres of louping-ill virus are excreted in the milk of infected goats, transmission in this way is possible.

CONTROL

Control of louping-ill may be achieved either by vaccinating sheep or by preventing them becoming infected by control of the tick.

Prophylactic methods that have been advocated include the use of both live virus and formalin-inactivated vaccines derived from infected sheep brains, infected mouse brain, or chick, sheep and baby hamster kidney (BHK 21) cell cultures. The value of administering immune serum has been assessed in an extensive field trial. The vaccine presently available is inactivated virus propagated in BHK 21 cells, concentrated by membrane filtration and incorporated in an oil-based adjuvant. It is administered as a single subcutaneous injection in the neck. To control the disease on farms where it is endemic, it is generally the practice to vaccinate all the ewe lambs that are to be retained for breeding, either in the autumn or in the following spring before ticks become active. In addition, all purchased sheep should be vaccinated at least 28 days before exposure to tick-infested pasture. Finally, where the disease occurs for the first time, it may be considered prudent to vaccinate the whole breeding flock.

Another control strategy is suggested from a consideration of the epidemiology of louping-ill. From detailed analysis of biological and molecular characteristics of louping-ill virus, it is apparent that the virus has been introduced to the British Isles within the past 300–500 years [6]. Furthermore, appraisal of the history of the sheep industry and observations on the distribution of the tick support the view that louping-ill virus probably has been dispersed essentially by the activity of sheep husbandry. Provided this conclusion is valid, it follows that the maintenance of louping-ill virus depends largely on the availability of susceptible sheep, and that elimination of such animals will result in the eradication of louping-ill. The tick represents only a temporary reservoir of infection as transtadial but not transovarial transmission occurs, and a tick population should become 'clean' of louping-ill virus in a period of 2 years in the absence of re-infection. Thus, two methods of eradication are suggested, namely the physical removal of sheep from the environment or systematic vaccination of the whole flock over a period of 2 years. There is evidence from islands off the west coast of Scotland to suggest that both methods are effective. It is, however, generally impractical to remove sheep entirely from a grazing for a period of 2 years and mass vaccination also is probably not justified unless the possibility of the re-introduction of infection, by tick-infested wild or domestic animals, can be ensured. In addition, although the life cycle of the tick is completed in 3 years in optimal conditions, in the cooler upland grazings this may be extended to 5 or 6 years. Thus, the period during which louping-ill virus transmission is suppressed may have to be extended.

A final method of control is directed at reducing the prevalence of, or eradicating, the tick. The attraction of such an approach is that not only would louping-ill be controlled, but other tick-associated conditions also would be eliminated. In the absence of dips that have long residual properties, dipping alone is unlikely to achieve a marked effect unless the interval between dipping is short. As an alternative to ectoparasiticidal dips for sheep and lambs, 'pour-on' formulations have given very encouraging results and may have an important role in controlling tick parasitism. In a trial employing a cypermethrin pour-on as a single application to ewes and lambs, tick numbers on the treated animals were markedly reduced throughout the 8-week trial [7]. However, progressive reduction in tick numbers and disappearance of diseases associated with tick parasitism can be achieved by a combination of strategic grazing and acaricidal treatment. In practice, during the periods of greatest tick activity, sheep are grazed on improved pasture on which ticks do not survive, then dipped or otherwise treated before moving on to unimproved grazing after the peak of tick activity.

Such approaches to tick control succeed only if there are few alternative hosts available on which the adult stage of the tick can feed. In some areas, wild species such as hares and deer host large numbers of ticks, so, unless they too can be removed from the pasture, these methods may be inappropriate. However, where coordinated programmes have included massive reduction in mountain hare numbers, the objective of reducing the tick population has been achieved. It is essential also that vigilance in tick control procedures is maintained, as regeneration of a large tick population can result in the re-introduction of all the tick-associated diseases into an entirely susceptible sheep population, which may have devastating results. Where the combined interests of improving grouse stocks and sheep health have been coordinated in ambitious programmes employing vaccination and tick-suppression, both objectives have been achieved. Such programmes require a high level of dedication from all involved as well as education of both farming and shooting interests. However, as pressures grow to maximize sustainable economic productivity in the absence of farm animal subsidy suppression of louping-ill virus to ensure that losses in the red grouse population are minimized is becoming a priority.

It is clear, therefore, that care must be exercised in selecting the appropriate control strategy or combination for each individual situation.

ZOONOTIC IMPLICATIONS

It is essential that precautions are taken to reduce the risk of infection. Those engaged in the slaughter of sheep from enzootic areas should wear rubber gloves. Post-mortem examination of suspected animals should be conducted with great care to avoid accidental inoculation or the creation of aerosols. In the laboratory, all work with the virus should be performed in a safety cabinet. It is strongly advised that all goats used for milk production in enzootic areas should be vaccinated. This will reduce the risk of infection to those handling the milk. Pasteurization renders the milk safe by destroying the virus.

Individuals likely to be regularly exposed to the virus, particularly laboratory workers, should be vaccinated against tick-borne encephalitis (FSME vaccine, Immuno AG A-200 Vienna, Austria). Human louping-ill virus infection in Britain is rarer than tick-borne encephalitis virus infection in man in continental Europe. The reason for this is not clear but may be related to the increased metabolic activity of the tick in the relatively high temperatures of the region where tick-borne encephalitis occurs frequently in humans.

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37

Listeriosis

P.R. Scott

Listeriosis is one of the commonest neurological disease of adult sheep in the UK and is primarily, but not exclusively, a winter–spring disease caused by feeding spoiled grass silage contaminated with *Listeria monocytogenes*. The natural reservoirs of *L. monocytogenes* appear to be soil and mammalian gastrointestinal tracts, both of which contaminate vegetation. Grazing animals ingest the organism and further contaminate vegetation and soil. Animal-to-animal transmission occurs via the faecal–oral route.

CAUSE

Clinical disease is caused primarily by *L. monocytogenes*; *L. ivanovii* causes only abortion in ruminants. Other species are generally non-pathogenic. *Listeria* spp. are Gram-positive rods, 1–2 μm long and 0.5 μm wide, grow in temperatures ranging from 3 to 45°C, in aerobic or microaerophilic but not anaerobic conditions, and within a pH range of 5.6–9.6. *L. monocytogenes* is an intracellular pathogen capable of multiplication within monocytes and macrophages following escape from their phagosomes. The process is enabled by listeriolysin O, a secreted haemolysin crucial to the bacterium's virulence. Intracellular multiplication is followed by intercellular spread [1].

Because it is a serious food-borne pathogen *L. monocytogenes* is much studied [2, 3], with new information emerging on aspects of sero-, phage and molecular typing [4].

CLINICAL SIGNS

Encephalitis

Listeriosis of adult sheep also occurs sporadically in growing cattle and goats with encephalitis the most readily recognized form. Sheep aged 18–24 months are most commonly affected due to molar teeth eruption facilitating infection of buccal lesions. In northern Europe, listeriosis is classically seen in sheep fed poorly conserved grass silage used to supplement rations during late gestation. Listeriosis can very occasionally be encountered in young lambs which have access to silage. Cases of ovine listerial myelitis are very uncommon.

The clinical course in sheep and goats is often rapid; death may occur 24–48 hours after onset of clinical signs. With aggressive antibiotic therapy and supportive care the recovery rate can be up to 30 per cent. In cattle, the disease course is less acute with a higher treatment response approaching 50 per cent.

Lesions are localized in the brainstem and the signs indicate unilateral dysfunction of the third to seventh cranial nerves, and occasionally other nerve nuclei. Initially, affected animals are anorexic, depressed [involvement of ascending reticular activating system (ARAS)], disoriented and may propel themselves into corners or under gates. This propulsive tendency must be differentiated from head pressing behaviour observed with a diffuse cerebral lesion such as ovine pregnancy toxaemia. Ipsilateral hemiparesis often results in animals supporting themselves against walls/pen divisions. Sometimes, there is knuckling of the ipsilateral fetlock joint and dropping of the elbow joint which should be differentiated from radial nerve paralysis. Affected animals may move in a circle towards the affected side (vestibulo-cocchlear nucleus), but this is by no means pathognomic of listeriosis. The synonym of 'circling disease' is unhelpful when describing listeriosis. Facial nerve [cranial nerve (CN) VII] paralysis presents with drooping ear, the muzzle pulled to the normal side, flaccid lip and lowered upper eyelid (ptosis) with narrowing of the palpebral fissure on the affected side. There is unilateral lack of blink response. There is profuse, almost continuous, salivation with food material impacted in the cheek of the affected side due to trigeminal nerve (CN V) paralysis which also results in loss of skin sensation of the face. Terminally, affected animals fall and, unable to rise, lie on the same side; involuntary running movements are common.

Other syndromes

Uterine infection and abortion at any stage of pregnancy, without distinguishing features, is fairly common (see Chapter 19). Cases of septicaemia with associated fever and malaise may occur in adults, younger animals and neonates in flocks experiencing listeric abortion.

PATHOLOGY

Listeria that are ingested or inhaled tend to cause septicaemia, abortion and latent infection. Those that gain entry to tissues have a predilection to localize in the intestinal wall, medulla oblongata and placenta; or to cause encephalitis via minute wounds in the buccal mucosa.

Listeric encephalitis

The encephalitis is essentially a localized infection of the brainstem that occurs when *L. monocytogenes*

ascends the trigeminal nerve or following haematogenous spread. There are few gross lesions apart from some congestion of meninges. Microscopic lesions are confined primarily to the pons, medulla oblongata and anterior spinal cord, the characteristic feature being a focus of inflammatory cells with adjacent perivascular cuffs consisting predominantly of lymphocytes, with histiocytes, plasma cells and occasional neutrophils. In severe cases lesions may coalesce.

Septicaemic (visceral) listeriosis

Septicaemic (visceral) listeriosis is found in young ruminants before the rumen is functional. The septicaemic form affects organs other than the brain, the principal lesion being focal hepatic necrosis. In adults a marked, extensive haemorrhagic enteritis may be present.

Abortion

The uterus of all ruminants is susceptible to *L. monocytogenes* at all stages of pregnancy, which can result in placentitis, metritis, fetal infection and death, abortion, stillbirths, neonatal deaths and, possibly, viable carriers. The aborted conceptus shows few visible lesions and is prone to early autolysis.

DIAGNOSIS

Diagnosis of encephalitic listeriosis in the live animal is based on a thorough neurological examination. Occurrence of listeriosis is not solely dependent on silage feeding, and can occur at any time of year. Samples of lumbar cerebrospinal fluid (CSF) can readily be collected from sheep under local anaesthesia [5] to support the presumptive diagnosis and guide prognosis. Details of the procedure and diagnostic/prognostic indicators have been provided [5–7]. Briefly, a needle of appropriate length and gauge for the individual animal is carefully inserted through the surgically prepared lumbosacral site (between L6 and S2 dorsal spines) to enter the dorsal subarachnoid space. Gentle cautious aspiration will provide 1–2 ml of CSF for laboratory analysis. Cases with a CSF protein concentration under 1.5 g/l and a low total white cell count warrant aggressive antibiotic therapy and supportive care as they are more likely to survive.

Culture of lumbar CSF is useful only in cases of neonatal bacterial meningitis.

Listeriosis can be confirmed only by isolation and identification of *L. monocytogenes.* Specimens of choice are brain from animals with central nervous system (CNS) involvement and aborted placenta and fetus. If primary isolation attempts fail, ground brain tissue should be held at 4°C for several weeks and recultured weekly. Selective media for isolation from faeces, water and feedstuffs may be adopted for clinical specimens [8]. Serology is not used routinely for diagnosis because many healthy sheep have high titres of anti-listeria antibodies.

Differential diagnosis

Listeriosis can be differentiated from pregnancy toxaemia and polioencephalomalacia (both diffuse cerebral lesions) by careful clinical examination noting that peripheral facial nerve paralysis may be present as a consequence of lateral recumbency or trauma. Brain abscesses and coenurosis typically present with space-occupying lesions of one cerebral hemisphere with circling, contralateral blindness and proprioceptive deficits, and no cranial nerve deficits.

The unilateral signs of trigeminal and facial paralysis, when present, help to differentiate listeriosis from peripheral vestibular lesions (eye drop and spontaneous nystagmus, no trigeminal nerve signs) and basillar empyema (cranial nerve signs variable often affecting both sides, pupillary light reflexes/menace responses often absent).

EPIDEMIOLOGY

Listeriosis is primarily, but not exclusively, a winter–spring disease of silage-fed ruminants. *Listeria* spp., including *L. monocytogenes*, are widely distributed in the agricultural environment including surface soils and herbage, allowing natural contamination of silage. The less acidic pH of spoiled silage enhances multiplication of *L. monocytogenes*. Outbreaks may occur within 10 days of feeding poor-quality silage. Removal or change of silage in the ration often halts the

appearance of disease but cases can still occur for 2 weeks or so. Encephalitic cases are more common than abortion and septicaemia is observed only occasionally.

Listeric encephalitis may recur on the same premises in successive years. The number of animals clinically involved in an outbreak is usually less than 1–2 per cent but in exceptional circumstances may reach 30 per cent in a flock of sheep or goats and 10 per cent in cattle.

TREATMENT AND CONTROL

L. monocytogenes is susceptible to penicillin, ampicillin, ceftiofur, erythromycin and trimethoprim/sulfonamide. The drug of choice is penicillin with high doses required to achieve minimum bactericidal concentrations within the brain but recovery depends on early detection and aggressive antibiotic treatment. If signs of encephalitis are severe, death usually occurs despite treatment. Penicillin G should be given with the first dose up to 300 000 iu/kg body weight intravenously followed by more conventional dose rates (44 000 iu/kg) daily for 5 consecutive days. The high cost of such initial treatment means that sheep are more commonly treated at dose rates of 100 000 iu/kg. Intravenous injection of 1.0 mg/kg dexamethasone at first presentation remains controversial but there is clinical evidence that this regimen achieves higher success rates than antibiotic alone. Supportive therapy, including fluids and electrolytes by orogastric tube, is required for animals having difficulty eating and drinking.

In an outbreak, affected animals should be segregated. If silage is being fed, use of that particular silage should be discontinued. Spoiled silage should be discarded routinely. As the disease is much more prevalent in 1–2 year olds than other age groups, consideration should be given to feeding hay if available. Corn ensiled before being too mature, or the use of additives for grass silage, is likely to produce a more acid pH, which discourages multiplication of *L. monocytogenes.*

Vaccination

Results with vaccines in sheep are limited [9] and the sporadic nature of the disease questions the cost-benefits of vaccination.

ZOONOTIC IMPLICATIONS

L. monocytogenes is a well-recognized food-borne human pathogen. Although relatively rare, around 150 cases of human listeriosis per year are formally recorded in the UK, with a case mortality rate in excess of 20 per cent. Chill-like symptoms of fever, headache, nausea with or without vomiting are the main features and, exceptionally, septicaemia and meningitis. Infection in pregnant women can result in abortion, stillbirth or severe disease of the newborn. The elderly, the very young, pregnant women and immunocompromised individuals are at highest risk of life-threatening listeriosis.

Unpasteurized dairy products are the main source of human infection but others sources include uncooked food of animal origin and raw vegetables as the wide environmental distribution of *L. monocytogenes* affords opportunity for their contamination. It is to be noted that tolerance of low temperatures allows *L. monocytogenes* to maintain slow growth during refrigeration.

Attention to personal and kitchen hygiene, food preparation, storage and working procedures, and avoidance of unpasteurized dairy products are sensible preventive measures.

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38

Other nervous diseases

P.R. Scott

This chapter describes the more common neurological diseases of sheep, except listeriosis which is covered in Chapter 37. Early recognition of illness by the shepherd, accurate clinical diagnosis and appropriate therapy by the veterinary surgeon are emphasized.

SWAYBACK

Swayback is a congenital condition affecting newborn lambs; delayed swayback (enzootic ataxia) most commonly affects lambs aged 2–4 months.

Cause

Swayback is associated with low copper status of the dam and/or growing lamb. In the UK the condition occurs within well-defined areas, usually upland and hill pastures where it is often related to pasture improvement including liming, fertilizer application, and re-seeding. Swayback occurs more commonly after mild winters because less supplementary feeding is provided to sheep during mid and late gestation.

It has been proposed that copper deficiency reduces the activity of copper-dependent and certain other enzymes, including cytochrome oxidase, to levels below which the cell is unable to maintain the metabolic requirements of structure, growth and function (see Chapter 54).

Clinical signs

Congenital form

Severely affected lambs are small and weak, and may be unable to raise themselves or maintain sternal recumbency. Depressed corneal and pupillary relexes and blindness [1] have been reported. Some affected lambs show fine head tremor which is increased during periods of activity such as feeding. Less severely affected lambs have normal birth weights, are bright and alert but have poor coordination and difficulty finding the teat, leading to starvation. Inadequate passive antibody transfer predisposes these lambs to infection of the gastrointestinal tract, liver, joints and meninges after bacteraemia.

Delayed form

In the delayed form of swayback (enzootic ataxia) the lambs are normal at birth but show progressive weakness of the pelvic limbs from 2 to 4 months of

Figure 38.1: Delayed swayback in a 6-month-old Texel X Blackface wether. Note the weakness of the pelvic limbs.

age (Figure 38.1). Signs are often first noted during gathering or movement when affected lambs lag behind the remainder of the flock. The pelvic limbs are weak with reduced muscle tone and reflexes, and show muscle atrophy.

Pathology

Macroscopic lesions are inconsistent; in some congenital cases the cerebral white matter may contain sharply demarcated, fluid-filled cavities or gelatinous transformations with indistinct boundaries traversed by fine glial or vascular strands. These lesions occur most frequently at the tips of the white matter cores of parietal and occipital gyri.

Lesions pathogonomic of swayback are microscopic and present in the brainstem and spinal cord. Nerve cell changes are most evident in the large neurons of the red and vestibular nuclei, the reticular formation, and the ventral horns of the spinal cord [2]. In goat kids the cerebellar cortex is involved more frequently than in lambs [3]. The changes consist of swelling, vacuolation and chromatolysis proceeding to a 'hyaline-like' necrosis.

In the spinal cord altered nerve fibres mainly occupy the peripheral zones of the dorsal and ventral parts of the lateral funiculi and the sulco-marginal funiculi. There is pallor of myelin in the affected areas and a positive Marchi reaction. There is evidence of Wallerian-type degeneration [4], myelin degradation [5] and reduced myelin synthesis [6].

Diagnosis

Clinical diagnosis is based on clinical findings, and flock details such as geographical area, copper administration and supplementary feeding during mid-gestation. Differential diagnoses of the congenital form of disease include border disease, septicaemia and hypoglycaemia/hypothermia. Compressive lesions affecting the thoracolumbar spinal cord (T2–L3) resulting from vertebral empyema are common in 2–4-month-old lambs following neonatal bactaeremia [7], and tick-borne fever and tick pyaemia on infested upland and hill pastures. The clinical course associated with thoracolumbar vertebral empyema (T2–L3) is progressive over 4–7 days with upper motor neuron signs to the pelvic limbs and, as such, this condition differs from the lower motor neuron signs typically observed in enzootic ataxia. Lesions compressing the thoracolumbar spinal cord cause an elevated protein concentration in lumbar cerebrospinal fluid (CSF) [7–9] which aids differentiation from enzootic ataxia. Pelvic limb paresis has been reported in sheep with thoracolumbar spinal cord lesions associated with *Sarcocystis* spp infestation [10] and coenurosis [11].

Diagnosis is confirmed by histopathological examination of the brain and spinal cord, and supported by plasma and liver copper determination (see Chapter 54). Care must be exercised that cases of enzootic ataxia have received no copper supplementation prior to liver copper determinations.

Treatment, prevention and control

Treatment of lambs with congenital swayback is hopeless and affected lambs must be humanely destroyed for welfare reasons. There is limited evidence that copper supplementation of lambs with enzootic ataxia slows progress of the condition. Lambs with enzootic ataxia should be confined in small paddocks to allow appropriate supervision and fed a high concentrate ration in an attempt to achieve marketable weights.

There is a great breed variation in susceptibility to copper toxicity; Texel and certain rare breeds such as the Soay and North Ronaldsay are very susceptible. Indeed, reports have detailed copper toxicosis in Texel sheep which had received neither supplementary feeding nor parenteral copper administration [12, 13].

Prevention of swayback by copper supplementation must be carefully considered including such factors as the prevalence of confirmed or suspected swayback cases in the flock, breed of sheep, supplementary feeding during gestation and geographical area, including soil analysis. Determination of serum copper and copper-dependent enzyme concentrations, such as superoxide dismutase, in pregnant ewes provides some indication of copper status but liver copper concentration is the most useful measurement. The subcutaneous or intramuscular injection of chelated copper presents the most convenient method for supplementation of extensively managed ewes during mid-gestation but is not without risk.

POLIOENCEPHALOMALACIA

Synonym: cerebrocortical necrosis (CCN)

Polioencephalomalacia (PEM), an acute neurological disease of weaned lambs, is also seen sporadically in adult sheep. The disease is characterized by blindness, initial depression and dorsiflexion of the neck (Figure 38.2) progressing rapidly to hyperexcitability, convulsions and opisthotonus.

PEM is the outcome of impaired cerebral cellular metabolism brought about by deficiency of thiamine (vitamin B1). Normally, the ruminal microflora synthesize adequate amounts of thiamine (and other members of the vitamin B complex) but, in cases of PEM, excessive ruminal production of thiaminase rapidly destroys thiamine, leading to deficiency. High concentrations of sulfide in the rumen have a similar effect.

Figure 38.2: Polioencepalomalacia affecting a greyface gimmer. Note the dorsiflexion of the neck.

Clinical signs

PEM is seen most commonly in weaned lambs aged 4–8 months, but disease does occur in adult sheep. Sucking lambs are rarely affected. Individual lambs are usually affected approximately 2 weeks after movement to another pasture or other dietary change; either event may be associated with routine anthelmintic treatment. During the early stages of PEM, affected sheep are blind and become isolated from the group and may wander aimlessly. When stationary, there is marked dorsiflexion of the neck ('star-gazing'). The condition deteriorates within 12–24 hours to lateral recumbency with opisthotonus. Affected sheep are hyperaesthetic to auditory and tactile stimuli which may precipitate seizure activity. Dorsomedial strabismus and spontaneous horizontal nystagmus are frequently present. Trauma to the superficial branch of the facial nerve on the dependent side may result in ptosis and drooped ear. Death follows within 3–5 days in untreated sheep.

PEM caused by sulfur toxicity

An outbreak of PEM affected 21 of 71 weaned lambs aged 4–6 months 15–32 days after they were introduced to an *ad libitum* concentrate ration containing 0.43 per cent sulfur [14]. The clinical signs were acute and included depression, bilateral lack of menace response and head pressing behaviour. Hyperaesthesia, nystagmus, dorsiflexion of the neck and opisthotonus were not observed. Response to intravenous treatment with vitamin B1 and dexamethasone was poor. No further cases were identified after all the remaining lambs were given a single intramuscular injection of vitamin B1. An outbreak of PEM was reported in a group of fattening lambs fed a ration containing ammonium sulfate as a urinary acidifier [15]. The condition has been induced in sheep within 3 weeks of introduction to experimental diets containing 0.63 per cent sulfur [16, 17].

Pathology

The cerebral hemispheres are swollen, pale and soft with yellow discoloration of some gyri, especially in the frontal, dorsolateral and dorsomedial areas of the cortex. The posterior vermis may appear cone-shaped due to herniation through the foramen magnum. Affected areas of the cortex may exhibit a bright white autofluoresence when cut sections of the cerebrum are viewed under ultraviolet light (Wood's lamp; 365 nm). This property has been attributed to the accumulation of lipofuchsin in macrophages [18] but not all PEM cases fluoresce. Definitive diagnosis relies on the histological findings in the cortical lesions of vacuolation and cavitation of the ground substance with astrocytic swelling, neuronal shrinkage and necrosis.

The pathology of sulfur toxicity cases includes widespread and severe areas of malacia in the brain with a very clearly defined periphery. Areas of the thalamus and mid-brain are also involved but no lesions are observed in the cerebellum or hippocampus [14].

Diagnosis

Diagnosis is based on clinical findings and response to parenteral administration of thiamine. Common differential diagnoses include focal symmetrical encephalomalacia in weaned lambs, and pregnancy toxaemia [19], acute coenurosis and listeriosis in adults. Diagnostic biochemical parameters for PEM including thiaminase activities in blood, rumen fluid or faeces are rarely used in farm animal practice.

Treatment, prevention and control

During the early clinical stages the treatment response to high doses of thiamine (10 mg/kg twice daily), administered intravenously on the first occasion, is generally good. Successfully treated sheep are able to stand and commence eating after 24 hours, although normal vision may not return for 5–7 days. Treatment should be continued for 3 consecutive days. The intravenous injection of high doses of soluble corticosteroid such as dexamethasone (1 mg/kg) at the first treatment to reduce cerebral oedema remains controversial. The sporadic occurrence and good treatment response of PEM cases mean that prevention measures are rarely attempted under most grazing systems in the UK.

Although the treatment response in cases of PEM in lambs fed diets containing high levels of sulfur was poor, no further cases were observed after all remaining at-risk lambs received an intramuscular injection of 20 mg/kg thiamine [14]. This field observation is consistent with the results of experimental work in which PEM did not develop in animals fed high sulfur-containing diets supplemented with vitamin B1 [16, 17].

BACTERIAL MENINGOENCEPHALITIS

Bacterial meningitis occurs sporadically in young lambs, rarely exceeding 0.5 per cent, although the true incidence of disease has not been accurately determined because affected sheep may simply be found dead and such losses not thoroughly investigated.

Causes

Bacterial meningoencephalitis most commonly affects young lambs aged 2–4 weeks after localization of bacteraemia arising from the upper respiratory tract or intestine. Failure of passive antibody transfer predisposes neonates to bacteraemia [20] with subsequent localization of pathogenic bacteria. The umbilicus as a route of infection remains uncertain as few affected lambs have omphalophlebitis lesions [21]. Furthermore, the 2–4-week interval between neonatal umbilical infection and clinical signs of meningitis suggests another route of bacterial invasion. There is little experimental evidence to support the hypothesis that the umbilicus is the major portal of entry for pathogenic bacteria [22]. An investigation of polyarthritis in kids associated with *Klebsiella pneumoniae* indicated that enteroinvasion was the probable route of infection [23]. Studies in neonatal lambs with watery mouth disease revealed bacteraemia in 34 per cent of neonates with severe disease [24]. *Escherichia coli*, *Mannheimia* (*Pasteurella*) spp., *Staphylococcus pyogenes* and *Arcanobacterium pyogenes* have been isolated from clinical cases of meningoencephalitis. *E. coli* was the most common isolate from septicaemic calves [25].

Clinical signs

Twenty lambs with a confirmed diagnosis of meningoencephalitis had a median age of 3 weeks with a range from 3 days to 6 months; 90 per cent of lambs were 1–8 weeks old [21]. The initial clinical signs included depression, hunger and failure to follow their dam, with affected lambs found behind shelters and hedgerows. Some lambs may have an abnormal gait including walking sideways or backwards. Affected lambs are rarely pyrexic. The head is often held lowered in rigid extension and gentle forced movement of the neck is resisted. Affected lambs are hyperaesthetic to tactile and auditory stimuli. Antibiotic injection, especially by the intravenous route, often evokes abnormal vocalization. Episcleral congestion and dorsomedial strabismus are consistent findings. Menace response may be reduced or absent but can be difficult to interpret in depressed or stuporous lambs. Lambs may present in sternal recumbency with the thoracic limbs held in rigid extension and dorsiflexion of the neck. More advanced cases present in lateral recumbency with seizure activity, opisthotonus and odontopresis common during the agonal stage of the disease. There may be evidence of concurrent bacterial infection of other organ systems, particularly the limb joints.

Diagnosis

Diagnosis is based on a thorough clinical examination. Differential diagnoses include cerebellar hypoplasia, hepatic necrobacillosis, starvation/hypothermia/exposure complex, and septicaemia. Lumbar CSF can be collected readily using hypodermic needles [26–28]. Gross inspection reveals a turbid sample caused by the huge influx of white cells, and frothy appearance after sample agitation due to an increased protein concentration. Laboratory analysis reveals an average 100-fold increase in white cell concentration comprised mainly of neutrophils (neutrophilic pleocytosis) and fivefold increase in protein concentration [21]. CSF glucose concentration determined by urinary dipstick is negative and can be used as a field test to support visual inspection, but caution must be exercised in the interpretation because hypoglycaemia is common in neonatal lambs suffering from starvation.

Culture of lumbar CSF is rarely rewarding. *Escherichia coli*, *Mannheimia* (*Pasteurella*) spp., *Staphylococcus pyogenes* and *Arcanobacterium pyogenes* have been isolated from meningeal swabs taken at necropsy.

Treatment, prevention and control

The treatment response in cases of bacterial meningoencephalitis is hopeless and affected lambs should be humanely destroyed once the diagnosis has been confirmed by CSF inspection [27]. Antibiotics that could be used include either trimethoprim/sulfonamide combination or ceftiofur. It is recommended that antibiotic therapy is continued for 4–6 weeks but this would be cost-prohibitive except for valuable breeding stock. The role of high doses of soluble corticosteroid, such as 1.1 mg/kg dexamethasone, remains controversial in the treatment of bacterial meningoencephalitis in humans and animals. Control should be directed at general preventive measures for all bacterial infections in the perinatal period which include ensuring adequate transfer of passive antibody and high standards of environmental hygiene. The isolation of Gram-negative enteric bacteria from bacteraemic neonates supports the hypothesis that the early environment is an important source of infection [20].

BRAIN ABSCESSATION

Brain abscesses are relatively uncommon in mature sheep but are more often diagnosed in lambs aged 3–8 months. It is unusual to find other evidence of pyogenic disease except for the almost ubiquitous mild docking and/or castration lesion caused by elastrator rings. Clinical signs progress slowly and result more commonly from the space-occupying nature of the lesion from than the associated inflammatory response. The lesion more often affects one cerebral hemisphere and, as a consequence, the animal presents with contralateral blindness but normal pupillary light reflexes and contralateral proprioceptive deficits.

Clinical signs

Depression is a fairly consistent finding, often with the head turned towards the chest (Figure 38.3). There

Figure 38.3: Brain abscess affecting the right cerebral hemisphere in a 4-month-old lamb. Note the head depression and poor body condition, indicating the chronic nature of the lesion.

may be compulsive circling but affected sheep often stand motionless or appear trapped with the head pushed into the corner of the pen. Circling behaviour is more commonly encountered in coenurosis and listeriosis. The gait may appear ataxic. Proprioceptive deficits, with hyperflexion of the fetlock joint of the contralateral limb, are commonly observed.

Basillar empyema (pituitary abscess) does occur in sheep but the clinical features have been more accurately defined for cattle [29]. The most common clinical findings in cattle included depression, anorexia and ataxia followed by head-pressing, dysphagia often associated with bilateral trigeminal nerve involvement, blindness and loss of pupillary light reflex (PLR). Unlike listeriosis (Chapter 37), deficits are often mixed, involving cranial nerves from both sides (L facial nerve but R trigeminal nerve and loss of PLR in R eye), and may be bilateral (e.g. both L and R trigeminal nerves).

Diagnosis

Diagnosis is based on a careful neurological examination. Abscessation is more common in sheep under 6 months and may result from pyogenic infection in the neonatal period. Evidence of other localized bacterial infection is uncommmon and, unless present, there are no changes in routine haematology values, fibrinogen or serum globulin concentrations. Lumbar CSF analysis has revealed a small increase in protein concentration and increased white cell concentration [26, 28]. Coenurosis is uncommon in sheep under 6 months of age.

VESTIBULAR LESIONS

The vestibular system helps the animal maintain orientation in its environment, and the position of the eyes, trunk and limbs with respect to movements and positioning of the head. Clinical signs of vestibular disease depend upon whether there is unilateral or bilateral involvement, and whether the disease process involves the peripheral or central components of the vestibular system [30]. Unilateral peripheral vestibular lesions are commonly associated with otitis media and ascending infection of the Eustachian tube is not uncommon in growing lambs. There may be evidence of otitis externa and a purulent aural discharge in some cases but rupture of the tympanic membrane is not a common route of infection. Haematogenous spread may result from a localized infection elsewhere in the body. There may be a history of head trauma including fighting injuries in rams. Unilateral peripheral vestibular lesions with associated facial nerve paralysis should be differentiated from listeriosis because there are different treatment regimens and control measures.

Sheep with unilateral vestibular disease present with a head tilt towards the affected side and horizontal nystagmus with the fast phase directed away from the side of the vestibular lesion although the nystagmus may regress with time. Circling behaviour towards the affected side may be present. Eye droop on the affected side is usually present. Ipsilateral facial nerve paralysis frequently results from otitis media causing ptosis and drooping of the ear.

Treatment, prevention and control

Mannheimia (*Pasteurella*) spp., *Streptococcus* spp. and *Arcanobacterium* spp. have been isolated from infected lesions. A good treatment response is achieved with 5–7 consecutive days of treatment with procaine penicillin (44 000 iu/kg) when disease is recognized during the early stages.

SPINAL CORD LESIONS

Traumatic and infective lesions of the vertebral column causing spinal cord compression and dysfunction are common in sheep. Infective lesions are more common

in lambs between 1 and 3 months old but can occur in all age groups [31, 32]. Vertebral body abscesses occur as a sequel to an infectious focus elsewhere but macroscopic evidence of localized infection of another organ system is uncommon [7, 31]. Iatrogenic lesions caused by dosing gun injuries and those associated with intramuscular injection of a potentially irritating substance into the neck muscles may give rise to infective lesions which track into the cervical spinal canal. Traumatic lesions involving the cervical region occur in rams caused by fighting injuries prior to the seasonal breeding period. Tumour conditions affecting the spinal cord, such as meningioma [34], have been reported occasionally. Under field situations it may prove difficult to undertake a satisfactory examination in large rams and heavily pregnant ewes which are recumbent. Metabolic conditions causing recumbency and altered mentation, including ovine pregnancy toxaemia and hypocalcaemia, should be carefully considered in recumbent ewes during late gestation [19].

Causes

Arcanobacterium pyogenes and *Staphylococcus* spp. have been isolated from typical lesions [32]. In field studies prolonged antibiotic therapy prior to necropsy has prevented meaningful bacteriological investigations [7].

Clinical signs

The clinical signs depend on the degree and location of spinal cord compression. Localization of a spinal cord lesion is achieved by evaluating the reflex pathways. In mild cervical lesions (C1–C6) there is ataxia and weakness involving all four limbs; usually the pelvic limbs are more severely affected. There are hopping, placing and conscious proprioceptive deficits. The thoracic and pelvic limb reflexes are increased (upper motor neuron signs). Severely affected sheep may not be able to maintain sternal recumbency and must be supported.

A lesion in the region C6–T2 results in flaccid paralysis of the thoracic limbs with loss of reflexes because the lesion is at the level of the reflex arc. There is spastic paralysis of the pelvic limbs and increased reflexes (upper motor neuron signs). Pyogenic lesions commonly involve the vertebral column in the region T2–L3, often at the thoracolumbar (T13–L1) junction. Affected sheep adopt a characteristic dog-sitting posture and may drag themselves using the thoracic limbs. The clinician's attention is immediately drawn to this abnormal posture because sheep always raise themselves with the pelvic limbs first. While the thoracic limbs have normal function there is spastic paralysis of the pelvic limbs with increased reflexes. Lesions in the region L4–S2 result in flaccid paralysis of the pelvic limbs with reduced or absent reflexes. Lesions affecting S1–S3 cause hypotonia of the bladder and rectum resulting in distension with urine and faeces, respectively.

Diagnosis

A careful neurological examination should identify the section of the spinal cord involved in the disease process. Rapidity of onset, duration and change in clinical presentation, in addition to age and recent management practices of the animal, such as mixing mature rams, may provide some useful information.

Lumbar CSF can be collected readily under local anaesthesia and analysed for protein and white cell concentrations [26]. Inflammatory lesions extending into the vertebral canal causing spinal cord compression result in a marked elevation in protein concentration but only slight increase in white cell count. Craniad CSF flow is prevented by the compressive lesion thus preventing equilibration in the lateral ventricles. Inflammatory compressive spinal cord lesions, such as an epidural abscess or extension of a vertebral body abscess, result in a significantly increased lumbar CSF concentration [7, 26]. This phenomenon caused by blockage of craniad CSF flow is not dissimilar to Froin's syndrome in humans [9] that has been reported as a result of localized spinal meningitis [34].

Once the suspected lesion has been localized to a region of the spinal cord, radiography may allow identification of vertebral osteomyelitis. Myelography can be undertaken under general anaesthesia but these more specific diagnostic procedures are too expensive except for particularly valuable breeding stock. Radiography may help to identify suspected fracture(s) of a cervical vertebra in valuable rams.

In general practice, a diagnosis of an infective lesion causing significant spinal cord compression is based on duration of clinical signs described above greater than 10 days without improvement despite

antibiotic therapy plus a lumbar CSF sample with an elevated protein concentration.

Treatment, prevention and control

The extent of the vertebral body osteomyelitis that precedes spinal cord compression and the appearance of neurological dysfunction is so severe that antibiotic treatment will not effect a cure and affected sheep should be humanely destroyed for welfare reasons. As disease occurs sporadically there are no specific prevention or control measures.

COENUROSIS

Synonyms: Bendro, gid, goggle-turn, staggers, sturdy, water brain

Coenurosis is an uncommon disease of the central nervous system in sheep in the UK, although during the 1980s slaughterhouse surveys in certain geographical areas reported prevalence rates of *Coenurus cerebralis* from 0.5 to 5.8 per cent [35]. Education programmes, increased treatment of farm dogs with effective anthelmintics and correct disposal of sheep carcasses have all combined to break the sheep–dog cycle.

Cause

Coenurus cerebralis is the larval stage of *Taenia multiceps,* a tapeworm that infests the small intestine of carnivores. Contamination of pastures grazed by sheep by dog faeces can result in larval invasion of the central nervous system and clinical disease. The life cycle is completed when the carnivorous definitive host ingests infested sheep's brain. Foxes are much less efficient than farm dogs in transmitting infection [36].

Clinical signs

Both acute [37] and chronic forms of coenurosis have been described, although chronic disease is more readily identified and more frequently reported.

Acute coenurosis has been reported in a flock of sheep introduced on to a pasture heavily contaminated by dog faeces. Clinical signs appeared within 10 days which ranged from mild to severe with death occurring within 3–5 days of onset of neurological dysfunction [37]. Acute coenurosis has also been reported in 6–8-week-old lambs in which clinical signs ranged from pyrexia, listlessness and head aversion to convulsions and death within 4–5 days.

Chronic coenurosis is more commonly reported in sheep aged 6–18 months in which it presents as an insidious onset and slowly progressive focal lesion of the brain. Only rarely has chronic coenurosis been reported in sheep over 3 years of age. The time taken from larval hatching, migration to the brain and evidence of neurological dysfunction varies from 2 to 6 months. The cyst is located in one cerebral hemisphere in 80 per cent of cases, the cerebellum in approximately 10 per cent, and affecting multiple locations in 8 per cent [38]. Individual cases of gid cyst within the spinal cord have been reported [38, 39].

Localization of the coenurus cyst

The presence of a cyst in the cerebral cortex causes loss of the menace response in the contralateral eye, thus blindness in the right eye indicates that the lesion is in the left hemisphere. Blindness can also be investigated by unilateral blindfolding. Unilateral proprioceptive deficits suggest a contralateral cerebral cyst, whereas bilateral deficits more likely indicate a cerebellar cyst. Blindfolding may exacerbate these deficits.

Compulsive circling behaviour is commonly observed in sheep with coenurosis. Narrow diameter circles (1–2 m) suggest involvement of the basal nuclei at a deep location within the forebrain whereas wide circles are suggestive of a more superficial location of the cerebral cyst. There is the tendency for sheep to circle towards the side of superficial cysts and away from the side of more deeply sited cysts. Depression and head-pressing behaviour occur with cysts involving the frontal lobe of the cerebrum. A head tilt towards the affected side may result if the cyst involves either the vestibular or cerebello-vestibular pathways.

Cerebellar lesions are characterized by dysmetria, ataxia but with preservation of strength, and wide-based stance [40]. Bilateral postural deficits and lack of menace response are usually also present with a cerebellar cyst. Deterioration of the clinical condition occurs more rapidly with a cerebellar cyst.

Diagnosis

Abscessation should be included in the differential diagnosis list but the clinical signs tend to remain static and do not deteriorate as occurs in chronic coenurosis. Listeriosis, louping-ill and polioencephalomalacia should be considered when formulating a diagnosis of acute coenurosis. Sheep with listeriosis present with depression, multiple cranial nerve deficits, and in many cases circling behaviour and head tilt. Polioencephalomalacia has an acute presentation with bilateral loss of menace response, hyperaesthesia to auditory and tactile stimuli, and, without appropriate treatment, rapid progression to opisthotonus. In certain situations an outbreak of ovine pregnancy toxaemia [41] may present with some of the clinical features of acute gid.

Ancillary tests, such as the intradermal injection of coenurus cyst fluid, do not give consistent results and false positive results are common. The presumptive diagnosis is confirmed at surgery after the lesion has been localized to either the right or left cerebral hemisphere, or the cerebellum, following a thorough neurological examination. Softening of the frontal bone, as a consequence of a generalized increase in intracranial pressure, may be palpable but is not a reliable guide to the precise location of the cyst. The bone softening may be either ipsilateral or contralateral to the cyst position and, in some cases, there is softening on both sides in the presence of only a unilateral cyst. Real-time B mode ultrasonography has been described as an aid to *C. cerebralis* cyst localization [42].

Pathology

During the acute phase of coenurosis pale yellow tracts are visible on the surface of the brain and in cut sections of brainstem and cerebellum. On microscopic examination the tracts comprise necrotic tissue surrounded by haemorrhage and leucocyte infiltration. Eosinophils and giant cells predominate in the inflammatory reaction surrounding these tracts.

In chronic coenurosis the increased intracranial pressure from the cyst compresses surrounding brain tissue and may result in softening of an area of the skull, but such changes may not occur in bone immediately overlying the cyst. Hydrocephalus may result from a coenurus cyst in a ventricle or the cerebral aqueduct. Increased intracranial pressure may cause herniation of the vermis of the cerebellum through the foramen magnum or the cerebrum may become herniated beneath the tentorium.

Treatment, prevention and control

Many farmers may elect to slaughter those sheep fit for marketing for economic reasons. A 74 per cent surgical success rate for removal of the coenurus cyst has been reported [43] but a higher figure, around 85 per cent, can be achieved after accurate localization of the lesion. A procedure for cyst removal is outlined in Chapter 74.

Control of coenurosis can be effected by regular anthelmintic treatment of farm dogs at 6–8-week-intervals with an effective taenicide and correct disposal of all sheep carcasses. Foxes are not considered to be an important definitive host of *T. multiceps* [36, 44].

SARCOCYSTIOSIS

Sarcocystis spp. are obligate two-host parasites; the two potentially pathogenic microcyst species in sheep (*S. arieticanis* and *S. tenella*) have either a sheep–dog or sheep–fox cycle [45]. A full account is given in Chapter 59. Other sporozoan parasites such as *Neospora caninum*, which has a probable cattle–dog life cycle, may also infect sheep (see Chapter 17).

MISCELLANEOUS DISORDERS

Dandy–Walker malformation

This malformation (agenesis of the caudal cerebellar vermis) has been reported in Suffolk sheep in the UK [46, 47]. The prevalence can be high, with 16 affected lambs from 22 ewes, and 17 lambs from 60 ewes [46]. Cases included twins in which either both lambs were affected or only one lamb, the unaffected twin lamb growing normally. The associated hypertensive hydrocephalus and doming of the skull frequently causes dystocia [46]. Many affected lambs are either stillborn or die during the neonatal period.

Pathology

There is marked distension of the ventricular systems including the lateral ventricles, third and fourth ventricles. The cerebellum is abnormal with no visible vermis [46]. Hydrocephalus with agenesis of the cerebellar vermis are consistent features of Dandy–Walker malformation.

Treatment, prevention and control

In an investigation of Dandy–Walker malformation in three flocks, the condition occurred only in Suffolk sheep and was associated with particular rams, although exposure to an unidentified teratogen could not be excluded [46]. Careful examination of breeding records in pedigree flocks may identify the affected lambs as the progeny of a ram introduced into the flock for that breeding season. It would be prudent that this ram, and his progeny, were not used for further breeding. It is possible that limiting the gene pool by selecting for scrapie resistance may increase the prevalence of this congenital abnormality.

Daft lamb disease

Daft lamb disease is a poorly defined disease of neonatal lambs with an uncertain mode of inheritance. Estimation of disease prevalence is limited by the poor disease definition, prevalence of other neonatal lamb neurological diseases with predominantly cerebellar signs and other causes of neurological dysfunction including hypoglycaemia in neonatal lambs, border disease and congenital swayback.

Clinical signs

The clinical signs observed within the first 2–3 days of life are typical of cerebellar disease including wide-based stance with lowered head carriage, ataxia and dysmetria but with preservation of strength. Severely affected lambs have difficulty in searching for the teat but suck vigorously when bottle-fed. Affected lambs should be humanely destroyed for welfare reasons.

Pathology

In the original description of the pathology of daft lamb disease [48] degenerative changes, swelling, pallor or hypochromasia, vacuolation and necrosis were observed among the Purkinje cells and type II Golgi cells of the cerebellar cortex, the nerve cells of the dentate nucleus, the central cerebellar nuclei and occasionally in the olives. However, such changes may not be a consistent finding [49].

Diagnosis

A tentative diagnosis of cerebellar disease can be based on the clinical findings but histological examinations are required to differentiate this disease from border disease, idiopathic hydrocephalus and congenital swayback. *In utero*, Akabane virus infection can cause congenital defects of arthrogryposis and hydranencephaly (see Chapter 63).

Control

Since the disease has a genetic basis, an increased disease prevalence will occur after the introduction of replacement breeding stock, usually a ram. The suspected heterozygote and all progeny should be sold for slaughter and not kept as breeding replacements.

Cerebellar abiotrophy

This familial syndrome has been described in a Charollais sheep in the UK [50], and there have been further reports of this condition in 3–4-month-old Charollais lambs (E.M. Milne, 1997, personal communication). Clinical signs of progressive cerebellar abiotrophy may be present from birth [51, 52] or occur in adults [53]. More recent reports in the UK have described lambs with a normal gait for the first 4–8 weeks of life followed by progressive deterioration (E.M. Milne, 1997, personal communication) [50]. The clinical signs include lowered head carriage, intention tremors, a wide-based stance, ataxia, and dysmetria but with preservation of strength. The pelvic ataxia may result in the animal falling over, especially when turning quickly. Fine muscle fasciculations may be present in the neck and head regions resulting in movement of the overlying skin. On arousal, the muscle fasciculations may become more pronounced and resemble coarse muscle tremors causing vigorous jerking movements of the head. The head tremors occur between one and three times per second and persist for a long period after arousal.

Pathology

The cerebellar weight is more than 8 per cent of brain weight indicating no cerebellar hypoplasia. The major histological findings are widespread degeneration of Purkinje cells with associated hypocellularity of the granular layer and degeneration of myelin in cerebellar foliae and peduncles. There is widespread loss of Purkinje cells with individual remaining cells being angular and showing condensed, eosinophilic cytoplasm and loss of nuclear detail. There is no evidence of cytoplasmic vacuolation.

Treatment, prevention and control

Clinical diagnosis is based upon history of progessive deterioration in neurological function with signs indicative of a cerebellar lesion. *In utero* infections of the fetus causing cerebellar dysplasia, such as border disease virus (see Chapter 18), present with neurological signs at birth. Confirmation of the diagnosis of cerebellar abiotrophy requires histological demonstration of widespread Purkinje cell degeneration in the cerebellum. The possible inherited nature of this metabolic defect [53] stresses the importance of an accurate diagnosis especially in a stud ram which may contribute significantly to the genetic profile of the flock [50].

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Part VIII
Diseases of the feet and legs

39

Diseases of the feet

J.R. Egerton

Lameness is often seen in flocks of sheep at pasture and under more intensive conditions. The lameness observed varies from mild and transient to severe and persistent. Early, accurate diagnosis of the cause of lameness is an essential prerequisite for decisions on the level of intervention required to correct the problem. Excessive intervention may be unnecessary and detrimental to the welfare of the sheep. It is important also to recognize that lameness is often the presenting sign with foot-and-mouth disease (Chapter 40), which may be present simultaneously with foot-rot. Other viral infections including contagious ecthyma and bluetongue disease also cause lameness (Chapters 42 and 60, respectively).

Bacterial infections of the skin and other tissues of the hoof are the most common causes of lameness in sheep, and result in distinguishable clinical entities: toe abscess (TA), heel abscess (HA), ovine interdigital dermatitis (OID), contagious ovine digital dermatitis (CODD), benign foot-rot (BFR) and virulent foot-rot (VFR). Some characteristics of these diseases are summarized in Table 39.1.

Other bacterial infections that may cause lesions and/or lameness in the feet include dermatophilosis (Chapter 46) and erysipelas or post-dipping lameness (Chapter 41). Acute lameness involving all feet occurs with the laminitis that follows grain engorgement. Acute lameness may result in sloughing of the hooves.

Table 39.1: Characteristics of major bacterial diseases of the feet of sheep

Disease	Tissue affected	Associated bacteria	Lameness severity ϕ	Prevalence (per cent)
Toe abscess	Sensitive laminae	Non-specific	++++	1–5
Heel abscess	Subdermal, joint capsule *PII–PIII, bones, ligaments	*F. necrophorum, A. pyogenes*	++++	1–10
Ovine interdigital dermatitis	Interdigital skin	*F. necrophorum, A. pyogenes*	0/+	5–30
Benign foot-rot	Interdigital skin	OID flora, avirulent *D. nodosus*	0/+	5–100
Virulent foot-rot	Interdigital skin, sensitive laminae of soft, hard horn of heel, sole and wall	OID flora, virulent *D. nodosus*	0/+/++/+++	5–100
Contagious ovine digital dermatitis	Skin of coronary band, sensitive laminae of hard horn of wall	Treponemes	+++	5–40

Severity score: 0 normal gait, + mild lameness, ++ moderate or intermittent lameness, +++ marked persistent lameness; recumbency, ++++ severe lameness; carrying of affected foot; recumbency.
* P, phalynx.

FOOT-ROT

Synonyms: contagious foot-rot, hoof rot, piétin (French), pedero (Spanish), rotkreupel (German)

Foot-rot is a specific disease of sheep and other ruminants [1], which under suitable environmental conditions, is highly contagious. The prevalence in highly susceptible sheep approaches 100 per cent. Some forms of foot-rot are characterized by chronicity, although remission occurs without treatment in a proportion of animals. In less severe forms of the disease, spontaneous remission is much more likely to occur.

Foot-rot occurs in all major sheep-producing countries. In Australia and New Zealand, foot-rot is a major disease of the Merino breed, but also occurs in British breeds and their crosses with Merinos. Sheep exported from Australia and New Zealand have been associated with outbreaks of foot-rot in sheep indigenous to Bhutan, Malaysia and Nepal.

Cause

Foot-rot is a multifactorial disease [2]. The primary site of infection, the interdigital skin, must be devitalized before bacterial invasion occurs. This is usually the result of prolonged exposure to wetness and faecal contamination. Prior or simultaneous infection with *Fusobacterium necrophorum* is necessary to facilitate epidermal invasion by *Dichelobacter nodosus*, the transmitting agent of foot-rot. *F. necrophorum*, which is voided in the faeces, is normally present in the environment of sheep. In contrast, *D. nodosus* is a strict parasite and its only known habitat is the epidermal tissues of the hooves of ruminants affected with foot-rot. It does not persist in the environment for more than a week. *D. nodosus* is a Gram-negative, non-sporing anaerobe. Commonly, flocks free of foot-rot acquire the disease from affected sheep introduced to the flock. Sheep straying from affected, neighbouring flocks are a common source of infection. When affected sheep and goats are herded together, *D. nodosus* transfers readily from one species to the other [3].

Treponemes have been associated with foot-rot in sheep for at least 60 years [1], but without any clear evidence of their causal or contributory involvement. However, the recovery of these bacteria from bovine digital dermatitis [4] and from sheep with severe acute dermatitis of the digits [5] has prompted renewed appraisal of their aetiological significance in diseases of the feet of ruminants.

Clinical signs

Lameness is the sign that usually indicates the presence of foot-rot in the flock. The proportion of affected animals exhibiting lameness and the degree of lameness are determined by the severity of the outbreak. Even in severe outbreaks, some affected animals may not be lame and it is important to base prevalence estimates in affected flocks on the examination of all feet of an appropriate sample of the flock.

Characteristically, sheep with severe foot-rot are recumbent for much of the time. Feeding is restricted, body weight reduces and wool quality is adversely affected. Affected rams do not mate readily, and ewes in poor body condition have lower lambing percentages. Decubitis ulcers may be present on recumbent sheep. Fly strike (myiasis) may occur in affected feet and transfer to the fleece when sheep are recumbent.

Relative to the normal hoof (Figure 39.1 in the colour plate section) foot-rot lesions vary from mild inflammation of the interdigital space to extensive separation of the soft horn of the heel and sole (see Figure 39.2 in the colour plate section) and, in some cases, all the horn overlying the sensitive laminae. Serum exudes from the affected tissues, and there may be a necrotic film on the surface. Where sensitive laminae are affected, the necrotic tissue, when mixed with serous exudate, has a characteristic odour. In individual flocks, it is possible to recognize clinically different manifestations of foot-rot. At either end of the spectrum of severity of disease are virulent and benign forms of foot-rot, which may be defined as follows.

Virulent foot-rot

Two features characterize VFR. In well-established outbreaks there is a high prevalence of infection. Importantly, among affected animals, many have severe lesions. These result from advancement of infection from its initial site in the interdigital skin into the sensitive layers underlying the soft and hard horn of the hoof. These under-running infections are the ones most likely to cause lameness, recumbency and weight loss. Some cases of VFR have infections

confined to the interdigital skin and, again, lameness may occur. Some animals remain unaffected.

In circumstances where the environment is favourable, sheep are fully susceptible and virulent strains of *D. nodosus* are involved, VFR is easy to recognize. The examination of a few sheep will disclose the severe under-running infections characteristic of the disease. Where environmental, host or bacterial factors combine to limit the full expression of VFR many more sheep may need to be examined to establish an accurate estimate of the proportion of cases with severe infections. In making these assessments an objective and repeatable scoring system, based on the extent of advancement of the infection determined by the examination of all feet, should be used [6]. Lameness is not an accurate criterion for the presence or absence of foot-rot.

Where regulatory programmes for foot-rot management exist [7] the presence of more than 1 per cent of cases with severe infections may result in a diagnosis of VFR. There has been concern that in another environment these outbreaks of low virulence may be much more aggressive. The evidence so far is that a change to a more favourable environment does not enhance the expression of these less virulent outbreaks [8]. Earlier, outbreaks like these had been classified as intermediate foot-rot (IFR) [9] and in them the expected prevalence of severe cases was 1–10 per cent. A decision by regulatory authorities in Australia to eliminate IFR as an acceptable classification of foot-rot resulted in the diagnosis of such outbreaks as VFR.

Where regulatory control is absent the decision on what constitutes VFR should be based on the economic and welfare impacts of the outbreak on the flock. This decision should be made by an experienced veterinarian in consultation with the owner and should include consideration of the impact of the disease on neighbouring flocks and in the flocks of potential purchasers. As a working rule, if more than 10 per cent of the flock is severely affected, action should be taken.

Benign foot-rot

BFR is a disease in which, even when the prevalence is high, the proportion of animals with severe, i.e. under-running infections, is very low. Of the affected sheep, 1–2 per cent (perhaps uniquely susceptible ones) will have separation of the horn, but most will have infections confined to the interdigital skin.

In chronic infections of the interdigital skin, especially in VFR, single or multiple verrucose projections may occur as an aberrant response to inflammation. Similarly, granulomatous lesions may occur on the feet of sheep where dermal tissue has been injured by over paring. These sequelae respond to recommended treatments for foot-rot (see Figures 39.2a and b in the colour plate section).

Pathology

The pathology of foot-rot has been summarized [9]. Essentially, the bacterial invasion causes a recurrent degeneration of the stratum granulosum of the epidermis with an associated hydropic degeneration of the epidermis and vacuole formation. Leucocytes invade the affected areas of the epidermis. The role of the different bacteria in the pathogenesis of foot-rot is not clear, but the elimination of *D. nodosus* from lesions either by specific immunization or antibiotic therapy will result in a cure of most affected animals.

Diagnosis

The clinical diagnosis of the type of foot-rot in a flock has been described above. In differential diagnosis TA, HA and shelly toe (white-line disease) should be considered. Confirmation of foot-rot in individual cases can be achieved by microscopy of Gram-stained smears of necrotic material thinly spread on a slide. *D. nodosus* has a characteristic morphology, a large rod usually with terminal swellings. Many other bacteria, Gram-positive and Gram-negative, are observed in the smears, but few, if any, leucocytes. The presence of *D. nodosus* does not necessarily indicate the occurrence of VFR. If VFR cannot be inferred by clinical examination alone, *in vitro* tests of *D. nodosus* may assist diagnosis. The elastase test [9] and the gelatin gel test [10] assist in the characterization of isolates as virulent or benign. The intA gene of *D. nodosus* is correlated with virulence [11]. In the field, sheep have simultaneous infections of both benign and virulent isolates. It is essential, therefore, to characterize multiple isolates from outbreaks that are clinically uncertain, especially in areas and flocks from which more virulent strains have been eradicated. In the range between BFR and VFR there are

many outbreaks that are more difficult to classify. Clinically, it is important to assess the impact of the disease on the flock as a unit: if many sheep have severe infections, intervention is necessary for the whole flock; if only a few animals (or none) are badly affected, less rigorous and less costly intervention is required. Assessment of the disease in the flock can be made by examining a sample of sheep sufficient to be confident about the prevalence of severe infections. In assessing the proportion of severe cases, it is desirable to know whether the disease has expressed itself maximally in the flock. Early in an outbreak of VFR, most cases could still have lesions confined to the interdigital skin. Re-examination a few weeks later and the comparison of recorded foot scores [6] will help confirm or modify the first diagnosis.

Epidemiology and transmission

Outbreaks of foot-rot result from the occurrence of a number of host, environmental and microbial factors. Some breeds are more susceptible than others. Within breeds, there are more-resistant individuals and this resistance is heritable [12]. Exposure to environmental conditions that devitalize the interdigital skin is a necessary cause of foot-rot. When the stratum corneum of the tissue is hydrated and hyperkeratotic, it is susceptible to invasion by *F. necrophorum*. Damage resulting from the invasion allows the establishment of *D. nodosus* if it is present in the environment. The severity of foot-rot depends, in part, on virulence factors in infecting strains of *D. nodosus*. Multistrain infections may occur.

The incubation period of foot-rot is 10–14 days when humidity is high and the ambient temperature is above 10°C. Clinical expression is complete after 14 days. Thereafter, again depending on the host, the environment and the infecting bacteria, cases may persist, regress or heal completely. There is evidence that some animals that apparently recover from foot-rot, either naturally or after treatment, are carriers of *D. nodosus*. These carriers may relapse and re-infect unaffected members of the flock when environmental conditions are suitable.

Goats, cattle and deer are also susceptible to foot-rot. *D. nodosus* strains isolated from goats can cause either benign or virulent foot-rot in sheep [3]. Those from foot-rot and other foot conditions in cattle are benign for sheep. There are insufficient data from deer to indicate whether they are hosts for organisms virulent for sheep.

Treatment, prevention, control and eradication

The form and intensity of treatment depends on whether or not benign or virulent foot-rot is present in the flock.

Benign foot-rot

Foot bathing without paring is sufficient to control the lameness that may be present. Walking sheep through footbaths 6 m long and 10 cm deep, which contain 10 per cent zinc sulfate solution, is adequate. In environments particularly favourable for foot-rot, walks through footbaths should be repeated weekly until conditions improve, or signs of lameness abate.

Virulent foot-rot

Cases that have separation of either soft or hard horn respond to either topical or parenteral treatment. If topical treatment is preferred, all hoof-overlying necrotic tissue must be removed either with secateurs or a sharp knife. This procedure is slow and laborious and it is also painful. Over-zealous paring can cause greater damage than the disease. If paring is inadequate, topical treatment is not successful. After paring, good cure rates can be achieved by walking sheep through footbaths of 10 per cent zinc sulfate and returning sheep to a dry environment.

Antibiotics injected intramuscularly at adequate rates (Table 39.2) cure as many cases of virulent foot-rot as combined paring and topical treatment. Withholding antibiotics for appropriate periods before slaughter should be observed. Parenteral therapy removes the necessity for paring other than to establish a diagnosis, but the therapeutic response of sheep treated parenterally is highly dependent on their being kept in a dry environment for 24 hours after treatment. Returning animals to wet pasture after treatment inhibits diffusion of antibiotics to the affected tissues.

Both topical and parenteral treatment, properly administered, will cure 90–95 per cent of cases. Treated animals should be examined after 3–4 weeks to identify animals that have failed to respond and should be disposed of.

Table 39.2: Recommended single doses of parenteral antibiotic treatment of virulent foot-rot

Antibiotic	Dose	Withholding period (days)
Procaine penicillin/dihydrostreptomycin sulfate	70 000 U/kg + 70 mg/kg	28
Oxytetracycline LA 200 mg/ml	1 ml/10 kg	42
Lincomycin HCl Monohydrate/spectinomycin SO_4 tetrahydrate (Lincomycin base 50 mg/ml, Spectinomycin base 100 mg/ml)	1 ml/10 kg	28
Erythromycin base 200 mg/ml	0.2 ml/10 kg	3

In those countries where it is not subject to regulation, owners may elect either to control VFR in their flocks or to eradicate it.

Where flock management is based on the regular introduction of sheep from other flocks and where the foot-rot status of the introduced sheep is not known, control may be the best option. The impact of foot-rot on a flock can be reduced to acceptable levels by regular foot-bathing with or without vaccination. If control is the goal, paring should be limited to those sheep in which the flock control system has failed. For welfare reasons, these animals will need intensive treatment or they should be culled from the flock.

Immunization with multivalent vaccine can achieve two effects; it prevents transmission and it also accelerates healing in a high proportion of affected animals. The duration of immunity is relatively short – about 12 weeks – after the recommended schedule of two doses. Vaccination should be timed to precede those periods when transmission is most likely to occur. If the transmission period is long, revaccination may be required. Revaccination requires one dose only.

Eradication of foot-rot is achievable because *D. nodosus* does not persist in the environment. Before eradication is adopted as a goal, it is essential that a diagnosis of VFR is confirmed as, in commercial flocks at least, eradicating BFR does not warrant the expense involved. Furthermore, benign strains of *D. nodosus* are very difficult to eradicate from flocks, perhaps because there is a higher proportion of unrecognizable carriers. When eradication is the goal, its achievement depends on complete removal of *D. nodosus* from the flock. This can be done most effectively by disposal of the whole flock, resting pasture and barns for 7 days, then replacing with sheep known to be free of VFR.

Alternatively, those animals that have foot-rot should be identified by rigorous inspection of feet and either disposed of or treated to eliminate infection. When a diagnosis of VFR has been made, all cases in the flock, irrespective of the degree of under-running present, should be regarded as cases of VFR. Preferably, affected and unaffected animals should be identified when transmission is unlikely. This avoids the risk of including cases in incubation with the unaffected component of the flock. Affected and unaffected animals should be separated if there is a risk of transmission. Unaffected animals should be kept in an environment known to be free of *D. nodosus*. To confirm their freedom from foot-rot, the unaffected animals should be re-examined twice at intervals of 3–4 weeks. If affected animals are treated, rather than being culled, they too should be re-examined at least twice. Animals that do not respond to treatment should be culled. Animals that are cured can be returned to the unaffected group.

During a flock eradication programme, foot-bathing of unaffected sheep is contraindicated, because a major aim is to identify sheep with foot-rot. Topical treatment may temporarily prevent this. Eradication at the flock level is achievable with well-planned and executed programmes. The chances of success will be governed by the prevalence of infection at the outset and by the skill and application of the personnel involved. Before a programme is commenced, control measures should be used to reduce prevalence to less than 10 per cent and systems for the prevention of reinfection, by neighbouring or purchased sheep, established. Vaccines based on the few antigenic strains present in affected flocks are much more potent than those which include all known strains. Experimentally, the use of these specific vaccines has accelerated eradication in difficult environmental conditions [13, 14].

CONTAGIOUS OVINE DIGITAL DERMATITIS

This acute infection of the digits of sheep [5] is associated with the presence of spirochaetes similar to those isolated from bovine digital dermatitis and human periodontitis [15]. The role of these organisms in the pathogenesis of contagious ovine digital dermatitis (CODD) has not been established nor have studies been published on the relationship of these spirochaetes to those previously described in cases of foot-rot in sheep [1].

Clinico-pathological signs

Acute lameness is the presenting sign of the disease. This is sometimes severe and the affected foot is carried. The disease develops initially as a circumscribed ulcerative and proliferative lesion of the skin at or above the coronary band. Separation occurs at the junction of the coronary band and the wall of the hoof, and the infection invades the sensitive laminae underlying the horn. In advanced cases the hoof of the affected claw may slough leaving a florid, haemorrhagic digit (Figure 39.3 in the colour plate section). In contrast to foot-rot, only one claw on one foot is usually affected [16].

Point prevalence of CODD is high; up to 40 per cent of sheep were affected in a series of six flocks not concurrently infected with foot-rot [17]. The cumulative prevalence observed in another flock was 80 per cent [5].

Descriptions of the lesions observed and resulting clinical signs may be complicated by the simultaneous occurrence of foot-rot and CODD in some flocks [17]. Apart from the distinguishable sequence of events in the development of CODD cases and the infrequency of interdigital lesions, the absence of *D. nodosus* from affected tissues is another indicator of CODD.

Treatment and control

Failure of flocks to respond to treatments for foot-rot, e.g. foot-bathing in formalin or zinc sulfate, may be the initial indicator of its presence in a flock. Topical application of a solution of lincospectin soluble powder is effective [18]. Because the distribution of CODD in UK has not been determined, careful attention to foot health in purchased sheep before addition to the flock is warranted.

FOOT 'ABSCESSES'

Foot abscess and digital suppuration were used to describe suppurating infections of the feet of sheep that needed to be distinguished from the contagious disease foot-rot [1]. Later [19], these 'abscesses' were shown to include two distinct clinico-pathological entities: 'toe abscess' (lamellar suppuration) and 'heel abscess' (infective bulbar necrosis). Radiological evidence [20] indicates that 'heel abscess' cases frequently also involved the joint capsule, bones and ligaments of the distal interphalangeal joint. Because the sites of infection and tissues affected help to distinguish these entities they will be referred to subsequently as toe abscess (TA) and heel abscess (HA) although neither is, pathologically, an abscess.

Toe abscess (lamellar suppuration)

This is an acute, purulent or necrotizing infection, usually involving only one digit. The prevalence in affected flocks is usually very low, at <5 per cent. TA is not contagious. It is confined to the sensitive laminae under the hard horn of the toe region. The disease is caused by pyogenic or necrotizing bacteria that gain adventitious entry to the laminae through breaks in the horn of the wall, along the white line or through separation at the coronary band. The lameness observed may be severe with the affected digit being carried. Identification of the site of infection may need careful palpation and localized application of pressure. The pain associated with TA is relieved and the infection cured by careful removal of all horn overlying the affected laminae.

Heel abscess (infective bulbar necrosis)

HA is an acute infection due to *F. necrophorum* and *Arcanobacterium pyogenes*, usually involving one digit of the foot. In most cases, infection begins in the interdigital skin and extends into the deeper structures to involve the distal interphalangeal joint, its capsule, and associated ligaments and tendons [18].

Predisposing factors

Prior damage to the interdigital skin is necessary to allow deeper bacterial invasion of the subcutaneous tissue and the joint capsule of PII–PIII. Experimentally, the disease has been reproduced by housing sheep in dirty conditions, following damage to the interdigital skin. Continuous exposure to wet pasture and faeces induces ovine interdigital dermatitis (OID) in many sheep. In HA, the bacteria responsible for OID, *F necrophorum* and *A. pyogenes*, invade beyond the epidermis and infect and necrotize the deeper tissues of the heel including the joint capsule of PII–PIII. Other predisposing conditions include stones and prickly or abrasive vegetable matter. In South Africa, tick bites are considered to be a causal factor (see Chapter 65). Unskilled paring of TA cases may accidentally introduce infection to the joint capsule of PII–PIII. The disease is most common in heavy ewes in wet conditions, particularly if the ewes are driven on roads or on stony areas when OID is present. In New Zealand, young rams seem particularly susceptible and outbreaks occur after the impaction of the interdigital cleft with mud and faeces.

Clinical signs

The predominant sign is acute lameness, often to the extent of carrying of the affected foot and extended periods of recumbency. HA is characterized by marked swelling, a feature absent in TA and in foot-rot. Once infection becomes established in the joint, some permanent damage is inevitable. Initially, there is marked oedema and inflammation of the interdigital skin with necrosis and fissure formation in some areas. Sinus tracts, continuous with the joint and the interdigital cleft, form along the abaxial coronary border (see Figure 39.4 in the colour plate section). Discharge from these sinuses becomes increasingly purulent and less necrotic as the disease progresses. In some cases, in addition to damage to the phalanges, the axial collateral ligaments rupture. Usually, the digit heals within 8 weeks to a stage where no infection remains, but some permanent deformity, swelling and disability persist. The prevalence is generally low and seldom rises above 10 per cent. Much of the importance of the disease arises from the severity of lameness and because it primarily affects rams, reducing their capacity to serve, or ewes close to lambing when they are predisposed to pregnancy toxaemia.

Treatment

The bacteria responsible for foot abscess, *F. necrophorum* and *A. pyogenes*, are sensitive to penicillin. However, by the time most cases are recognized, the joint is already damaged, and some disruption of its structures has occurred. Bandaging affected feet will support the joint and its ligaments while healing proceeds. Surgical drainage is not recommended.

Limiting exposure to conditions which predispose to OID may reduce the prevalence of HA. Foot-bathing in 10 per cent zinc sulfate to reduce the severity of OID may also be an option.

OVINE INTERDIGITAL DERMATITIS

This condition, which occurs in sheep continuously exposed to wet pasture, is due to invasion of the interdigital skin by *F. necrophorum*. There is a mild dermatitis involving all or part of the skin between the claws of one or more feet. In some cases, circumscribed areas of erosion or even ulceration may be seen. Microscopy of affected tissues reveals many different Gram-staining bacteria, and filaments of *F. necrophorum* may be recognized. By definition, *D. nodosus* is absent from cases of OID.

OID alone is of minor clinical significance. Its importance is its association with the pathogenesis of both foot abscess and foot-rot. In cases of the former, *F. necrophorum,* which is normally restricted to the interdigital skin, invades the subcutis and the capsule surrounding the joint between the terminal phalanges. In foot-rot, OID predisposes to invasion of the skin by *D. nodosus* if it is present in the environment. The mild and transient lameness caused by OID usually does not warrant treatment.

STRAWBERRY FOOT-ROT

Cause

The agent considered to be responsible is *Dermatophilus congolensis*. However, the virus of contagious ecthyma (orf) has been implicated in the disease. Chronic exposure to a moist environment is a major contributing factor. Thus, in Australian flocks where

lumpy wool (dermatophilosis) is relatively common, strawberry foot-rot is prevalent only in exceptionally wet seasons.

Clinical signs

The circumscribed, heaped scabs that are characteristic of *D. congolensis* infections in the woolled skin appear about the coronary band and elsewhere about the lower limbs. Later, the lesions coalesce. Mechanical damage to these proliferative scabs results in fissure formation and the exposure of raw, bleeding tissue superficially like mashed strawberries (see example in Figure 71.5 in the colour plate section). If there is superinfection by pyogenic bacteria, purulent exudate may be observed. Little lameness is present and the lesions regress spontaneously over 6–8 weeks.

Diagnosis

Strawberry foot-rot is more likely to be confused with contagious ecthyma (orf) than foot-rot. Orf is common in the digits and the proliferative hyperkeratosis is similar (compare with Figure 42.3 in colour plate section). Demonstration of the presence of *D. congolensis* in smears of affected tissue will assist confirmation of diagnosis.

Treatment and prevention

While *D. congolensis* is susceptible to a number of antibiotics, the disease does not usually warrant this form of treatment. If serious outbreaks occur regularly in particular flocks, vaccination against orf might be considered. Foot-bathing in antibacterial astringent solutions may be helpful.

SHELLY TOE

Synonyms: clover burn, separated wall, white line disease

Superficially, shelly toe resembles some cases of VFR because of the apparent separation of the hard horn of the abaxial wall from the underlying tissues (Figure 39.5 in the colour plate section). However, there is an intact and functional wall underlying the superficial one. The cause of this condition is unknown but may follow a loss of integrity of the white line. Shelly toe is common in the feet of Merinos.

Lameness does not occur unless impaction of the space between the two layers of hard horn with mud and faeces exerts pressure on the underlying soft tissues. Necrosis and/or exudate, a constant feature of VFR, is unusual. Treatment is not necessary unless serious impaction has occurred. This can be relieved by cutting away the outer layer of horn.

ZOONOTIC IMPLICATIONS

F. necrophorum has been isolated from human wound infections, so cuts and abrasions acquired during examination of affected animals should be cleaned and treated with antibacterial agents. *D. nodosus* is not a human pathogen.

Both *D. congolensis* and contagious pustular dermatitis (contagious ecthyma) organisms, implicated in strawberry foot-rot, cause unsightly skin infections in humans. Care should therefore be taken in handling affected animals and in the laundering of protective clothing.

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Foot-and-mouth disease

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Foot-and-mouth disease (FMD) is a highly infectious vesicular disease of cattle, sheep, goats, pigs and wild ruminants. It is the most important disease constraint to international trade in livestock and animal products.

CAUSE

The disease is caused by a virus belonging to the *Aphthovirus* genus of the family Picornaviridae and so is non-enveloped and has icosahedral symmetry. The virus particles range in diameter from 24 to 28 nm. The virion has a core of single-stranded RNA approximately 8400 nucleotides long that is translated to yield a polyprotein which is processed into structural and non-structural proteins. The capsid consists of 60 copies of each of the four structural proteins 1A, 1B, 1C and ID, respectively. The virus is sensitive to acid (below pH 6) and to alkali (above pH 9), but resistant to detergents and to ether, alcohol and chloroform. Seven immunological serotypes are recognized: O, A, C, SAT 1, SAT 2, SAT 3 and Asia 1. Formerly over 60 subtypes were recorded, but field isolates are now related: (1) to current virus strains in vaccines to determine the most appropriate vaccine to use; and (2) to reference virus strains from past outbreaks to determine the source of infection [1, 2].

CLINICAL SIGNS

The incubation period in sheep following infection with FMD virus is usually 3–8 days but can be as short as 24 hours following experimental inoculation, or as long as 12 days, depending on the susceptibility of the sheep, the dose of virus and the route of infection. Generally, the first sign observed in a flock of sheep is lameness, which quickly involves an increasing number of animals. Affected animals are reluctant to walk, anorexic and dull, and may lie away from the others. They are febrile and their affected feet are hot and painful when handled. During the lambing season the first signs in an infected flock may be the death of young stock due to heart failure. Lambs may die before they develop vesicular lesions [3].

Typically, early mouth lesions comprise erosions. Fluid-filled vesicles in the mouth are unusual and, if they occur, are transient, as the superficial epithelium is very thin and easily ruptured. The most common site for erosions is on the dental pad [4], in particular where the incisors touch (see Figure 40.1 in the colour plate section). Erosions are preceded by blanched areas of necrosis in the epithelium. Other sites where erosions may be seen are on the gums, the hard palate, the lips and the tongue (see Figure 40.2 in the colour plate section). Occasionally, they may be found in the nostrils. Generally, healing takes place very rapidly by serofibrinous in-filling. The sharp margination of erosions is lost after about 3 days and the lesions progressively change into scars [5].

Affected feet are warm and painful to the touch. Vesicles may be found in the interdigital space (see Figure 40.3 in the colour plate section), at the bulbs of the heel and along the coronary band. It is usually necessary to carefully reflect the hair just above the digits to see the lesions along the coronary band. Rupture of foot vesicles may lead to secondary infection and foot-rot is often present at the same time. Lameness may be sufficiently severe that affected sheep hobble on their knees or remain recumbent.

The severity of lesions in FMD can be influenced by the amount of physical trauma to predilection sites. Foot lesions may be severe or mild depending on whether sheep are kept on hard surfaces or soft pasture, and oral lesions may be severe if sheep are

fed coarse fodder. These findings underline the importance of making a complete examination of sheep, including the feet, when cases are suspected.

Occasionally, vesicles may be seen on the teats, vulva and prepuce. Affected rams are unwilling and unable to serve. In the early stages of the disease milking ewes are usually agalactic. Milk production may resume, but total yield is reduced. Mastitis may be a sequel. Loss of condition is evident among animals in the flock.

The severity of the disease varies considerably with the breed of sheep, the virulence of the strain of virus and the environmental conditions. With strains of low virulence there may be little to see, especially in sheep with dark pigment. On numerous occasions following epidemics serological surveys have demonstrated that infection must have been present in certain sheep flocks even though this was not recognized at the time, presumably because the clinical signs were mild or inapparent. This conclusion is supported by experimental findings, in which over 27 per cent of sheep were shown by seroconversion to have been infected yet they did not develop lesions [6]. FMD virus does not usually cause mortality in adult sheep. However, when pregnant ewes are infected, abortion and death may occur [7]. FMD virus has a high affinity for the cardiac tissue of young stock, including lambs, and commonly causes their death through multifocal mycardial necrosis and heart failure. The mortality rate among lambs may be very high (over 90 per cent). During the 1989 type O epidemic in Tunisia, which started in the lambing season, more than 50 000 lambs succumbed [8].

PATHOLOGY

The route by which FMD virus initiates infection in a flock can be related to the exposure dose, the husbandry system and the mechanism of introduction of virus on to the premises. Sheep are highly susceptible to infection by the respiratory route [9] and by the introduction through breaks in the epithelium, e.g. through foot lesions caused by foot-rot. After entry by the respiratory tract, or through the epithelium, virus multiplies locally before entering the bloodstream. It then becomes disseminated throughout the body to all organs and tissues, including the predilection sites, where it gives rise to vesicular and/or erosive lesions, or, in lambs, to lesions in the myocardium [10].

The pathological changes in the epithelium occur first in the cells of the stratum spinosum and consist of intracellular and intercellular oedema, necrosis and granulocyte infiltration. Some lesions evolve as vesicles, resulting in separation of the epithelium from the underlying connective tissues with interposition of fluid, whereas others progress to necrosis without the accumulation of fluid. In lambs dying of heart failure there is a lympho-histiocytic myocarditis which, in hyper-acute cases, can lead to death within a few hours. Macroscopically, the heart is pale and soft with scattered greyish spots of variable size, mainly in the ventricles. In other cases death occurs later and the heart lesions are more obvious to the naked eye. They consist of greyish stripes, mainly in the left ventricle and in the interventricular septum. The typical appearance has been termed 'tiger heart'. Microscopically, there is necrosis of the myocardial fibres and a granulocyte infiltration.

Around 45 per cent of convalescent sheep may become persistently infected for up to 8 weeks and a small number of animals may carry virus for up to 9 months. Such animals are commonly known as 'carriers', where a carrier is defined by the recovery of live virus from the pharynx at 28 or more days post-infection. The virus persists in the tonsillar tissue and samples can be collected using a 'probang' sampling cup which recovers mucus and superficial epithelial cells from the oesophageal–pharyngeal region [10].

DIAGNOSIS

The diagnosis of FMD is based on the clinical signs, together with laboratory examination to establish the serotype of the causal virus. Because of its highly infectious nature and potential for rapid spread, FMD is a notifiable disease in most countries and investigations after the first reports are carried out by the official veterinary service. It is important that if there is any suspicion of FMD it should be reported as soon as possible to the appropriate authority; otherwise, economically damaging spread can occur.

For laboratory diagnosis, the clinical sample of choice is epithelial tissue taken from animals showing

early lesions, and as much material as possible should be collected. It may be necessary to examine several animals in order to find lesions suitable for sampling. To avoid injury to personnel collecting the samples, as well as for animal welfare reasons, it is recommended that animals are sedated before any samples are collected. Where there is insufficient epithelium, blood should be collected without anticoagulant for serum examination and with heparin for virus isolation; also oesophageal–pharyngeal samples. In the absence of evident lesions, blood sampling for virological examination may be targeted towards pyrexic sheep. In the case of lambs found dead from suspected myocarditis, samples of at least 1 g should be collected from the ventricles and atria. If there are lactating ewes, samples of their milk should be collected. If other susceptible species are present on the farm, they should be examined and samples collected from any which have suspicious signs [11].

Samples from suspected cases of FMD must be handled with extreme care to prevent further spread. The outside of sample containers must be disinfected with an approved disinfectant and they must be securely packed to prevent spillage or leakage of the contents. Packages must be clearly labelled, they must be transported according to national and international regulations, and they should be sent only to a laboratory that has been designated as a national, regional or world reference laboratory for FMD. Details are provided in the Office International des Epizooties (OIE) *Manual of Standards for Diagnostic Tests and Vaccines* [11].

In an approved laboratory the clinical specimens are tested for the presence of FMD viral antigen. The diagnostic test most commonly used for this purpose is an indirect enzyme-linked immunosorbent assay (ELISA) [11]. A positive result will also identify the serotype. If insufficient antigen is present to give a conclusive result by ELISA the virus is grown in a sensitive cell culture system such as primary bovine thyroid cells and repeat tests carried out. Virus isolation from uncoagulated blood samples, or from milk or oesophageal–pharyngeal samples is attempted in cell culture. Alternatively, tests for viral RNA can be performed using the reverse transcriptase, polymerase chain reaction (RT-PCR) method [12]. The experience of the Food and Agriculture Organization World Reference Laboratory for FMD at Pirbright is that more samples are detected positive by RT-PCR than by virus isolation. However, a major deficiency of RT-PCR is that occasionally it fails to detect some FMD viruses with mutations in the region of the genome targeted by the primers or probe or else (as in the case of SAT serotype viruses) may detect them with a reduced sensitivity. This problem may be addressed by improving our knowledge of the sequence diversity in the primer and probe target regions and/or combining RT-PCR tests directed at different parts of the viral genome (Dr David Paton, personal communication).

Serum and milk samples can be examined for antibodies against FMD virus by the liquid phase blocking ELISA (LPBE). The sensitivity of this test is close to 100 per cent and the specificity is around 95 per cent. Samples giving inconclusive results are tested by a virus neutralization test (VNT). The relatively low specificity of the LPBE makes the method unsuitable for large-scale sero-surveillance as many confirmatory VNTs will be required. As an alternative the solid-phase competitive ELISA (SPCE) has been developed. This test has both high sensitivity and specificity and is suited for the rapid processing of large numbers of samples [13]. A variety of tests is available for the detection of antibodies to the non-structural proteins of FMD virus [14, 15] including commercially available kits. These provide a good indicator of past infection with FMDV, regardless of vaccination status and have the advantage of being able to detect antibodies elicited by all FMDV serotypes. However, they are not yet fully validated for use in sheep.

A positive result from the initial antigen-detection ELISA takes about 3 hours from the time of receipt of the sample if it contains a sufficient quantity of viral antigen. However, if it is necessary to grow the virus in cell culture, 48 hours or even longer may be required to obtain a result. At least two passages in cell culture are required before a sample can be declared negative. Tests for the detection of virus and antibody may be used in the laboratory or 'on-farm' [10].

Once the priority tasks of laboratory confirmation and serotype identification have been accomplished the next important activities are to characterize the virus isolate antigenically and genetically. The purpose of antigenic characterization is to determine what vaccine would be most appropriate should it be decided to use vaccination as part of the control measures [16]. Genetic characterization is performed by comparing the partial nucleotide sequences of a given field isolate with reference strains held in a virus bank. Generally, investigations are focused initially on the

sequence of the 1D coding region. Subsequently, the sequencing of other regions of the genome may be undertaken. The establishment of identity or close relationship between an isolate and a reference strain or strains is highly indicative of an epidemiological association [17]. By applying the RT-PCR method it is possible to sequence and analyse an isolate within 2 days. The identification of the origin of an outbreak is especially important for a country which is normally free of FMD so that preventive measures can be taken to ensure that the risk of a repetition is eliminated or at least greatly reduced.

EPIDEMIOLOGY AND TRANSMISSION

FMD occurs throughout the world. At present, North and Central America, Australia and New Zealand remain free of the disease. Certain countries are recognized by OIE as having disease-free zones within their territory, e.g. Colombia, Republic of South Africa and Namibia. Serotypes O, A and C occur in South America; O, A, C and Asia 1 in the Middle East, the Indian subcontinent and the Far East. In Africa O, A, C, SAT 1, SAT 2 and SAT 3 are found. Information about the current status of a country can be obtained by consulting the OIE website [18].

The spread of FMD virus can occur in a number of ways; by direct contact between infected and susceptible livestock; by contact with or feeding on infected meat products and milk; by infected semen or embryos; by the airborne route; or by mechanical carriage on people, domesticated or wild animals, birds, vehicles and fomites [1, 9, 10]. Sheep are more likely to be infected by direct contact, by the airborne route or through contact with contaminated people or carriage in contaminated vehicles. Spread is rapid where sheep are kept in close contact as in feedlots, markets, abattoirs and on vehicles. It will be slower when the rate of contact is less, for example when sheep are under extensive systems of management.

Virus production and excretion in sheep infected by inoculation or direct contact have been shown experimentally to fall into three phases. In the first phase virus replicates rapidly in the sheep, reaching a peak and then declining. Traces of viral RNA are isolated in the second phase. During the third phase virus and viral RNA are found in the oesophageal–pharyngeal region (carriers). Development of antibodies later in the first phase results in a dramatic decline in virus excreted. Airborne virus is recovered from infected sheep early in the first phase. The length of each phase depends on the strain of virus and the weight of infection. For example with O UK2001 virus (isolated during the 2001 FMD epidemic) the first phase lasted 7–8 days with peaks of virus in the blood at 2–3 days, in nasal swabs at 3–4 days and in aerosols on the second day. The second phase lasted 1–3 days with viral RNA being isolated from nasal and rectal swabs. The third phase (carriers) commenced at 4 weeks. Antibodies developed at 4–5 days in phase 1 [19].

The role of sheep in the transmission of FMD can be related mainly to the first of the above phases since this is when they are most infectious. Sheep may, however, appear clinically normal at this time, especially in the early stages or when infected by strains of low virulence. FMD may not be detected on a farm until infection has spread from sheep and caused lesions in cattle as happened in the 1967–8 and 2001 epidemics in the UK [20]. Extensive spread can therefore result before the presence of infection is realized. Sheep have been implicated on many occasions as the disseminators of FMD both between and within countries. Examples are provided by Donaldson [8] and include: the introduction of FMD into Canada by sheep imported from the UK in 1875; the 1978 and 1983 type A epidemics in Morocco; and the 1994 type O epidemic in Greece. Infected sheep imported from South America were the most likely cause of the 1978 epidemic in Morocco. In the 1983 Moroccan epidemic infected sheep from Spain entered Morocco through the Spanish enclave of Ceuta and caused a series of outbreaks extending from the north of the country to Agadir in the south-west. Clinical disease was identified only in cattle but a serological survey showed that many sheep (and goats) had been infected. The 1994 type O epidemic in Greece was linked to the smuggling of sheep from Asiatic Turkey to Lesbos. FMD was not diagnosed initially on Lesbos but when sheep were transferred to mainland Greece cattle became infected and the disease was recognized. Another example was the North African epidemic of 1989–92 which started during the winter of 1989 in Tunisia and then swept westwards into Algeria and Morocco. The majority of the spread was attributed to the uncontrolled

movement of large numbers of sheep, especially around the time of religious festivals when there was a surge in the demand for sheep meat.

The type of husbandry and the relative numbers of livestock in a region can influence the importance of the role of sheep in the epidemiology of FMD. In the Middle East, India and other parts of Asia sheep have been responsible for the maintenance and spread of FMD. In the UK, before the 2001 epidemic, sheep were not so often involved in outbreaks; generally, FMD was seen first in pigs and cattle and subsequently spread among the sheep population. In 2001 pigs became infected and spread FMD to sheep which subsequently carried infection throughout the UK [21]. Historically, the most usual routes of entry of FMD virus to the UK have been by infective bones or offal from overseas or by airborne spread from northern France and the Low Countries.

PREVENTION AND CONTROL

FMD is subject to national and international control, and the measures taken depend on whether the country is free from the disease, is subject to sporadic outbreaks or has endemic infection. The categories defining whether a country or zone can be classified as FMD-free, with or without vaccination, or is infected can be found in Chapter 2.2.10 of the OIE Terrestrial Animal Health Code [22].

In countries free of the disease, animals to be imported from countries considered to pose a risk should be quarantined and subjected to tests to ensure freedom from virus and antibody. Importation of meat products may be prohibited or only deboned meat or heat-treated products allowed. Milk and milk products must be heat-treated and safeguards must be taken to ensure that semen and embryos do not originate from infected animals. If FMD enters a country hitherto free, movement of animals should be immediately banned or restricted. Vehicles carrying animals must be disinfected. Veterinarians and people handling or coming into close contact with animals must disinfect themselves and their equipment before going to other premises. Suitable disinfectants are those that contain acid or alkali. A 4 per cent solution of sodium carbonate is a highly effective, cheap and non-toxic disinfectant especially when used after initial washing to reduce the amount of organic matter that is present.

The policy is to slaughter the clinically affected and all other cloven-hoofed animals on the affected holding. This measure eliminates that particular flock or herd as a source of virus for spread by the airborne and other routes and also the possibility that carrier animals will remain. Pigs excrete more virus as an aerosol than cattle and sheep, and sheep are less likely than cattle to be infected by the airborne route and to pass on infection over distance than cattle. Priority at the start of an epidemic should be given to the slaughter of pigs followed by cattle. Provided movement controls and biosecurity are maintained, the slaughter of sheep may be delayed until virus and antibody tests are completed [23].

In some countries normally free of disease a decision may be taken to vaccinate when FMD enters (emergency vaccination). The pros and cons of employing emergency vaccination have been reviewed [24]. Priority should be given to vaccinating cattle for the reasons given earlier [23]. The vaccine is made from virus grown to high titre in cell culture and concentrated by filtration or precipitation, inactivated by treatment with aziridine, and adjuvanted with either aluminium hydroxide and saponin or mineral oil. Vaccines formulated with either type of adjuvant have been used successfully to protect cattle and sheep. The measures that a country or zone will have to take to recover its free status following the use of emergency vaccination are specified by OIE [22].

In many countries, where disease is endemic or sporadic or there is a risk of its introduction, routine prophylactic vaccination is practised. Generally, cattle are the principal species vaccinated prophylactically in campaigns to control the disease. For example, Uruguay eradicated the disease by intensively vaccinating its cattle population of around 10.6 million animals, while leaving the sheep population, numbering 19.6 million animals, unvaccinated. When the vaccination of the cattle stopped there were no recrudescent outbreaks. One year after ceasing all vaccination Uruguay was recognized as being free from both the disease and the virus. There are circumstances, however, in which the vaccination of sheep is indicated: where they are at risk of being infected, such as when they form part of an immune belt ('buffer zone') [8] between an infected and a free area; and during emergency ('ring') vaccination around an outbreak. With routine prophylactic vaccines, some protection is given after one dose of vaccine, but two doses at an interval of 2–4 weeks are

preferable. Vaccination should be repeated at 4 months to 1 year later, depending on the weight of infection to which the sheep might be exposed. FMD vaccines may be given at the same time as other sheep vaccines. Animals to be exported to countries where FMD occurs may require to be vaccinated with a vaccine containing the appropriate virus strains. Further information on the vaccines and their use may be obtained from the manufacturers.

Research on novel vaccines using new molecular biological techniques has had limited success but efforts in this direction are continuing [2].

ZOONOTIC IMPLICATIONS

FMD in people is very rare. Thirty-seven cases have been described, in which, in addition to clinical signs, diagnosis was confirmed by virus isolation, the presence or rise in antibody or a combination. Clinical signs included fever, sore throat, headache and general malaise, followed by vesicles on the hand, foot or mouth. The incubation period varied between 1 and 10 days, with 2–7 days for the majority of cases. Infection resulted from close contact with affected cattle or pigs, with virus entering through broken skin, being ingested in infected milk or being inhaled in aerosols [25]. No cases have been reported in people associated with affected sheep, but this does not rule out sheep as a possible source of infection and the development of vesicles in persons after contact with affected sheep should be reported to the appropriate authorities.

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41

Arthritis

G.H. Watkins

Arthritis is inflammation of the joints and, although the term applies to fibrous, cartilaginous and synovial joints, it is the latter that are most commonly affected in sheep. A number of pathogenic mechanisms can cause inflammation of the joints but infection, particularly with bacteria, is most important in sheep. Arthritis may follow local trauma to a joint and infection may track into joints from local wounds but usually infection is haematogenous [1]. This has two important consequences: firstly, arthritis in sheep usually affects more than one joint. Secondly, due to the relative incompetence of their immune system, lambs are much more likely to develop infectious arthritis than adult sheep. 'Joint-ill' is often used to describe polyarthritis in lambs but as this term encompasses disease caused by several different pathogens, it is more helpful to consider the various arthritides according to the causal organism.

Arthritis has a severe adverse affect on the welfare of affected sheep, both directly through pain in inflamed joints and indirectly because affected lambs lose mobility and may starve or become prey to predators. Although there are no recent data on the economic consequences of arthritis, these are probably considerable. Affected lambs that do not die in the acute stage require intensive and prolonged therapy, which disrupts management during the lambing period, are usually stunted and reach slaughter weight after a delay of many weeks, if at all. In British abattoirs arthritis is a common cause of condemnation of lambs [2].

ARTHRITIS IN YOUNG LAMBS

Streptococcal arthritis

Streptococci are the most common cause of arthritis in lambs in the UK: in a survey in England and Wales they

were isolated from at least one arthritic joint of 80 per cent (of 212) of sheep under 1 year old [4]. Although streptococcal arthritis is an important disease in parts of the former Soviet Union, it has not been reported as a common disease of lambs elsewhere.

Cause

The causal streptococcus, an α-haemolytic member of Lancefield's group C, shares many cultural characteristics with *Strep. dysgalactiae* subsp. *dysgalactiae* of cattle but its taxonomic status is not certain.

Clinical signs

Only lambs younger than 4 weeks of age are affected [3]. Older lambs are not susceptible, probably due to the development of bacteriostatic activity in blood [4]. The first sign is usually lameness in one or more limb, although it is not uncommon for affected lambs to be recumbent or appear to be 'wasting away'. In the acute stage, joints are not grossly enlarged, becoming distended with pus only in the chronic stages, usually 1–2 weeks after onset of signs. Only a minority of affected lambs exhibit gross omphalitis.

Pathology

There is an accumulation of watery, yellowish pus in the joints, most commonly the carpi and tarsi, (Figure 41.1) often only apparent when joints are incised at post-mortem.

The articular cartilage is not grossly eroded but periarticular oedema and haemorrhage often affect the subcutaneous and intermuscular connective tissues. Histologically, large numbers of bacteria are present in the capillaries of the synovial membrane and, frequently, in the blood vessels of the epiphysis and metaphysis, from where they may spread to cause widespread osteomyelitis or even necrosis of infected bone. Streptococci occasionally spread to the epiphysis from the diaphysis via the small blood vessels that traverse the growth plate (physis) and from there they may penetrate the joint space by forming microscopic channels devoid of matrix within the articular cartilage. Joint infection results in a mixed inflammatory exudate in synovium and bone but not in cartilage. Neutrophils predominate initially and

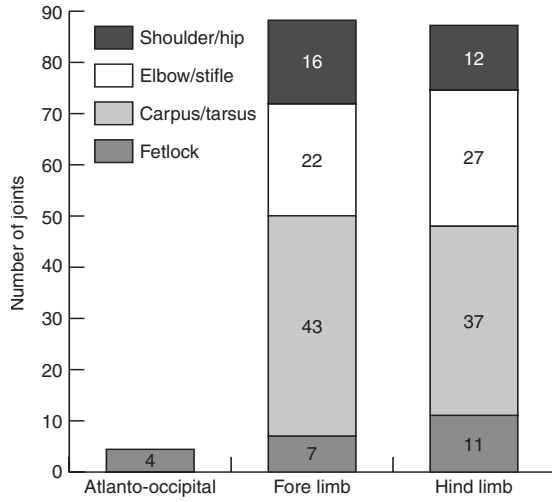

Figure 41.1: Macroscopically affected joints in 27 lambs with streptococcal arthritis.

the numbers of macrophages, lymphocytes and plasma cells increase in the chronic stages.

Other occasional lesions include abscesses in the liver and pharynx, endocarditis and necrotizing myocarditis. There is often histological and bacteriological evidence of ascending infection of the umbilical vessels, which are usually grossly normal.

Diagnosis

When lambs are lame, a diagnosis of arthritis is usually straightforward and joint fluid may be aspirated for bacteriological examination. When recumbency or ill-thrift is the main feature, diagnosis is often difficult and the disease must be differentiated from nutritional muscular dystrophy, spinal abscess, delayed swayback, border disease, nephrosis and weakness due to starvation.

Epidemiology and transmission

Morbidity in affected flocks is usually between 2 and 15 per cent but may be up to 50 per cent in severe outbreaks. Mortality is variable: 50 per cent has been recorded but is usually much lower and can be zero if affected lambs are detected, treated promptly and nursed well. Recumbent lambs in paddocks are often

killed by predators if they are not detected and housed at an early stage.

The epidemiology of streptococcal arthritis is imperfectly understood. Lambs are likely to acquire infection during their first 2 weeks of life when they are in lambing sheds or paddocks. The source of infection for most lambs is usually a contaminated environment. The bacterium survives exceptionally well on dry straw and on wool, less well on wet, faecally contaminated straw, grass and the hairless skin of ewes, and not at all on the surface of wooden and metal hurdles [5]. Some ewes within a flock with streptococcal arthritis carry bacteria in the vagina and possibly in other sites and are probably responsible for the original environmental contamination. Lambs born to such ewes may acquire infection when they pass through the vagina at birth, when they are licked dry or when they are attempting to locate a teat.

Treatment and prevention

Treatment is by parenteral administration of antibacterial agents. As some strains of the causal streptococcus are resistant to tetracyclines the drug of choice is probably penicillin G which should be given for 10 days. Response is largely dependent on the duration and extent of inflammatory changes. Full recovery is rare but treated lambs eventually reach slaughter weight, usually many weeks after unaffected lambs.

At present, there are no satisfactory means of preventing streptococcal arthritis and control will not be possible until factors that predispose lambs to the disease are known. Some measures, while not guaranteeing freedom from disease, should be implemented in an outbreak. The navel should be dipped in disinfectant as soon as possible after birth and again after the lamb has been licked dry. Tincture of iodine is probably the disinfectant of choice but the dip cup must be kept free of organic material and disinfectant regularly replaced if the cup itself is not to become a means of spread of infection. Stomach tubes used for the administration of colostrum should be disinfected between lambs. Lambing pens should be cleaned and well strawed to reduce the likelihood of transfer of infection between successive ewes and lambs. Most affected flocks have a high stocking density of ewes in lambing sheds or paddocks and it should be reduced if streptococcal arthritis recurs year after year.

Staphylococcal arthritis (tick pyaemia)

Cause

Haemolytic strains of staphylococci that produce coagulase are responsible for this disease but have not been further characterized. Concurrent infection with *Anaplasma phagocytophilum,* the cause of tick-borne fever (Chapter 51) which results in profound lymphopenia and neutropenia, is thought to increase the susceptibility of lambs to staphylococcal infection.

Infection is introduced when the lamb is bitten by the tick *Ixodes ricinus* [6] seeking a blood meal. In the UK, this occurs in the spring when ewes and lambs are put on to tick-infested grazings. Staphylococci resident on the skin or contaminating tick mouth parts colonize the bite site, resulting in bacteraemia, usually followed by localization in joints. There may be localization and abscess formation in other organs.

Morbidity varies from year to year and between farms but is usually around 5 per cent. Lambs are affected when between 2 and 10 weeks old, become lame and fail to thrive. Neurological signs occur if staphylococci localize in the central nervous system.

Treatment is often unrewarding owing to irreversible joint changes and the presence of staphylococci within abscesses elsewhere in the body. Control is by removing the tick vector, by antibacterial prophylaxis or, most successfully, both [7]. Tick control is by dipping or spraying ewes and lambs with synthetic pyrethroids or organophosphate acaricides to coincide with the 'spring rise' of ticks (Chapter 51). Ovine staphylococci are susceptible to penicillins and to tetracyclines; long-acting formulations given to lambs of about 3 weeks of age prior to exposure to ticks reduce the incidence of staphylococcal arthritis. A single injection of long-acting oxytetracycline also prevents tick-borne fever for about 3 weeks, so increasing resistance of lambs to staphylococcal infection.

Other infectious causes

Escherichia coli, *Erysipelothrix rhusiopathiae* and *Arcanobacterium pyogenes* may infect the joints of young lambs, although they do so less commonly than streptococci. Infection is usually via the umbilical vessels from a contaminated environment and there are often hepatic abscesses in addition to polyarthritis. Morbidity is usually less than in streptococcal arthritis

and, unless there are major failures of husbandry, arthritis due to these bacteria is usually sporadic rather than occurring as an outbreak.

Diagnosis and prevention are as for streptococcal arthritis. Treatment is with is appropriate antibacterial drugs.

ARTHRITIS IN OLDER LAMBS

Arthritis due to *Erysipelothrix* infection

Cause

The cause is *Erysipelothrix rhusiopathiae*, especially serotypes 1 and 2. A slender, non-motile, non-sporing Gram-positive rod, *E. rhusiopathiae* is a facultative anaerobe, is easily cultured under standard laboratory conditions and is a commensal or pathogen of a wide range of vertebrates and invertebrates. A review of the infection in sheep is available [8].

Clinical signs

Lambs between 2 and 6 months are affected. Mortality can be up to 40 per cent but is usually less than 10 per cent. The first signs are lameness or stiffness and pyrexia but usually without depression or recumbency. Affected joints are not severely swollen, and careful palpation and observation may be needed to detect the arthritis. When the disease becomes chronic swelling around affected joints and muscle wastage are more obvious.

Pathology

In the acute stages of disease, the joint fluid is predominantly fibrinous with variable numbers of infiltrating polymorphonucleocytes. There are no obvious erosions of the articular surfaces but they may be covered in sheets of fibrin. In the chronic stages, there is less joint distension but increased thickening of the joint capsule due to fibrosis and oedema. The villi of the synovial membrane are elongated and branched and contain aggregates of lymphocytes and plasma cells. Pannus formation is not extensive and is confined to the periphery of articular cartilage.

Diagnosis

A tentative diagnosis made from the clinical signs and history, particularly the age of affected lambs, should be confirmed by laboratory examination. In the acute stages, the organism may be cultured from affected joints, either directly after death or by aspirating synovial fluid but is unlikely to be recovered in the chronic phase of the disease. Detection of high titres of antibody to the bacterium by the slide agglutination test is currently the most useful way of confirming a diagnosis in lambs with chronic arthritis.

Epidemiology and transmission

Like the *Streptococcus* responsible for arthritis in young lambs, *Erysipelothrix* is able to survive for long periods, probably many years, in the environment but, unlike the former, it has a wide host range and infection is not confined to sheep. Lambs probably acquire infection from contaminated organic matter in the soil but the routes by which they do so are not known. Soil is usually contaminated by other infected sheep, but pigs, turkeys and other birds have also been implicated as original sources of infection.

Treatment, prevention and control

Penicillin is the drug of choice. Therapy is most likely to be successful if instituted in the early stages of the disease. A formalin-killed, aluminium hydroxide-adjuvanted vaccine is available for use in adult sheep and lambs when there is known to be a high risk of infection. Two injections, given 2–6 weeks apart, elicit immunity and also colostral antibody that passively protects young lambs. There is cross-protection between serotypes of *E. rhusiopathiae* as the immunogenic and the serotype-specific antigens are distinct.

Post-dipping lameness

This disease, a special form of *E. rhusiopathiae* infection, has been described in most areas of the world where sheep are kept and dips are used.

Clinical signs, pathology and diagnosis

The first sign is invariably severe lameness, affecting most of the flock. Affected sheep are pyrexic. The

coronary band and skin of the interdigital area and lower limb are reddened, swollen and hot owing to cellulitis of the coronary band which spreads distally into the sensitive laminae and proximally and subcutaneously to the fetlock. Bacteraemia and polyarthritis occur only occasionally.

The history and clinical signs are highly suggestive and laboratory confirmation in the acute stage is by culture of *E. rhusiopathiae* from swabs of the cut surface of the coronary band. Serology is likely to be of value only if there is secondary polyarthritis.

Epidemiology, treatment and control

Post-dipping lameness may occur in sheep of any age that have been dipped within the preceding 2–7 days. The source of infection is faecally-contaminated dip tank fluid, in which *E. rhusiopathiae* may multiply rapidly. Thus, sheep are most at risk if they are immersed in dip solution used a few days previously.

If it is necessary to re-use a dip, an antibacterial substance should be added, preferably on the first day, before it is used. The antibacterial must be compatible with the ectoparasiticide and the dip formulation and most manufacturers specify which substances should be used. Penicillin G is probably the drug of choice for treatment and should be given over sufficient time to eliminate bacteria completely or bacteraemia and polyarthritis may ensue. Vaccination may be considered when there is a high risk of post-dipping lameness.

OTHER INFECTIOUS ARTHRITIDES

Arthritis associated with the chronic phase of contagious agalactia is covered in Chapter 15.

Chlamydial arthritis has been reported in rapidly growing feedlot lambs in mid and western North America, where it may occur as outbreaks of high morbidity [9]. The disease has not been confirmed in the UK and it is not an important cause of arthritis in continental Europe. The causal organism is *Chlamydophila pecorum*, which does not cause abortion but may cause conjunctivitis, often concurrently with arthritis.

Brucella melitensis and, less commonly, *Br. abortus* may localize in joints and other synovial structures but arthritis is not usually the main clinical feature of ovine brucellosis (Chapter 20).

Severe, bilateral arthritis of the carpal joints has been described in ewes infected with maedi-visna virus [10]. The disease is covered in Chapter 31.

NON-INFECTIOUS ARTHRITIS

Severe osteoarthritis of the elbow joint resulting in extensive osteophyte production has been described in adult sheep from 2 to 5 years of age. The disease may be either unilateral or bilateral, affect either sex, although predominantly rams, of a variety of breeds. There is no treatment [11].

Osteochondrosis, predominantly of the shoulder and elbow joints, has been reported as a consequence of large feed intake and growth rate in 4-month-old rams [12].

ZOONOTIC IMPLICATIONS

E. rhusiopathiae can infect humans, and care should be taken to cover and disinfect any wounds or skin abrasions after contact with affected sheep.

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Part IX
Diseases of the skin, wool and eyes

42

Orf

H.W. Reid and S.M. Rodger

Synonyms: Contagious pustular dermatitis (CPD), contagious ecthyma, contagious pustular stomatitis, malignant aphtha, sore mouth, scabby mouth

Orf is a virus disease affecting the skin primarily around the mouth, udder and coronet, and is prevalent in all countries where sheep and goats are raised. Characteristically, affected animals develop scabs, which may become extensive and develop into proliferative wart-like lesions, while, in some outbreaks, extensive necrotizing oral lesions also occur, which may extend down the oesophagus. Disease is seen most frequently in young lambs and feeder animals, although older sheep may be affected also, particularly on the udder, and a venereal infection has been described. In addition to sheep, goats and related species, humans also are susceptible to infection through contact with infected animals or fomites.

CAUSE

The disease has been recognized as an entity since the last century and was shown to be caused by a specific virus in 1923. The virus is a poxvirus belonging to the genus *Parapoxvirus*. It is a large DNA virus (260×150 nm) and has a characteristic basketwork arrangement of microtubules, which can be observed by electron microscopy. The full nucleotide sequence has been determined and is available for the construction of primers for amplifying viral genome by polymerase chain reaction (PCR).

Orf virus infectivity is retained in dried scabs for long periods at cool temperatures (23 years at +7°C), but at higher temperatures, and if the scabs are moist, infectivity is lost relatively rapidly. The virus is partially resistant to ether, but extremely sensitive to treatment with chloroform. Recommended disinfectants for destroying infectivity include iodophors and 2 per cent formalin, but most proprietary preparations are adequate provided surfaces are cleaned before application.

Strain variation between isolates from the UK, the USA, France and Australia could not be detected in cross-protection trials, but some other reports are at variance with this conclusion. However, in view of the difficulties inherent in performing such tests, these data are unconvincing. There is also conflicting evidence for antigenic variation between strains from studies using polyclonal antisera in complement fixation, neutralization and agar gel precipitation tests. The technical problems in the application of these tests to studies of orf, however, make interpretation problematic. However, employing a panel of monoclonal antibodies, several laboratory-adapted isolates were shown to be different. In addition, several studies employing restriction enzyme analysis of the viral DNA indicate that there is considerable heterogeneity between isolates, but this has not been related to antigenic diversity [1]. Such diversity was predicted by researchers [2], who failed to find differences between isolates using a serum neutralization test but, on the basis of electrophoretic analysis of the structural polypeptides, placed 11 isolates into four groups. Despite these observations and the variety of clinical manifestations presented in different orf outbreaks, there is no evidence to suggest that field isolates of different virulence exist.

The biological significance of this diversity is still unclear, and it should be noted that a single New Zealand isolate is employed as a vaccine both in the Antipodes and in Europe. It is concluded that, despite limited genomic and antigenic variation among orf virus strains, this is not reflected in either pathogenicity or in the capacity of different types to cross-protect.

Marked variation in the severity of outbreaks and anatomical distribution of lesions is attributed to environmental predisposing factors.

Antigenically, the other parapoxviruses – bovine papular stomatitis virus, milker's nodule virus and pseudocowpox virus – appear closely related to each other and to orf virus, which tends to support the view that biologically significant antigenic diversity within orf virus isolates is unlikely. Adaptation of orf virus to grow in cell cultures is generally difficult and associated with loss of virulence, which has been an impediment to investigations and a limitation to laboratory diagnosis.

CLINICAL SIGNS

The disease primarily affects animals under 1 year old, in two peaks, the first in spring shortly after lambing and again in 3–4-month-old lambs (see Figure 42.1 in the colour plate section). Older animals do, however, become affected and the condition may occur at any time of the year. Morbidity usually approaches 100 per cent but in most outbreaks mortality is negligible.

The clinical manifestations of infection in sheep are strikingly variable: in many cases infection may go undetected, while, at the other extreme, it may be severe, with mortality approaching 80 per cent [3]. Characteristically, however, infection establishes only where the skin or mucous membrane has been traumatized. Thus, following scarification and experimental application of virus, signs of infection are restricted to the lines of scarification, the essential target appearing to be the freshly regenerated epithelium, which appears within 24 hours of the trauma.

The first signs are of local erythema associated with the formation of papules, which rapidly develop to a vesicular then pustular stage that gives rise to scab formation within a few days. Removal of the scab at this stage frequently causes haemorrhage of the proliferating underlying dermis, which appears as papillomatous projections. As lesions resolve, scabs will become dry and are shed within approximately 28 days of infection, leaving no scar. In natural cases of orf, proliferation often gives rise to wart-like outgrowths, which may go on to produce extensive cauliflower-like growths that can persist for long periods (see Figure 42.2 in the colour plate section). Such lesions are particularly associated with infection of the poll of rams and may extend to involve the ears as well.

In young lambs, lesions are most frequently seen around the mouth and nostrils, often originating at the commissures of the lips. These generally appear as scabby lesions but, in a proportion, proliferative lesions can become infected with bacteria, which may prolong the course of the disease and result in more extensive involvement. Significant mortality does not occur unless the buccal cavity is also affected, when mortality can be severe. Lesions are most frequent along the gums, associated with the erupting teeth, the hard palate and dorsum of the tongue, and may extend to involve the oesophagus. These lesions, initially papuloerosive and whitish with a surrounding zone of hyperaemia, rapidly become necrotic and slough, and may resemble the ruptured vesicles of foot-and-mouth disease. The resulting ulcers on the hard palate and tongue can be up to several centimetres in diameter.

In most epizootics in lambs, the disease characteristically spreads very rapidly. This results from ewes that have developed udder lesions from their affected lambs refusing to allow their lambs to suck. These hungry lambs will attempt to steal milk from other ewes, thus spreading the virus. A possible serious secondary effect of these lesions in the ewe is the risk of acute staphylococcal mastitis, which results in the loss of the affected half udder and, not infrequently, in the death of the ewe (see Chapter 15).

Infection may also involve the thigh, axilla, poll, genitalia, lower limbs and coronet, the latter frequently taking the form of verrucose masses, which are easily abraded and prone to haemorrhage (see Figure 42.3 in the colour plate section). Such lesions around the coronet, sometimes referred to as 'strawberry foot-rot', may be exacerbated by infection with *Dermatophilus congolensis* (see Chapter 39).

The venereal form usually appears soon after the rams are turned out, and spreads rapidly within the flock. In the ewe it begins as small pustules at the vulva on the skin–vaginal mucosa junction, and, in the ram, at the preputial orifice. At both sites, the lesions show a tendency to spread and form shallow ulcers, which may become infected by *Fusobacterium necrophorum* giving a 'floor' of necrotic tissues to the ulcer. Affected rams rapidly become reluctant to mate, and the sequel can be disruption of breeding, with a consequent prolonged lambing.

PATHOLOGY

Replication of orf virus is restricted to proliferating epithelial cells and therefore damage to the skin or mucous membranes typically precedes infection. Within 24 hours of trauma to the skin, regrowth of the epidermis occurs from the margins of the area of damage and from epidermal cells surrounding surviving hair or wool follicles [4]. By 48 hours after infection vacuoles develop in the cells of the regenerating epidermis, and after about 5 days progressive intracellular oedema and cytoplasmic swelling results in ballooning degeneration, accompanied by infiltration with polymorphonuclear neutrophils, lymphocytes and plasma cells. Intracytoplasmic inclusions may be seen in infected cells. Grossly, these changes are associated with intense local erythema and the formation of small vesicles that subsequently develop into pustules. The stratum corneum containing the pustule ruptures, with eventual formation of a scab that is firmly attached and interdigitated with proliferative epidermis. Removal of the scab at this stage results in bleeding from the raw, spongy base. Although papillomatous growths are not a feature of experimentally induced orf, they frequently develop in natural orf and may become extensive. They consist of marked pseudo-epitheliomatous hyperplasia and granuloma formation, which may persist for several weeks or even in some cases many months. Resolution of uncomplicated lesions results in complete re-epithelialization, which leaves no scar, a process that takes about 6 weeks.

Lesions inside the mouth do not form scabs but appear as raised reddened or greyish areas surrounded by an intensely hyperaemic zone.

DIAGNOSIS

A diagnosis of orf on clinical grounds may be reached when the typical papillomatous lesions are present around the lips and nostrils of lambs. Frequently, however, the clinical picture is atypical and laboratory confirmation is required. Historically, the most convenient and rapid method for confirming infection is to examine material prepared from a moist active scab by electron microscopy for evidence of the characteristic virus particles. However, PCR is now the obvious test of choice of which two have been developed. One used for diagnostic specimens from ruminants is a semi-nested PCR which amplifies a 235 bp product of the BZL gene. The other, for human diagnostic material, amplifies a conserved viral RNA polymerase gene. However, where there is a requirement to differentiate between wild-type viruses and vaccine strains, primers for a more variable region should be selected. Virus isolation is not routinely carried out, as scab material tends to be toxic for tissue culture cells and a number of blind passages are required before typical cytopathic effects are observed. Agar gel precipitation tests and complement fixation tests to detect viral antigen have also been described, but are insensitive. Evidence of infection may be difficult to confirm in old scabs but some long-standing proliferative lesions have abundant virus particles.

Several serological tests to measure the humoral antibody response have been described but, as infection is prevalent in many sheep populations, it is difficult to relate the presence of antibody to the clinical manifestations. Antibody responses are generally poor to initial infection. A delayed-type hypersensitivity response has been recommended as a sensitive method for detecting animals that have been exposed to infection, but is unlikely to be adapted for practical management of the disease.

EPIDEMIOLOGY AND TRANSMISSION

Spread is through direct contact with infected animals or virus-contaminated fomites, and infection will establish only at sites where skin is traumatized. Thus, primary lesions are largely around the lips of sucking lambs, teats of suckling ewes, the poll and prepuce of rams, and coronet and lower limbs of those grazing areas where there is prickly vegetation. The lesions tend to remain localized at these predilection sites and only occasionally do they occur elsewhere.

Infection spreads rapidly in a flock, with most animals becoming affected within a few weeks. Outbreaks will last for 6–8 weeks, and generally do not reappear until there is a fresh crop of susceptible lambs. Survival of infection between outbreaks is assumed to be in the form of virus contained in scabs. However, while infectivity is retained in scabs that are dry for prolonged

periods, infectivity in wet material is rapidly lost [5]. Thus, virus in scabs exposed to the normal British weather conditions are unlikely to survive between epidemics, but infectivity will survive in scabs that remain dry in buildings. In addition, persistently infected animals showing no clinical disease have been described, and it is possible that such animals contribute to inter-epidemic survival of the virus [6]. The lesions on such persistently infected animals may not be readily detected. Thus, apparently normal animals may have trivial lesions that are capable of being a source of infection for other fully susceptible animals. All isolates of orf virus collected from a farm over an 18-year period appeared to be of the same strain on the basis of restriction enzyme analysis of the DNA, but isolates made in the nineteenth year were distinct. It is concluded that the same strain persisted on the farm for 18 years and was replaced by the introduction of a new strain in the nineteenth year of the study [7]. This finding would appear to confirm that orf can persist probably in the buildings and handling facilities between epidemics. It should be noted also that the scabs shed following vaccination contain fully virulent virus, which will contribute to the pool of infectivity. Re-infection some weeks after recovery from the initial infection is possible.

CONTROL

Various treatments have been tried but results generally have been disappointing. Emollient preparations containing broad-spectrum antibiotics may be useful in certain cases, e.g. on scabs or the lips of lambs. Antibiotics in aerosol form have been claimed to shorten the course of the disease by limiting secondary bacterial infections but, in larger flocks, it is unlikely that the results achieved would justify the labour involved. A number of proprietary treatments as well as homeopathic preparations are available. Although there are advocates for these treatments, the course of orf outbreaks is unpredictable and none has been shown to have any beneficial effect in experimental trials. In some outbreaks, it may be possible to identify predisposing factors, such as herbage, likely to traumatize legs or lips. Very wet conditions can predispose to severe lesions on the legs, and build-up of infection in housing through the lambing period can result in particularly high challenge and severity of disease. Steps taken to ameliorate these environmental factors are likely to be beneficial.

Control and prevention is by use of live vaccine applied to a site unlikely to produce chronic lesions, e.g. inside the thigh, behind the elbow or on the caudal fold. It must be emphasized that the virus is live and virulent, and should not be used in closed flocks in which the disease is not known to occur because of the risk of introducing the infection to the flock. All vaccines available in the UK are derived from scabs collected from experimentally scarified lambs. Such vaccines have a limited shelf life. A cell culture vaccine has been developed but is not yet licensed for use outside Australia [8]. The site of vaccination is the inside of the thigh except in ewes shortly before lambing, where persistent lesions could infect the udder. In this case, the recommended site is behind the elbow. Some also advocate this site in young lambs, so that they are less likely to transfer infection to the lips through sucking the site of vaccination. The caudal fold is not recommended because of the possible enhanced risk of secondary infection. It is difficult to assess the degree or duration of the immunity produced, as a number of variables are involved, the most important being the weight of the challenge.

The time chosen for vaccination should be governed by the expected timing of the clinical disease. Where the disease does recur annually, ewes should be vaccinated behind the elbow 6–8 weeks before lambing. The flock should be managed so that the scabs resulting from the vaccination are not shed in the lambing area, thus contributing to the pool of infectivity available to infect the lambs. Late summer outbreaks may be controlled by vaccinating lambs some 6 weeks before the expected time of the appearance of the disease.

To date, all effective vaccines have employed fully virulent virus. Future developments will depend on the isolation of mutant viruses, which lack certain genes of virulence but which retain immunogenicity. Such an approach is currently being pursued and is likely to depend on a detailed molecular understanding of the virus before specific candidate viruses will become available.

The venereal form of the disease does not commonly become endemic in a flock, and a routine examination of ewes and rams should be undertaken before they are mixed to ensure that no lesions are present in vulva or prepuce. The appearance of the disease in

the flock should, with careful shepherding, be seen early in the outbreak and an attempt made to limit its spread, as it may well be confined initially to one group of ewes and one or two rams. All rams that have had possible contact should be isolated, and the group of ewes carefully examined. Any showing lesions should be removed. The remainder should be isolated for 10 days then re-examined, and any showing lesions added to the infected group.

Clean rams may then be introduced to the clean ewe group. Where groups are large or mixing has occurred, this procedure is unlikely to be successful. During an outbreak of this form of the disease, vaccination is not recommended.

Isolation of affected sucking lambs and their dams reduces the weight of infection, but this is rarely worthwhile because of its rapid spread. Vaccination may be advisable and has been reported to arrest the spread in groups of young lambs. If vaccination during an outbreak is considered necessary, and commercial vaccine is not available, an autogenous vaccine can be prepared and used in the flock, subject to national regulations.

ZOONOTIC IMPLICATIONS

Orf is the most frequently diagnosed viral zoonosis in the UK and occurs mainly in those directly handling sheep, particularly when bottle-feeding lambs, shearing and slaughtering sheep. However, infection also can be transmitted by fomites and, sometimes, contact with sheep or goats is difficult to establish. In addition, parapoxvirus infection of humans may be due to infection from cattle (milker's nodule) or wildlife, particularly seals, in the UK.

Virus infection will establish following introduction through the epidermis, thus the fingers of those feeding lambs are particularly at risk. Following an incubation period of 3–7 days, the maculopustular reaction establishes, surrounded by an erythematous rim. This tends to increase in size, with a weeping surface and central vesiculation and pustulation. The lesion then crusts, overlying a papillomatous surface that is liable to be haemorrhagic if the crust is detached at this stage. The crust then dries and will detach after 6–8 weeks, leaving no scar. Normally only a single lesion is present and spread to other areas does not usually occur.

Secondary bacterial infection of orf lesions can cause complications, but can normally be controlled through antibiotic application. Lymphangitis and lymphadenitis of the draining lymphatics is a frequent complication and may be associated with flu-like symptoms. Less frequently, infection is associated with a generalized reaction, including widespread maculopapular eruption and erythema multiforme. Extensive lesions have been described in those with burns received at the time of infection and in immunosuppressed patients, which have resulted in the development of 'giant orf', resembling pyogenic granuloma and requiring amputation of the affected digit. Patients with atopic dermatitis may also be more vulnerable to infection. Diagnosis is often made on the basis of clinical presentation and history. Virus isolation is not generally successful, but characteristic parapoxvirus particles can be observed in scab or vesicular fluid collected early in the course of infection. The most convenient laboratory confirmation is amplification of viral DNA by PCR. Histological examination of biopsy material will differentiate orf infection from other proliferative skin disorders. Recovered patients will have antibody to orf virus, which is most readily detected by enzyme-linked immunosorbent assay.

Any immunity is of short duration and re-infection occurs readily. Normally, infection can be avoided through good hygienic practices and the wearing of protective gloves when handling infected animals or raw sheep products. Individuals who may be more vulnerable to infection, owing to immunosuppression or other factors, should avoid contact with sheep or goats. It should also be recognized that the vaccine used for protecting sheep is a fully virulent virus and operators must take great care not to self-inoculate while vaccinating.

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43

Sheep pox

R.P. Kitching

Synonmys: capripox, sheep and goat pox

Sheep pox is a malignant pox disease of sheep, characterized by fever, multiple non-vesicular swellings on the skin and mucous membranes, rhinitis, conjunctivitis, respiratory distress and death. The disease is present in Africa, north of the equator, Turkey, the Middle East, India, Nepal and parts of China. Sheep pox is caused by strains of *Capripoxvirus*, indistinguishable antigenically and genomically from strains that cause a clinically similar disease in goats, goat pox.

The distribution of sheep pox and goat pox is almost identical, although only one disease may be reported when the virus spreads into new areas, reflecting the host preference of individual strains of *Capripoxvirus*. In 1984, goat pox spread from India into Bangladesh [1], and there have been frequent incursions of sheep pox into Europe from Turkey: for example, into Italy in 1983, Greece in 1988, 1995, 1996, 1997 and 2000, and Bulgaria in 1995 and 1996, and more recently into Russia from the south of the country. Sheep pox is absent from North and South America, Australia, New Zealand, Japan and countries of South-east Asia other than Vietnam which recently experienced an outbreak of goat pox following importation of animals from China.

CAUSE

*Capripoxvirus*es are large, enveloped, double-stranded DNA viruses, forming a separate genus within the family Poxviridae. All strains of *Capripoxvirus*, including those that cause lumpy skin disease in cattle, will replicate in sheep. Depending on the strain of virus and breed of sheep, the response can vary from a barely discernible local lesion at the site of intradermal or subcutaneous injection to a large local reaction that spreads to other sites on the skin and internal organs, resulting in death. Some strains are equally pathogenic in sheep and goats, but most produce clinical disease in only one species. Individual strains can be identified only by the fragment pattern produced on agarose gel following treatment of the purified

DNA with a suitable restriction endonuclease [2]. Certain characteristic patterns have been recognized for sheep strains and goat strains [3], but some strains produce patterns intermediate between the two, and probably reflect the ability of strains of *Capripoxvirus* to recombine in the field. Inter-strain differences have been detected among small numbers of sheep and goat isolates from defined geographical regions [2, 4] but their wider significance has yet to be established.

Although very susceptible to sunlight, *Capripoxvirus*es can persist for many months in a cool, dark environment.

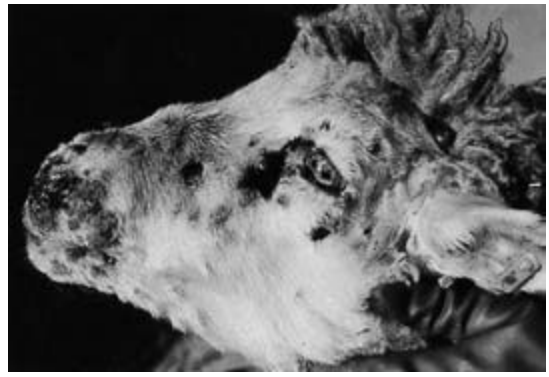

Figure 43.1: Sheep pox, showing lesions on face and crusty nasal discharge.

CLINICAL SIGNS

The incubation period of sheep pox following contact between an infected and susceptible animal is 8–13 days. Infection is usually by the aerosol route, but virus also can be spread mechanically by insect bite or experimentally by intradermal or subcutaneous injection, in which case a reaction can be seen at the site of infection within 4 days. The rectal temperature rises to 40°C or higher and, in the following 2–5 days, macules (1–3 cm diameter areas of hyperaemia) appear on the skin, particularly in the groin, axilla and perineum. Macules develop into hard swellings or papules, which rarely have a fluid-filled cap. Some particularly susceptible breeds of sheep, such as the Soay, die before the appearance of the characteristic clinical signs. The papules on the mucous membranes of the nose, eyes, mouth, mammary glands, vulva and prepuce quickly ulcerate, leading to rhinitis, conjunctivitis and blepharitis and a secondary mucopurulent discharge and mastitis (Figure 43.1). The superficial lymph nodes, in particular the prescapular nodes, become enlarged and pressure on the trachea from the enlarged retropharyngeal nodes can interfere with breathing. Lesions are not restricted to the skin. Ulcerating papules may be seen at post-mortem examination on the mucosa of the turbinates, hard and soft palate, trachea, oesophagus, abomasum and large intestine. Papules may be present also on the tongue, rumen, kidney cortex, liver and testicles. However, the large number of hard, pale sometimes haemorrhagic lesions found throughout the lobes of the lung are usually the most obvious and probably the most likely cause of death (see Figure 43.2 in the colour plate section). All the body lymph nodes are enlarged and may be haemorrhagic and contain microabscesses.

If the affected sheep survives the initial acute stage of the disease, between 5 and 10 days after their initial appearance, the papules form scabs, which persist on the skin for a further 2–3 weeks and then are shed, leaving scars, most obvious on the face. In tropical regions, secondary fly strike can result in permanent blindness or death. Recovery from severe disease is slow, and characterized by bouts of fever, pneumonia and anorexia. Abortion is rare unless there are secondary or additional infections.

PATHOLOGY

Infected cells are characterized by the presence of intracytoplasmic inclusion bodies, which are sites of virus replication. They are particularly apparent in histological sections of the skin and mucous membranes in glandular cells. The virus also replicates in macrophages, and can be isolated from the blood buffy coat during the viraemic stage of the disease. During the later stage, as the affected animal develops an immune response, the blood vessels supplying the papules thrombose, the papules become necrotic and the virions in the affected cells concentrate in a matrix of virus-induced protein within the cytoplasm. This material may protect the virions as the cells are shed into the environment and could provide a

future source of infection if inhaled by a susceptible animal.

DIAGNOSIS

The clinical signs of severe sheep pox are pathognomonic. Generalized contagious ecthyma or orf (a parapoxvirus infection) is rare. Confusion could occur between mild sheep pox and orf or even insect bites. The virus is concentrated in the skin lesions. A biopsy sample should be collected early in the course of the disease and a portion ground to make a 10 per cent suspension. This suspension is suitable to coat an electron microscopy grid for rapid diagnosis; the *Capripoxvirus* virion is approximately 270–290 nm, brick-shaped and covered in short tubular elements and, although it appears identical to *Orthopoxvirus* virions such as vaccinia, it is distinct from parapoxvirus virions, which are more oval in shape, and covered in a continuous tubular filament. For virus isolation, the supernatant of centrifuged suspension or blood buffy coat can be inoculated on to cultures of primary lamb testes or kidney cells. The remainder of the biopsy material should be placed in 10 per cent formalin for histology.

Field isolates of *Capripoxvirus* may require up to 12 days to produce a recognizable cytopathic effect on cell culture. If none is seen by day 14, a further passage should be made. Staining the culture or histological section with haematoxylin and eosin or a more specific inclusion body stain will, in positive cultures or sections, reveal eosinophilic intracytoplasmic inclusion bodies, variable in size and up to half the size of the nucleus, surrounded by a clear halo. Cultures may also be used for immunofluorescence or enzyme-linked immunosorbent assay. The agar gel immunodiffusion test has been used to detect *Capripoxvirus* antigen, but cross-reactions may be seen with parapoxvirus antigen.

At present there are no specific and sensitive serological tests for *Capripoxvirus* antibodies. The virus neutralization test lacks sensitivity, particularly using sera from mildly affected or vaccinated sheep, while the agar gel test cross-reacts with antibody to parapoxvirus. Research to improve the serological test using the plasmid-expressed P32 *Capripoxvirus* antigen is in progress [5]. All the available diagnostic tests for *Capripoxvirus* antigen and antibody are described in detail in the Office International des Epizooties (OIE) *Manual of Standards for Diagnostic Tests and Vaccines* [6].

EPIDEMIOLOGY AND TRANSMISSION

Transmission of sheep pox is usually by aerosol following close contact between a susceptible and clinically affected animal. Animals that die with acute disease before showing typical clinical signs and animals with only very mild, localized infections rarely transmit disease. The epidemiology is very similar to that of human smallpox and, like smallpox, most transmission occurs from severely affected individuals during the stage when ulcerated papules are present on the mucous membranes to the start of papule necrosis, when the antibody response can be detected. There is no transmission during the 'prepapular' stage, and the infectivity of the virions present in the scab material is not known. Although biting insects have been shown to transmit *Capripoxvirus* under experimental conditions, there is no evidence that they are significant in the epidemiology of sheep pox [7]. Occasionally, in the field, sheep pox can take up to 3 months to infect a group of 12 animals, but can infect a complete flock of 200 in less than a month, reflecting the many additional factors, other than the strain of *Capripoxvirus,* that influence transmission and virulence; factors such as breed of sheep, housing, humidity, feeding abrasive fodder, presence of other diseases that affect mucous membrane integrity, such as orf, peste des petits ruminants, foot-and-mouth disease.

The epidemiology of sheep pox in endemic regions is characterized by a range of scenarios, all of which suggest that the disease requires a certain minimum size population in order to persist. In countries such as the Yemen Arab Republic, sheep pox is always present on the low coastal plain, and in areas of the central plateau where sheep numbers are high, but is usually absent from the isolated mountain villages [8]. In the densely sheep-populated areas, disease is therefore seen in the young animals as they lose their maternal antibody and contact the virus, whereas sheep pox can be seen in all age groups in the isolated villages, usually associated with the introduction of new animals from a lowland market. Recovery

from sheep pox is complete, immunity following infection is effectively lifelong and no carrier stage is recognized, so that the virus must constantly pass from an infected to a susceptible animal to survive. A similar situation can be seen in the markets outside Khartoum, in Sudan, where animals are collected together from villages across the country. Here also, sheep pox can be seen in animals of all ages, as many of them have their first contact with *Capripoxvirus*, even though they have always lived in a sheep pox endemic country.

Although it is always possible to find individual animals with typical generalized sheep pox in endemic areas, the local breeds do appear to have some genetic resistance and, in uncomplicated cases, death is rare. However, when these breeds are gathered into feedlots in attempts to increase their productivity, the additional 'stress' appears to overcome their innate protection, or possibly it is the combination with additional infections acquired during mixing that makes them more susceptible, and mortality can be over 50 per cent. Similarly, when European or Australian breeds are imported into endemic regions without protection from contact with local animals, morbidity and mortality can approach 100 per cent.

The involvement of goats in the epidemiology of sheep pox has been controversial. Certainly, there are strains of *Capripoxvirus* that can transmit between the two species and cause disease in both. However, the majority of strains show a host preference and where investigations have taken place, such as during the 1985 outbreak in Cyprus, only one species was affected, with no evidence of involvement of the other. Similarly, a recent outbreak in Greece and Bulgaria only affected sheep and, although not adequately examined, there was no evidence for transmission to goats or their association with persistence of the disease.

TREATMENT, PREVENTION AND CONTROL

There is no effective treatment other than supportive therapy with antibiotics and fly control. Prevention in countries free of sheep pox is directed towards prohibiting the importation of live sheep or goats or their products from pox-endemic regions [9]. Prevention in endemic areas relies heavily on vaccination; however, countries such as Australia, which are free of disease but which export to sheep pox-infected areas, do not allow the use of live attenuated vaccines on their territory, for fear of losing their disease-free status. Vaccines against sheep pox, like all other pox vaccines are live. Inactivated vaccines, at best, provide only short-term immunity. Immunity is predominantly cell-mediated, and the humoral response appears to be of secondary importance, a consequence of which is that a replicating antigen provides a more effective immunogen. In addition, the *Capripoxvirus* exists in two forms, one in which the virus is surrounded by host cell membrane, as occurs when the virus is naturally released from an infected cell, and a second form in which this membrane is absent, as is seen in virions released by freeze–thawing an infected cell culture. These two forms have different surface antigens, and therefore sheep vaccinated with an inactivated preparation produced by freeze–thawing cell cultures would have no resistance to aerosol challenge from an infected sheep producing membrane-bound virions.

Several strains have been used as attenuated vaccines, for example the 0240 Kenya sheep and goat pox strain used in sheep and goats, the Romanian and RM-65 strains used in sheep and in cattle, and the Mysore and Gorgan strains used in goats [6]. Most of these are interchangeable between species, providing protection against all *Capripoxvirus* infections. However, the 0240 should not be used in cattle and, as a general rule, any new vaccine strain should be tested first on local target animals in various stages of production to ensure that the strain is fully attenuated.

Protection against disease is effectively lifelong following vaccination with the 0240 strain, although, experimentally, virulent challenge virus will replicate at the site of subcutaneous inoculation in an animal vaccinated 2 years previously. Immunity is sufficient to prevent any generalization. The vaccine is very cheap to produce, and in control programmes should be used annually in all sheep and goats, regardless of age. Young animals may be protected for up to 6 months by maternal antibody, depending on the immune status of their mothers; but all young animals should be included in the vaccination campaign; those without maternal antibody will respond and be protected, while those with maternal antibody will not develop any active immunity but can be vaccinated and protected the following year. Also, older animals missed in one annual round will probably be included in the next. The object of any vaccination campaign must be to reduce

the number of susceptible animals below that required to maintain the virus. Movement restrictions and quarantine will provide additional control by preventing the only susceptible animals – those young animals losing their maternal protection and not covered by vaccination – from contacting any remaining infected animals.

There is reluctance to use vaccine to eradicate outbreaks of sheep pox in areas or countries previously free of the disease, as this interferes with trading status for a period of time, whereas control by slaughter of affected animals and movement restrictions quickly restores disease-free designation. Movement control is essential, as the long incubation period allows sheep pox to remain hidden while animals are moved, even though the very obvious clinical signs should make rapid identification by the veterinary authorities automatic. There is an urgent need for a sensitive and specific serological test to identify those animals that have had only mild or subclinical infection, in order to provide evidence of freedom from infection, for disease control and trading purposes. Ideally, such a test would also distinguish animals that had been vaccinated from those that had recovered from infection.

ZOONOTIC IMPLICATIONS

Although there have been two reports in the literature that *Capripoxvirus* can transmit to humans, these can be discounted. *Capripoxvirus* does not infect people.

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44

Caseous lymphadenitis

G.J. Baird

Synonyms: pseudotuberculosis, maladie caséeuse, verkazende lymphadenitis, CLA, CA, 'cheesy gland'

To the veterinary pathologist *Corynebacterium pseudotuberculosis*, the causative organism of caseous lymphadenitis (CLA), has a valid claim to being the perfect parasite. Once established in the body the bacterium can evade the host immune system with apparent ease and remain there for the rest of an animal's life. If left unchecked, the disease is contagious

enough to infect the majority of sheep within a flock, yet rarely causes enough harm to kill an animal. Even away from its ovine host the pathogen is well equipped for long-term survival in the environment. The consequence of this for the veterinary clinician is that CLA is a disease that in most instances must be managed rather than eradicated.

The global range of *C. pseudotuberculosis* includes much of Europe, Australasia, North and South America, Africa and the Middle East. In many of these regions caseous lymphadenitis has been an established and problematic infection of sheep for many decades [1]. It has been suggested that the origins of the infection may lie in Europe and that its spread around the world followed the exportation of livestock, especially Merino sheep, by the eighteenth-century colonial powers. CLA became a politically significant issue in the 1920s when large numbers of mutton carcasses imported into Britain were found at meat inspection to be badly affected by the disease. This led to efforts by several exporting countries to control the previously neglected disease.

CAUSE

The *Corynebacteriaceae* are now considered to belong to the actinomycete family and *C. pseudotuberculosis* shares many of the classic features of its genus. It is non-motile and generally appears on direct smears as a short rod of some 0.5–0.6 μm by 1.0–3.0 μm – although a large degree of pleomorphism is possible. The organism has a tendency to stain Gram-positive and groups of the bacteria are also inclined to form a characteristic palisade or 'Chinese letter' arrangement on smears.

At a temperature of 37°C *C. pseudotuberculosis* will grow under both aerobic and anaerobic conditions. On solid media the bacterial colonies are pale grey, dry and friable in consistency, and may be moved freely over the surface with the point of a probe. Visible growth tends to be limited within the first 24 hours of incubation, with colonies becoming fully defined by 48 hours and reaching a maximum diameter of some 3 mm after several days. Bacterial growth benefits from the addition of serum or whole blood to the nutrient media. When whole blood is used, a narrow band of red cell haemolysis is evident in the agar around each colony, although this may not appear until 48–72 hours of incubation.

CLINICAL SIGNS

C. pseudotuberculosis infections are classically associated with the formation of pyogranulomas [2]. These lesions present in two main forms, which may co-exist within the same animal. The external form of the disease, also known as superficial or cutaneous, is characterized by abscessation of the palpable lymph nodes of the body (see Figure 44.1 in the colour plate section). Occasionally, external lesions may appear as subcutaneous abscesses, not directly associated with lymph nodes. However, this type of lesion is uncommon, even in heavily infected flocks.

The visceral form of the disease is associated with the development of abscesses within the internal lymph nodes and other organs, their principal location being the lung parenchyma.

Mastitis due to *C. pseudotuberculosis* is encountered occasionally and is most likely to represent an extension of infection from the adjacent supramammary lymph node. This may take the form of an acute suppurative mastitis, or appear as chronic encapsulated abscesses within the mammary gland. Lesions in other organs such as liver, kidneys and scrotum also tend to be encapsulated abscesses containing a thick caseous material [3].

PATHOLOGY

No naturally occurring avirulent strain of *C. pseudotuberculosis* has been described, yet the mechanism of the organism's pathogenicity remains poorly understood. To date, research has focused on two recognized virulence factors identified as phospholipase D (PLD) and mycolic acids. Isolates of *C. pseudotuberculosis* in which the gene for PLD has been deleted are incapable of causing the classical lymph node abscesses of CLA in sheep. Similarly, the presence within an animal of specific antibody to PLD has been shown to greatly limit the progress of the clinical disease. Although PLD possesses a number of potentially significant biological activities, its ability to increase the permeability of vascular endothelial membranes

has received most interest. This causes leakage of plasma from blood vessels into the surrounding tissues and thence into the lymphatic drainage. This effect may play an essential part in the early stages of infection, allowing *C. pseudotuberculosis* to be carried by escaping tissue fluids, away from the site of inoculation and towards the local drainage lymph node. Other significant actions have been proposed in which PLD may protect the bacterium from destruction by opsonization and phagocytosis by white cells.

In liquid media and aqueous solution *C. pseudotuberculosis* has a tendency to form clumps. This has been related to the fact that the organism does not possess a capsule, but instead carries a waxy lipid coat of mycolic acids external to the cell wall. The waxy coat has cytotoxic properties and provides the organism with mechanical and possibly biochemical protection from hydrolytic enzymes within lysosomes. In turn, this enables the bacterium to survive phagocytosis and to exist in the host as a facultative intracellular parasite, a capacity almost certainly essential in the organism's migration from the point of entry to the eventual site of the lesions.

Numerous routes of entry have been used to induce experimental caseous lymphadenitis infections. However, in natural infections the predominant route of entry is believed to be through breaks in the skin. Entry via the oral cavity has been suggested, as has the umbilicus in neonates. In contrast, the more distal parts of the intestinal tract are not believed to provide a portal of entry for the organism, even in the presence of parasitic damage. It would also appear that entry of infection via the respiratory tract, although a theoretical risk, is of negligible importance.

The rarity of simple subcutaneous CLA lesions suggests that once the organism has gained entry through the skin it is not well suited to establishing persistent infections there, but must progress to the local drainage lymph node and beyond if infection is to be sustained. Spread of infection to the local drainage lymph node occurs rapidly following inoculation and, in a proportion of cases, is followed by a further extension of infection via the blood or the lymphatic system, leading to the development of lesions within other organs. Pulmonary lesions most commonly take the form of encapsulated abscesses (see Figure 44.2 in the colour plate section) similar to those seen in the lymph nodes, although more extensive bronchopneumonia is also recorded. The generally random distribution of lesions within the lung is consistent with haematogenous or lymphogenous spread. In animals with pulmonary abscesses, CLA lesions are occasionally encountered in the associated mediastinal and bronchial lymph nodes (see Figure 44.3 in the colour plate section), implying an additional step in the transit of the organism from the lung parenchyma.

The nature of the lesions in CLA means that chronic disease is the rule rather than the exception. Thus, viable bacteria may be found within abscesses several years after initial infection. Reactivation of disease is also encountered, such that lesions may develop at new sites after a period of apparent remission.

Once colonized by the organism, a lymph node undergoes a short period of generalized inflammation, with PLD the likely initiator of this lymphadenitis. Within as little as 24 hours a number of micro-abscesses appear, subsequently becoming more numerous, expanding and coalescing to produce larger purulent foci encapsulated by connective tissue. In the early stages of the lesion the pus is soft and semi-fluid with a greenish tinge but, over time, takes on a more plastic or solid form. As the lesion expands the capsule undergoes a repeated process of necrosis and reformation. Small nodules of calcification form within the purulent material, which becomes much paler as a result. These calcified foci may be laid down in concentric layers to give the lesions a lamellated ('onion ring') appearance (see Figure 44.4 in the colour plate section). Single lymph node abscesses may reach a diameter of 15 cm, although 3–5 cm is more common. Lesions within other organs and tissues typically have a similar gross appearance to those found within the lymph node and it seems likely that their formation follows a similar pattern.

In many parts of the world, including North America, South Africa, Australia and New Zealand, the external form of CLA in sheep most commonly affects the superficial lymph nodes of the torso, principally the prescapular and prefemoral [1]. The implication of this is that the initial point of entry for the pathogen is in the areas that drain to those particular nodes – in other words the skin of the torso. In contrast, as CLA has emerged in the UK the principal lymph nodes to be affected have been those of the head and neck – the parotid, submandibular and retropharyngeal, with other superficial nodes less commonly affected [4]. Once again, the implication seems to be that the pathogen is gaining initial entry to the animal through the skin of the head or ears, via the oral cavity, or perhaps by way of the upper respiratory

tract – leading to lesions in the lymph nodes draining those structures.

DIAGNOSIS

The diagnosis of CLA is based on the identification of *C. pseudotuberculosis* following bacterial culture of the purulent contents of lesions. In sheep, the presence of abscesses within external lymph nodes is highly suggestive of the condition, particularly if several animals are similarly affected. Although other bacterial pathogens such as *Actinobacillus licheniformis*, *Arcanobacterium pyogenes* and, in some countries, *Staphylococcus aureus* subsp. *anaerobius* are capable of producing a suppurative lymphadenopathy, these infections tend to be more sporadic in nature and are rarely seen as flock outbreaks.

While isolation of the organism remains the gold standard, there are frequently occasions when this may not be advantageous or indeed possible. The puncture of CLA abscesses will release pus on to the animal's skin or into its environment, presenting a risk of transmission of infection to other animals. Chronic external lesions that have ruptured, frequently become fibrosed, and may contain little pus and few viable organisms. In contrast, animals affected by a purely internal form of the disease show no visible lesions that may be sampled, but remain a potential source of infection to others. Much research interest has therefore focused on serological tests that offer an opportunity to identify animals subclinically affected by CLA without recourse to bacterial culture. Several assays have been developed for this task, most of which are based on the detection of a humoral response to PLD exotoxin. It is the enzyme-linked immunosorbent assays (ELISAs), first developed in the early 1980s, which have come closest to providing levels of sensitivity and specificity required of a diagnostic test [5].

EPIDEMIOLOGY AND TRANSMISSION

The predominant means by which CLA enters a naïve flock is through the introduction to the group of an infected carrier animal. However, anecdotal reports from a number of countries suggest that on rare occasions outbreaks occur in previously unaffected closed flocks some weeks after the visit of a contract shearer – the suggestion being that the organism was carried on to the premises on the shearer's kit.

Much research effort has focused on how *C. pseudotuberculosis* organisms, very often contained within thick-walled abscesses, might be transferred from one animal to another. Very large numbers of bacteria are present within the purulent discharge from a CLA lesion, with estimates of between 1×10^6 and 5×10^7 viable organisms per gram of pus. The rupture of superficial abscesses can therefore contaminate the adjacent skin, fleece and immediate environment with huge numbers of bacteria. Other animals may then be exposed to infection during social interaction, feeding and other behaviours, all accentuated by the ability of the organism to survive within the environment.

In Australia, where most transmission studies have been conducted, the process of shearing is regarded as the principal predisposing factor in the initiation of CLA infections. The spread of infection at this time is also known to take place in the absence of any discharging superficial lesions. Sheep with pulmonary CLA lesions have been implicated as the predominant source of infection in such circumstances, based upon epidemiological observations and on the isolation of the organism from the airways of sheep with lung abscesses. The assumption is that a small number of animals with pulmonary lesions can infect many others by an aerosol route – accentuated by conditions of close contact and the skin abrasions frequently caused during shearing [2]. This shearing-associated skin damage is believed to provide the most common route of access for the organism to the subcutaneous tissues and thence to the lymphatic drainage. In addition to aerosol contamination, if superficial lesions are damaged pus may be transferred mechanically from animal to animal by the shears. The organism is also known to survive in certain dip solutions for up to 24 hours and contaminated plunge or recycling shower dips may be a source of infection, particularly for recently sheared sheep.

As already noted, the location of CLA lesions in sheep in the UK tends to be quite different from the clinical presentation observed in other parts of the world. It has also been widely reported that prevalence of disease amongst rams is often greater than among ewes [6]. As a consequence, the accepted model in

which shearing plays the central role in initiating new infection is unlikely to apply. It is more likely, therefore, that other factors such as housing, methods of feeding and the management of rams play a part in the epidemiology of the disease. Risk factors such as fighting injuries, ear tagging and consumption of contaminated feed have all been suggested.

Global prevalence and economic significance

The concern with which CLA is regarded is markedly different around the world. In the sheep industries of the southern hemisphere the infection is both widespread and highly prevalent. A general familiarity with the condition in the farming and meat industries means that the condition causes no more than moderate concern. However, in countries such as The Netherlands, the UK and Ireland, the emergence of disease in recent years has produced much greater alarm – at the very obvious lesions seen in sheep and at the relentless progress of which the infection is capable.

Farm- and abattoir-based research aimed at establishing disease prevalence rates has been conducted in several countries in recent years. In large parts of Australia most flocks are infected with CLA. Prevalence rates of over 50 per cent among adult sheep have been recorded in some regions, although programmes of vaccination have led to a steady decrease in rates in recent years. In the western USA average disease prevalence amongst adult ewes has been estimated to be more than 40 per cent, while similar studies conducted in Canada have found the prevalence of clinical CLA to range from 21 to 36 per cent amongst culled adult sheep. Published prevalence data do not exist in most European countries, although a serologically based study conducted in Britain suggested that as many as 18 per cent of flocks in the terminal sire sector showed evidence of infection [6].

CLA is recognized as a significant cause of financial loss to the sheep industries of a number of countries. Most of this economic loss relates to condemnation or trimming of affected carcasses at meat-inspection, with CLA the leading single cause of such losses globally.

Systemic infection by *C. pseudotuberculosis* is acknowledged to have some negative effect on the production of the infected animal, although there is no clear consensus on its overall significance. Work in Australia has established a detrimental effect on wool production, but no evidence of a similar effect on live weight gain. In contrast, in North America the consequences of infection are considered to be more significant. There, the visceral form of infection with *C. pseudotuberculosis* has been associated with the so-called 'thin-ewe syndrome' described as chronic emaciation, despite good appetite and the absence of parasites or specific clinical signs – although the published data on the syndrome are limited. In the UK, sporadic cases of a similar chronic ill-thrift associated with severe systemic CLA have been reported.

TREATMENT, PREVENTION AND CONTROL

In the laboratory *C. pseudotuberculosis* is sensitive to a range of antibiotics, but clinical cases of CLA are generally refractory to antibiotic therapy. This is assumed to be a consequence of the thick encapsulation of lesions and the similarly thick and caseous nature of the contained pus. The intracellular nature of the organism during parts of the disease process is also believed to confer protection from the effects of antibiotics. Surgical treatment of external lesions has been suggested as an alternative to culling in the case of particularly valuable animals. Whether the lesion is removed surgically or simply lanced and flushed out daily until healed, a 4–6-week course of parenteral antibiotic has been recommended to reduce the likelihood of recurrence. However, this course of action is unreliable, since it depends upon the antibiotic removing all pathogens from the treated lesions and assumes that no internal lesions are present.

Its lipid coat renders the organism relatively resistant to destruction within the farm environment, where it may remain viable for many weeks. Although most common disinfectant preparations such as calcium hypochlorite, formalin and cresol solutions are effective in killing the organism, the presence of organic material extends the exposure time required to produce a bacteriocidal effect.

In much of the world, measures to control CLA in sheep are based on the use of vaccines. These fall into three categories. Autogenous vaccines, consisting of washed and formalin-killed bacteria, are the sole products currently licensed for use in the UK. Elsewhere, they have been largely replaced by vaccines

based on a toxoid of concentrated, purified and formalin-inactivated PLD. The regular use of such vaccines within an infected flock has the potential to decrease CLA prevalence by 60–80 per cent within 5–6 years. Finally, in some countries, a third category of vaccine is available which contains PLD toxoid combined with whole killed bacterial cells.

Disease eradication and control through the use of serology has received considerable attention in The Netherlands, principally in the dairy goat sector in which a successful CLA Health Scheme now exists based on the use of an ELISA test [7]. An equivalent scheme, proposed for the control and eradication of CLA in the Dutch sheep sector, foundered because of poor test sensitivity in certain flocks. An ELISA developed in the UK is now used in a voluntary ram monitoring scheme.

ZOONOTIC IMPLICATIONS

Reports of human infection with *C. pseudotuberculosis* are relatively few, with most recorded in workers regularly exposed to sheep, such as shepherds, shearers, abattoir workers and butchers [8]. Human cases tend to have a chronic course and present as a localized suppurative granulomatous lymphadenitis, often associated with a period of flu-like symptoms and increasing lethargy. Treatment with systemic antibiotics is generally unrewarding and the majority of cases are resolved only after surgical excision of the affected lymph node. It has been noted that lymph node abscesses may not always be cultured following excision from human subjects. This, coupled to the fact that axillary lymphadenitis is not uncommon in shearers, has led to speculation that human infections may be under-reported in some countries.

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45

Staphylococcal skin infections

P.E. McNeil

CAUSE

Staphylococci are spherical, resilient bacteria that are able to grow with or without the presence of oxygen (i.e. under aerobic and anaerobic conditions). They inhabit the skin and mucous membranes of animals and humans, and have been recovered from the skin, nostrils, mouths and vaginas of sheep; lambs can acquire staphylococci within the first few days of life.

The pathogenicity of these common organisms depends on a battery of virulence factors including surface proteins that enable tissue colonization, enzymes that facilitate tissue invasion and surface factors and biochemical properties that inhibit engulfment of the organisms by phagocytic cells and enhance their intracellular survival if phagocytosis occurs. The nature of the organisms is such that they provoke a marked inflammatory cell (neutrophil) response and resultant skin lesions are generally characterized by the presence of pus (pyoderma).

A number of species and subspecies of staphylococci have been classified. Coagulase-producing strains are considered to be more pathogenic than coagulase-negative staphylococci, although the latter are regarded as potential opportunistic pathogens. Pathogenic strains are carried by a proportion of healthy individuals. For example, a study in French farms revealed nasal carriage of *Staphylococcus aureus* in 29 per cent of dairy ewes [1]. Such organisms are a potential source of infection for skin and other organs, milk and meat. The development of skin disease is believed to depend on disturbance of the normal balance between bacterial virulence factors and host defences, e.g. by alteration in the local microenvironment and/or physical breach of the skin surface. In most cases, staphylococcal skin disease is localized to the area around the point of entry of the organisms but potentially fatal bacteraemia, septicaemia or toxaemia may develop if the bacteria and/or their toxins enter the blood stream.

Several manifestations of staphylococcal skin disease have been described in sheep and associated with *S. aureus*, either alone or in combination with other organisms. Many cases are mild and of little clinical significance but severe disease occurs occasionally.

The various clinical forms of staphylococcal skin infections are described below.

Periorbital eczema

Synonyms: eye scab, facial dermatitis, necrotic ulcerative dermatitis, staphylococcal dermatitis

First described over 30 years ago, this condition is a severe, ulcerative and necrotizing, dermatitis affecting adult sheep (not to be confused with photosensitization in cattle, sheep and goats reported as facial eczema in Australia, New Zealand and South Africa). In a detailed study [2], the causative agents of periorbital eczema were identified as 'dermopathogenic' strains of *S. aureus* that produce alpha, beta and gamma haemolysins as well as coagulase and other virulence factors. The disease could be reproduced by inoculation of large numbers of the organisms into damaged skin and did not require the presence of orf virus infection for the development of the disease.

Typically, the condition is found in late winter or spring and has been linked to skin trauma during close contact, e.g. during trough feeding. It appears to be contagious with up to 50 per cent of a flock being affected. Affected individuals can present an alarming appearance with a bloody discharge on the face and adhering to the wool (see Figure 45.1 in the colour plate section). Close inspection reveals localized areas of deep ulceration and necrosis that discharge

blood and pus. Surrounding tissues are swollen, inflamed and devoid of hair. Areas of skin most likely to be affected are over bony prominences of the face, especially the orbital rim, base of horns, ears and nasal and maxillary bones. As healing progresses, scabs form over the ulcers. Repair by granulation tissue takes place over 3–4 weeks in individual sheep. Further trauma will dislodge scabs resulting in further bleeding and slowing of the healing process. In a flock, the course of the disease may extend to several months.

Similar facial lesions have been reported in lambs with concurrent contagious pustular dermatitis (orf) virus, *S. aureus* and *Arcanobacterium pyogenes* infections [3]. *S. aureus* was considered to have played a major role in the pathogenesis of the disease. It was postulated that trauma of the lips and nose by thistles might have contributed to the spread of infection to those sites.

Trauma by thistles has also been implicated in the development of staphylococcal dermatitis on the legs of sheep [4].

Ulcerative staphylococcal dermatitis affecting the teats and udder of housed lactating ewes has been linked to trauma by sucking lambs and/or the barley straw used for bedding [5]. In that outbreak, more than two thirds of the sheep in one pen had dermatitis and 31 per cent (11/35) of the affected individuals (4.3 per cent of the flock) developed staphylococcal mastitis.

Folliculitis

Folliculitis is inflammation of the intact hair/wool follicle. Coagulase-positive staphylococci have been isolated from small pustular lesions (plooks) on the skin of the muzzle or beneath the tail of lambs. Groups of transient lesions may arise and disappear over a period of weeks [6].

Rarely, more severe infections with rupture of the hair follicle (furunculosis) have been recorded; a predisposing factor in the development of severe dermal lesions was thought to be self-trauma due to the pruritus associated with psoroptic mange [7].

Impetigo

Impetigo is inflammation of the interfollicular skin with development of intra-epidermal pustules. These superficial lesions are generally mild and of little clinical consequence unless mastitis ensues [6]. Udder impetigo in sheep has been linked to folliculitis in the lambs and is thought to be due to cross-infection and contamination of superficial abrasions.

Scalded skin syndrome

Staphylococcal scalded skin syndrome (SSSS) is a blistering, exfoliative, skin disorder that affects young children and immunocompromised adults. It is caused by strains of *S. aureus* that produce exfoliative exotoxins. Haematogenous spread of the toxin in vulnerable individuals (i.e. those lacking specific antitoxins) can lead to severe, potentially fatal, disease. This disorder is rarely recorded in animals (although exudative epidermitis due to *S. hyicus* in pigs is somewhat similar). A generalized exfoliative disease in association with acute renal failure has been described in lambs [8] and a pure culture of *S. aureus* was isolated from the lesions.

PATHOLOGY

Staphylococci are thought to gain entry to the skin via microabrasions or larger areas of trauma. Persistence and extension of infection and the development of disease then depend on the presence of virulence factors and the production of exotoxins. Haemolysins and other enzymes appear to contribute to the development of ulcerative and necrotic lesions. Serine proteases are responsible for the severe blistering and exfoliation of SSSS but may also be involved in milder lesions of epithelial cell separation, e.g. in impetigo. The target of these exotoxins has recently been confirmed as one of the intercellular adhesion molecules of the epidermis, desmoglein-1 [9].

The gross appearance of staphylococcal skin lesions ranges from small, superficial pustules that do not involve the hair follicles (impetigo) through follicular pustules (folliculitis) and dermal nodules (furunculosis) to deeply excavating ulcerative and necrotic lesions (cellulitis). Occasionally, discrete abscesses are also attributed to staphylococci, e.g. *S. aureus* ssp. *anaerobius* [10].

Histologically, lesions are characterized by infiltration of neutrophils and by some degree of tissue

destruction and accumulation of cell debris (pus formation). Bacterial cocci may sometimes be demonstrated within these areas of suppurative inflammation by Gram's stain.

DIAGNOSIS

Diagnosis depends on isolation of causative organisms from suitable samples; ideally, this should be by aspiration from intact pustules or nodules or by surgical excision of deep tissue. Swabs taken from the surface of exudative or open lesions often yield a mixture of organisms that is difficult or impossible to interpret.

EPIDEMIOLOGY AND TRANSMISSION

By itself, the presence of staphylococci on healthy skin is unlikely to be harmful. However, as indicated previously, disruption of the local microenvironment or skin trauma can trigger development of disease. In periorbital eczema, close head contact, e.g. in communal trough-feeding, is a predisposing factor. Facial and head lesions and their associated discharges are ready sources of infection for contact sheep and, over time, up to 50 per cent of the flock may be affected. Healing can take place over several weeks but, experimentally, re-infection can occur after 40 days. The disease has been known to affect some flocks in consecutive years.

Local trauma by thistles and by sharp bedding straw have been implicated in initiation of staphylococcal infection of the legs and mammary glands respectively.

TREATMENT, PREVENTION AND CONTROL

Little or no treatment is required for lesions of impetigo and superficial folliculitis. Mild topical cleansing (and drying) of the skin and cleaning of the environment may aid control by reducing the bacterial load and minimizing direct and indirect transmission of staphylococci.

Deep lesions of furunculosis and necrotizing dermatitis generally respond well to injections of procaine penicillin although the presence of penicillin-resistant strains is increasingly recognized and thus potentiated penicillins or other antimicrobials may sometimes be required. However, staphylococcal strains exhibiting antimicrobial multiresistance are much less common in animal isolates than in clinical human isolates [11].

Prevention of severe staphylococcal dermatitis depends on minimizing predisposing factors, e.g. by providing adequate trough space for sheep at feeding times and by eliminating other potential causes of skin trauma where possible. Elimination of carriage of pathogenic or potentially pathogenic staphylococci is likely to prove impracticable.

ZOONOTIC IMPLICATIONS

S. aureus is a significant human pathogen, causing disease by a variety of mechanisms and exhibiting increasing antimicrobial resistance. Direct transmission of pathogenic strains from animals to man (and vice versa) is possible but seems to be less of a hazard than either endogenous infection or spread of human-derived strains from one person to another. The presence of toxin-producing staphylococci in milk, milk products and meat is a potential cause of human food poisoning. Staphylococcal skin infections, as well as nasal carriage by animals and humans, are possible sources of such contamination. Attention to personal hygiene, wearing of protective gloves (and masks) and hand-washing are sensible precautions.

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46

Bacterial and fungal infections of the skin and wool

J. Plant

In recent years, skin diseases of sheep have become more widely recognized. This coincides with the removal of regulatory controls on sheep lice and sheep scab. Sheep producers are now seeking advice on the cause and control of fleece derangement and skin diseases in their flocks.

DERMATOPHILOSIS

Synonyms: mycotic dermatitis, lumpy wool, cakey wool.

This bacterial infection of the skin is due to *Dermatophilus congolensis*. It affects woolly and non-woolly areas and sheep of any age. It also affects other animals, including pigs and cattle and is transmissible to humans.

Dermatophilosis is economically important to the sheep industry, not only because of damage to the skin and to the fleece, but also because it is a major pre-disposing cause of fly strike, especially in younger sheep.

Cause

The disease is caused by a filamentous bacterium, *D. congolensis*, which attacks the epidermis of the skin. Short, Gram-positive branching filaments and clusters of cocci may be seen in abundance in smears of lesions.

Clinical signs

In the early stages of the disease, a serous exudate produced by the skin infection at the base of the wool fibres results in matting of the fibres and formation of hard, thick, dry crusts in the staple. In chronic infections, as the wool fibre grows out, the crusty exudates persist, forming hard dry 'pegs' in the staple. These may not be obvious until the wool is handled (see Figure 46.1 in the colour plate section).

In persistent or chronic infections, the lesion may extend along the staple towards the tip. In cases of self-cure, the crusty exudate separates from the skin and there is a band of normal wool beneath the crusts. In some sheep, a carrier state develops with small crusty lesions up to 0.5 cm in diameter occurring on the skin of the face, nose and ears.

Lambs in the first few weeks of life are very susceptible to infection. Lesions are more common over the muzzle and ears, probably due to close contact with the ewe at feeding (see Figure 46.2 in the colour plate section). Some lambs can develop a generalized infection affecting the skin over the back and sides. The serous exudates form a crust over the skin, making it very painful if the lamb attempts to move. Death usually follows as a result of secondary bacterial infections or from fly strike [1].

Strawberry foot-rot is also caused by *Dermatophilus* infection. This is characterized by dry crusts on the skin above the coronet. Removal of the crust exposes a proliferative granulomatous lesion. In susceptible flocks, it may be also be associated with contagious pustular dermatitis (orf) infections, affecting the pasterns or hock.

Pathogenesis

When exposed to moisture, the coccoid forms in scabs develop flagella and become highly motile zoospores in the moist environment. These zoospores are attracted towards the skin. Infection is more likely where there has been prior damage to the lipid zone, e.g. following heavy rain, dipping, shearing or bacterial infections such as fleece rot. Under suitable conditions, the zoospores invade the epidermis and wool follicles, developing hyphal filaments. The proliferation of the hyphal forms results in a severe inflammatory response with serous exudation and cornification of the dermis. There is matting of the wool fibres and separation of scab material from the skin. In chronic infections, especially where there is frequent wetting of the skin, the continued re-infection of the new epidermis results in a thick, laminated scab composed of alternate layers of cornified epidermis and exudate. These grow out with the wool staple.

Subsequent wetting of sheep that have not developed a good immunity can result in re-infection of the skin beneath existing scabs or the transmission of infection to other parts of the body.

Predisposing factors

The coccoid form can persist in dry scabs for several years, but the zoospore is viable for only a few days. In the carrier animal, scabs can also act as a continuing source of infection in the flock.

Outbreaks are associated with the presence of infected sheep, a wetting event that allows the coccoid forms to develop into the highly motile zoospores, and contact between infected and susceptible sheep. Susceptible sheep include animals of any age not previously exposed to infection. Adult sheep introduced from drier areas into an infected flock in a wetter climate are just as susceptible as younger sheep in the same flock. Young lambs in the first few weeks of life are particularly susceptible to infection. Infection is commonly spread when sheep are put through plunge or shower dips. Not only is the fleece wet, but the dip wash allows for the transmission of infection between sheep. There is also likely to be some damage to the protective lipid layers of the skin, allowing the motile zoospores to set up the infection. Severe outbreaks in lambs have been seen when ewes and lambs have been dipped together in the same dip. Spread is enhanced when wet sheep are held together in yards immediately after dipping.

Spread can also occur whenever infected and wet sheep come into contact with each other, e.g. jetting or shedding sheep in cold wet weather or yarding wet sheep for routine husbandry procedures. Young lambs can also be infected by close contact with an infected mother when sucking or when the ewes and lambs are yarded in the early morning for lamb marking, whilst they are still wet from dew.

Diagnosis

In most flocks, the disease can be diagnosed by clinical examination of the fleece, with the typical lesions being detected in the staple. In some flocks, it may

only be detected at shearing or when sheep are being handled for other reasons. Concurrent infections can occur with orf, especially on the pasterns and feet.

Laboratory confirmation is best made by taking impression smears from the underside of moist scabs removed from active lesions on the skin. Staining with Giemsa or Gram will demonstrate the characteristic coccoid and branching filamentous forms.

Examination of a number of sheep in the flock will allow an accurate determination of the time of infection in relation to wool growth. Sheep with lesions within 1–2 cm of the tip of the staple that extend down towards the skin indicate infection shortly after shearing. This often coincides with dipping. If lesions start half-way down the staple, then one needs to consider the management practices in the flock at that time that have provided the opportunity for a wetting event.

Treatment

In most sheep, the development of a good immunity and the lack of favourable conditions for the continued re-infection of the skin result in self-cure, with the lifting of the scabs off the skin as the wool fibres grow out. In some sheep, active lesions will persist and they may require treatment.

Antibiotics given at least 8 weeks before shearing will cure the disease and allow affected sheep to be cleanly shorn. The recommended treatment is long-acting oxytetracycline (20 mg/kg) given by intramuscular injection. A previous treatment using a penicillin/streptomycin combination (70 000 units of penicillin and 70 mg streptomycin/kg body weight) is no longer available in some countries. Animals should be kept dry for a few days after treatment, as they are still susceptible to re-infection from the crusty material that is attached to the wool above the skin lesion.

In affected flocks, attention needs to be given to preventive fly treatments as the serous exudate is attractive to the gravid female fly and provides ideal conditions for the development of the maggots.

Control and prevention

In most flocks, the disease can be controlled by eliminating or reducing the wetting event responsible for the spread of infection. This will involve avoiding yarding wet sheep for any reason. During dipping, young lambs should be dipped first, the ewes later and any obviously infected sheep should be dipped last. In flocks where there is a continual problem, zinc sulfate can be added to the dip wash at the rate of 10 kg/1000 litres of dip wash, provided that the zinc sulfate is compatible with the insecticide formulation being used. Wet sheep should not be held together after dipping but should be let out of the yards as soon as possible and not brought together again until the fleece is dry.

Most sheep will develop an immunity resulting in a self-cure and resistance to subsequent re-infection. Research is seeking development of a suitable vaccine [2]. Severely affected sheep should be culled from the flock, but eradication from a flock is not considered feasible because of the difficulty in detecting all carriers and the survival of the organism in scabs in the environment.

FLEECE-ROT

Synonym: water stain (see later for canary stain, a different condition)

Cause

Fleece-rot is an exudative dermatitis caused by bacteria, usually *Pseudomonas aeruginosa*. Other bacteria that can cause fleece-rot include other *Pseudomonas* spp., *Corynebacterium* spp., staphylococcal infections and *Bacillus cereus*. Active infection causes a discoloration of the fleece, the colour depending on the bacteria present. Green or brown banding of the staple is usually associated with *P. aeruginosa* infection while blue banding is caused by *P. indigofera*. Whilst the discoloration may have some effect on the value of the wool, the main economic loss is the result of the increased susceptibility of affected sheep to fly strike on the body.

Pathogenesis

P. aeruginosa is present on the skin and fleece of normal sheep. It has the ability to multiply rapidly and to inhibit the growth of other organisms. If the skin of the sheep is kept wet for 3–4 days as a result of rain, then the epithelial debris, exudates from the sweat glands and the lipid coating the skin result in a medium ideal for bacterial growth. In susceptible sheep, an exudative dermatitis develops that may extend up the

staple. Depending on the bacteria that are present, the fleece may have a discoloration ranging from light brown or yellowish to green, greenish-brown, brown or red [3].

Predisposing factors

The major predisposing factor is a period of wet weather with warm temperatures to provide the conditions on the skin suitable for the growth of bacteria, especially in sheep with 4–6 months' wool. Young sheep, not previously exposed to these conditions, are more susceptible to infection. There is also a genetic predisposition to fleece-rot, with some sheep more susceptible to infection and thus to body strike. Sheep with an open fleece and poor staple structure are more prone to wetting to skin level, setting up the high humidity and moisture conditions suitable for the multiplication of bacteria.

Clinical signs

In most affected sheep, there are no obvious external signs. On opening the fleece of affected sheep, there is discoloration of the wool, sometimes with evidence of a serous exudate at the base of the staple. Where there have been several episodes suitable for the development of the skin lesion, there may be several distinct bands of discoloration in the staple. Some sheep may show evidence of skin irritation with rubbing and biting, whilst there is an active dermatitis lesion. The exudative dermatitis is not only attractive to gravid female flies, but also provides moisture for eggs to hatch and soluble protein for the first instar larvae to feed. It is often associated with body strike when environmental conditions are suitable for flies.

Control and prevention

Heritability estimates for susceptibility to both fleece-rot and fly strike are estimated in the range of 0.35–0.40 [4], which provides scope for selection of resistant breeds and strains within breeds. In flocks where fly strike, associated with fleece-rot, is a problem, consideration needs to be given to management factors that avoid exposing susceptible sheep to long periods of wet weather. This may involve changing the time of shearing.

Vaccination against *P. aeruginosa* was tried some years ago [5]. It was effective in those flocks where fleece-rot was caused by *P. aeruginosa*, but in many flocks other organisms were also involved and the vaccine was not effective.

CORYNEBACTERIUM SPP. INFECTION (BOLO DISEASE)

Cause

This problem has been confirmed as a cause of a localized skin disease in woolly sheep [6]. The affected areas are well defined with dark-grey to almost black spots, patches or bands visible on or in the fleece. They are more common on the neck and shoulders, but can affect the back. The wool in affected areas is visibly shorter, less dense and fragile. At the base of the staple, there are yellowish- to greyish-white scaly deposits. In acute cases, there are circumscribed reddish-purple skin lesions, with a tender skin that cracks when handled. On shorn sheep, affected areas have a chalky white appearance. The disease has been reproduced by applying suspensions of a *Corynebacterium* spp. to the skin. However, if there was wetting of the skin post-infection, the lesions were not produced. The disease does not require the warm, wet conditions that are necessary for the development of fleece-rot or dermatophilosis.

Histologically, the skin lesions include superficial and follicular hyperkeratosis, acanthosis and sebaceous gland hyperplasia and hypertrophy. The epidermis is thickened, with only a few remaining collagen fibres separating the follicles and sebaceous glands in affected areas.

OTHER CONDITIONS

Corynebacterium pseudotuberculosis infection

Infection can result in chronic discharging fistulae from infections in the lymph nodes of the head and neck and the prescapular and prefemoral lymph nodes. The lesions are more commonly seen in older sheep (see Chapter 44).

Arcanobacterium pyogenes infection

This is a cause of subcutaneous abscesses and is often confused with *Corynebacterium pseudotuberculosis* infection. The lesions may not involve the lymph nodes and are more commonly seen in younger sheep.

Pseudomonas aeruginosa dermatitis

P. aeruginosa can cause a severe, progressive, necrotic dermatitis, affecting the woolled areas and the wool-free areas of the legs or scrotum. It is usually seen within 6 weeks of shearing, associated with persistent wet weather or with dipping 3–4 weeks earlier [7]. The lesions appear as extensive ulcers, covered by thick crusty scabs. Removal of the scab reveals foul smelling, greenish purulent material, involving the dermis.

Treatment is not usually successful, because of the nature of the lesion. Many years ago, acriflavine was used with some success in affected rams.

Staphylococcal dermatitis

This is characterized by a severe pyoderma in the head region, especially around the eyes, ears and nose. Suppurative, thick-walled ulcers develop caused by *Staphylococcus aureus* infection (Chapter 45).

Recently, a severe generalized staphylococcal scalded skin-like disease, associated with renal failure, has been reported in lambs [8]. There was a generalized skin exfoliation with widespread ulceration. Lesions were bilateral and symmetrical and affected lambs had enlarged, pale, oedematous kidneys. Pure cultures of *S. aureus* were isolated from the lesions.

Staphylococcal folliculitis

This occurs in lambs and is usually associated with a β haemolytic, coagulase-positive staphylococcus. It is characterized by transient pustule formation on the non-woolly areas, especially around the lips and perineum. Outbreaks are often associated with small pustules on the udder of nursing ewes (sometimes referred to as mammary impetigo). The lesions heal spontaneously and are not considered a major problem (Chapter 45).

Actinobacillosis (leathery lips, cruels, king's evil)

This is a chronic granulomatous infection of the skin, subcutaneous tissues and regional lymph nodes of the head caused by *Actinobacillus lignierisi*. It is more common in adult sheep and may be associated with wounds or skin abrasions or grass-seed penetration of the skin and oral mucosa. Affected sheep have chronic granulomatous abscesses with extensive fibrosis in the subcutaneous tissues of the cheeks, lips and nose. The lesions often discharge to the exterior with a thick greenish-yellow pus.

The diagnosis is confirmed by the demonstration of the characteristic club colonies of the organism in smears taken from lesions.

Fleece discoloration caused by bacteria

This is usually the result of a dermatitis caused by *Pseudomonas* infection. There is a leakage of serum setting up a suitable environment for bacterial growth. *P. aeruginosa* produces a green, brown or red discoloration, *P. indigofera* a blue colour. *Chromobacter* spp. can also be involved.

Pink rot

This occurs after a period of prolonged wetting and is characterized by a bright pink discoloration of the wool staple. It is caused by a *Bacillus* spp. infection of the wool fibre. The wool fibres are bound together in a creamy pink mass in the middle of the staple. It is usually detected at shearing.

Ringworm (club fungus)

This is usually caused by *Trichophyton verrucosum* [9] or *Microsporum canis* [10]. In the USA, there are reports of outbreaks in sheep being prepared for exhibition or sale. They are often associated with the washing of the fleece of sheep as part of their preparation for show.

Clinically, there are crusty, wart-like lesions on the face and ears, resembling mange. It is often associated with an intense pruritis. There may be circular

areas of alopecia with thickish grey scabs and it can be confused with orf.

Transmission is by direct contact with infected animals or contaminated equipment. There are reports of outbreaks after shows and sales where susceptible sheep have been exposed to infection. The disease is diagnosed by the demonstration of the characteristic fungal spores in skin scrapings and hair from the periphery of active lesions.

Most affected animals recover spontaneously within 4–6 weeks and treatment is not normally attempted. Spread of the disease is best controlled by isolation of affected animals and avoiding contact with contaminated pens and equipment.

Black fungus tip

This is characterized by abnormal black coloration in the top 1.5 cm of the staple. It is caused by infection of the wool fibres with a fungus, *Peyronella glomerata*. The pigment produced is not scourable, but affects only a small part of the fibre. Infection is usually associated with previous damage to the wool fibre.

OTHER NON-INFECTIOUS CONDITIONS

Diffuse yellow coloration

This is common in the long-woolled breeds and is thought to be caused by pigments secreted by the skin glands. There is recent evidence to suggest that it is an inherited condition [11].

Canary stain

This is a diffuse unscourable yellow stain seen after periods of high temperature and humidity. It is a diffuse staining of the staples as distinct from the banding usually associated with fleece-rot caused by bacterial infection of the skin. It is normally associated with a high suint content of the fleece and the presence of lanaurin, an insoluble yellow pigment in suint.

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47

Sheep scab (*Psoroptes ovis*)

P. Bates

Synonyms: ovine psoroptic mange, psoroptic scabies, psoroptosis

Sheep scab is found in most of the sheep-rearing countries of the world (with the exception of Australia, New Zealand, Canada and the USA). Infestations can be debilitating with high morbidity, and fatalities can occur through loss of condition, malnutrition, secondary infections and hypothermia. The disease is also of considerable economic significance because of poor animal growth, fatalities, and the downgrading of wool and leather. It should be noted that the term 'sheep scab' is sometimes applied incorrectly to infestation with *Sarcoptes scabiei* (Chapter 48).

CAUSE

Sheep scab is an acute or chronic form of allergic dermatitis caused by the non burrowing, astigmatid mite *Psoroptes ovis* (Figure 47.1). The mite is just visible to the naked eye, the adult female is pearly white and globular and approximately 1 mm in length. The mite can remain viable off the host for 15–17 days [1] and still be capable of infesting sheep. The life cycle takes 14 days in ideal conditions and consists of the egg, hexapod larvae, octopod protonymphs and tritonymphs, and adult males or females. Moulting between instars lasts 12–36 hours. Once fertilized, the adult female will not mate again but will live for an average of 40 days, laying one or two eggs daily.

Figure 47.1: Scanning electron micrograph of *Psoroptes ovis.*

Host specificity

There are no hosts for *P. ovis* in the UK, other than sheep. *P. ovis* can infest cattle restrained from grooming, during which period the mite is still infestive to sheep [2]. Psoroptic mange in cattle is not endemic to the UK, but is a major problem in mainland Europe and the USA. There have been three cases of psoroptic mange in cattle in the UK since 1980, all traced to animals imported from Europe. Mites from the last case in 1984 were shown to be non-infestive to sheep and subsequently were identified as a different species of *Psoroptes* (*P. natalensis*) specific to cattle. The ear mite, *P. cuniculi,* on the other hand, has been isolated from goats, horses and domestic rabbits. *P. cuniculi* does not appear to be present in wild rabbits in the UK and is not infestive to the bodies of sheep, although mites isolated from the ears of rabbits have been shown to establish in the ears of sheep [2].

CLINICAL SIGNS

Early disease is characterized by low mite numbers and very small lesions. Mites feed within hours of

contact and initially the lesion is a small raised vesicle filled with clear fluid. Excess serous fluid, leaking from the vesicle, dries to form a yellow scab about 1 cm in diameter, often with a moist, faintly green, periphery. Early lesions are virtually undetectable, with the mite adjusting to the new host and the host becoming sensitized to mite allegens. The lag phase can last 20–25 days for artificial infestations of a strain of *P. ovis* of 'medium virulence'. Sheep with early infestations may show no signs or be restless, rubbing against fence posts, etc., exhibit soiled and stained areas of wool (particularly on the shoulders), head tossing and deranged or tagged fleece. Sheep with subclinical scab can look perfectly normal and can unknowingly be introduced to a flock [3].

Later stages of infestation are characterized by a rapid increase in mite numbers and lesion spread. Scab mites (being semi-liquid pool feeders) cannot feed on the hardened scab and, with the hyperkeratinized skin and build-up of toxic faecal material, are forced to aggregate at the periphery of the expanding lesion. The lesion gradually spreads outwards as the mite population increases. Rubbing and head tossing become more excessive, areas of wool loss may appear, together with open, bleeding wounds. Sheep rapidly lose condition and epileptiform fitting may result [3].

Early disease is confined to the dorsal and lumbar areas but, if the condition remains untreated, may extend over the whole sheep, down the flanks and limbs and also on to the head, face and tail (Figure 47.2). In the later stages of this phase, 'flaker' sheep may occur, characterized by extensive wool loss, usually on the flanks and withers, the denuded areas covered in a pavement of flakey ('cornflake') scabs, overlying thousands of active mites. During this phase mites are more likely to pass to other sheep, either by direct contact or via fomites. In the later stages of the rapid growth phase, mites also begin to migrate to the 'cryptic sites' (the pinnae, infra-orbital fossae, inguinal fossae and perineum) and the external auditory canal (EAC) [3].

Eventually, the disease enters a decline phase. Mite faeces are still bound to the dried scab and will continue to elicit irritation as long as the scab is in contact with the skin. The general appearance of the lesion changes, the active moist edge becomes indistinct and scaly and aural haemotomas and secondary bacterial infections may occur. Mite populations decrease due to the lack of feeding sites and the immune response of the sheep. Mite-specific immunoglobulin and leucocytes attack the mid-gut cells of the mite (which also form the peritrophic membrane of *P. ovis*), inhibiting nutrient absorption and ultimately egg production. The lack of feeding sites and the host immune response may also force the mites to disperse at random over the entire body and many continue to reach the cryptic sites [3].

Figure 47.2: Sheep with extensive sheep scab.

After the decline phase it is not unusual for the mite population to die out completely and for an animal to make a full natural recovery without treatment. New wool growth begins in previously denuded areas and the scab continues to lift away from the skin as the wool grows. Other sheep, however, appear to recover completely but may be harbouring small populations of mites, under dry scabs or in the cryptic sites, waiting to reinfest the sheep once normal skin conditions are restored [3].

TRANSMISSION AND PATHOGENICITY

Sheep scab can be contracted via contact with live mites in tags of wool or scab attached to brambles, bushes, etc., but is usually contracted through forced sheep-to-sheep contact at market, in livestock lorries, etc. Shearing combs and cutters and contaminated clothing can also spread scab.

It is not known exactly how *P. ovis* feeds. The mite possesses long, barbed chelicerae, capable of piercing and scraping the skin, as well as a sponge-like

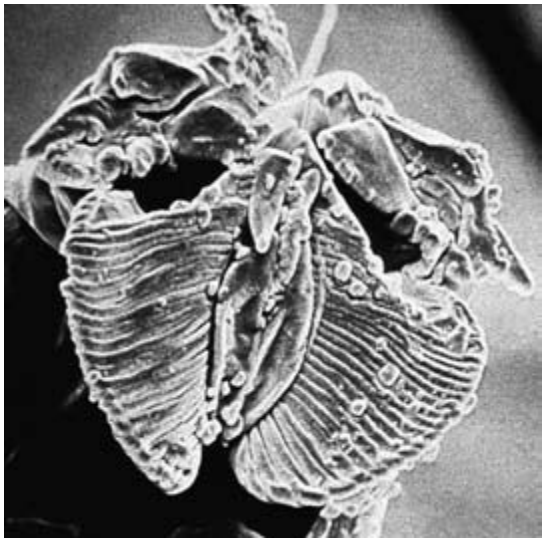

Figure 47.3: Scanning electron micrograph of the mouth parts of *Psoroptes ovis.*

'lapping' organ (the pseudorutella) (Figure 47.3). It is thought the mite grazes the skin around the moist periphery of the lesion, taking in nutrients with the serous exudate, skin secretions and lipid. The clinical signs of sheep scab are caused only partly, if at all, by the direct action of mite feeding. Sheep scab is a form of allergic dermatitis initiated by mite faeces (and possibly the cuticle) which *P. ovis* exploits. The heat and humidity produced by the inflammation forms the microclimate needed for mite survival and the leakage of serous exudate forming the basis of the mites' nutrition [3].

Seasonality

Sheep scab is a winter disease, with most cases occurring between September and April, although a significant number of cases do occur in the summer months, particularly on animals still full fleeced (lambs, hoggs, etc.) and on 'ridges' of longer fleece on poorly shorn sheep. These sheep can infest ewes with an adequate fleece length. Sheep scab mites will actively migrate to the 'cryptic sites', but only on sheep with extensive disease and then more often in the winter than the summer [4]. Shearing can stop the progress of disease (either temporarily or permanently) by removing the microclimate, leaving the mites exposed to dehydration [5].

Eac infestations with *Psoroptic* mites

Scab mites can migrate into the EAC, with no actual scab lesions in the pinna itself and with the body lesion terminating only as far as the withers [2]. Surveys at the Veterinary Laboratories Agency, Weybridge, Britain, have shown that scab mites (*P. ovis*) infest the EAC of 26 per cent of sheep with extensive scab and that these mites can survive plunge dipping. It is postulated that two species of *Psoroptes* mite infest sheep: *P. ovis* on the body and *P. cuniculi* in the ears [2]. *P. cuniculi* has been found deep within the ears of sheep in Britain, with no recent history of sheep scab [6]. These mites were located within tubes of scab within the last centimetre of the EAC, next to the tympanic membrane. Ear mites are morphologically identical to the sheep scab mite, but do not initiate clinical scab. In adult sheep, infestations may be without clinical signs or result in aural haematomas, violent head shaking and ear rubbing, leading to excoriation and wounding of the ear and ear base. Lambs show plaques of scab (often bloody) on the external ear cleft, excoriation of the ear base, ear scratching with the hind feet and inflammation of the external aspects of the horizontal canal. In all cases the internal pinnae are clear of typical psoroptic scabs.

Population variation in *P. ovis*

Populations of the scab mite can vary in their speed of lesion production, rate of mite population increase, irritation, otoacariasis, efficacy of resistance to ivermectin and acaricide [7]. Some populations cause slow (chronic) disease, covering the sheep in 8–10 months, with few or no signs. Other populations can be very fast (acute), taking only 4–5 weeks to cover an animal, with an associated high degree of irritation and mortality. There are differences also in the efficacy of single injections of ivermectin: chronic populations can be almost eradicated but significant numbers of acute strain mites can survive treatment. A repeat injection of ivermectin, however, will eradicate all strains of sheep scab mite, highlighting the need to administer ivermectin correctly, with two injections 7 days apart [8].

Figure 47.4: Pedicel of *Psoroptes ovis*.

DIAGNOSIS

Differential diagnosis from other sheep ectoparasites (lice, keds, blowfly strike, ticks, etc.) and skin conditions (scrapie, mycotic dermatitis, etc.) is essential. The positive diagnosis of scab requires the isolation and identification of the *Psoroptes* mite (Figure 47.4). Visual examination of the lesion may reveal the larger adult females at the edge of the lesion, but in most cases it is necessary to take skin scrapings from this area. Wool should be clipped (and stored for differential diagnosis). Scrapings should be taken with the blade held at an acute angle, shaving rather than scraping off the outer epidermis, as opposed to being held at right angles and scraping until the skin oozes blood, as for the diagnosis of *Sarcoptes* mites. Samples should be transferred immediately into small tubes that can be securely stoppered or into 'self-sealing' envelopes. Samples can be examined directly under a dissecting microscope for the presence of live mites or after processing in hot potassium hydroxide [9].

TREATMENT AND PREVENTION

If possible, all new stock, agisted sheep and shared tups should be isolated for at least 3 weeks prior to mixing with the main flock and observed regularly for signs of scab and other ectoparasites. Veterinary advice should be sought if an ectoparasite is suspected and the parasite(s) must be identified professionally. Isolated sheep should not be released into the main flock until treatment is completed and the infestation has been shown to be cured. Fencing must be effective in preventing straying on or off the property and direct contact with neighbouring sheep. On common or unfenced grazing cooperation must be sought with neighbouring properties to attain equal standards of health. All flocks should be treated simultaneously.

Plunge dipping

Clinical scab can be cured by plunge dipping in wash containing the organophosphate (OP), diazinon or the synthetic pyrethroid (SP), high *cis*-cypermethrin (HCC). OP and some SP dips are effective after a single dipping but some SP formulations require a second dip after 14 days [10]. Dipping for 30 seconds is adequate for the control of lice, blowfly or ticks but sheep need to be immersed for 60 second (with the head under twice) to eradicate sheep scab. Twice the amount of acaricide is absorbed by dipping for 60 seconds compared to 30 seconds. In all baths, sheep must be kept moving: the swimming action displacing the air in the fleece, thus aiding dipwash penetration. OP dips are effective after a single dipping.

The sheep scab mite can live off the host and remain infestive for 15–17 days [1]. 'Approved dip' formulations (licensed prior to 1992) containing the OP diazinon are guaranteed to protect against reinfestation for at least 3 weeks on sheep with 1.0 cm of fleece. In reality, this would be considerably longer on fully fleeced sheep. Consequently, sheep dipped in one of the 'approved dips' can be returned to an infested pasture, yard or barn directly after dipping, without risk of reinfestation. SP formulations licensed after 1992 (i.e. HCC) are no longer required to offer protection against scab, although some do. Sheep treated with SP formulations that do not claim protection against scab must not be returned to infected pasture after dipping.

Systemic endectocides

Systemic endectocides (macrocyclic lactones – MLs) have both acaricidal and anthelmintic properties. At present, MLs are administered as injections. Correct doses (according to body weight) control ectoparasites anywhere on the body through the ingestion of the active ingredient. At present doramectin, ivermectin and moxidectin are the only authorized MLs for sheep in the UK.

Some MLs require two injections, 7 days (ivermectin) or 10 days (moxidectin) apart, to kill mites emerging from eggs or from a moult and to eradicate acute populations of mites. The washing process and drying of the microclimate seen in dipped sheep is not generally observed after the use of an injectable ML. Consquently, lesions may take longer to resolve, even after a second injection [11]. Animals can still exhibit severe bouts of irritation and hypersensitivity as long as the lesions or part of the lesion, is still in contact with the skin (even though the mites themselves have been killed). Irritation decreases as the fleece grows, lifting the scab away from the skin. MLs have varying periods of protection. The prolonged period for complete resolution of clinical disease increases the chances of sheep rubbing and thus contracting residual mites from the environment.

Acaricide resistance

In South America the sheep scab mite developed resistance to the organochlorine, lindane (γHCH/γ BHC), in 1962 and to the OP, diazinon, in 1965. In the UK, scab mites developed resistance to the SP, flumethrin [12], with side resistance to HCC [13]. Scab mites have developed resistance to the OP, propetamphos [14], but side resistance was not shown to diazinon [13]. At present there are no recorded cases of ML resistance in the sheep scab mite in the UK or anywhere else in the world.

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48

Other ectoparasitic conditions

P. Bates

Ectoparasites can seriously affect sheep productivity, with reduced milk and meat yields, the downgrading of wool and leather, and requirement for expensive control programmes. Ectoparasites also seriously compromise the welfare of flocks and individual sheep, often resulting in prosecutions for animal cruelty.

Permanent ectoparasites, spending their entire life cycle on the sheep (e.g. mange mites, keds and lice) are seasonal, with peak populations occurring in the winter or early spring. Semi-permanent ectoparasites, with at least one life stage free-living (e.g. adult blowflies, headflies and nasal bot flies) are active mainly in the warmer months of spring and summer. Shearing will remove most permanent ectoparasites, and thus affect their seasonality.

Transfer of permanent ectoparasites occurs when animals are closely herded or penned together and in the close contact between mother and young within the first few hours of birth. Chewing and sucking lice (*Bovicola* spp. and *Linognathus* spp.), keds (*Melophagus ovinus*) and most mange mites (*Chorioptes* spp., *Sarcoptes* spp., *Psorobia* spp. and *Demodex* spp.) are host-specific. The sheep scab mite, *Psoroptes ovis*, has been cultured on rabbits and cattle restrained from grooming, although these transmissions are unlikely to occur under field conditions in the UK.

MITES

Mange is a form of allergic dermatitis caused by mites and characterized by skin encrustation, alopecia and pruritus. Five genera of parasitic mite can cause mange in sheep (*Chorioptes* spp., *Demodex* spp., *Psorobia* (*Psorergates*) spp., *Psoroptes* spp. and *Sarcoptes* spp.) all demonstrating a high degree of host specificity (Table 48.1). *Psoroptes ovis* causes a form of mange known as sheep scab, which is described in detail in Chapter 47. Other terms used for non-psoroptic mange are scabies, itch and acariasis.

SARCOPTIC MANGE

Synonyms: *Sarcoptes scabiei*, head mange, sarcoptic scabies

Cause

Sarcoptic or head mange in sheep, caused by *Sarcoptes scabiei* var. *ovis*, has been recorded in Europe, Africa, the Middle East, the Balkans, India and South and Central America but has never been recorded in the UK. *Sarcoptes* spp. mites are round bodied, with short legs. In adult mites leg pairs one and two project beyond the body edge, and suckers on long unjointed stalks are present on leg pairs one, two and four in males and leg pairs one and two in females.

Epidemiology and pathogenicity

S. scabiei var. *ovis* on sheep is found on the sparsely haired parts of the body, such as the face and ears. Mites burrow into the epidermis and feed on tissue fluids, which causes irritation and consequent scratching, leading to inflammation and exudation which forms crusts. Small foci of infection do not appear to affect the health of an animal adversely but can be more serious if the condition spreads.

Table 48.1: Ovine mange: differentiation of mite species

	Mites			Lesions	
Mange type	Species	Life cycle (days)	Length (μm)	Early locations	Characteristics
Psoroptic (sheep scab)	*Psoroptes ovis*	10–12	♀ 600 ♂ 500	Withers, flanks and rump	Central crust, alopecia
Sarcoptic (head scabies)	*Sarcoptes scabiei* var. *ovis*	17	♀ 300–600 ♂ 200–400	Head	Thick, crusty, wrinkled, alopecia
Psorobic (itch mite)	*Psorobia ovis*	28–35	♀ 190 ♂ 170	Flanks, thigh	Dry, scurfy, 'deranged' fleece
Chorioptic (foot mange)	*Chorioptes bovis*	19–23	♀ 400 ♂ 300	Feet, pasterns, scrotum	Crusty, brown, thickened, fissured
Demodectic (follicular mange)	*Demodex ovis*	Not known	♀ 214 ♂ 170	Follicles and sebaceous glands over entire body	Normal to nodular, pustular
	Demodex aries	Not known	♀ 339 ♂ 324	Eyelids, prepuce, vulva, neck, back	Relatively non-pathogenic

CHORIOPTIC MANGE

Synonyms: *Chorioptes bovis*, chorioptic mange, foot mange, scrotal mange

The cause

Chorioptes mites possess broad bowl-shaped suckers on very short unsegmented stalks (pedicels) (Figure 48.1). Foot and scrotal mange due to *C. bovis* has been recorded in low incidence in Australia and New Zealand. The parasite was recorded as infesting the pasterns of sheep in the UK in the late 1960s, and was thought to have been eradicated from sheep following 18 years of compulsory dipping against sheep scab (*Psoroptes ovis*). However, chorioptic mange was found again in 2000 [1] and is now not uncommon in the UK.

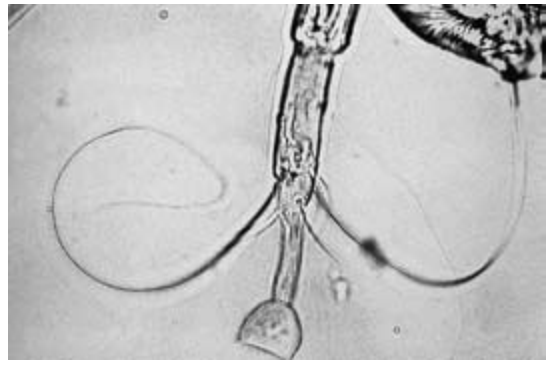

Figure 48.1: *Chorioptes* pedicel. (Copyright, Veterinary Laboratories Agency, Weybridge.)

Epidemiology and pathogenicity

Chorioptes mites infest the woolless areas, particularly the lower parts of the hind legs and scrotum, and can decrease fertility by causing inflammation of the scrotal skin. *C. bovis* does not pierce the skin, but feeds on skin debris leading to a yellow–brown lesion with haemorrhaging fissures resulting from allergic reactions to the mites or mite by-products. Intense itching causes foot stamping and biting. The complete life cycle takes about 3 weeks, with ovigerous females living for 3 weeks, while non-ovigerous females and adult males may live for 7–8 weeks.

PSOROBIC (PSORERGATIC) MANGE

Synonyms: *Psorobia ovis, Psorergates ovis,* itch mite

Cause

Psorobia (*Psorergates*) *ovis* appears to affect only Merino sheep and has been reported only in Australia, New Zealand, South Africa, South America and the USA. Adult *Psorobia* can be recognized by the radial arrangement of their legs around a more or less circular body, each leg having an inward curving spine on each femur.

Epidemiology and pathogenicity

Most mites are found under the stratum corneum in the superficial layers of the skin of the sides, flanks and thighs, feeding on the exuding fluid. The infested area is dry and scurfy, wool fibres break easily, with the remaining wool coming together as ragged tufts. Irritation causes the sheep to rub and kick the affected area and chew its fleece, resulting in 'fleece derangement' and downgrading of the wool clip [2]. Affected sheep may become 'tolerant' after 1–2 years but can still remain infested.

Infestations spread slowly and may affect 15 per cent of sheep in a neglected flock. Female *P. ovis* lay few eggs in their lifetime, hatching to larvae with small legs. Three larval stages follow, the legs becoming progressively larger with each moult and, by the adult stage, the legs are well developed and the mites are motile. Adults of both sexes are very small (200 μm) and the life cycle is completed in 4–5 weeks. Adult *P. ovis* are very sensitive to desiccation, dying within 24–48 hours when removed from their host. Transmission occurs during the brief period after shearing. Considerable mortality of mites was observed up to and including day 9 after shearing which suggests that fluctuations of temperature and solar irradiation following shearing create considerable stress for the *P. ovis* population, and that the first 2 weeks after shearing could be an appropriate time for control.

DEMODECTIC MANGE

Synonyms: *Demodex ovis, Demodex aries*, demodecidosis, follicular mange

Cause

Demodex mites have short legs and elongated, striated, 'cigar-shaped' bodies. Two species of *Demodex* have been isolated from sheep. *Demodex aries,* an innocuous commensal of the follicles and sebaceous glands of the feet, face, eyelids, ears, prepuce and vulva [3], and *Demodex ovis,* which lightly parasitizes the hair follicles and sebaceous glands of primary hairs over the entire body, with highest populations occurring on the neck, flanks and shoulders.

Epidemiology and pathogenicity

Infested follicles become distended with mites, mite exuvia, eggs and epithelial cells, forming nodules. Pyogenic bacteria may convert these nodules into pustules. Skin with advanced lesions is thick and scaly, alopecic, nodular or pustular. Itching may stimulate kicking, biting and rubbing of the lesions. In general the disease is of low incidence and of little importance.

DIAGNOSIS OF MANGE

The positive diagnosis of mange requires the isolation and identification of the specific mite. Visual examination of the lesion may reveal the larger mites (e.g. *Psoroptes*), but in most cases it is necessary to take skin scrapings from the edge of visible lesions. Wool should be clipped above the lesion edge (and stored for the differential diagnosis of mycotic dermatitis). In general, for mites living on the skin surface (i.e. *Psoroptes* or *Chorioptes*), scrapings should be taken with the blade held at an acute angle, shaving rather than scraping off the outer epidermis. *Demodex, Psorobia* or *Sarcoptes* are found burrowing into the skin and the scalpel blade should be held at right angles and the skin scraped until it oozes blood. Samples should be immediately transferred into small tubes that can be securely stoppered or into 'self-sealing' envelopes. Samples can be examined directly under a dissecting microscope for the presence of live mites or after processing in hot potassium hydroxide [4].

TREATMENT OF MANGE

The macrocyclic lactones (MLs) – doramectin, ivermectin, moxidectin – are highly effective against

Table 48.2: Ovine lice: differentiation of species

Names	Length (mm)	Colour	Feeding	Location	Incubation (days)	Life cycle (days)
Body louse (*Bovicola ovis*)	1.2	Pale yellow to red/brown	Chewing	Upper sides, withers	9–10	24–36
Blue body louse, face louse (*Linognathus ovillus*)	2.5	Blue/grey	Blood sucking	Face, body	11–13	35
Foot louse (*Linognathus pedalis*)	2.0	Blue/grey	Blood sucking	Hairy legs, scrotum	17	43

Sarcoptes. Ivermectin administered in the spring effectively reduces the numbers of *Psorobia* to a low level but gives poor control when administered in the autumn. Single subcutaneous injections of ivermectin are effective against chorioptic mange although, in large-scale studies, subcutaneous injections of 200 μg/kg given singly or repeated after a 2-week interval were not effective. No conclusions could be drawn on the efficacy of intramuscular injections of doramectin (300 μg/kg) for the control of chorioptic mange due to the small number of mites identified. The use of doramectin for elimination of *Chorioptes bovis* may be limited by the feeding behaviour of the mites [1].

Two plunge dippings at a 10-day interval in the organophosphate (OP) acaricide phoxim, at 0.2 per cent and a 1.0 per cent flumethrin pour-on (2.0 ml/kg) have also been shown to be effective in controlling sarcoptic mange in sheep. Spring dipping in a mixture of rotenone (75.6 ppm) and cypermethrin (19 ppm) is extremely effective, but mite populations resurged after autumn treatment.

LICE (PHTHIRAPTERA)

Synonym: pediculosis

Infestations of lice constitute a chronic dermatitis, characterized by constant irritation, itching, rubbing, tagging and biting of the fleece. Lice probably occur in all sheep-producing countries but, with the exception of wool-producing Australia, attract little attention. Three species of louse infest sheep (Table 48.2): the chewing louse *Bovicola ovis* (formerly *Damalinia ovis*) and the blood-sucking lice *Linognathus ovillus* (the face louse) and *L. pedalis* (the foot louse). Infestations can cause considerable, but unmeasurable losses from unthriftiness, retarded growth and damaged wool and leather.

THE SHEEP BODY LOUSE

Synonym: *Bovicola* (*Damalinia*) *ovis*

Cause

Mammalian lice can be divided into the blood-sucking lice (Anoplura), identified by their narrow head and the chewing (erroneously called 'biting') lice (Mallophaga – 'wool eaters'). As their name suggests chewing lice feed on skin debris and hair, and can be identified by their wide heads (containing the musculature and mouthparts necessary for this method of feeding). The sheep chewing louse *B. ovis* is a small, pale to red/brown insect with a broad head and chewing mouthparts, which feeds on epithelial scales, wool fibres and skin debris (Figure 48.2).

Epidemiology, transmission and pathogenicity

B. ovis is an obligate parasite but its bionomics are greatly influenced by climate. The egg stage lasts 1–2 weeks, nymphal stages 1–3 weeks with the total time from egg to egg being 3–5 weeks. Adults can live for up to a month. Chewing lice have a low intrinsic rate of increase and spread slowly among sheep [5, 6]. The prevalence of chewing lice is seasonal, with most

Figure 48.2: Sheep body louse (*Bovicola ovis*). (Copyright, Veterinary Laboratories Agency, Weybridge.)

cases occurring in the winter, although infested sheep have been recorded in the summer. The severity of infestation appears to depend on the breed, fleece length and overall health of the host together with the ambient climate. Australian Merinos can carry heavy infestations of *B. ovis* resulting in severe irritation. Sheep breeds in the UK appear to carry lower populations of lice with little or no irritation. Mortalities caused by external factors such as excessively hot or wet weather or management practices can be reflected in louse populations for 6 months or more [7]. Suitable fleece fibres and skin temperatures are required for infestations to establish and progress. Populations of lice are influenced by fleece length, with high populations observed on sheep with long fleeces [8].

The normal sheep skin temperature is 37.5°C, the temperature at which peak *B. ovis* oviposition occurs. In anatomical areas of low temperature (e.g. legs and tail) oviposition is inhibited. At a fleece thickness of 30–100 mm most eggs are laid within 6 mm of the skin surface, even when the fleece is 100 mm deep few eggs are laid more than 12 mm from the skin surface. In fleeces where the temperature ranges from 38°C at the skin surface to 15°C near the tip of the fleece, 69 per cent of the mobile population (nymphs and adults) are within 6 mm of the skin surface and only 15 per cent more than 12 mm from the skin. When the tip of the fleece is shaded and warmed, adults and third-stage nymphs can come to the surface. It is under these conditions that *B. ovis* spreads within a closely herded flock. Thus, lice spread quickly within flocks in hot climates (e.g. Australia) and more slowly in more temperate climates (e.g. the UK). Populations of *B. ovis* are limited by a number of factors including shearing, when 30–50 per cent of the population can be lost. During the winter, when lice populations thrive, the numbers on a sheep can increase from 400 to 4000 by the spring.

Heavy infestations of lice are also associated with animals in poor health and/or maintained in unhygienic conditions. Populations are influenced by sheep body condition, the lower the body condition score the higher the population of lice [8]. It seems likely that lice exploit an animal already out of condition due to concomitant infections or bad husbandry. No significant differences in lamb percentages and lamb weights at weaning between louse infested and louse-free sheep were observed over a 4-year period [9]. It was observed that the most prolific source of lice on a particular property was a crippled, bottle-fed lamb and a group of ewes diagnosed with ovine progressive pneumonia [10]. It has been observed that populations of *B. ovis* increased during winter on sheep on a low plane of nutrition [11]. Thus, chewing lice can be a significant indicator of underlying welfare problems within a flock. Irritation due to modest infestations is enough to provoke scratching and rubbing with damage to fleece and hides. Immune responses to *B. ovis* can result in the nodular skin defect known as 'cockle', downgrading the value of the leather.

All species of louse (*Anoplura* or *Mallophaga*) are generally considered to be host-specific but studies in Australia have reported that *B. ovis* can survive and apparently breed on Angora cross goats penned with lousy sheep [12]. However, chewing lice of Angora goats (*B. limbata*) do not appear to be infestive to sheep [13].

The clinical signs of chewing lice can be confused with that of sheep scab (*Psoroptes ovis*) and thus possible resistance may occur in both ectoparasites if they are not professionally identified and the correct treatment applied. Sheep can also present with mixed infestations of sheep scab and chewing lice.

THE FACE LOUSE

Synonyms: *Linognathus ovillus,* blue body louse, face louse

Figure 48.3: Face louse (*Linognathus ovillus*). (Courtesy of Novartis Animal Health.)

Cause

The face louse or blue body louse (*L. ovillus*) has been recorded in Australia, France, New Zealand, the USA, the UK and probably all other sheep-rearing countries. *Linognathus* lice can be identified by their thin elongated head and piercing mouthparts, that penetrate the skin and suck blood (Figure 48.3).

Epidemiology and pathogenicity

L. ovillus can be found on both the haired and woolled areas of the face. As populations increase infestations can spread over the woolled skin of the entire body. Dense accumulations of *L. ovillus* on the face can discolour white hair or wool to a definite grey.

THE FOOT LOUSE

Synonym: *Linognathus pedalis*

Cause

L. pedalis is morphologically similar to *L. ovillus* and occurs in Africa, Australia, the USA and South America. Like foot mange (*Chorioptes bovis*), the foot louse may have succumbed to former annual compulsory scab dipping and has not been recorded in the UK for at least 20 years.

Epidemiology and pathogenicity

L. pedalis inhabits the haired skin between the hooves and knees and hocks, usually forming stationary clusters (often reaching several hundred insects per square centimetre). Heavy infestations may spread on to the woolled areas of the abdomen and scrotum. Adaptation to woolless areas of the sheep limbs allows *L. pedalis* to survive low environmental temperatures twice as long as *L. ovillus.* Lambs can be infested with *L. pedalis* within 48 hours of birth. Heavy infestations cause foot stamping and biting, and can produce lameness.

TREATMENT OF LICE INFESTATIONS

The control of chewing lice in the UK has in the past been an adjunct to former autumn compulsory scab dipping and lice were almost eradicated from mainland UK, with pockets of infestation remaining on some Scottish islands and isolated areas of Dartmoor, the Lake District and the Pennines. With the deregulation of sheep scab in 1992, lice have become prevalent on nearly all hill grazings in the UK. Plunge dips containing the organophosphate (OP) diazinon or the synthetic pyrethroid (SP) high *cis*-cypermethrin (HCC) are extremely effective against chewing lice. Spot-on or pour-on formulations of SPs (alphacypermethrin, deltamethrin or HCC) are also licensed for the control of *B. ovis* [14]. In Australia it is recommended that louse control treatments be administered to off-shears (i.e. within 30 days of shearing), when louse populations are at their lowest [15].

Linognathus spp. can also be controlled through the use of OP/SP plunge dips or SP spot-on/pour-ons but, as they are blood feeders, they are susceptible to the systemic macrocyclic lactones (MLs – ivermectin, doramectin, moxidectin) administered either by injection or oral drench.

Insecticide resistance

Resistance to plunge dips containing the organochlorine γHCH (γBHC, lindane) developed in populations of *B. ovis* in Cumbria, Lancashire and Yorkshire in the mid-1960s. Failure of synthetic pyrethroid (SP) pour-ons and plunge dips to control *B. ovis* were first reported in Australia and New Zealand in the 1980s.

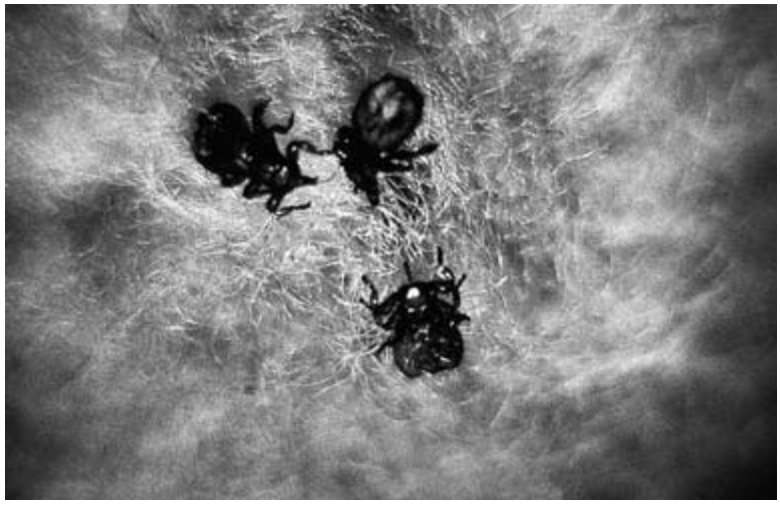

Figure 48.4: Sheep ked (*Melophagus ovinus*). (Courtesy of Novartis Animal Health.)

Initially, resistance factors (RFs) were only 26-fold, but this was sufficient to prevent pour-ons working effectively. By 1991 a population of *B. ovis* was identified with an RF of 642-fold to cypermethrin, with side-resistance to other SPs. With the UK's deregulation of sheep scab and the resultant widespread increase of lice, confirmation of SP resistance in lice was predicted. A pilot study, using an *in vitro* bioassay, investigating SP susceptibility within four UK populations of *B. ovis* demonstrated RFs to deltamethrin of 10.4-, 5.4-, 2.2- and 1.0-fold. This and subsequent investigations indicate that an RF of more than 10.4-fold implies resistance to SP applied as a pour-on or spot-on [14]. In all cases, the bioassay was carried out against deltamethrin, although HCC was also involved, along with deltamethrin, in later cases.

FLIES

Hippoboscid flies

Synonyms: *Melophagus ovinus,* sheep tick, sheep ked

Cause

The ked (*Melophagus ovinus*), a wingless hippoboscid fly, is a blood-feeding ectoparasite of sheep in temperate countries and in the cooler highlands of the tropics, but is absent from the hot, humid tropics. Adults are red–brown insects, 4–6 mm in length, with a broad head and stout piercing mouthparts (Figure 48.4).

Epidemiology and pathogenicity

Keds spend their entire life cycle on the sheep. The female is viviparous, retaining the larva within a modified oviduct until it is fully grown, depositing it as an immobile pre-pupa, that pupates once attached to the wool. Pupae are deposited when the female is 13–14 days old, with subsequent larvae deposited every 7–8 days. In its lifetime of 4–5 months a female will produce about 15 larvae, a comparatively slow rate of increase for an insect. Deposited larvae pupate within 6 hours and the duration within the pupal stage is 20–26 hours. Thus, a cycle from newly emerged adult female to emergence of an adult of the next generation is 5 weeks. Pupae develop over a relatively narrow range of temperature (25–34°C) with optimal development at 30°C. Puparia are glued to the fleece and carried away from the skin as the fleece grows. Temperature at skin level will be close to 37°C but considerably cooler nearer the fleece tip. Puparia are therefore deposited in areas where a suitable temperature will be found during the 3 weeks of pupal development. In hoggs, over 50 per cent of the pupae are found in the neck region, while 60 per cent of

adult *M. ovinus* are found on the forelegs and flanks. On lambs, puparia are concentrated on the hind legs, neck and belly, although substantial numbers of adults are found on the flanks and forelegs.

Adult keds are blood-feeding and large numbers can gradually exsanguinate the host and cause variable degrees of anaemia. Excreta can stain the wool and downgrade the fleece. Large numbers can cause restlessness, the sheep biting, kicking and rubbing the affected areas, mechanically damaging the fleece.

HEAD FLY

Synonyms: *Hydrotea irritans*, head fly, broken head, black cap

Cause

Hydrotea irritans is a muscid fly, similar in size to a house fly, with an olive green abdomen and orange–yellow wing bases. The fly occurs throughout the UK, although head-fly-related damage has been recorded only in the north of England and the Scottish borders.

Epidemiology and pathogenicity

The fly produces only one generation of adults a year. Eggs are laid in late July and September, in soil with a dense cover of vegetation, usually on the edge of (particularly coniferous) woodland. Eggs hatch within 7 days and the carnivorous larvae feed and grow until late autumn, when development ceases, only to resume again the following spring. Pupation occurs in May, with adults emerging after a minimum of 4 weeks. Flies are active only during the day and will not take to the wing in windy conditions. Adults and larvae are prone to dessication and tree cover offers protection from the sun, with flies venturing out for short distances to feed. Horned sheep with hairy faces (e.g. Blackface, Swaledale, etc.) are most susceptible. Flies feed at the base of the horn but also on nasal and lachrymal secretions using their rasping mouthparts. Lesions at the skin–horn junction of young sheep and wounds resulting from fighting in rams also attract head flies.

Swarms of flies initiate head shaking and rubbing the head against the ground, undergrowth or scratching with the hind feet. Trauma may produce breaks in the skin of the poll with exudation of blood and serum that attracts more flies. Continual feeding at the periphery of the lesion can lead to possible loss of large areas of skin from the head ('broken head' or 'blackcap'). In severely affected flocks, 50 per cent of ewes and 90 per cent of lambs can show lesions. Secondary infestions and, on occasions, blowfly strike may occur. Wounds usually heal following the cessation of fly activity.

TRAUMATIC MYIASIS

Synonyms: cutaneous myiasis, maggot fly, blowfly strike, blowfly myiasis

Cutaneous myiasis is the infestation of living tissues with the larvae of true flies (Diptera). In sheep, infestations, colloquially termed blowfly strike or maggot fly, constitute a major disease problem throughout the world.

Causes

The larvae of three species of fly commonly parasitize sheep in the UK: *Lucilia sericata* (the 'greenbottle'), *Phormia terrae-novae* (the 'black blowfly') and *Calliphora erythrocephala* (the 'bluebottle'). These species are not obligate parasites and large numbers are associated with the environmentally useful tasks of faecal and carcass decomposition. In other sheep-rearing areas of the world (Australia, New Zealand, South Africa, etc.) the obligate parasitic blowfly *Lucilia cuprina* is the major strike species.

Clinical signs

Signs of blowfly strike include agitation and dejection. In breech strike, infested sheep stamp their hind legs, shake their tails vigorously or gnaw and rub at the breech. As lesions develop a distinctive odour is noticeable and the wool becomes matted and discoloured. If the infestation remains untreated the affected area increases and wool is shed from the

Figure 48.5: Blowfly strike. (Courtesy of Novartis Animal Health.)

centre, accompanied by signs of constant discomfort (Figure 48.5). Research from Australia, where strike due to *L. cuprina* is a serious problem, has demonstrated that many strikes go unnoticed, with a ratio of 13:1 covert to overt strikes.

Epidemiology, transmission and pathogenicity

Blowflies have low population densities, with under 1500 flies per square mile and only gravid females are attracted to sheep. Blowfly myiasis in the UK can be classified as body strike or breech strike. In body strike, flies are attracted to sheep by the odours of excessive 'sweating' and/or decaying organic matter in the fleece, usually over the loins, shoulders, flanks, neck, back, throat or abdomen. In breech or tail strike, flies are attracted to fleece contaminated with urine and/or faeces and are particularly associated with scouring. Other forms of strike include foot strike, head strike occurring in horned breeds with accumulation of dirt and grease at the horn base or through wounds from fighting or de-horning, and pizzle strike, occurring in the wool around the opening of the prepuce. Analysis of strike cases in 1995 demonstrated that over 70.9 per cent of strike cases occurred on the breech or tail, 19.7 per cent of strikes on the body with foot strike accounting for 11.4 per cent of cases.

Blowflies attack in waves, classified as primary, secondary or tertiary fly waves. Primary flies (*Lucilia* and *Phormia*) oviposit on damaged or soiled areas of fleece. The larvae crawl to the skin which they eventually lacerate and digest using anterior hooks present on the oesophageal skeleton and secretory proteolytic enzymes. First instar (L_1) *Lucilia* larvae damage skin mainly through the action of proteolytic enzymes, although *L. cuprina* is more invasive than *L. sericata*. On close examination, the strike lesion appears as a foul-smelling area of moist brown wool, often with early stage maggots visible. Larvae in primary strike cases may or may not invade the living tissue. Secondary flies (*Lucilia* spp., *Phormia* spp. or *Calliphora* spp.) are attracted by the smell of the primary lesion. *Calliphora* rarely initiate strike on their own. Similarly, the third wave of flies is attracted by the increasing lesion and secondary bacterial infection. If unchecked, extensive infestations of secondary, tertiary or further waves of flies occur and sheep can die a quick agonizing death. Lipid-soluble ammonia, excreted by *Lucilia* larvae, is absorbed through the skin directly affecting the heart, lungs and brain. In other parts of the world, species of *Chrysomia* or *Sarcophaga* can be secondary flies.

In a survey of sheep farmers in England and Wales, 80 per cent reported at least one case of blowfly strike in their flocks, with an estimated half a million sheep struck annually [16]. An average of 1.6 per cent of sheep was reported to be struck within flocks. The prevalence of blowfly strike is weather-dependent, with most cases of body strike occurring during periods of high humidity or warm periods after heavy rain. In south-east England strikes can occur any time between March and December. Breech strike depends less on weather as the moisture supplied by urine and/or scouring is sufficient to attract flies.

Adult flies can fly 10 miles and are not greatly impeded by wind speed. Oviposition begins 5–9 days after emerging from pupae and 2000–3000 eggs can

be laid in nine or ten batches over a 3-week period, depending on temperature. Any source of ammoniacal decomposition will induce flies to oviposit. Eggs hatch within 24 hours into L_1 larvae if the fleece humidity is optimal. Eggs are continually being deposited on the fleece, but L_1 larvae cannot survive in wool with a moisture content below 70–90 per cent. Later instars are less dependent on humidity. Total development can take 5–11 days on a carcass but only 3 days in a case of body or breech strike or even shorter in wound strikes. Larvae leave the sheep to pupate in the soil and remain in the pupa for 3–21 days under summer conditions. Over-wintering pupae remain inactive until the soil temperature rises above 7°C.

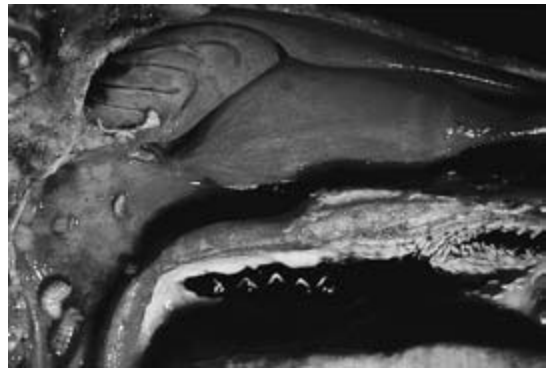

Figure 48.6: Third instar (L_3) *Oestrus ovis* larvae infesting nasal cavity. (Copyright, Veterinary Laboratories Agency, Weybridge.)

NASAL MYIASIS

Synonyms: *Oestrus ovis,* nasal bot fly, oestrosis

Cause

The nasal bot fly (*Oestrus ovis*) is a parasite of sheep throughout the world.

Clinical signs

In the UK, signs of oestrosis are most prominent in May, appearing in flocks as early as March but still recorded in November. Signs include nasal discharge (rhinitis), sometimes haemorrhagic, sneezing, wheezing breath, snorting, head shaking, unthriftiness, rubbing noses against the ground, head tossing, nervous excitability, 'gadding', and sometimes blindness, pneumonia and death. Larvae (particularly L_1) can also be recovered from animals not demonstrating any clinical signs. Clinical oestrosis can be confused with scrapie, psoroptic otoacariasis or even sheep scab.

Epidemiology and pathogenicity

Adult flies are larviparous, depositing L_1 larvae, 1.3–3.5 mm in length, into the nasal cavity. L_1 larvae migrate to the frontal, maxillary or palatine sinuses (via the ethmoid process) where they moult into the second (L_2) instar and eventually into the third (L_3) instar (Figure 48.6). L_3 larvae leave the sinuses, after approximately 35–40 days (in the summer) to be sneezed out to pupate in the soil. The pupal period can last 17–70 days and is extremely temperature-dependent. Exposure to temperatures below 16°C and above 32°C are lethal and the optimum temperature has been recorded as 27°C. In the UK, there appears to be only one wave of adult flies, consisting of two overlapping generations. The first generation originates from an overwintering population of larvae emerging in May and June which then deposits L_1 larvae in June or July. These larvae form the basis of the second generation, depositing L_1 larvae in August, September or October [17]. Larvae deposited by the second generation remain quiescent as L_1 larvae in the nasal turbinates until the following spring, when they continue their development.

An abattoir survey for the whole of Britain revealed a national incidence of 0.75 per cent with *O. ovis* was most prevalent in the warmer counties south of latitude 52˚ which may represent the northernmost range of the species. Veterinary Surveillance Report (VIDA) returns record a prevalence of 8.7 per cent for England and Wales, and 11.8 per cent in the counties where *O. ovis* was found. Localized surveys covering South Wales and south-west England recorded prevalences of 0.5 per cent and 0.75 per cent, respectively, but a survey in Surrey and West Sussex revealed a prevalence of 16.6 per cent. In one flock, shown to be infested, 51.3 per cent of ewes presented signs of oestrosis. In the same flock, ram lambs, confirmed as infested with L_1 larvae in October, did not show signs

of oestrosis. Areas further north could be foci of infestation, through the importation of infested sheep from the endemic south with warm summers allowing the establishment of temporary populations.

Oestrosis is more frequent in older ewes with over half reported to show signs in late summer. The prevalence of *O. ovis* varies from year to year. Higher incidences of infestation may follow years with unusually hot summers and thus a greater number of active adult flies challenging sheep.

SARCOPHAGID FLIES

The family Sarcophagidae includes the obligatory parasitic species, *Wolfahrtia magnifica,* which occurs in Greece, North Africa, Asiatic Russia, Asia Minor and Central Europe. Females are larviporous, depositing 120–170 L_1 larvae on wounds or body openings, ear, nose and eyes. Once deposited the larvae mature quickly, burrowing into tissue, causing extensive damage, the results of which are often fatal. The larvae are fully grown within 6–7 days and leave the host to pupate in the soil.

TREATMENT AND CONTROL OF FLY PROBLEMS

Sheep keds can be controlled by plunge dipping in wash containing the OPs diazinon, propetamphos or the SPs, flumethrin or HCC or the use of SP-based pour-on (backline) treatments. The systemic endectocides (ivermectin, doramectin or moxidectin) are also effective.

For head flies, pour-ons containing cypermethrin or HCC or spot-ons containing deltamethrin are effective in preventing attack.

Currently, the control of blowfly strike is through the prophylactic use of insecticides, either the OPs applied as a plunge dip or the SP, HCC, applied either as a plunge dip or pour-on. The insect growth regulators, cyromazine and dicyclanil, are highly effective against blowfly strike, but have no efficacy against other sheep ectoparasites.

Shearing temporarily reduces the risk of strike, but susceptibility increases as the fleece grows. Crutching and dagging, the removal of the soiled wool from around the breech and inside the hind legs, is an effective control of breech strike. The operation is best started in early April and must be repeated every 4–6 weeks to remain effective. Tail docking will also reduce the incidence of breech strike. Trimming wool around the the opening of the prepuce of rams will reduce the incidence of pizzle strike. In Australia, where the Merino is the principal breed, the techniques of modified and radical mulesing are used to amputate skin from each side of the perineum to remove the wrinkled skin of the breech and widen the bare surface of the perineum.

Some evidence points to the possibility that some attraction factors are hereditary and breeding ewes and rams that are continually struck should be culled, as should ewes with deformed genital openings, where urine is directed onto the fleece or ewes with narrow breeches that favour soiling.

Good husbandry in preventing other skin infections will greatly benefit strike control. Mycotic dermatitis (lumpy wool, *Dermatophilus congolensis*) can be a predisposing factor. Excessive chewing of the fleece in order to relieve irritation by ticks or lice can increase fleece moisture and, together with bites and open wounds through excessive rubbing, can attract blowflies. Helminth parasites should also be controlled to reduce the prevelance of scouring. In Australia, the incidence of breech strike was reduced from 50 to 5 per cent after anthelmintic drenching. Control of scouring caused by changes in diet and digestive disturbances due to lush grass is also essential. Unlike other species of blowfly, *L. sericata* will not enter areas of low light intensity, consequently they are rarely encountered in houses or barns.

Oestrosis can be controlled through the use of doramectin-, ivermectin- or moxidectin-based injections or oral drenches containing closantel, ivermectin or moxidectin.

Resistance

Resistance to the organochorine insecticide, dieldrin, was reported in *L. cuprina* in Australia in 1958, New Zealand in 1961 and South Africa in 1964, and *L. sericata* in Ireland in 1968. Evidence has demonstrated that resistance can last a long time, with dieldrin-resistant populations of *L. cuprina* still detectable in Australia 24 years after the widespread

use of the insecticide. OP resistance in *L. cuprina* appeared in Australia in 1965 and South Africa in 1968. There has been no recorded case of OC, OP or SP resistance in *L. sericata* in the UK.

ZOONOTIC IMPLICATIONS

Some mites, such as *Sarcoptes scabei* from sheep, can infest humans temporarily, producing a pruritic rash of red blisters, particularly on the skin of the inner forearm. The infestation is usually short-lasting and the rash disappears within 7–15 days.

Not uncommonly in the UK, *Oestrus ovis* may deposit L_1 larvae in the human eye (but not the nasal cavities). The larvae rarely develop beyond L_1.

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49

Photosensitization

A. Flåøyen

Synonyms: yellowses, saut, plochteach, alveld, facial eczema, geeldikkoop

Photosensitization in sheep is a problem both of economic and welfare importance in various parts of the world [1, 2]. Over 500 000 head of small ruminant livestock were reported to have been photosensitized in severe outbreaks of geeldikkop (literally 'yellow-thick-head') in South Africa, and production losses due to severe outbreaks of facial eczema in livestock from New Zealand have been calculated to be in the order of NZ$100 million (approximately £34 million) [1]. In some years in some flocks in Norway up to 30–50 per cent of the lambs will suffer from alveld (elf-fire) after ingestion of bog asphodel (*Narthecium ossifragum*) [2].

CAUSE

Normally photosensitization diseases are divided into three categories [1].

Type I: primary photosensitization

This arises when the photosensitizing agent is absorbed directly from the digestive tract and transported by the blood to the skin, where it causes the characteristic lesions after sunlight exposure.

Type II: photosensitization due to aberrant pigment synthesis

In this case the photosensitizing substance is a pigment, not normally found in animals, that is produced endogenously by an aberrant metabolic process. Alternatively, the aberration may be the excessive formation of a pigment that is ordinarily produced only in small, harmless amounts.

Type III: hepatogenous photosensitization

This occurs when a toxin, normally produced by a plant, fungus or alga, causes liver damage or dysfunction, resulting in the retention of the photosensitizing agent phytoporphyrin (the name phylloerythrin has been abandoned in favour of the new name) [3]. Phytoporphyrin is a metabolic product of chlorophyll produced by rumen micro-organisms, which normally is conjugated by the liver and excreted into bile. Some types of lesions or dysfunction lead to depression or cessation of hepatic elimination of phytoporphyrin, which therefore can reach the skin where photochemical reactions with sunlight occur.

All economically important photosensitization diseases of sheep are of type III.

Most of the photosensitizing agents generate oxidative reactions that require molecular oxygen for the reaction to proceed. Sunlight in a given wavelength range corresponding to the absorption spectrum of the photosensitizing molecule excites the molecules and transfers their energy, either directly to biomolecules or to oxygen. If the reaction is with oxygen, singlet oxygen or oxygen radicals are generated causing pathological changes. Such changes include abnormal cell division, alteration in membrane permeability and active transport processes, interference with glycolysis and cellular respiration, disruption of protein and DNA synthesis, mithochondrial damage, lysosomal damage, and cell death [4]. Phytoporphyrin is located and probably causes damage mainly in the Golgi apparatus and mitochondria [5].

CLINICAL SIGNS

In general, only unpigmented sheep or sheep with unpigmented areas of skin become photosensitized. The clinical signs are similar regardless of the type of photosensitizing agent [1, 6] (Figure 49.1). The most salient clinical signs are restlessness, head shaking, scratching of the face and ears with the hind feet, and rubbing of irritated skin against the ground or trees. The skin changes develop rapidly and include oedema and reddening. The eyelids, muzzle and lips become swollen and turgid. However, the most obvious signs in serious cases are thickened, oedematous, heavily drooping ears. Signs progress to include seepage of sticky, honey-coloured serum from the thickened skin that then forms extensive scabs that mat the covering hair after 1 or 2 days. Secondary bacterial infection of the skin is a normal finding in severe cases. In addition, jaundice often is visible in animals suffering from hepatogenous photosensitization.

Photosensitized sheep will try to avoid exposure to direct sunlight and are often found hiding under trees, bushes or rocks. Photosensitized lambs are often separated from their dams. The condition is painful and affected animals stop eating and drinking. Many will die from dehydration and/or as a result of secondary infections if not put in a dark place and given appropriate treatment. In severe cases, lesions of the eye and surrounding tissues interfere with vision and animals may be drowned in bogs and streams. In animals surviving the acute phase of the disease, the affected skins dries and cracks and the ears curl, and part, or all, of the ear may be lost from necrosis.

Figure 49.1: A 10-week old lamb photosensitized after ingesting bog asphodel (*Narthecium ossifragum*).

IMPORTANT PHOTOSENSITIZATION DISEASES

Yellowses, saut, plochteach or alveld

This is the most important photosensitization disease of sheep in the British Isles and the rest of northern Europe. It is a hepatogenous photosensitization associated with grazing of the lily, bog asphodel (*Narthecium ossifragum*) (Figure 49.2 in the colour plate section). Bog asphodel is a loose-to-dense clonal, perennial herb, up to 40 cm tall, with a creeping rhizome. The plant occurs on oligotrophic, mesotrophic, and eutrophic peat deposits in Scandinavia (69°42′N) to the British Isles, The Netherlands, Belgium, north-western Germany, western and central France, northern Spain and eastern Portugal [6]. Photosensitization of sheep grazing this plant has been reported in the British Isles, Norway and the Faroe Islands [1, 2, 7]. The disease occurs normally only in lambs 2–6 months of age and rarely is seen in adult sheep. Breed differences in susceptibility to the disease have been demonstrated [1]. Differences in microsomal enzyme activities have been suggested to explain the observed variation in susceptibility between lambs and adult sheep and lambs of different breeds. However, lambs have been found to ingest more *N. ossifragum* than their dams (unpublished results), thus the difference in susceptibility between lambs and adult sheep may simply be dose related. Typical of the disease is its sporadic occurrence, as bog asphodel does not always seem toxic to grazing sheep and it is difficult to reproduce the disease during dosing experiments. The first cases occur in May, increase in incidence during June and July, and normally cease in August. There is no firm evidence concerning the distribution of the disease in the British Isles, and its overall economic significance is unknown (P. Gould, personal communication). However, in some areas up to 10 per cent of lambs may be affected (Chapter 56). In Norway,

the disease occurs all along the west coast of Norway up to 69°42′N and, in several areas, is a major threat to the sheep industry. Up to 30–50 per cent of lambs can be affected in severe outbreaks. The incidence of the disease in Norway is much greater during cold and rainy summers. It is thought that the plant is more toxic in cold and rainy weather than in warm and dry weather.

It has been suggested that steroidal saponins in bog asphodel cause the liver lesions, which result in retention of phytoporphyrin [2], At least eight other plants containing steroidal saponins are also reported to cause hepatogenous photosensitization of sheep [2]. These are *Agave lecheguilla* (causes photosensitization in sheep in the USA), *Tribulus terrestris* (South Africa, USA, Argentina, Australia and Iran), *Brachiaria decumbens* (Australia, Malaysia, Indonesia, Nigeria and Brazil), and five types of millet [*Panicum dichotomiflorum* (New Zealand), *P. schinzii* (Australia), *P. miliaceum* (New Zealand), *P. coloratum* (USA and South Africa) and *P. virgatum* (USA)]. Whether the saponins are the sole cause of the diseases or not is still debated, but all the diseases are characterized by an accumulation of sapogenin crystals in hepatocytes, in and about the bile ductuli and in the bile ducts. The saponins exist as glycosides in the plants, which on ingestion are hydrolysed by the ruminal micro-organisms into free sapogenins (a saponin is a sapogenin conjugated to a sugar group at C3 of the steroidal skeleton). The two sapogenins of bog asphodel, sarsasapogenin and smilagenin, are further converted into epi-forms, absorbed from the jejunum, transported via the portal vein to the liver, conjugated with glucuronic acid and excreted into the bile as β-D-glucoronides, either of episarsasapogenin or epismilagen [2]. The livers of lambs that become photosensitized after ingesting bog asphodel contain increased concentrations of conjugated sapogenins [8].

Normally, there are no macroscopic lesions in the livers of lambs suffering from the disease, except for the yellow discoloration due to jaundice that can be seen in some cases. The histopathological changes are dominated by single cell necrosis of hepatocytes, portal fibroplasia and bile duct proliferation [2, 8]. Crystalloid clefts can be found in bile ducts or Kuppfer cells in some cases.

In addition to photosensitization of sheep, bog asphodel has been reported to cause nephrotoxicity in cattle in Northern Ireland [9] and in Norway [10]. The nephrotoxin has been identified to be 3-methoxy-2(5*H*)-furanone [11]. Sheep are also susceptible to 3-methoxy-2(5*H*)-furanone, but field cases have not been reported, probably because sheep develop increased tolerance to the nephrotoxin [9].

Geeldikkop

Tribulus terrestris is a highly nutritious plant that is often grazed by sheep in the Karoo area of South Africa, where it sporadically causes severe outbreaks of geeldikkop [12]. The disease has also been diagnosed in the USA, Argentina, Australia and Iran. Typically, outbreaks occur when *T. terrestris* wilts during the hot, dry spells that follow summer rains. The plant contains at least nine sapogenins, but only crystals of β-D-glucoronides of episarsasapogenin and epismilagen have been found in the bile. The pathology of geeldikkop is similar to that of yellowses/alveld.

Facial eczema

Sporidesmin produced by the saprophytic fungus *Pithomyces chartarum* cause photosensitization in sheep, cattle, goats and fallow deer [1]. The disease occurs in animals grazing improved pastures and causes severe problems in New Zealand. It is also diagnosed in Australia, South Africa, the USA, Argentina, France, Spain, Uruguay and Paraguay. The disease occurs only in specific weather conditions when *P. chartarum* grows rapidly and sporulates freely during periods when high humidity is combined with grass-minimum temperatures of 12°C or greater on two consecutive nights. Icterus, periportal fibroplasia and bile duct proliferation are the dominating findings of animals suffering from facial eczema. Degeneration and necrosis of hepatocytes can also be seen.

Lantana camara intoxication

L. camara is native to central America and Africa and has become distributed throughout many tropical and subtropical areas of the world. Intoxication of ruminants has been reported from Australia, India, Africa and the Americas [1, 12], but intoxications also probably occur in most countries where the plant grows. The toxic compounds of *L. camara* causing hepatogenous photosensitization are pentacyclic triterpene acids, of which lantadene A and B are the most important. Icterus is common in *L. camara*-intoxicated

animals. Characteristic histological findings in the liver include swelling and degeneration of hepatocytes, single cell necrosis of hepatocytes, bile duct proliferation and fibrosis.

Microcystis aeruginosa intoxication

Sporadic outbreaks of hepatogenous photosensitization caused by a cyclic heptapeptide produced by *M. aeruginosa*, a cosmopolitan freshwater, blue–green alga, have been reported in South Africa, North America and Norway [1, 12]. Massive hepatic necrosis occurs in peracutely intoxicated sheep. In less severe cases, moderate to severe fatty degeneration of hepatocytes and necrosis of single and small foci can be present. Fibrosis and bile duct proliferation are present in more chronically affected sheep.

Hypericism and fagopyrism

Both conditions are primary photosensitization diseases (type 1). Hypericism occurs in animals ingesting plants of the genus *Hypericum*; St John's wort (*Hypericum crispum*) is the most common cause of the disease [1]. Cases have been reported in western USA, Australia, Europe, New Zealand and Iraq. Hypericin, the photosensitizing pigment of *Hypericum*, is present in minute glands located on different plant organs in various species. The definitive photodynamic action of hypericin that causes photosensitization is unknown.

Fagopyrism occurs in sheep ingesting buckwheat (*Fagopyrum esculentum*) [1]. Fagopyrin, a pigment found mainly in the flowers and seeds, normally causes the disease. However, all parts of the plant (fresh and dry) are capable of causing photosensitization. The chemical structure of fagopyrin is similar to the structure of hypericin.

Type II photosensitization is reported to occur as an inherited congenital disease in Southdown and Corridale breeds of sheep.

DIAGNOSIS

There are no commercial tests available to determine whether or not photosensitization is present, thus the diagnosis must be based on the clinical signs, which are practically the same in all photosensitization diseases regardless of cause. In some cases it can be difficult to distinguish between photosensitization and other dermatitides. The serum activities of aspartate aminotransferase (AST), glutamate dehydrogenase (GLDH) and γ-glutamyl transferase (GGT) normally are increased in animals suffering from hepatogenous photosensitization. In each case the aetiological diagnosis must be based on information of the diet and age of the affected animal, as well as the climate and the time of year. A spectrophotometric method for detection of phytoporphyrin in plasma or sera has been developed. It has so far only been used for experimental purposes [13].

TREATMENT, PREVENTION AND CONTROL

Animals suffering from photosensitization should be put into a dark place immediately to avoid further reaction of the photosensitizing agent to sunlight. The animals will often have difficulties eating and drinking, and it is very important to prevent dehydration. In some cases, in the early phase of the diseases, glucocorticoids and antihistamines will reduce the swelling of the head and facilitate healing. Antibiotics may be administered to avoid septicaemia and severe bacterial dermatitis.

The only known way of preventing the diseases, except for facial eczema, is to keep the animals away from the dangerous pastures. Facial eczema can be prevented by breeding for resistance, oral dosing with zinc and spraying of pastures with fungicides [1].

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Ocular diseases

B.D. Hosie

INFECTIOUS KERATOCONJUNCTIVITIS

Synonyms: contagious keratoconjunctivitis (CKC), contagious ophthalmia, inclusion body keratoconjunctivitis, pink eye, heather blindness, snow blindness

Infectious keratoconjunctivitis occurs in sheep throughout the world. The vast majority of outbreaks are dealt with by flock-masters without veterinary assistance. While the disease appears to be painful and distressing to the affected sheep, it usually is of little economic consequence except for the cost of treatment. However, in the Alps, sheep act as a reservoir of infection for wild Caprinae such as chamois and ibex, among which mortality can reach 30 per cent when blind wild animals fall from cliffs or starve to death [1]. All ages of sheep may be affected, and cases occur at all seasons of the year. Pregnancy toxaemia and agalactia are possible sequelae in heavily pregnant ewes that cannot feed properly through blindness.

Cause

Mycoplasma conjunctivae is now considered the primary causative agent of infectious keratoconjunctivitis in domestic and wild sheep and goats. This organism has been detected in cases of infectious keratoconjunctivitis throughout the world, including Europe, America and Australasia. There are several reports of experimental reproduction of keratoconjunctivitis following intra-ocular instillation of *M. conjunctivae* [2–4]. The incubation periods varied from 1 to 21 days, probably reflecting a variation in the titre of the inoculum. Experimental challenge generally produces a milder keratoconjunctivitis than is seen in the field, except for one study in which the clinical signs were described as being identical to field cases [2]. Neither time of onset nor the severity of the clinical signs was influenced by corticosteroid [2]. Experimental work has demonstrated the persistence of *M. conjunctivae* in the eyes of sheep for up to 3 months after apparent recovery. This persistence of latent *M. conjunctivae* infection is consistent with isolation of the organism from both infected and uninfected eyes of sheep in field studies. The lipoprotein S adhesin (LppS) of *M. conjunctivae*, encoded by the LppS gene, is strongly implicated in the adhesion of *M. conjunctivae* to lamb joint synovial cells [5]. LppS is likely to play an important role in mycoplasma– host cell interactions and is a virulence factor for *M. conjunctivae*.

There were occasional reports of chlamydia and other mycoplasma species as the cause of outbreaks of infectious keratoconjunctivitis. However, experimental attempts to reproduce the disease with chlamydia gave variable results, and the classical techniques employed to culture and identify mycoplasma are cumbersome and require specialized technical expertise. The possibility that *M. conjunctivae* failed to grow cannot be excluded.

Bacteria

Many different genera of bacteria have been isolated from both clinically normal and affected eyes of sheep. However, *Branhamella ovis* and *Escherichia coli* occur more frequently in affected eyes and, in one study *Staphylococcus aureus* occurred more frequently in mildly affected eyes [6]. These bacteria may monopolize the primary lesion caused by *M. conjunctivae* and cause a more severe form of ovine keratoconjunctivitis. However, experimental evidence is lacking for the proposition that *B. ovis* and *M. conjunctivae* act concurrently to cause ulceration.

Clinical signs

All descriptions of the clinical appearance of ovine keratoconjunctivitis are similar regardless of the causal agent identified. One or both eyes may be affected. Four clinical stages of ovine keratoconjunctivitis are recognized [7]:

- *Stage I*: hyperaemia of the palpebral and the bulbar conjunctival vessels, serous lachrymation, increased blinking and blepharospasm. The corneo-scleral junction may show congestion in preparation for vascular migration (pannus) into the cornea. Most cases do not progress beyond this stage but regress spontaneously.
- *Stage II*: continuation of stage I, characterized by corneal inflammation with blood vessels and pannus spreading from the corneo-scleral margin. Early keratitis causes great irritation and, consequently, the blepharospasm and lachrymation are more obvious. Again spontaneous regression also occurs (see Figure 50.1 in the colour plate section).
- *Stage III*: progresses from stage II to a more mucopurulent keratitis with extension of migrating blood vessels and a more purulent lachrymal discharge. Sometimes shallow corneal ulceration is apparent, which affects vision. These cases are very obvious and usually receive treatment.
- *Stage IV*: corneal ulcer develops and vision is lost. Pus may be present in the anterior chamber of the eye (hypopyon). These cases are slow to resolve even with treatment. Corneal scarring may persist permanently following resolution.

Lymphoid follicle hyperplasia causes the conjunctiva and third eyelid to appear granular and nodular. Frequently, the disease is less severe in lambs and most cases do not progress through all the stages described. Corneal ulceration also is rare in lambs. Relapses in treated and naturally recovered animals commonly occur in infectious keratoconjuncivitis.

Pathology

In the acute stages of *M. conjunctivae* infection, the conjunctiva and cornea are infiltrated with large numbers of neutrophils and small numbers of plasma cells and lymphocytes. In one study, the neutrophil count rose 1 day after inoculation to a peak 8 days after inoculation [2]. It then decreased from day 13 to return to negligible values after day 25. Samples of conjunctival cells can be taken readily by scraping the conjunctival fornix with a rounded, blunt spatula after local anaesthesia by the installation of 0.4 per cent oxybuprocaine hydrochloride. The cells are then smeared on to slides, fixed and stained by the Giemsa method. The presence of large numbers of unipolar, bipolar or tripolar bodies of approximately 250–1100 μm in diameter closely associated with epithelial cells is typical of *M. conjunctivae*. These bodies specifically fluoresce when antiserum to *M. conjunctivae* is used.

Diagnosis

Infections are by far the most common cause of keratoconjunctivitis in sheep. The clinical diagnosis is readily made after foreign bodies and entropions are eliminated. The differential diagnoses include pine or cobalt deficiency, pasteurellosis and bluetongue. The diagnosis is made usually on the clinical signs and response to specific treatment.

The ready availability of molecular techniques has transformed the approach to the detection of *M. conjunctivae*. Its isolation from ocular swabs transported in mycoplasma transport medium requires appropriate high-quality artificial media and scientists highly skilled and experienced in the culture and identification of mycoplasma. Now swabs should be moistened in saline or mycoplasma transport medium before they are gently rubbed over the eye and inserted into the fornex behind the third eyelid. They can then be sent in a protective sleeve to the laboratory for *M. conjunctivae* detection using molecular techniques.

Various molecular techniques for the detection of *M. conjunctivae* are described. One is a nested technique with a *Mycoplasma* genus-specific primer pair in the first step followed by a specific polymerase chain reaction (PCR) assay based on unique sequences of the *rrs* genes (16S rRNA) of *M. conjunctivae* from the second [8]. Another is based on PCR of the 16S rRNA gene with *Mycoplasma*-specific primers followed by separation of the PCR product according to primary sequence using denaturing gradient gel electrophoresis [9]. *M. conjunctivae* is one of the 67 species detected and differentiated using this single generic test.

Detection of specific antibodies to *M. conjunctivae* by an indirect enzyme-linked immunosorbent assay (ELISA) test is used for diagnosis and epidemiological studies [10]. Serospecific antigens of *M. conjunctivae* were separated from cross-reacting antigens by the extraction of Tween 20-soluble membrane proteins. The protein extract was used to coat the microtitre plates.

All the bacteria associated with ovine keratoconjunctivitis are readily cultured aerobically on 5 per cent blood agar. Identification follows standard bacteriological techniques.

Epidemiology and transmission

M. conjunctivae is probably widespread, endemic and self-maintaining in sheep populations throughout the world. In Switzerland, an indirect ELISA revealed a flock seroprevalence of almost 90 per cent with 57 per cent of sheep in positive flocks being seropositive [11]. Mixing sheep with mild or inapparent latent infections is the principal means of transmission of keratoconjunctivitis between and within flocks. The disease is most readily spread when sheep are in close contact, during feeding at troughs, close yarding, transportation or when ewes are herded together for mating. Flies that frequent the head and eyes are believed to play an important role in transferring *M. conjunctivae* from sheep to wild Caprinae [1].

Outbreaks occur at all seasons of the year but can be more obvious in the winter, when sheep are gathered for mating, feeding or housing. Cases in adult sheep in the summer may be more severe, perhaps exacerbated by the greater intensity of sunlight.

Treatment

A great many topical and parenteral antibiotic preparations are used to treat infectious ovine keratoconjunctivitis. Critical evaluation of various treatments in several field outbreaks found that the most effective was a single intramuscular injection of a long-acting formulation of oxytetracycline [12].

Products containing chloramphenicol were ineffective, while those containing penicillin and dihydrostreptomycin gave a slow response. This outcome is not unexpected given the pathological significance of *M. conjunctivae* in infectious ovine keratoconjunctivitis. While products containing chloramphenicol or penicillin are likely to have an effect on secondary bacterial infections, they are unlikely to have any effect on mycoplasmas.

Experimental studies [13] have shown that a single intramuscular injection of oxytetracycline dihydrate given at the onset of clinical signs halted further development of clinical conjunctivitis. If treatment was started when the conjunctivitis was at its most severe, clinical cure was effected with 4 days. However, therapeutic treatment did not eliminate *M. conjunctivae* infection. This latent or carrier state may provide a source of infection for uninfected animals and an explanation for the relapse of recovered cases.

Infectious ovine keratoconjunctivitis typically causes more severe clinical signs in adult sheep than lambs. A single intramuscular injection of a long-lasting formulation of oxytetracycline is therefore recommended for the treatment of adult sheep with the additional application of aureomycin topical powder for severe cases. While parenteral oxytetracycline is also effective for the treatment of sucking or weaned lambs with infectious keratoconjunctivitis, treatment with aureomycin topical powder is generally sufficient. A single application proved sufficient in a hill flock [14], but several daily applications may be required to treat intensively managed lambs. Ideally, affected animals should be isolated following treatment, as relapses are common and antibiotic treatment does not eliminate the causal organisms. However, in some circumstances, such as a severe and widespread outbreak, parenteral treatment of all animals with a long-acting formulation of oxytetracycline should be considered in order to suppress the infection. More modern antimicrobials such as danofloxacin and tilmicosin are effective in the treatment of other disease conditions caused by mycoplasmas. However, their use cannot be recommended at present as no *in vivo* or *in vitro* studies have been undertaken with *M. conjunctivae*.

Prevention

Infectious keratoconjunctivitis occurs sporadically in many flocks, and purchased animals, such as rams, may introduce the disease into previously unaffected flocks. Sheep to be introduced to such flocks should be kept isolated for at least 2 weeks while they are checked for evidence of ocular disease. Although prophylactic treatment with parenteral oxytetracycline may reduce the titre of any infection carried by these animals, it will not eliminate latent *M. conjunctivae* infections [13].

Control

In lambs, the disease is usually so mild that control is not attempted. However, mixing of affected and unaffected groups is undesirable while clinical disease is present and, subsequently, while latent carriage is likely to be widespread. The control of flies and provision of adequate space in houses and particularly at feed troughs is also recommended.

BRIGHT BLINDNESS

A possible consequence of prolonged ingestion of bracken (*Pteridium aquilinum*) is bright blindness, a progressive retinal degeneration. Animals over 2 years of age are most commonly affected and incidence is highest in animals 3–4 years old (see Chapter 56).

OPHTHALMITIS ASSOCIATED WITH *LISTERIA MONOCYTOGENES*

An ophthalmitis preceded by conjunctivitis due to *Listeria monocytogenes* infection has been described in cattle [15] and sheep [16] fed baled silage from hoppers or ring feeders. Clinical examination revealed blepharospasm, cloudiness of the cornea and a swollen, folded iris with pupillary construction. In some sheep, floccular material was present in the anterior chamber of the eye and a catarrhal conjunctivitis. *L. monocytogenes* was isolated from conjunctival swabs using standard bacteriological techniques. The response to topical treatment was poor but, when combined with parenteral ampicillin, resolution was obtained within 2 weeks [16]. The condition

may be prevented by reducing ocular contact with silage through feeding in troughs.

PARASITIC KERATOCONJUNCTIVITIS

Thelazia californiensis, the nematode eye-worm, is sometimes found in the conjunctival sac or on the surface of the cornea and may cause discomfort and contribute to the development of conjunctivitis. Its pathogenic importance is uncertain. The life cycle is indirect, with flies (*Musca* spp.) acting as intermediate hosts. They deposit larvae of the worms in the conjunctival sac when feeding round the eyes. Systemic anthelmintics are likely to be effective in removing these nematode parasites, but reliable data are lacking.

DEVELOPMENTAL ABNORMALITIES

Entropion

Congenital entropion is a common cause of traumatic keratitis in neonatal lambs. Examination reveals the lower lid rolled inwards, causing the hairs to rub on the surface of the cornea. There is a secondary keratoconjunctivitis, epiphora and squinting. The severity is variable. Mild cases can be treated by a subconjunctival injection of an antibiotic such as a penicillin, which everts the lid. Michel clips can be used to staple a vertical fold of the lower eyelid in more severe cases. In the most severe cases, surgical removal of a strip of skin from the lower eyelid is advisable. The operation requires anaesthesia and disinfection of the site prior to surgery (see Chapter 74). Entropion is an inherited condition, and rams that produce offspring with entropion should not be used for further breeding.

Palpebral coloboma

This is a rare condition in which Manx Laughton sheep that possess four horns appear to be most susceptible. The upper eyelids are usually affected by coloboma (splitting). The mode of inheritance is unclear.

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Tick-borne diseases

Z. Woldehiwet

Although numerous viruses and rickettsiae have been isolated from ticks, only a few cause disease in sheep. Other than louping-ill (see Chapter 36), the most important are described in this chapter.

TICK-BORNE FEVER

Tick-borne fever (TBF) fever is a rickettsial infection in areas of Europe where sheep are exposed to vector ticks, particularly *Ixodes ricinus* (Figure 51.1). The disease was first described in 1932 in Scotland, but infection of sheep, cattle and other ruminants has since been shown to occur in other parts of the UK, Scandinavia and other regions of northern Europe and also in higher-altitude regions of southern Europe, including parts of Italy, Spain and the Balkans. The importance of the infection as a cause of abortion and as a predisposing factor in other infections (e.g. tick pyaemia, pneumonic pasteurellosis, listeriosis, louping-ill) for sheep farming in UK and Scandinavia has long been recognized, but evidence of infection and disease in non-ruminant species in Europe and USA has recently been found, with variants of the same species of granulocytic *Anaplasma* species, including clinical disease in humans, dogs, horses and cats. It is known that infection also occurs wherever infestation with *I. ricinus* or other competent vectors (e.g. *I. scapularis*, *I. pacificus*, *I. trianguliceps*) of reservoir hosts (including rodents) occurs, including parts of the Atlas and Taurus mountain ranges, across the northern hemisphere in North America and as far as the Pacific coast of Russia.

Figure 51.1: *Ixodes ricinus*, male on right and larger female on the left. (Courtesy of Dr A. Walker.)

Cause

Rickettsiae in the genus *Anaplasmataceae*, which multiply in cytoplasmic vacuoles predominantly in

granulocytes, are the casual agents of TBF. Different variants of *Anaplasma phagocytophilum* (formerly known as *Ehrlichia* [*Cytoecetes*] *phagocytophila*) are now considered to cause TBF in ruminants [1], while other variants of the same species cause human granulocytic ehrlichiosis (HGE), equine granulocytic ehrlichiosis (EGE) and canine granulocytic ehrlichiosis (CGE) in the USA and some parts of Europe [2].

Clinical signs

After natural exposure to ticks, the incubation period is 4–8 days. The main clinical sign is an acute febrile reaction which lasts for about 7 days, with temperature exceeding 41°C during the first 3–4 days of bacteraemia when the organisms can be detected in the neutrophils and eosinophils. During the febrile period, appetite is usually depressed; respiratory and pulse rates are elevated and coughing may be present. In non-pregnant animals, the importance of TBF is usually a serious exacerbation of other infections. Early researchers had observed that sheep and cattle with TBF displayed a range of clinical signs that were attributable to secondary infections, resulting in serious losses due to tick pyaemia, respiratory infections or systemic viral, bacterial or fungal infections. In pregnant ewes, abortion is a frequent sequel to infection, and the mortality rate in aborting ewes may be significant. In lambs, tick pyaemia is the most common complication of TBF, with up to 30 per cent of TBF-infected lambs developing a crippling lameness and paralysis following bacterial (usually staphylococcal) septicaemia, with joint localization resulting in lameness and paralysis if intervertebral localization occurs. Studies in the UK and Scandinavia have clearly established that TBF variants of *A. phagocytophilum* are immunosuppressive, resulting in several other disease syndromes including pneumonic pasteurellosis, septicaemic listeriosis and exacerbation of other viral (parainfluenza type 3 virus, orf, louping-ill), bacterial (*Chlamydophila abortus*, *Clostridium* spp.) and fungal infections.

Pathology

The most important pathological effects of TBF are the leucopenia and other haematological effects which accompany the febrile reaction. The leucopenia is due to an early lymphocytopenia and a prolonged neutropenia. A high proportion of neutrophils and eosinophils is infected with the organism during the period of bacteraemia, and during this period both B and T lymphocyte subsets in the peripheral blood are reduced. An initial reduction in the number of monocytes is usually followed by a period of a rapid reactive monocytosis and thrombocytopenia. The haemogram returns to normal after 2–3 weeks. Gross pathology is unremarkable unless secondary infections have significant outcome.

Diagnosis

The presence of high fever in animals that have been recently moved into tick-infested pastures or woodlands is one of the first indications of TBF in sheep. Exposure may also follow the introduction of ticks by sheep bought in from tick-infested pastures. However, the presence of other clinical signs such as tick pyaemia in lambs and respiratory and other signs affecting several animals and other secondary infections a few days after being introduced to tick-infested pastures are good indicators of TBF. The severe leucopenia, particularly the prolonged neutropenia, which accompanies the disease, is also a good indicator of infection. In some cases, abortion storms may occur, particularly when pregnant ewes are moved to tick-infested pastures during the final stages of pregnancy. In young lambs, the main clinical signs are likely to be those of tick pyaemia, the most common and serious complication of TBF. Confirmation of infection is based on the demonstration of typical inclusions in peripheral blood granulocytes and, occasionally, monocytes. The demonstration of specific DNA using primers specific to the 16S rRNA and other genes is increasingly being used to confirm diagnosis and for epidemiological studies in animals and vector ticks. Variants of the organism that cause TBF in sheep have recently been cultivated in tick cell lines *in vitro*, but this method is unlikely to be of diagnostic value because primary isolation takes 2–4 weeks. A rising titre of antibodies by immunofluorescent antibody test (IFAT) or by other serological methods can be indicative of recent infection but since exposure to the pathogen is common in some areas, a single sample is of little use.

Epidemiology and transmission

Infection is transmitted by nymphs and adult ticks; attachment of 24–48 hours is required for infection. Vertical transmission does not occur or is inefficient. The natural cycle of infection probably involves free-living ruminants, including deer and possibly rodents and other reservoirs. Recovered animals, which may harbour infection for up to 2 years after recovering from primary TBF, are thought to serve as reservoirs of infection as well.

Treatment, prevention and control

A high proportion of the 30 million or so sheep population in the UK is thought to live in the hilly, often tick-infested areas and hundreds of thousands of lambs develop tick pyaemia annually. Most of the lambs that develop tick pyaemia die or are of low economic value. A significant portion of the sheep that develop TBF may also die from other secondary infections and losses due to TBF-related abortions can be significant.

Oxytetracyclines are very effective for the treatment of uncomplicated and acutely febrile cases of TBF, resulting in a rapid decline in the fever within 24–48 hours.

Current control strategies are based on the reduction of tick infestations when sheep are turned out into pastures and the use of long-acting antibiotics as a prophylactic measure given before animals are moved from a tick-free environment into tick-infested pastures. As abortion storms of up to 90 per cent are common in naïve pregnant ewes, in-lamb ewes should not be brought on to tick-infested pastures. As a vaccine is unavailable, the reduction of ticks by regular dipping or pour-on application of synthetic pyrethroids may help to mitigate losses. Another alternative is a controlled exposure resulting in subsequent immunity through the introduction of ewes to infested pastures during low-risk (non-pregnant) periods. If it is necessary to introduce pregnant ewes or young lambs to tick-infested areas, prophylactic use of long-acting tetracyclines could be considered [1], although treatment would need to be repeated until infection of the majority of the animals had occurred. If tick pyaemia is a problem, a change of grazing should be considered for ewes with lambs at foot to reduce the level of infestations, or the use of pour-on acaricides. In marginal areas, pasture management may greatly reduce tick numbers on the pastures, but animals are at risk from ticks from feral animals or by sheep that gain access to infested pastures or woodlands through broken fences.

HEARTWATER

Former synonym: cowdriosis

Heartwater (HW) is an important constraint to small ruminant husbandry and improved production in much of sub-Saharan Africa, where transmission occurs by ticks of the genus *Amblyomma*. In susceptible breeds of sheep, infection is acute or peracute, with high mortality rates, particularly in sheep brought in from non-endemic regions. In west, east and central Africa, the principal vector is *A. variegatum* (Figure 51.2) and in South Africa, *A. herbeum*. Infection and potential disease appear to occur wherever nymphs and adults of *Amblyomma* species are present. In some countries (e.g. Ethiopia, Kenya and Sudan), such species include *A. lepidum* and *A. gemma*, whose distribution extends into semi-arid habitats. In parts of southern and northern Africa, conditions are too dry, and possibly cool, for vector survival, and in most countries vector distribution is uneven, which increases the possibility for disease occurrence in domestic and introduced stock. The disease is also present in several Caribbean islands, including Guadeloupe.

Cause

HW is caused by *Ehrlichia ruminantium* (formerly *Cowdria ruminantium*). The organism is genetically

Figure 51.2: *Amblyomma variegatum*, male on left and female on the right. (Courtesy of Dr A. Walker.)

and antigenically related to the rickettsiae in the genus *Ehrlichia* (*E. canis*, *E. chaffeensis*) [2]. *E. ruminantium* multiplies in cytoplasmic vacuoles within endothelial cells *in vivo* and *in vitro* and within neutrophils *in vivo*. Antigenic strain variation appears to be important; there is a lack of cross-immunity between isolates; small ruminants (particularly goats) appear to develop a more effective level of immunity than cattle.

Clinical signs

After tick infestation, the incubation period is 10–30 days, with an average of 14 days for transmission by nymphs and slightly longer for adult ticks. Sheep frequently undergo an acute febrile reaction with the temperature exceeding 41°C within 2 days of onset and a clinical course lasting 3–8 days. The clinical disease may range from peracute, with death after a rapid onset of pulmonary oedema and pleural and pericardial effusions, often with nervous manifestations, to subclinical with mild depression and transient febrile episodes. Severe respiratory embarrassment, manifested by abdominal breathing and anxious expression, frequently develops 3–4 days after the onset of fever, which usually leads to death within 24 hours, even after treatment with antibiotics. Other signs include loose faeces, fits, hypersensitivity to sound, chewing movement and other nervous signs, but not all signs are always observed. Mortality and morbidity rates may vary according to the strain of *E. ruminantium* and the breed and age of animal. Subclinical and mild cases without nervous signs are common in local indigenous ruminants and in very young animals of all breeds [3]. Mortality is above 50 per cent in non-indigenous breeds of sheep. After introduction of sheep to *Amblyomma*-infested areas, cases may occur rapidly, but in some sheep infections may not be apparent for months or even years. This may be related to rates of tick infestation and rates of infection of vector ticks and to sheep being not the preferred hosts for *Amblyomma* ticks if cattle or other ungulates are present.

Pathology

In sheep, gross pathology is variable. The main and characteristic finding at post-mortem is the presence of significant quantities of transudates in the pericardium ('heartwater') and in the thoracic and peritoneal cavities. The liquid frequently clots after exposure to air. Other lesions may include oedema of the lungs and the mediastinal and perirenal tissues, congestion of the liver, distension of the gall-bladder, moderate splenomegaly and petechiae and haemorrhages in various organs.

Diagnosis

Clinical signs are often atypical and, even when the most characteristic nervous signs are present, a long list of other diseases must be considered for differential diagnosis. Careful clinical examination may indicate respiratory embarrassment from pleural effusion, muffled heart sounds and dullness on percussion. The organism is often present in the neutrophils of peripheral blood but it is difficult to demonstrate this in fresh blood smears; 24–48-hour culture of neutrophils may increase the chances of detection. Ante-mortem diagnosis can also be attempted by microscopic examination of brain cortex smears obtained by biopsy. It is now also possible to detect specific bacterial DNA in peripheral blood or brain cortex by polymerase chain reaction (PCR).

HW can usually be diagnosed with some certainty after death. The findings at post-mortem are usually suggestive, but should be confirmed by examination of stained smears of the brain cortex for the presence of typical intracytoplasmic clusters in endothelial cells, by *in vitro* culture in endothelial cells or by PCR. For simplicity and safety at necropsy, brain smears can be made from match-head-sized pieces of cerebral cortex aspirated via a 14G needle through a nail hole punched in the cranium. In all cases, a careful analysis of history, including the recent sudden exposure to *Amblyomma* ticks of susceptible breeds of sheep brought in from HW-free areas is an important consideration.

Epidemiology and transmission

E. ruminantium is transmitted by nymphs and adults of the vector ticks. Ticks infected during the previous instars (larvae or nymphs) maintain their infection during the trans-stadial period and infection may be passed also through the sexual route, but transovarian transmission is not thought to be efficient. The

natural cycle of infection probably involves free-living ruminants, including buffalo, but in many parts of Africa this role may now mainly involve domestic stock. Recovered animals can be carriers of infection for extended periods and, in cattle, this has been postulated as a mechanism in the maintenance of enzootic stability. Compared to cattle maintained under the same conditions, sheep appear to be exposed to infection at a lower rate; this may reflect lower rates of tick infestation in sheep.

Treatment, prevention and control

The onset and course of the disease is rapid and once marked nervous signs and haemorrhagic diarrhoea have developed, treatment is ineffective. Treatment should not be delayed until laboratory confirmation is established. Tetracyclines are effective when given before the onset of nervous manifestation. Long-acting tetracyclines are preferred, making it possible to treat animals with only one injection.

A blood stabilate vaccine is used in Zimbabwe and South Africa, but it may not protect against other strains of *E. ruminantium*. The use of this vaccine is also hampered by the need for cold-chain facilities and treatment of reacting animals. Intensive research is underway to develop recombinant vaccines against HW and other intracellular rickettsiae. Where vaccination is not available, control of HW is based on the intensive or strategic use of acaricides in cattle and valuable sheep and goats. This usually involves weekly or monthly dipping with acaricides, which have 7–10 days' activity. Selecting for genetic resistance in sheep by cross-breeding with indigenous breeds of known disease resistance to HW and 'controlled natural immunization', by turning susceptible animals to tick-infested pastures followed by early antibiotic treatment during the early phases of febrile reaction, are other options of control. Prophylactic use of long-acting tetracycline preparations may also be considered if exposure to *E. ruminantium* challenge can be predicted to occur during a given period.

NAIROBI SHEEP DISEASE

Nairobi sheep disease (NSD) is a tick-borne viral disease of small ruminants in east and central Africa, characterized by fever, diarrhoea and high mortality rates. Serological evidence also suggests that the agent or closely related viruses may be present along the Indian Ocean seaboard of southern Africa as far as northern Zululand. A related virus (Ganjam virus) causes infections in sheep, goats and humans in India.

Cause

NSD virus (family Bunyaviridae, genus *Nairovirus*) is transmitted by *Rhipicephalus* ticks, trans-stadially and transovarially. *R. appendiculatus* is considered as the principal vector in Kenya and Uganda but *R. simus* and *R. pulchellus* have also been associated with NSD in some regions of east Africa, suggesting that other *Rhipicephalus* spp. may also be competent vectors.

Clinical signs

The onset of clinical NSD starts 5–6 days after the introduction of susceptible animals to pastures infested with *R. appendiculatus*. The first signs of disease are characterized by a steep rise in body temperature to above 41°C. This is quickly followed by watery diarrhoea, which is fetid in nature and containing mucus. As the disease progresses, the temperature rises above 41°C and the animals strain continuously; the faeces may contain frank blood. As nasal discharge and conjunctivitis are also common features of NSD, it can easily be confused with peste des petits ruminants (PPR) (Chapter 58). Mortality rates can be higher than 90 per cent, with death usually occurring within 2–7 days of clinical onset.

Pathology

Mucosal haemorrhages in the gastrointestinal tract, extending from the folds of the abomasum to the colon, are the most prominent features. The haemorrhages in the caecum and colon, which may look like striations, are prominent features of NSD, but similar lesions may also be present in PPR. In prolonged cases of diarrhoea, dehydration may be evident and the mesenteric lymph nodes may also be enlarged and swollen. In some cases severe glomerulotubular nephritis may be evident.

Diagnosis

The presence of the typical clinical signs and post-mortem lesions in sheep with a history of exposure to ticks in endemic areas of NSD can be useful in establishing a tentative diagnosis. However, some of the clinical signs can occur in cases of PPR, and NSD must also be differentiated from other tick-borne infections. Exposure to ticks may also result in HW at the same time with NSD affecting some animals, if *Rhipicephalus* and *Amblyomma* ticks are present in the same area, as is frequently the case in parts of east Africa, but HW is usually characterized by nervous manifestations despite the possible occurrence of diarrhoea and tenesmus. If death occurs at all ages, particularly lambs, Rift Valley fever (Chapter 62) must also be considered.

NSD can be confirmed by isolation of the virus in culture or the demonstration of viral antigens or nucleic acids by reverse transcription-PCR. The demonstration of rising antibody titres by virus neutralization of by enzyme-linked immunosorbent assay (ELISA) may indicate recent infection.

Epidemiology and transmission

Sheep and goats appear to be the natural reservoir hosts of the virus. The apparently low level of disease in endemic areas of east Africa has been attributed to the high exposure rate of lambs during the period in which they are protected by maternal antibody. Introduction of sheep from non-endemic areas, or the exposure of sheep after the waning of maternally derived immunity can lead to outbreaks of NSD, with mortality reaching as high as 90 per cent for indigenous breeds. Disease can also occur when, following prolonged rainfall, the distribution of vector ticks becomes wider than usual.

Treatment, prevention and control

Prognosis is poor, but supportive therapy may be justified for individual breeding stock. NSD can be controlled by maintaining enzootic stability, which requires early exposure to vector ticks or by strict vector control. In regions of east Africa where east coast fever is endemic the control of NSD has been based on the intensive or strategic use of acaricides. However, the reduction of *R. appendiculatus* has been reported to result in the emergence of sheep flocks which are highly susceptible to HW. The use of vaccines is likely to be the most effective method of control of NSD, but none is available commercially.

OVINE ANAPLASMOSIS

Synonyms: tropical anaplasmosis of small ruminants

Anaplasmosis is generally a benign, but persistent rickettsiosis of sheep and goats, which is endemic in tropical and subtropical Africa, Asia, parts of southern and central Europe and the western USA [4]. Infected animals remain as carriers of the infection for life.

Cause

Two species of *Anaplasma*, *A. ovis* and *A. mesaeterum*, are known to infect sheep and goats. *A. ovis* is more widespread and more pathogenic to sheep. Goats and deer are also susceptible to infections. *A. mesaeterum* was recently recognized, mainly in Europe, and appears also to be more pathogenic to sheep than goats. *A. ovis* is antigenically and genetically related to the bovine pathogen *A. marginale*.

Clinical signs

Clinical signs are usually mild or inapparent despite the bacteraemia lasting for nearly 2 weeks. The clinical phase lasts for 1–2 weeks and is characterized by anaemia resulting in pale mucous membranes, increased heart and respiration rates and laboured breathing following exercise. Recovery is often slow and infected animals remain as carriers for life; splenectomy and stress may provoke clinical signs, increased levels of bacteraemia, severe anaemia, jaundice and, usually, death.

Pathology

The main findings at post-mortem are associated with anaemia. The carcass is usually emaciated, with increased amount of fluid in body cavities.

Diagnosis

The presence of typical inclusion bodies in erythrocytes of sheep and goats with anaemia is sufficient to establish diagnosis, but the detection rate in carrier animals is usually low. *A. ovis* is found at the margins of the red cells while *A. mesaeterum* lies at the centre of the cell. Detection of antibodies by IFAT or ELISA using *A. ovis* or *A. marginale* antigens can be used for serological diagnosis in a flock and specific DNA can also be detected in the blood of infected animals by PCR using primers specific to the 16S rRNA or other genes.

Epidemiology and transmission

Vertical transmission has been demonstrated to occur by the transplacental route, and ixodid and argasid tick vectors are involved. Mechanical transmission by biting flies appears likely, although experiments were unsuccessful with sheep keds. The prevalence rates appear high in endemic areas.

Treatment, prevention and control

Because of the benign nature of the clinical disease, control measures are not usually practised on indigenous sheep and goats, but when exotic breeding stock are introduced to an endemic areas they should be monitored for any signs for 2–3 months; they can be given long-acting tetracyclines as a prophylactic measure. The drugs of choice are oxytetracyclines.

No vaccines are currently used but cattle can be immunized with live attenuated *A. marginale* or *A. centrale*.

MALIGNANT THEILERIOSIS OF SMALL RUMINANTS

Synonym: (malignant) ovine theileriosis

The benign form of theileriosis in sheep has a wide distribution, with *Theileria ovis* being present in Europe, the Middle East, Africa and parts of Asia, while *T. separata* is known to be present only in Africa. On the other hand, the more virulent form of ovine theilerosis, which is caused by *T. lestoquardi*, is endemic in the Middle East and is probably present in regions of north Africa and parts of China where bovine theilerosis due to *T. annulata* is endemic. In susceptible indigenous sheep moved from non-endemic to endemic areas, losses due to malignant theilerosis can be as high as 40 per cent and even higher in imported exotic sheep.

Cause

The main cause is the protozoan parasite *Theileria lestoquardi* (formerly *T. hirci*), but a different species of *Theileria* (*Theileria* sp. 1 and sp. 2) is reported to cause a serious fatal disease in small ruminants in northern China.

Clinical signs

In acute infections, the disease is characterized by loss of appetite, fever, nasal and ocular discharges, and swelling of superficial lymph nodes. Anaemia, icterus and blood-staining on faeces may be present also. Death may occur suddenly without any clinical signs becoming apparent. Recovery from disease is often slow and subacute disease, characterized by recurrent fever and anaemia, is also common in endemic regions. The clinical signs are less severe in lambs.

Pathology

Anaemia, enlargement of the liver, spleen and lymph nodes, and pulmonary oedema are common findings at post-mortem.

Diagnosis

The disease usually occurs after the introduction of susceptible sheep to endemic areas. Acute infection, which is characterized by fever, icterus and haemoglobinuria, can be confirmed by the demonstration of schizonts on smears prepared from lymph node biopsy or from lymphoid tissues at post-mortem. Piroplasms in erythrocytes are indicative of recent or non-recent infection but cannot be distinguished from the agents which cause the benign form (*T. ovis*). The detection

of specific DNA by PCR to amplify segments of the small subunit ribosomal RNA (ssu rRNA) is more specific and can be used to differentiate the virulent strains [e.g. *T. lestoquardi*, *Theileria* sp. 1 and sp. 2 (China)] from *T. ovis* and *T. separata* but it cannot distinguish between recent and chronic infection. Detection of antibodies by IFAT using homologous or heterologous (e.g. *T. annulata*) antigens can also be used to establish serological diagnosis in flocks, but the method lacks specificity.

Epidemiology and transmission

T. lestoquardi has been shown to be transmitted by *Hyalomma anatolicum anatolicum* but *Rhipicephalus bursa* has also been implicated and other ticks of the genus *Hyalomma* are likely to be competent vectors. The species of *Theileria* which causes fatal disease in ruminants in northern China is transmitted by *Haemaphysalis qinghaiensis*.

Treatment and control

Parvaquone and analogues, which are effective against bovine theilerosis, may also be effective against malignant theilerosis of small ruminants. The use of pour-on acaricides may be recommended to prevent infection of imported susceptible breeding sheep. An attenuated cell-culture-derived vaccine is used in Iran.

OVINE BABESIOSIS

Babesiosis of sheep and goats is widespread in most tropical and subtropical countries and in Europe and the Middle East. The main causes of babesiosis in sheep are *Babesia ovis*, *B. motasi* and *B. crassa* [5]. *B. ovis* is more pathogenic than *B. motasi* and *B. crassa*. They are transmitted vertically and from stage to stage by ticks of the genera *Rhipicephalus*, *Dermatocenter*, *Ixodes* and *Haemophysalis*.

Adult sheep and goats are more susceptible to infection than lambs and kids. The clinical signs may range from acute and severe to mild and chronic. The main clinical features are similar to redwater in cattle infected with *B. bigemina* or *B. bovis*, which is characterized by fever, jaundice, haemoglobinuria and anaemia.

Diagnosis is usually based on the presence of anaemia and other clinical signs in an endemic area and the demonstration of typical protozoa in peripheral blood. Cases that die suddenly may be confused with anthrax but ovine and bovine babesiosis is characterized by jaundice and haemoglobinuria. Mild cases of anaemia without haemoglobinuria must be differentiated from other causes (e.g. *Anaplasma ovis*). In milder cases, definitive diagnosis can only be established by the demonstration of the typical piroplasms in the red blood cells. The piroplasms of *B. ovis* are smaller than those of *B. motasi.* Serological methods are also frequently used for the diagnosis of subclinical babesiosis and for epidemiological studies but they lack specificity. The detection of specific DNA by PCR to amplify segments of the small subunit ribosomal RNA (ssu rRNA) using primers specific to *B. ovis* were recently reported; this method is very sensitive and specific [6].

Treatment based on diminazene aceturate or imidocarb diproprionate is reported to be effective. Dipping is not regularly practised but pour-on application may be used to protect imported breeding stock. The use of vaccines awaits further development.

OTHER TICK-BORNE INFECTIONS OF SHEEP

Ehrlichia ovis has been associated with occasional but severe outbreaks of disease in sheep in west Africa (Senegal, Mali) but it appears to be of little consequence elsewhere. Stress appears to be an important predisposing factor.

ZOONOTIC IMPLICATIONS

Few of the tick-borne ovine pathogens described in this chapter are of zoonotic concern; the following summarizes present knowledge.

Anaplasma

Some variants of *A. phagocytophilum* can cause the potentially fatal illness, human granulocytic

ehrlichiosis (HGE), in the USA and in some parts of Europe. However, to date there have been no documented cases of human disease associated with this bacterium in the UK, despite some serological evidence. HGE is characterized by flu-like symptoms within 5–10 days of exposure to a tick bite. As in TBF, the immunosuppressive effects may lead to life-threatening complications from exacerbation of bacterial or fungal infections, and hospitalization is frequent. Cases are undoubtedly greatly under-reported because of insufficient awareness by practitioners and, at the time of presentation, secondary infection may be more clinically important.

There is no evidence of zoonotic infection with *A. ovis, A mesaeterum* or *A. marginale*; unlike *A. phagocytophilum*, their host range appears to be limited to ruminants.

Ehrlichia

There is no evidence of zoonotic infection with *E. ruminantium* but humans have been shown to be susceptible to infection by the related organism *E. chaffeensis*.

NSD virus

Accidental infection is reported to result in fever, joint aches and general malaise. Antibodies against NSD virus have been found in human sera and other related viruses (e.g. Ganjam virus) are known to cause similar syndromes.

Theileria and Babesia

No cases of human infection have been reported.

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Part X
Metabolic and mineral disorders

52

Pregnancy toxaemia

N.D. Sargison

Synonyms: ketosis, twin lamb disease

Pregnancy toxaemia is a common disease of undernourished, stressed ewes carrying multiple fetuses, generally triplets and quadruplets, associated with a failure to adapt to the increasing metabolic demands of fetal growth during late pregnancy. All sheep breeds and management systems are affected, but the incidence is highest in lowground flocks of cross-bred ewes, typically involving 1–2 per cent of the ewes in well-managed flocks and up to 10 per cent in undernourished flocks. The mortality rate is high and treatment is expensive and generally unsuccessful unless the disease is recognized promptly. Control of pregnancy toxaemia therefore depends on effective flock management.

CAUSE

Pregnancy toxaemia results from the failure of ewes to meet the increasing demands for glucose of their multiple fetuses during the final 6 weeks of pregnancy. The particularly high susceptibility of ewes to pregnancy toxaemia is related to the fact that the total weight of their gravid uterus containing triplet fetuses may be as high as 27 per cent of their body weight at mating [1]. Clinical signs are often precipitated by some factor that reduces dietary energy intake, such as concurrent disease or poor nutritional management. The early clinical signs are essentially those of a hypoglycaemic encephalopathy, while advanced stages of the disease are associated with ketoacidosis and fatty liver changes.

In common with other ruminants, ewes' glucose requirements are mostly met by gluconeogenesis, rather than by direct absorption of carbohydrates. Dietary carbohydrates are first fermented in the rumeno-reticulum to acetic, propionic and butyric acids. Propionate is absorbed and metabolized to glucose in the liver. Acetate is metabolized in body tissues to long-chain fatty acids which are stored as lipids, and butyrate is mostly converted to β-hydroxy butyrate (BOHB) and absorbed. Certain amino acids derived from dietary protein and protein catabolism are also important in gluconeogenesis.

During the final 6 weeks of pregnancy, the gravid uterus occupies an increasing amount of the abdominal cavity, with a corresponding reduction of the rumeno-reticulum volume. Consequent lower dietary intakes, combined with the increasing fetal demand for glucose, mean that the energy requirements of late-pregnant ewes carrying multiple fetuses cannot be met by their dietary carbohydrate supply. Ewes cannot modify the glucose requirements of their fetuses, so when the fetal requirements are not met from the diet, they must draw on their body reserves. Under these circumstances, lipids are mobilized and free fatty acids produced are taken to the liver and oxidized via the tricarboxylic acid cycle to provide energy as acetyl coenzyme A (acetyl CoA). This biochemical pathway is dependent on a constant supply of oxaloacetate from propionate, which cannot be met due to the reduced dietary carbohydrate intake, leading to incomplete oxidation and production of aceto-acetyl CoA. Hydrogenation of aceto-acetyl CoA results in the formation of BOHB and acetoacetate, with acetone formed by decarboxylation [2]. While these ketones can be metabolized, their excessive production, combined with liver insufficiency due to the accumulation of triglycerides that accompanies lipolysis, leads to their accumulation proportional to the dietary carbohydrate deficit. During the final 6 weeks of pregnancy, plasma concentrations of BOHB less than 0.8, 0.8–1.6 and greater than 1.6 mmol/l represent adequately

nourished, moderately undernourished and severely undernourished ewes, respectively [3].

BOHB and acetoacetate are strong acids. Furthermore, prolonged urinary excretion of ketone bodies results in the loss of sodium and potassium ions, lowering the plasma alkali reserve. The resulting ketoacidosis causes dyspnoea and exacerbates the hypoglycaemic central nervous system depression, progressing to an irreversible stage, where there is dehydration and uraemia, often compounded by fetal death and autolysis *in utero*, toxaemia and metritis.

Prolonged hypoglycaemia leads to hyperactivity of the adrenal glands, with increased cortisol secretion, antagonism of the action of insulin and effective inhibition of glucose utilization.

CLINICAL SIGNS

Affected ewes are mostly in low body condition, although the disease is occasionally seen in excessively fat ewes, associated with stressful husbandry. During the early stages of the disease, affected ewes separate themselves from the rest of the flock, are reluctant to move and appear unaware of their surroundings, but are ambulatory and may show some interest in good-quality feed. Clinical signs of depression, unilateral or bilateral absence of a menace reflex, salivation, ear twitching and fine muscle fasciculations causing movement of the overlying skin of the face become more pronounced as the disease progresses. Ewes become sternally recumbent, stuporous and anorexic.

Some ewes die within a few days of the observation of clinical signs, while others survive for up to 10 days, becoming soaked in their own urine and developing extensive decubital sores. Death of fetuses *in utero* sometimes causes a temporary improvement in the condition of the ewe, but subsequent autolysis results in a rapid deterioration. Ewes may start to lamb, but the cervix seldom fully dilates and only decomposing fetal membranes are presented. Untreated cases which progress to a state of recumbency are invariably fatal.

PATHOLOGY

The gross post-mortem findings of pregnancy toxaemia are non-specific. Carcases are usually emaciated, with multiple fetuses in various states of decomposition *in utero*. The liver is swollen, pale or orange and friable, indicative of severe hepatic lipidosis. The adrenal glands may be hypertrophied and pale, or hyperaemic.

Histologically, the parenchymatous cells of the liver are distended with fatty globules. It has been estimated that the liver may contain 30 per cent fat on a wet weight basis, indicating triglyceride saturation of the liver cells.

Lesions consistent with hypoglycaemic encephalopathy have been described in the brain consisting of astrocytic nuclear swelling, hypertrophy and proliferation, and cerebrocortical neuronal necrosis, together with vacuolation of the cerebral and cerebellar white matter [4].

DIAGNOSIS

There is a need to establish an accurate diagnosis early, in order to expedite control measures. The clinical signs observed in the early stages of pregnancy toxaemia may also be seen in cases of listerial meningo-encephalitis, hypocalcaemia and polioencephalomalacia. Support for a differential diagnosis of ovine pregnancy toxaemia is provided by blood biochemistry, in particular the demonstration of BOHB concentrations greater than 3.0 mmol/l. The plasma glucose concentrations for confirmed cases of ovine pregnancy toxaemia range between 0.5 and 7.0 mmol/l (normal 2.2–4.0 mmol/l), with the degree of hypoglycaemia neither related to the severity of presenting clinical signs, nor to the ewe's ability to respond to treatment [5]. In many cases, plasma glucose concentrations are notably higher than the normal range, suggestive of poor glucose homeostasis and insulin insensitivity in stressed and ketotic pregnant ewes. Plasma glucose concentrations also rise significantly following fetal death. About 20 per cent of ewes with pregnancy toxaemia are also mildly hypocalcaemic with serum calcium concentrations less than 1.9 mmol/l, possibly associated with poor hydroxylation of vitamin D in ewes with severe hepatic fatty damage.

Post-mortem findings of multiple fetuses *in utero* and severe hepatic lipidosis are not specific, occurring secondary to prolonged anorexia of numerous aetiologies. Heavily pregnant ewes all show some

degree of fatty liver change, so the diagnosis of pregnancy toxaemia cannot be based on gross post-mortem findings alone. BOHB remains stable in aqueous humour for up to 48 hours following death and provides a useful diagnostic guideline, concentrations greater than 0.8 mmol/l supporting a diagnosis of pregnancy toxaemia [6].

Studies to produce prognostic indicators for pregnancy toxaemia have been mostly unrewarding. The serum fructosamine concentration, which reflects the ewe's energy status over the preceding 3–4 weeks, has been suggested as a prognostic index [7], but does not take into account an individual ewe's ability to respond to hypoglycaemia.

TREATMENT

Treatment must be instigated early during the course of the disease, otherwise it is invariably unsuccessful. Successful therapy depends on the ability to raise cerebrospinal fluid glucose concentrations, before irreversible changes occur in the brain. Ewes should be individually penned and offered fresh concentrates, good-quality hay and clean water. Various treatments including oral glycerol, glucose, propylene glycol [8] and concentrated rehydration solutions [9], intravenous glucose, anabolic steroids, B vitamins, insulin and recombinant bovine somatotropin (rBST) have been advocated. Anabolic steroids and rBST cannot be used in UK flocks and insulin injections are both unproven and uneconomic. It may take several days for a sustained rise in plasma glucose concentration to occur in ketotic sheep following daily intravenous glucose injections or oral dosing with glucose precursors such as propylene glycol or glycerol.

The treatment regime advocated by many UK veterinary practitioners includes oral administration three to four times daily of 160 ml of a concentrated dextrose and electrolyte rehydration solution. This is claimed to be effective in 68 per cent of cases, although the accuracy of the diagnosis of pregnancy toxaemia in the report [9] might be questioned. When oral rehydration solutions are administered to sucking lambs or calves, closure of the oesophageal groove causes their deposition in the abomasum and active absorption in the small intestine to cause rapid co-transport of water, glucose and sodium. The rationale for oral glucose–electrolyte therapy of ovine pregnancy toxaemia is based on this potential for rapid co-transport of glucose from the small intestine. However, when administered to adult sheep, concentrated oral rehydration solutions are mostly deposited into the rumeno-reticulum, where the glucose is rapidly metabolized by the microflora. It is therefore doubtful that significant amounts of glucose are rapidly absorbed following treatment with concentrated oral rehydration solutions.

An alternative approach to the treatment of pregnancy toxaemia involves removing the fetal glucose demands. Parturition can be induced in healthy ewes from about day 135 of pregnancy by injection of 16 mg of dexamethasone or betamethasone, with lambing about 36 hours later. Unfortunately, this method is less reliable in ketotic ewes, whose high endogenous cortisol levels may desensitize them to any drug-induced rise in corticosteroid concentrations. In particularly valuable ewes, Caesarean operation in the early stages of the disease may be an effective method of treatment.

In many cases, treatment is uneconomic and humane destruction should be considered to prevent further suffering whenever the prognosis is poor. In a study of 53 ewes lowground ewes with pregnancy toxaemia, only 33 per cent survived, despite intensive therapy including oral dosing with a concentrated rehydration solution and dexamethasone injection, with only 12 per cent of their potential lambs born alive [10]. The treatment response in hill ewes, which are often severely undernourished throughout pregnancy, is generally better than in lowground ewes. This is possibly because prolonged undernutrition results in compensatory intra-uterine growth retardation, decreased maternal thyroid activity and increased tissue insulin sensitivity.

The occurrence of pregnancy toxaemia usually indicates an urgent need to increase the energy nutrition of the flock. In the short term this can be achieved by introducing *ad libitium* treacle. In the longer term it may be necessary to increase the amount and/or quality of the concentrate feed, and/or change to higher quality hay or silage

PREVENTION

The poor treatment response of ovine pregnancy toxaemia cases and findings of the highest mortality rate

in ewes with the highest rates of tissue catabolism prior to the onset of disease support the need for flock preventive management. Correct feeding of the dam throughout pregnancy is essential. To achieve this, it is essential that ewes are mated in good body condition and correctly fed throughout pregnancy.

In order to ensure adequate body condition scores of 2.0–3.0 units on a scale of 1.0 (emaciated) to 5.0 (obese) during late pregnancy, ewes must be in target body condition score at mating of 3.0–3.5 or 2.5–3.0 for lowground and hill systems, respectively. While many factors contribute to ewe body condition score at lambing, ewes which are in poor body condition at mating are invariably in low body condition during late pregnancy and are predisposed to developing pregnancy toxaemia.

The traditional strategy of flushing ewes, involving provision of improved nutrition by means of a good grass sward or supplementary concentrate feeding for up to a month before and during the mating period must be adopted with caution. Under many management systems, the potential benefit of more lambs at lambing time is outweighed by the cost of nutritional management required to maintain multiple pregnancies, twin-bearing ewes in hill flocks and triplet-bearing lowground ewes being prone to pregnancy toxaemia.

Separation of ewes into different feeding groups based on: the results of real-time ultrasound scanning undertaken between days 45 and 90 of pregnancy to determine fetal numbers; keel marks to predict lambing dates; parity; and body condition score can serve to ensure adequate nutrition during late pregnancy, while avoiding wasteful overfeeding of late-lambing or single-bearing animals.

Dietary energy supply relative to metabolic demands of late pregnancy can be accurately determined by measuring ewes' serum or plasma BOHB concentrations. Experimental studies have determined the relationship between BOHB concentrations and nutritional metabolizable energy of single-, twin- and triplet-bearing ewes of different liveweights during different weeks of late pregnancy. While it is normal for the dietary metabolizable energy intake of late pregnant ewes to fall short of the requirements of twin and triplet litters, ewes with sufficient accumulation of colostrum in the udder and giving birth to healthy lambs of normal birth weight have serum BOHB concentrations of less than 1.1 mmol/l. Thus, the adequacy of metabolizable energy nutrition of late pregnant ewes can be confirmed by blood sampling a representative group of 10–12 ewes for BOHB concentrations [11].

A range of 3-OH butyrate concentrations is often encountered in a flock test, which is largely associated with fetal numbers. Thus, in those flocks where fetal numbers have not been determined, a target BOHB value of 0.8 mmol/l is used, while in flocks which have been ultrasound-scanned to determine fetal numbers, only twin- and triplet-bearing ewes are sampled and a value of 1.1 mmol/l is used. BOHB concentrations greater than 1.6 mmol/l in individual ewes represent severe metabolizable energy undernutrition, with the likelihood of pregnancy toxaemia developing as pregnancy advances, unless dietary changes are implemented. Sampling 3–4 weeks before the start of lambing, therefore, enables precise determination of any nutritional metabolizable energy deficit and adjustment of the energy concentration of the ration accordingly. When nutritional changes are required, further blood samples are collected about 2 weeks later to check on progress and further monitor any changes in ewe body condition scores [12].

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53

Deficiency of mineral macro-elements

A.R. Sykes

Of the major elements (those required in gram/day quantities), calcium (Ca) and magnesium (Mg) are probably the ones that clinicians are most regularly called on to deal with in sheep. In the case of Ca, hypocalcaemia is the most common manifestation of abnormal metabolism and arises not because of dietary deficiency but because Ca absorption in the ruminant is regulated endocrinologically through vitamin D metabolism and failures of this system to adapt to change in Ca demand. Over the past decade the major literature has centred on how dietary mismanagement may influence this regulatory process. Two main areas of investigation have been the manipulation of Ca fluxes in the body by changing dietary cation–anion balance, and therefore body fluid acid–base balance, and the importance of Mg status for the ability of the ruminant to maintain Ca homeostasis.

In the case of Mg, there have been few specific developments that would change the approach to prevention or treatment of clinical hypomagnesaemia which is usually, but not uniquely, observed around parturition. It is caused by failure of absorption to meet immediate demand, because there is no mechanism for rapid mobilization of the Mg in the skeleton. The major factors implicated, other than sudden reduction in intake due to environmental factors, are the effect of other dietary substances which depress Mg absorption. Much of the recent work has been directed towards a better understanding of the quantitative importance of these interfering factors through computer simulation modelling of Mg metabolism [1], which has demonstrated difficulties in scaling up from physiological studies of Mg transfer across membranes to whole animal Mg supply. They have queried the long-established assumption that Mg is absorbed mainly from the rumen, and pointed to the hind gut as an important site of absorption, and to how little is known about the regulation of absorption at that site. More recent re-analysis of data [2] has also questioned the long-held assumption that the sheep is a good model for Mg absorption in the dairy cow, by indicating a significantly greater ability for Mg absorption in the sheep. Why that should be is an interesting but, as yet, unresolved question.

The mechanism of absorption and precise requirement of sheep for phosphorus (P) have continued to

elude research workers [3, 4]. Reports of specific deficiencies in the British clinical literature are, however, rare. Deficiency of P is essentially a feature of animals offered low-quality forages and is most likely in rangeland situations, where senescent plant material, accumulated during periods of surplus pasture growth, is consumed during periods of little pasture growth. In that situation, a major problem is the differentiation between P and protein/nitrogen (N) deficiency, since N and P concentrations in those forages are highly correlated. Both elements (N and P) are required by rumen micro-organisms for cellulose breakdown and microbial protein synthesis and it is often difficult to judge which is rate-limiting [5]. On such forages, sulfur [5] can also be marginal and limit rumen bacterial activity.

Sodium (Na) and potassium (K) are highly conserved in body tissues. Both elements are absorbed readily and their excretion is highly regulated. There is still uncertainty about the effect of variation in their relative concentrations in the diet of ruminants, not so much from the standpoint of the requirement for these elements *per se,* but from the effect of a low Na:K ratio leading to high rumen K concentration and its effect on Mg absorption from this site. However, some natrophobic forage plants, such as lucerne or alfalfa, when grown on certain soil types, will allow spectacular live-weight-gain responses to supplementation with salt especially when weed species, which are generally natrophilic, are well controlled.

Sheep are particularly susceptible to nematode parasitism (Chapter 27), which changes the animal's ability to absorb P and possibly Ca. Only a *lack* of effect of parasitism on Mg metabolism has been recorded [6], and, although Na metabolism is spectacularly disturbed in parasitic infestations, change in dietary requirement as a consequence has yet to be clearly demonstrated [7]. The possibility of involvement of nematode infection must be included in the clinical assessment of major mineral status of animals.

CALCIUM AND PHOSPHORUS

More than 99.5 per cent of Ca and 75 per cent of P in the body are found in the skeleton in a ratio Ca:P of 2:1 as hydroxyapatite. The major role is to provide strength and rigidity to bone collagen. Ca in plasma ranges from 1.5 to 3.0 nmol/l, 50–60 per cent of which is weakly bound to albumin. Plasma phosphate (PO_4) has a wider range (2.0–4.0 nmol/l). The concentrations of both elements tend to be at the higher end of the range in young sheep with actively growing skeletons, and fall gradually as maturity is reached at 15–18 months of age. Ca has many functions outside the skeleton. It is involved in muscle contraction and the characteristic rumen stasis of Ca deficiency has been attributed to stimulation of receptors in the reticulo-rumen by parathyroid hormone which is involved in Ca homeostasis [8], as indicated below. It is involved in muscle protein synthesis and degradation through regulating the calpain/calpastatin group of protease enzymes. It is also involved in blood clotting and in nerve transmission. Hypocalcaemia can depress glucose production and enhance insulin resistance of tissues [9]. P is an important constituent of buffers that maintain osmolarity and acid–base balance in the body. It is involved in energy transfers in the phosphorylation and dephosphorylation of adenosine phosphate bonds, with a central role in energy and protein metabolism and particularly in gluconeogenesis [5]. More recently, P deficiency has been shown to interfere with the protein supply to the tissues from the digestive tract, low rumen P concentration being associated with a reduction in microbial protein synthesis [10].

Regulation of calcium and phosphorus metabolism

Maintenance of levels of Ca and P in extracellular fluid (ECF) that will sustain 'normal' mineralization of bone matrix and normal metabolic functions involves a series of endocrine reactions. Ca absorption from the alimentary tract by diffusion probably accounts for most Ca transfer in mature animals with a fully mineralized skeleton. However, a facilitated transport provides the major supply in animals with significantly increased Ca demands, such as during growth, late pregnancy or lactation. Vitamin D is critical to this process and to our understanding of Ca homeostasis. A schematic representation of this complex physiological system is given in Figure 53.1. Basically, vitamin D from two sources – the diet as vitamin D2 and insolation of 7-dehydrocholesterol under the skin as vitamin D3 – accumulates in the liver, where it is continually hydroxylated at the 25 position to

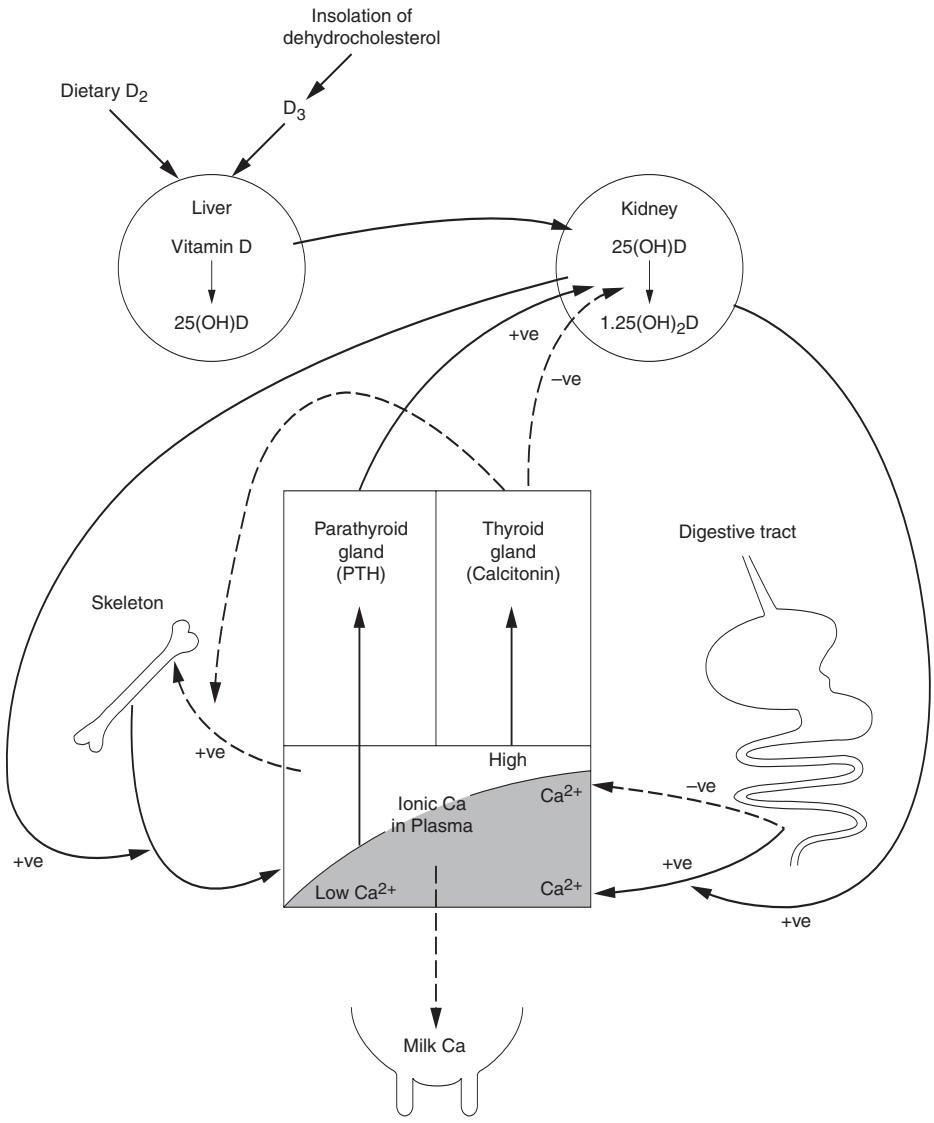

Figure 53.1: Schematic representation of mechanisms involved in calcium homeostatis.

form 25-hydroxycholecalciferol and exported into the circulation. Under the influence of parathyroid hormone (PTH), produced in response to low body fluid levels of Ca, it is further hydroxylated to 1,25-di-hydroxycholecalciferol. This compound has many physiological functions in the body, e.g. suppression of immune function [11] and in the growth and proliferation of cancer cells [12], but one is the stimulation of production of a Ca-binding protein in the alimentary tract to promote the facilitated transport component of Ca absorption. There is also recent evidence to suggest that the Ca transporter genes are upregulated by oestrogens through vitamin D-independent mechanisms [13].

There is evidence that 1,25-dehydroxycholecalciferol is involved in stimulating P absorption across the intestine of non-ruminants, but not in small ruminants. While active transport in those species is

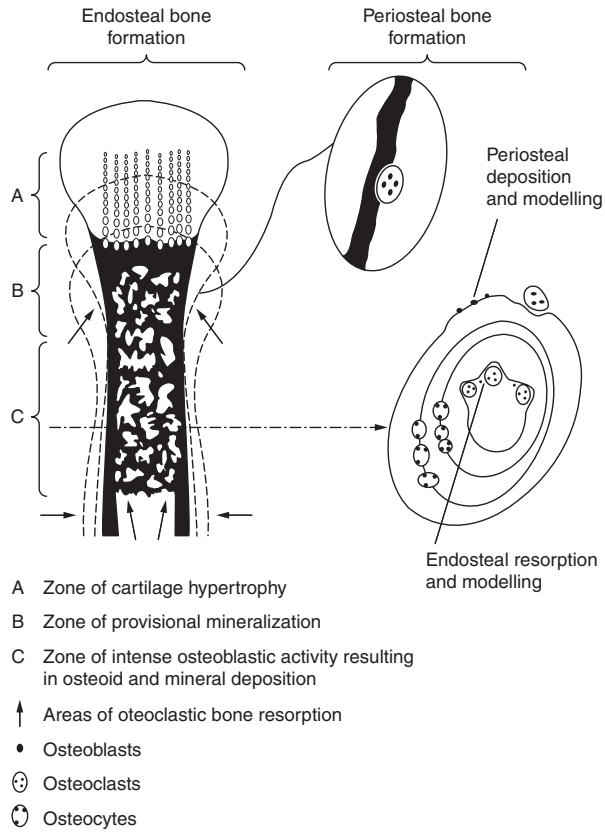

Figure 53.2: The two major processes in bone growth.

stimulated by P deficiency and hypophosphataemia, the mechanism is still unknown [3].

The skeleton

A feature of the regulation of Ca and P homeostasis is that, while mechanisms exist to maintain reasonably tight control of plasma and ECF concentrations, regulation of skeletal mineral content is poor. Thus, even animals with mineral intakes in excess of requirement during periods of high Ca and P demand, such as late pregnancy and early lactation, still lose considerable quantities of the mineral from the skeleton. Nutritionists now recognize that this is normal and that it is impossible to feed to maintain zero balance or prevent Ca loss during these periods.

Bone growth occurs by two major processes: cartilaginous and 'membranous' bone formation. In the first, which occurs in the distal and proximal extremities of long bones and vertebrae (Figure 53.2), cartilage cells hypertrophy, die and provide a provisionally mineralized matrix on to which bone-forming cells (osteoblasts) deposit osteoid or collagen. In the second process, bone cells in the periosteum secrete osteoid directly on to existing bone surfaces to provide 'membranous' bone (Figure 53.2). This osteoid, bathed in ECF, provides the matrix for mineralization with Ca and P. Some bone cells (osteocytes) become trapped in the mineralized matrix. This is a dynamic and continuing process in the growing animal and still occurs, although to a limited extent, after skeletal maturity, so that existing bone is continually being resorbed and deposited. In the growing animal, remodelling is particularly important for maintaining bone shape. Specialized multinucleate bone resorbing cells (osteoclasts) are recruited for this purpose.

It appears that these mechanisms operate so that increased demand for Ca simultaneously stimulates

receptors on the osteoblast to promote new osteoid deposition and stimulates osteoclasts to remove old bone, as well as increasing Ca absorption across the alimentary tract. Conceptually, increased demand leads to increased bone turnover, which results in the replacement of well-mineralized old bone with unmineralized new bone (osteoid). This subsequently mineralizes, but in the early stages net release of Ca from bone occurs to meet increased Ca requirements.

Implications for feeding

There are several important implications. First, hypocalcaemia is almost always likely to result from failure of the endocrine mechanism to promote influx of Ca from bone and the alimentary tract rather than from inadequate dietary Ca. Second, it is not possible to feed sufficient Ca to prevent endocrinologically driven bone loss. Third, it can lead to underestimates of the availability of Ca in forages unless animals are fed Ca in amounts that are less than the 'animal controlled' rate of absorption. Since forages are generally rich in Ca, many estimates suggest that Ca availability in forages is low, namely 30 per cent. However, maximum availability (A) can be written as $A = R/I$, where R is requirement and I is intake. If R is regulated by the animal, the availability of Ca from a forage can be obtained only when R is greater than I. This is rarely the case with common forages (Table 53.1), since dietary requirement is rarely greater than 3 g Ca/kg dry matter (DM) (Table 53.2). Concentrate supplements generally contain adequate quantities of cheap added Ca as limestone.

P homeostasis is no less complex. There is accumulating evidence [4] that animals respond to increase in P requirement by increasing intestinal absorption via an energy-dependent process. Unlike monogastric animals, in which this process has been shown to respond to 1,25-dihydroxycholecalciferol, this is not the case in small ruminants [3], and the mechanism is unknown. It is widely accepted that most dietary P (75 per cent) is absorbed. Indeed, it is a feature of ruminants that much of this P is recycled via saliva to the rumen. On most diets, probably four to six times as much PO_4 enters the rumen daily in saliva as from dietary sources. This cycle conserves rumen P and, as a consequence of a less than 100 per cent efficient reabsorption, P homeostasis is maintained via endogenous faecal P excretion. However, genetic differences have been shown between sheep, which can be predominantly salivary P regulators (low P absorbers) or predominantly urinary P regulators (high P absorbers). High P absorbers maintain homeostasis by regulating tubular P reabsorption at the kidney and therefore urinary P excretion. Uncertainty about the ability of all animals to absorb 75 per cent of dietary P still gives concern about the adequacy of diets for all animals at marginal dietary P content.

Table 53.1: Typical calcium and phosphorus concentrations in common foodstuffs (g/kg dry matter)

	Ca	P
Herbage, including hay and silage		
Grass dominant	7.5	2.8
Grass–clover swards	7.5	3.0
Lucerne	12.0–16.0	3.0–4.0
Forages and roots		
Kale	20.0	3.0–3.5
Swedes	3.0–4.0	3.0–3.5
Turnips	5.0–6.0	3.0–3.5
Cereals		
Barley	0.7–0.9	3.5–4.0
Oats	1.3–1.5	3.5–3.8
Maize	0.15–0.3	3.0–3.3
By-products		
Groundnut meal, decorticated	2.2	6.8
Linseed cake, expressed	4.1	8.6
Soya bean meal	7.3	3.0
Fish meal	50.0–60.0	35.0–40.0
Sugar beet pulp (dried)	6.0	0.8.1.0
Straws		
Barley	4.5	1.0
Oat	4.0	1.5

Interfering factors

Bone mineral content is not uniquely determined by Ca and P supply. Osteoid production is dependent on supply of protein as well as Ca and P. Thus, low bone mineral content can result from deficiency of bone matrix (osteoid) as well as bone mineral and, in each case, bone quality may be impaired. Thus, a low ash to volume ratio (A:V, g/ml), which indicates poor bone mineral, could arise from low bone matrix deposition (low fat-free, ash-free organic matter per unit bone volume, R:V), termed osteoporosis, or from poor mineralization of that bone matrix (low ratio of

Table 53.2: Calcium and phosphorus requirements of sheep (g/day)

	Feed quality (*q*)											
	0.5				0.6				0.7			
	Ca		P		Ca		P		Ca		P	
Growth												
Live weight (kg)	20	40	20	40	20	40	20	40	20	40	20	40
Rate of gain (g/day)												
0	0.7	1.0	0.5	1.0	0.6	0.8	0.4	0.8	0.6	0.7	0.2	0.4
100	2.5	2.6	2.0	2.6	2.4	2.4	1.7	2.1	2.3	2.2	1.3	1.5
200	–	4.5	–	4.6	4.1	4.0	3.1	3.6	4.0	3.8	2.5	2.6
Pregnancy (75 kg ewe with twins)												
Weeks before parturition												
8	2.6		2.9		2.4		2.5		2.2		1.6	
4	4.9		4.5		4.6		3.9		4.4		2.6	
0	7.6		6.1		7.2		5.4		6.9		3.5	
Lactation (75 kg ewe with twins)												
	7.6		8.7		7.0		7.7		6.7		5.4	

q, metabolizability of dietary gross energy, i.e. metabolizable energy/gross energy.

ash to organic matter ratio, A:R), termed osteomalacia. In sheep, an R:V ratio in vertebral bone of less than 300 and 250, in adult and young sheep, respectively, is indicative of osteoporosis, while an A:R ratio of less than 1.25 and 0.9, respectively, is indicative of poor mineralization. The different ratios for young and adult sheep represent relative rates of bone turnover and therefore the proportion of new bone. The bone changes resulting from Ca or P deficiency comprise changes to both R:V and A:R ratios, and the nature of the changes appear to be age (skeletal maturity) dependent.

Other diseases can have prolonged effects on the skeleton and limit the value of bone analysis in the diagnosis of Ca and P deficiencies. The best developed example is that of chronic parasitism, but even in this case the situation is complex. Nematode infections of the gastrointestinal tract induce protein deficiency because of the massive diversion of amino acid from body tissue growth (including the skeleton) to synthesis of proteins of the acute phase immune response, mucoproteins and gut epithelial cell replacement as part of the host response to infection and host tissue damage [14, 15]. Osteoporosis (low R:V ratio) is now a well-recognized consequence of parasitism. However, certain infestations, particularly those in the proximal small intestine due to *Trichostrongylus colubriformis* and *T. vitrinus,* will reduce P absorption [6] and lead to a combined osteoporosis with osteomalacia.

During the past two decades attention has focused on the need to provide pH balancing nutrients. The concept developed from the observations [16, 17] that incidence of milk fever in dairy cows decreased markedly in Europe with the switch from feeding hay to acid-preserved silages. Diets which predispose to an acidic rather than the conventional alkaline nature of ruminant urine are considered to be those in which the dietary cation–anion balance (DCAB), measured as (potassium (K^+) + sodium (Na^+)) − (chloride (Cl^-) + sulfate (SO_4^{2-})) in milli-equivalents (atomic weight ÷ valency)/kg DM, is below 100. Enhancing diets of dairy cows with Cl^-, for example as $CaCl_2$, has enhanced Ca absorption and bone Ca resorption possibly by enhancing the effectiveness of Ca-regulating hormones in the slightly lower body fluid pH achieved. However, DCAB values much higher than those on which responses to DCAB supplements have been achieved in the northern hemisphere are often seen in spring pasture in New Zealand and are associated with much lower incidence of milk fever, suggesting that there are many other factors at work. A more simple and cheaper

approach is to avoid parturition in areas in which heavy K fertilizer has been used for reasons adduced below.

Hypocalcaemia is a characteristic feature of the later stages of hypomagnesaemia. The activities of 1,25-dihydroxycholecalciferol and PTH are Mg-dependent and reduced at low plasma Mg concentrations and dietary Mg has been shown to stimulate Ca absorption in ovine small intestine *in vitro* and *in vivo* [18]. Recent modelling studies [19] suggest that the quantitative response of skeletal Ca resorption to change in plasma Mg concentration is curvilinear, being optimal at Mg concentrations of 0.9 mM, but decreasing by 50 per cent as Mg concentration falls to 0.6 mM and when it increases to 1.2 mM. In this context, high Mg intake might be responsible for clinical signs of hypocalcaemia, but feeding diets with high Mg content failed to precipitate hypocalcaemia [20].

Clinical signs

Osteodystrophies

The incidence of bone diseases appears to have decreased during the past few decades. Authentic reports are available on lameness in young sheep, which has been attributed to the lesions of rickets. Similarly, the osteoporosis – 'double scaup' or 'cappi' – recorded in northern Britain occurs rarely today. In all these cases, it was difficult to attribute these lesions specifically to Ca, P or vitamin D deficiency. Nonetheless, levels of vitamin D, from feed and insolation of dihydrocholesterol, can be critically low in breeding sheep around parturition and particularly in hoggets wintered without supplements, often on specialist pastures, but are responsive to vitamin D therapy. Therapy at that time of the year, however, is soon followed by pasture growth and improved nutrition and the situation will often resolve. Natural cycles of demineralization occur, particularly in breeding stock, but are of no consequence provided animals are well fed when they stop lactating (i.e. during the dry period). During the past four decades, financial support for agriculture and the application of research findings in sheep nutrition have increased the level of supplementary feeding and probably the levels of vitamin D in livestock. A return to less intensive systems of production would undoubtedly lead to an increase in incidence of these diseases.

Hypocalcaemia (parturient paresis, lambing sickness, moss ill, downer ewe syndrome)

This is economically the most important condition caused by Ca deficiency in sheep. Unlike its bovine equivalent, which invariably occurs at parturition, the condition can be found in ewes several weeks before lambing. Its occurrence is often associated with stressful conditions, e.g. the gathering of ewes for pre-lambing vaccinations. Ewes in the early stages of hypocalcaemia show incoordination and muscle tremors, but without the tetanic spasms associated with hypomagnesaemia. They generally fall with hind limbs extended and thereafter remain recumbent, with mild tympany, and pass into a comatose state. If untreated, the condition is normally fatal, although death is not as rapid as in hypomagnesaemia, generally occurring from a few hours to 2 days after occurrence of the first signs.

It is important to distinguish between hypocalcaemia, hypomagnesaemia (see below) and pregnancy toxaemia (Chapter 52). As with some forms of hypocalcaemia, pregnancy toxaemia occurs in the final weeks before lambing and many of the signs are similar, although ewes suffering from pregnancy toxaemia generally live longer. Serum Ca and plasma β-hydroxybutyrate determination in blood samples from affected individuals and representative numbers of the flock are of value in confirming diagnoses. At post-mortem examination, pregnancy toxaemia cases have a characteristic white liver indicative of fatty degeneration, as opposed to the absence of any macroscopic lesions in cases of hypocalcaemia. The rapid response of the hypocalcaemic ewe to Ca therapy contrasts with the poorer prognosis and frequent lack of response to treatment of the ewe with pregnancy toxaemia.

The hypocalcaemia of parturient paresis is caused by a breakdown of the homeostatic mechanisms, rather than by inadequacies of Ca intake or of poor reserves. Elevated concentrations of PTH and 1,25-hydroxyvitamin D (1,25-OHD) are invariably observed in cases of parturient paresis. Ewes suffering from parturient paresis respond very rapidly to the administration of Ca, generally given as a 200 g/l (20 per cent w/v) solution of Ca borogluconate. (This demonstrates that it is in fact the hypocalcaemia, and not the hypophosphataemia, that is the principal cause of the signs of parturient paresis.) Treated ewes generally resume normal behaviour and eating within an hour or less, but should be inspected frequently, as the condition can recur, necessitating further treatment.

At one time it was considered that hypocalcaemia in ewes was best prevented by feeding high levels of Ca and P during late pregnancy. Given our understanding of Ca homeostasis, this is counterproductive, as it is likely to reduce the mobilization of Ca from skeletal reserves. Feeding diets relatively low in Ca and normal in P before lambing, to stimulate Ca absorption and mobilization from bone, followed by an increase in dietary concentrations at parturition may reduce the incidence of post-parturient hypocalcaemia in dairy cows. However, this is impracticable in all but very intensive sheep systems. The involvement of hypovitaminosis D in hypocalcaemia is not clearly understood, although vitamin D status is known to fluctuate seasonally and can reach critically low levels during winter. Injections of 2000 IU/kg live weight will maintain adequate concentrations during a subsequent 3-month period [21].

Interest in providing diets that produce metabolic acidosis and lead to bone Ca mobilization is also current in dairy cattle nutrition [17]. A lower incidence of hypocalcaemia, claimed in research findings, has still to be confirmed in practice in animals on pasture. However, since this will require special diets, it seems unlikely to find a practical outlet in many sheep systems.

PHOSPHORUS

It is debatable, given the high dietary availability of P and the large reserve of P in the body, that simple clinical P deficiency exists in sheep in the UK. Adult sheep are particularly tolerant of moderate dietary P inadequacies for prolonged periods. Hypophosphataemia occurs in ewes with parturient paresis, but this is an effect rather than a cause of the condition, which responds readily to the correction of the associated hypocalcaemia. Nematode parasitic infection undoubtedly causes hypophosphataemia, particularly in young stock, and is readily corrected by effective parasite control. Where feed supplements such as straws or sugar-beet pulp (see Table 53.1) make up a major proportion of the diet, or are fed for prolonged periods, hypophosphataemia can result. In most situations these diets can, and should, be effectively supplemented, either by mineral mixtures in concentrate feeds or by simultaneous access to good pasture.

The signs of extreme P deficiency, when rumen and blood PO_4 concentrations fall below 1.0 mmol/l, achieved on diets containing <1 g P/kg of dry matter (DM), are reduced feed digestibility and intake. Depraved appetite or 'pica' can result, but is unlikely to be seen in sheep in the UK, except as a consequence of gross mismanagement of feed supplementation in intensive systems.

MAGNESIUM

Mg has several important physiological and biochemical functions in animals, not all of which have been demonstrated in ruminants. Mg is important in growth: it is necessary for optimal protein synthesis, probably being involved in the elongation of the polypeptide chain; in immunity, in lymphoblastic transformation and antibody synthesis; in muscular contraction, the Ca pump of the sarcoplasmic reticulum is Mg-dependent; the sensitivity of bone cells to the osteolytic agents PTH and 1,25-OHD is diminished in Mg deficiency and Mg stimulates Ca absorption in the small intestine [18].

Mg homeostasis is achieved by the kidney. A renal threshold exists (22 mg Mg/l plasma) below which Mg excretion is sharply reduced. The animal appears to have no ability to regulate Mg absorption. The young milk-fed lamb absorbs 95 per cent of dietary Mg from the large intestine. On the other hand, the ruminant lamb absorbs <30 per cent of Mg consumed and much of this from the rumen-reticulum. Indeed, research during the past 30 years has tended to accept this (implied) change in site of Mg absorption without question, and physiological studies have concentrated on the mechanism of absorption across the rumen wall and on the factors that influence the rates of absorption. Recent computer modelling approaches [1, 19] have begun to cast some doubts on this assumption, and several associated studies have suggested that the large intestine can be a major site of absorption (perhaps even providing compensatory absorption) when conditions limit absorption from the rumen-reticulum.

The rate of absorption of Mg from the rumen shows a curvilinear increase with increasing Mg concentration in rumen digesta, suggesting an assisted, but saturable, 'active' transport system. This process is energy- and Na-dependent, involving Na-adenosinetriphosphatase (Na-ATPase). An assisted transport system is necessary because the Mg ion, which is positively charged, has to be transported into the body

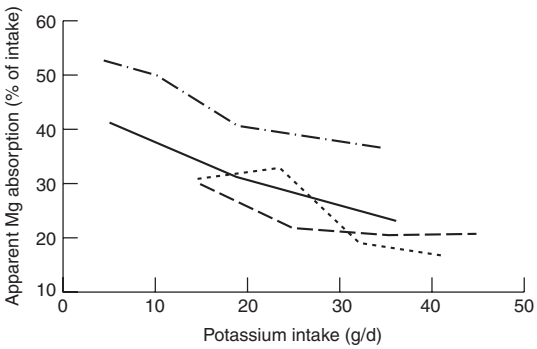

Figure 53.3: Effects of potassium intake on the availability of magnesium in sheep receiving diets containing different ratios of concentrates to roughage (data from several studies summarized in [26]).

from the rumen against an electrochemical gradient with a potential difference (p.d.) of about 40 mV, body positive [22]. At the same time, the p.d. facilitates Mg backflow down the electrochemical gradient from the tissue into the alimentary tract. Net absorption is sensitive to factors that change this p.d. and the rates on these processes, notably the concentration of K in rumen fluid. Variation in herbage K concentration from 10 g/kg DM, typical of mature forages or concentrate diets, to 40 g/kg DM, typical of rapidly growing leafy pasture, can increase p.d. from 40 to 60 mV, and reduce the proportion of dietary Mg absorbed from 40 to <20 per cent (Figure 53.3). On the other hand, low Na intake will have a similar effect, since, under these circumstances, rumen K will be elevated as a consequence of the substitution of K for Na in saliva. Sheep with hypomagnesaemic tetany [23] and which did not respond to supplementary Mg did recover when moved from severely Na deficient (0.4 g Na/kg DM) reseeded pasture to permanent pasture which could be expected to contain in excess of 1.0 g Na/kg DM, the probable dietary requirement of grazing sheep [24].

The proportion of Mg in the liquid phase of rumen digesta, and therefore available for absorption, is extremely sensitive to pH, and particularly at the normal pH of rumen contents, i.e. 6.8–7.0 (Figure 53.4) [25, 26]. Thus, changes in pH, as well as total Mg intake, can have large effects on the Mg available for absorption, and abrupt changes in diet can be significant. It is no coincidence that, for dairy cattle, spring turnout has been a critical time for hypomagnesaemia, when Mg intake, rumen pH and rumen K concentration could be changing simultaneously and significantly. Elevated rumen ammonia concentrations, as a consequence of the rapid breakdown in the rumen of large amounts of protein in leafy spring grass, has often been implicated. However, direct infusion of urea, to elevate rumen NH_3 concentration, has not been shown to affect absorption (L. Ryan and A. Sykes, unpublished observations) and this is in agreement with physiological studies [27]. Thus, while ammonia should, in theory, be toxic to energy-dependent transport systems, it seems more probable that speculation on the role of ammonia owes more to the close association between the concentrations of soluble protein and K in leafy pasture and the direct effect of K on p.d.

Preoccupation with the problems of Mg absorption from the rumen has tended to overlook the continuing importance (after rumen development) of the hindgut for Mg absorption. Computer simulation studies of Mg absorption, based on the physiological literature, had difficulty in reconciling predictions with actual Mg absorption measured in nutritional studies. Only when a component of absorption from the hindgut was included (using data parameters from rumen studies) did the studies reconcile, suggesting that the hindgut retains an important role in the ruminant. Subsequent nutritional studies [28] have clearly demonstrated that when Mg absorption from the rumen is impaired by high dietary intake of K, a substantial shift in Mg absorption from the rumen to the hindgut occurs (Figure 53.5).

Little is known about the transport mechanism involved or the conditions that influence Mg absorption from this site. However, the p.d. is much lower and the opportunity for effect of K much less, since much dietary K is absorbed in the intestine and excreted in urine. On the other hand, it has been shown that the solubility of Mg at critical pH in this region is consistently 30 per cent lower on grass diets than on hays or concentrates (Figure 53.4), whereas Mg solubility in the rumen is unaffected by diet. Understanding the factors that influence Mg absorption from the hindgut now seems to be a priority for development of methods to predict or manage problems with hypomagnesaemia.

The sheep has only very small Mg reserves with which to buffer changes in Mg absorption. Of these, 70 per cent is relatively tightly bound in the skeleton, where Mg is normally present in a Ca to Mg ratio of 50:1. The Mg, however, can be released only in association with general bone resorption. Thus, the young

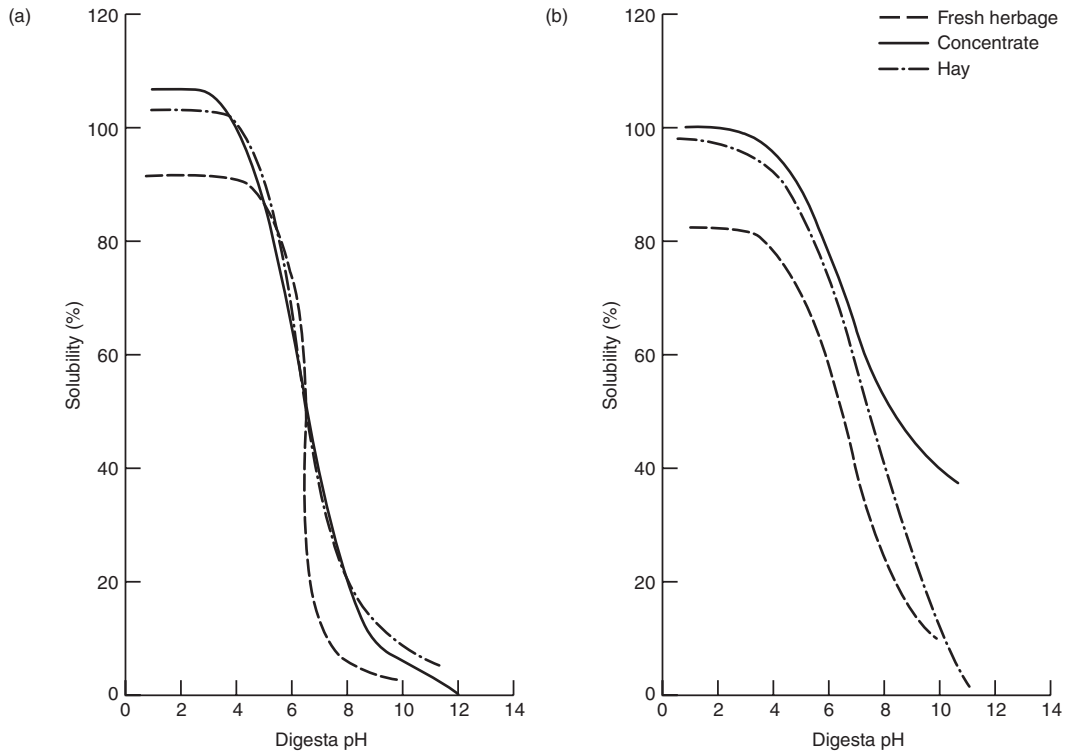

Figure 53.4: Relationship between digesta pH and Mg solubility in ruminal (a) and caecal (b) digesta from animals consuming fresh herbage, concentrate or hay diets. At normal caecal pH (7.0) Mg solubility on fresh herbage was 36 per cent, compared with 62 and 64 per cent on concentrate and hay diets, respectively [25].

animal with a high rate of bone turnover (see above) is more readily able to furnish Mg to the circulation than the adult. Indeed, diagnosis of Mg deficiency in young stock is aided by determination of the Ca:Mg ratio in bone. This can increase to 150:1. However, it is rare to see such a change in Mg-deficient adult stock. The ruminant is therefore extremely dependent on continual supplies of Mg in the diet and is very susceptible to changes in total DM intake.

There is now evidence that Mg can be removed from the circulation to other body compartments, and that this can precipitate clinical hypomagnesaemia. This is frequently associated with increased lipolysis, and factors that elevate plasma non-esterified fatty-acid concentrations, such as the stress of unaccustomed handling, transportation and cold exposure, can predispose to tetany. The turning-out to pasture of housed ewes shortly after lambing constitutes a stress and, as such, increases the risk of hypomagnesaemia. If weather conditions are adverse and the pasture has been fertilized with N and K, not only is Mg intake likely to be diminished by sheltering behaviour, and a lower proportion absorbed, but a smaller proportion of that absorbed is likely to be retained in extracellular fluid. The response is a decline in plasma Mg concentration from the normal of 22–24 mg Mg/l (1 mM). This in itself may be unexceptional and unimportant, even when values as low as 12–15 mg Mg/l are achieved. The critical body compartment for Mg in relation to the onset of hypomagnesaemic tetany is the cerebrospinal fluid (CSF), which is buffered against change in plasma Mg [29]. Thus, values of plasma Mg of 12–15 mg/l may be maintained for some time and be of little importance if CSF levels remain above 12 mg Mg/l (0.5 mM) (Figure 53.6). A concentration of 9–10 mg Mg/l in CSF appears to be critical for the onset of clinical signs, at least in cattle. This explains why low plasma Mg concentrations are not necessarily associated with clinical

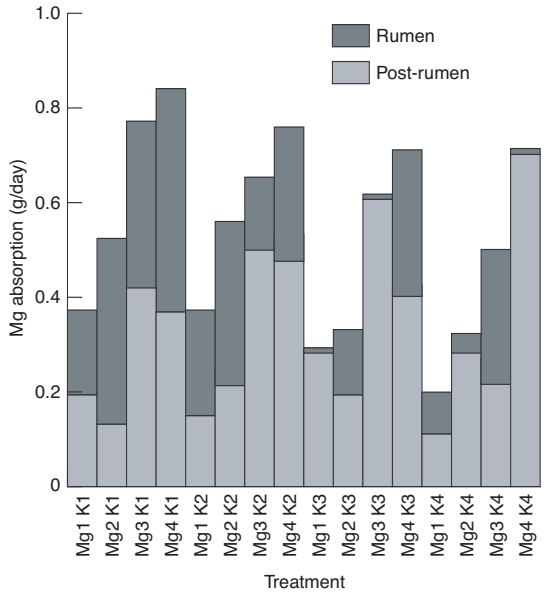

Figure 53.5: Effects of the infusion of increasing amounts of potassium (K1–K4) and magnesium (Mg1–Mg4) on the partitioning of magnesium absorption between the rumen and a post-ruminal site.

disease and why the onset of the latter occurs during inclement weather, which disrupts grazing patterns, reduces Mg and energy intakes, and causes body energy mobilization.

Hypomagnesaemia (grass tetany, grass staggers, lactation or magnesium tetany)

The acute form of hypomagnesaemia generally occurs within the first 4–6 weeks after lambing. Affected ewes exhibit hyperaesthesia and trembling, especially of the facial muscles. They appear unable to move, and others walk in an uncoordinated manner with a characteristic stiff-limbed gait. They collapse on the one side and show repeated tetanic spasms, with all four limbs rigidly extended, and with a characteristic extension of the head and neck. Death can be very rapid without the coma typical of hypocalcaemia. The condition develops extremely quickly, and very often the first indication of hypomagnesaemia is the finding of a dead ewe that had appeared normal some hours previously. Chronic hypomagnesaemia can occur in sheep subjected to poor nutrition during winter, but is less common than the acute form.

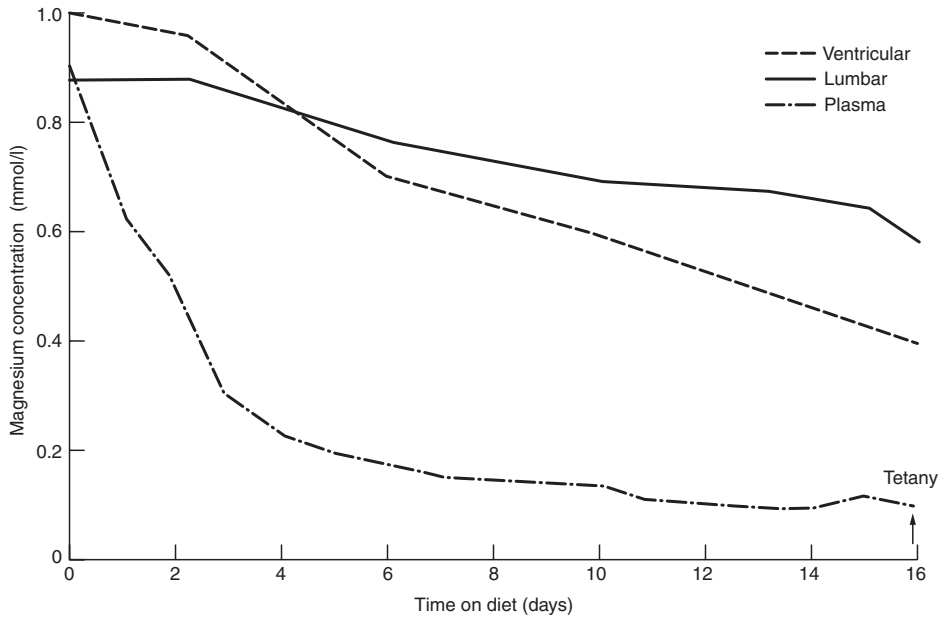

Figure 53.6: Changes in magnesium concentrations of ventricular and lumbar cerebrospinal fluid and plasma of a lactating cow offered a magnesium-rich diet. Adapted from reference [29].

Differential diagnosis from hypocalcaemia is based on clinical signs, the fact that hypomagnesaemia in its tetanic form generally occurs only in the post-lambing period, and from blood analysis. Other causes of central nervous disturbance that have to be distinguished from hypomagnesaemia include listeriosis, cerebrocortical necrosis and louping-ill in tick-infested areas.

A number of measures can be taken to lessen the risk of hypomagnesaemia. It has been advocated that adequate Na should be provided to stabilize the rumen Na:K ratio, and that the diet should have a high fibre content to encourage rumination and salivation, and a high energy content to prevent excessive rumen ammonia concentrations. The latter also promotes volatile fatty acid and carbon dioxide production, which facilitates Mg absorption. A high energy diet also serves to minimize lipolysis, and at the same time efforts must be made to avoid the imposition of physiological or environmental stress.

In addition to these husbandry measures, supplementary Mg should also be given when there is a risk of hypomagnesaemia. This is generally provided in the form of magnesium oxide (available commercially as calcined magnesite) supplied at 7 g/ewe/day. To encourage consumption, it is usually incorporated in a concentrate, and such is the potential loss from hypomagnesaemia that it is normally considered economic to feed concentrates solely as a carrier of the daily Mg allowance, even when supplementary energy and protein are not required. Alternative, but less satisfactory, forms of supplying Mg at times of risk include the foliar dusting of herbage with calcined magnesite and the provision of a high-Mg mineral mixture, either as a powder or liquid, to which ewes have free access. The addition of Mg to drinking water is a less effective means of administration when ewes are lactating.

Every effort should be made to supply ewes at risk with sufficient Mg in suitable form to prevent hypomagnesaemia, but care is needed as excessive Mg can cause scouring. Diets fortified with Mg should not be fed to male sheep, as this can result in the formation of magnesium ammonium sulfate calculi in the urethra, causing urolithiasis and possibly death following rupture of the bladder (Chapter 55).

Ewes affected with hypomagnesaemia, even in the comatose stage, can be treated successfully with a combined injection of Mg and Ca. This is given intravenously as a 200 g/l (20 per cent w/v) solution of Ca borogluconate with 30 g/l (3.9 per cent w/v) Mg chloride. An additional dose is given subcutaneously and, occasionally, a muscle relaxant may be indicated. Recovery is generally rapid but, as with hypocalcaemia, relapses are not uncommon and the treatment may have to be repeated, and here the prognosis is poor.

CHLORIDE, POTASSIUM AND SODIUM

Virtually 100 per cent of the K ingested by ruminant animals is rapidly absorbed from the rumen and small intestine, together with reabsorption of large quantities of K that are normally secreted in saliva. The concentrations of K in grass and other forages are, moreover, normally such that intake is many-fold greater than requirements. Between five and ten times the dietary Na intake is secreted in saliva and, together with dietary Na, is subsequently completely reabsorbed, mainly in the small intestine, but also in the large intestine.

Homeostasis of the two elements in the body is regulated at the kidney and cellular level [30]. K and Na, together with chloride (Cl^-), are intimately involved in the regulation of cellular and extracellular osmotic pressures. Of total body K, 90 per cent is found within the cell and 10 per cent in bone (7–8 per cent) and ECF (2–3 per cent), while 91 per cent of Na is extracellular and 9 per cent intracellular. The enzyme Na–K ATPase catalyses the hydrolysis of adenosinetriphosphate (ATP) to adenosinediphosphate (ADP). The energy generated by each mole of ATP is used to extrude three Na atoms from the cell and take two K atoms into the cell via specific protein channels in the cell membrane. This Na and K pumping accounts for at least 30 per cent of the animal's normal maintenance energy expenditure.

Chloride ions are present in higher concentrations in ECF than in the cell interior, and tend to diffuse along this concentration gradient into the cell. The interior of the cell is negatively charged relative to the exterior, maintained by the Na–K pump, and Cl^- ions are pushed out of the cell along this electrical gradient. An equilibrium exists at −70 mV, the normal resting membrane potential of the cell. The equilibrium potential for K is, however, −90 mV, so that the potential gradient relative to the resting potential is outwards, and K leaves the cell along this gradient. For Na, the reverse is the case, the equilibrium potential being +60 mV, so that Na naturally enters the cell. The active transport of K into and Na out of the cells against these electrical and

concentration gradients maintains cell Na and K. The amount of osmotically active solute in cells is thus closely regulated to maintain constant osmotic pressure.

The volume of ECF is similarly determined by the total amount of osmotically active solute in the ECF, largely Na and Cl^-, and primarily by Na, since this determines the movement of the latter. The concentration of Na in ECF is maintained by two mechanisms controlled from the hypothalamic centres of the brain, which operate on the kidney cortex. A fall in ECF Na concentration results in release of adrenocorticotrophic hormone (ACTH), which stimulates aldosterone production in the adrenal gland. Aldosterone sets in train a series of events that, at the post-receptor level, induces ribosomal protein production, one consequence of which is active transport of Na (sodium pump) from the tubular lumen of the kidney out of the urine (or the saliva, sweat or gastric juice) into the interstitium and hence back into the bloodstream. Aldosterone production is also stimulated in response to a fall in ECF osmotic pressure. Discharge by juxtaglomerular cells in the glomeruli produces renin. This increases the conversion of angiotension I to II, which acts directly on the adrenal cortex to produce aldosterone and conserve Na and, therefore, water.

There are therefore strong mechanisms for absorption of Na and K from the digestive tract and conservation within the body. Human diseases such as Cushing's syndrome, which reflect defects in these regulatory mechanisms, are not generally seen in farm animals. Even in diseases of the gastrointestinal tract, such as parasitism, in which secretion of Na and K may be increased by several orders of magnitude, these conservation mechanisms are still effective. Bacterial and viral enteric diseases can damage these mechanisms and lead to excessive Na excretion in faeces, with consequent Na depletion and dehydration. They are, however, readily reversed by treatment of the disease and electrolyte and water replacement therapy [31].

Signs of deficiency of Na are lack of appetite and consequent poor performance. Certain plants, particularly for example lucerne, are notoriously deficient in Na, a feature that is enhanced by practices such as silage-making, since plant extracellular material, also rich in Na, is lost in effluent. Crops grown in readily drained and volcanic soils are typically low in Na. Supplementation is readily achieved in grazing ruminants through salt blocks or rock salt, since animals, and particularly deficient animals, have a well-developed appetite for salt. Diagnosis of Na deficiency is best achieved by analysis of the ratio of Na:K in saliva, which is about 20 in normal animals and as low as 0.45 in Na-deficient animals.

SULFUR

The principal function of sulfur (S) is as a constituent of the amino acids, cysteine, cystine and methionine, but it also occurs in insulin and in certain vitamins. Sulfur is generally considered to be particularly important in sheep nutrition, as the S content of wool is about 40 g/kg (4 per cent), incorporated in the cystine molecules. The main sources of S in sheep diets are the S-containing amino acids, which are constituents of the animal's normal dietary protein. Rumen microorganisms require inorganic S; the consequence of deficiency is reduced microbial protein synthesis and the typical symptoms of inadequate protein supply – poor growth, depressed wool production and poor fleece characteristics including lack of crimp due to delayed keratin formation, but which are also a feature of prolonged and severe copper (Cu) deficiency. Despite the importance of S, and the responses that have been demonstrated to the provision of additional S to sheep, S deficiency as such is not normally given consideration, as it would invariably arise as a secondary effect of a more serious protein deficiency. A lack of S can, however, limit production when sheep are fed diets containing urea or other forms of non-protein nitrogen, and in such situations it is recommended that sodium sulfate be incorporated in the diet at a ratio of 1:9 by weight with urea.

Excessive S intake also has implications for rumen function. In this case it can combine with molybdenum (Mo) to form tetrathiomolybdates, which reduce the absorption of Cu and thus lead to an induced Cu deficiency (Chapter 54). This occurs, however, only in the presence of relatively high concentrations of dietary Mo and there is little risk of inducing Cu deficiency by the addition of S to diets low in protein and supplemented with non-protein nitrogen.

GENERAL

Sheep can tolerate moderate deficiencies of most major elements for a considerable period without

serious effects on their health or productivity, always provided that there is opportunity at some stage to replenish depleted mineral reserves. The most important exception to this generalization is, as indicated above, with respect to Mg, where provision of supplementary Mg should be made at critical times. Attention should also be paid to the provision of Ca (Table 53.2), and in particular to the avoidance of supplying high levels of dietary Ca in anticipation of a later increased demand. In many management systems, sheep receive some form of supplementary feeding before and during lambing, and this provides opportunities for a degree of regulation of dietary Mg and Ca intakes.

The possibility of a mineral deficiency should always be considered when investigating any signs of ill-thrift or poor production.

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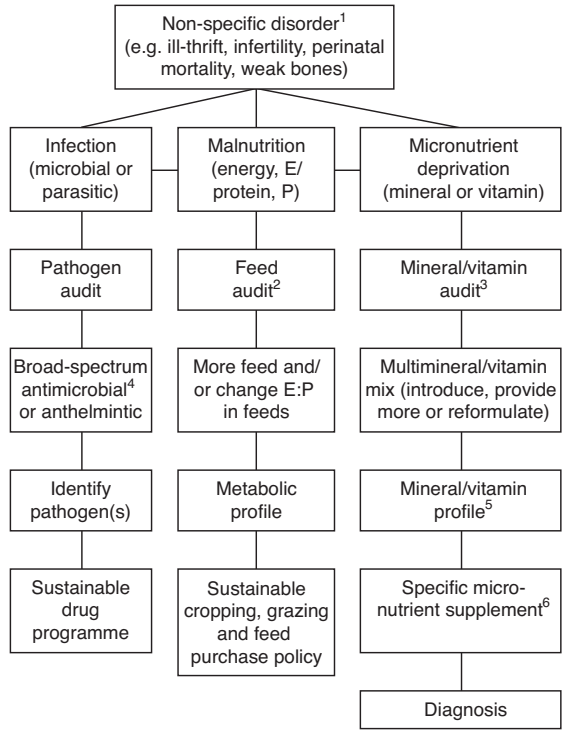
54

Micronutrient imbalance

N.F. Suttle and D.G. Jones

Many trace elements and vitamins are essential for optimum health and productivity, but the precise impact of deficiencies can be uncertain. Confusion can arise due to non-specificity of both clinical signs and the biochemical criteria used to support diagnosis: affected animals may simply appear unthrifty (i.e. 'pining'), while others remain healthy but have 'subnormal' blood biochemistry. A scheme for the differential diagnosis of trace element responsive disorders is given in Figure 54.1, and a diagnosis is supported when abnormalities are slow to develop and affect only a minority of the flock. *Disorder* is the last of four consequences to develop while insufficient trace elements are consumed (Figure 54.2): the first is *depletion* of any body reserves of an element; the second is *deficiency*, a lowering of its concentration in homeostatically controlled 'pools' such as the blood plasma; the third is *dysfunction*, a rate-limiting reduction in activity of



[1] First, rule out 'exotic' (e.g. plant, agrochemical or industrial poisons).
[2, 3] See references [44] and [45], respectively, for further details.
[4] While this may be common practice, it is preferable to establish sensitivity of the pathogen before selecting an appropriate antimicrobial and dosing regimen.
[5] See Tables 54.1 and 54.3 for best indices of trace element status.
[6] Some animals must be left untreated to confirm efficacy, and allow specific follow-up biochemical tests to be performed.

Figure 54.1: Scheme for the differential diagnosis of micronutrient disorders with non-specific signs; proceed vertically with a given line of investigation only while test results are positive**.**

one or more trace element-dependent or co-dependent enzymes. The commonly used biochemical indicators of trace element status monitor the first two phases, depletion and deficiency [1]. Accordingly, the proposed framework for interpretation of biochemical data (see Table 54.1) avoids premature diagnosis and overestimation of dysfunction and disorder. Response to a specific trace element supplement will often provide the surest diagnosis. This chapter summarizes the clinical and biochemical manifestations of cobalt (Co), copper (Cu), selenium (Se) and iodine (I) deficiencies, which continue to present the most important and widespread economic and welfare problems. Deficiencies of iron (Fe), manganese (Mn) and zinc (Zn) seldom occur naturally and are considered only briefly. As for vitamins, ruminal synthesis can be relied on to provide adequate amounts of all the water-soluble B group for both adults and suckled offspring, with the rare exceptions of thiamin (see Chapter 38) and vitamin B_{12}. Of the fat-soluble vitamins, deficiencies of E and D are the only ones likely to be encountered in forage-fed stock, forages being rich in β-carotene, the precursor of vitamin A. Vitamin D is addressed in the context of calcium and phosphorus disorders (see Chapter 53) leaving vitamin E to be covered in this

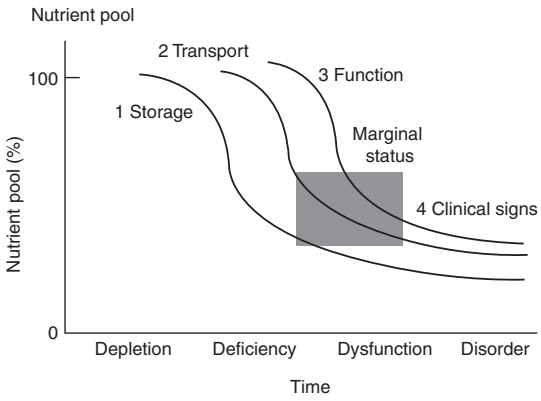


Figure 54.2: Sequence of events in sheep given an inadequate trace mineral supply: for Co, the sequence is (1) depletion of plasma B_{12} store; (2) further reduction in plasma B_{12} and fall in liver B_{12}; (3) fall in hepatic glucogenesis from propionate and/or methylation; (4) dry fleece, lachrymation, fatty liver, scabs on ears, anaemia. There is usually a zone of marginal status (shaded area), sometimes sustainable, where functions begin to fail but the animal remains healthy. (Adapted from reference [46].)

section alongside Se, with which it shares antioxidant function. Both the incidence and nature of lesions resulting from a particular deficiency may vary according to the stage of development at which deprivation occurs, the presence of other deficiencies and additional stresses imposed by environmental and/or management factors. Veterinarians should continue to be vigilant in looking for hitherto unrecognized aspects of deficiency.

COBALT DEFICIENCY

Cause

The only known biological role for Co in sheep, as in other animals, is as a constituent of vitamin B_{12} (cobalamin), which has coenzyme functions in the body. Pasture and food crops contain Co but not vitamin B_{12}, and ruminants rely on their rumen microbes to incorporate Co into the vitamin. Co deficiency is therefore synonymous with insufficient ruminal synthesis of vitamin B_{12}. Relationships between dietary Co, animal vitamin B_{12} status and deficiency signs are influenced by the concentration of Co in the diet; ingestion of extraneous Co (particularly as soil, which invariably contains far more Co than the herbage it supports), the efficiency with which Co is incorporated into 'true' vitamin B_{12} by the rumen microflora, the efficiency of vitamin B_{12} absorption and the metabolic demands placed on vitamin B_{12}-dependent functions. Absorption of vitamin B_{12} may be impaired by competition from physiologically inactive analogues that are also synthesized in the rumen. Moreover, parasitic infestation may reduce the synthesis of the intrinsic factor needed for vitamin B_{12} absorption, and impair the overall absorptive capacity of the mucosa. Only an approximate relationship, therefore, exists between disease and Co intake; problems can arise with dietary concentrations 50 per cent above [2] as well as below [3] those commonly regarded as nutritionally inadequate [0.08 mg Co/kg dry matter (DM)][1]. Dietary Co and plasma B_{12} concentrations are broadly related to available Co in the soil: values below 0.3 mg Co/kg soil, extractable with 0.43 M acetic acid, are often regarded as 'deficient' and are likely to arise in acid, Fe-rich and alkaline, Mn-rich soils [4]. The sandy soils of Sweden generally produce forages of low Co concentration [3].

Inadequate vitamin B_{12} absorption leads to reduced tissue concentrations of its two coenzyme forms. The first, methylcobalamin (Mecbl) assists methionine synthase, which recycles methyl groups via homocysteine, thus promoting methionine synthesis. In lambs deprived of Co, there is a rise in plasma homocysteine concentrations [5]. The relatively high susceptibility of sheep to Co deficiency may be due to their high S-amino acid requirement for wool growth [6]. Methionine supply ultimately influences DNA synthesis, and vitamin B_{12} deficiency may thus impair red cell maturation. Anaemia is, however, a late sign of deficiency in all species, due largely to the slow turnover of erythrocytes, and it is possible that the development of other cell types is impaired before anaemia is detectable. The second coenzyme form, deoxyadenosylcobalamin (Adocbl) assists methylmalonyl coenzyme A (MM CoA) mutase, and performs a key role in the energy metabolism of ruminants, facilitating the metabolism of propionate via succinate and the tricarboxylic acid cycle. Lack of Adocbl leads to the accumulation of MM CoA, which inhibits the β-oxidation of fatty acids mobilized to meet an energy deficit caused by anorexia: this partly explains the pathogenesis of the ovine white liver syndrome (OWLS) [7]. Mecbl deficiency may also play a part, homocysteine accumulation initiating the peroxidation of the

Table 54.1: Indicators of risk of trace element disorders (adapted from reference [46])

Copper*		Selenium*	
Diet (mg/mg)†		Diet (mg/kg DM)	**0.025**–0.05
S:Cu	500–**1000**		
Fe:Cu	50–**1000**		
Mo:Cu	0.3–**1.0**	Blood (nmol/l)	**500**–700 (**40**–56 μg/l)
Animal Plasma Cu (μmol/l)			
Neonate	**2**–4	Serum (nmol/l)	**400**–500
Others	**3**–9		
		Milk (μmol/l)	**50**–65
Liver Cu (μmol/kg DM)			
Neonate:	**200**–100	Blood GPX_1 (U/ml PCV @ 37°C)	**10**–15
Others:	**100**–300		
Cobalt*		**Iodine***	
Soil (mg/kg DM)		Diet (mg/kg DM)	
Extractable Co	<0.4	Summer	**0.05**–0.10
Mn	>1000	Winter	**0.10**–020
pH	<5 or >6.5		
		Goitrogens present in forages (leafy and root brassicas, clovers) and by-products (rapeseed meal, cassava meal), sometimes as mixtures of cyanogenetic and thioracil type, increase need for iodine	
Diet (mg/kg DM)			
Co	**0.05**–0.12		
Animal Serum B_{12} (pmol/L)			
Suckling	**100**–200	Thyroid weight (g FW/kg LW)	**0.5**–**1.0**
Weaned/adult	**250**–350		
MMA	7–14 μmol/l	Iodine (g/kg DM)	**1.2**–2.0
Liver B_{12} (nmol/kg FW)			
Suckling	**70**–140	Serum thyroxine (nmol/l)	**50**–60
Weaned/adult	**150**–300		
Milk B_{12} (pmol/l)		Serum or milk iodine (μg/litre)	**30**–40
	250–500		

* If the mean value for a set of samples falls within the marginal ranges given, there is a possibility of disorder that increases the closer that mean lies to the **bold** boundary limit – *keep stock 'off-limits'!*
† Where there are strong antagonisms between the elements, only the ratio between the agonist and antagonist reflects the 'copper value' of the diet.

DM, dry matter; MMA, methyl malonic acid; FW, fresh weight; LW, live weight; PCV, packed cell volume; GPX, glutathione peroxidase; B_{12}, vitamin B_{12}.

enlarged pool of labile fatty acids in the liver [7]. The prominence of anorexia as an early sign of deficiency has been related to impaired clearance of propionate from the bloodstream, but there may be a reduction in propionate synthesis by rumen microbial species that require vitamin B_{12} to produce propionate from succinate. In Co deficiency, methylmalonic acid (MMA) accumulates in plasma and becomes incorporated into

branched-chain fatty acids, which may alter lipid composition in both the liver and central nervous system (CNS) [5]. The two B_{12}-dependent pathways compete for absorbed vitamin and it is unlikely that both become rate-limited at the same time or that the critical pathway is the same in all Co-responsive disorders.

Clinical signs

The clinical signs of Co deficiency are non-specific but lambs are particularly susceptible, exhibiting loss of appetite, growth retardation, debility, emaciation and a watery discharge from the eyes (see Figure 54.3 in the colour plate section). In extreme cases, there is severe anorexia and anaemia: the latter is normochromic and normocytic and reflected by pale visible mucous membranes. Dramatic decreases in serum vitamin B_{12} concentrations can occur after weaning, and pastured lambs are most likely to become deficient in autumn. In many pastoral countries OWLS in lambs has been attributed to severe Co deficiency: scabby lesions may appear on the ears [2, 3], possibly caused by hepatogenous photosensitization (see Chapter 49). Fast growing Texel lambs with very low serum B_{12} concentrations (initial mean 173 and falling to <100 pmol/l) showed poor cell-mediated immune responses *in vitro,* and raised faecal egg counts during natural nematode infection although antibody responses to primary vaccination were not compromised [8]. In adult ewes, Co deficiency may be associated with secondary effects in the form of infertility and poor mothering, and there is evidence that Co deficiency in the dam can reduce the viability of her offspring [9].

Pathology

In severely affected animals, the carcass is extremely emaciated with no body-fat depots. There is bone marrow hypoplasia, and haemosiderin deposits are found in the spleen and sometimes in the liver. In OWLS, livers are grossly enlarged, fatty and friable. Histology reveals fatty changes in hepatocytes and bile ductules, and mesenchymal proliferation in portal areas. Ultrastructurally, mitochondrial abnormalities and accumulation of lipofuscin granules (products of lipid peroxidation) in the cytoplasm of hepatocytes are evident [10]. The neuropathy associated with vitamin B_{12} deprivation in humans is not a recognized feature of Co deficiency in sheep, although neuronal degeneration and demyelination have been reported in experimental cases.

Diagnosis

The major biochemical criteria used to support a diagnosis of Co deficiency have been subnormal concentrations of vitamin B_{12} in liver and plasma [11], but recent research has shown both to be poor indicators of Co responsiveness [12]. The relationship between them shows negative curvature, i.e. a plateau in liver vitamin B_{12} while plasma concentrations of the vitamin continue to increase. This is the opposite of the relationship between liver and plasma Cu, and indicates that plasma rather than liver serves as a store for vitamin B_{12} and monitors depletion. A recent analysis of individual lamb growth in a Co responsive flock in New Zealand showed that weaned lambs with <250 pmol B_{12}/l plasma were likely to suffer growth retardation [13]. This is a far lower threshold than that recommended previously on the basis of mean growth trial responses (<500 pmol/l), [11], but has now been confirmed by others [14]. Vitamin B_{12} concentrations in the liver correlate weakly with responses to Co therapy in suckling lambs. Mean values below 100 nmol/kg wet weight (WW) are indicative of deficiency. Although the threshold is higher for weaned lambs and adult ewes [12], liver and kidney Co concentrations are also low (<0.04 mg/kg DM [1, 3]). The relationship between individual plasma MMA and vitamin B_{12} concentrations is curvilinear, with MMA falling to 5 μmol/l or less as vitamin B_{12} rises above 375 pmol/ml [12, 15] in weaned lambs. Mean plasma MMA concentrations of 7–13 μmol/l can be regarded as marginal: as variation about the mean increases (to standard deviation >10), Co supplements are increasingly likely to improve health [12]. An advantage of an indicator of dysfunction, such as MMA, over indicators of depletion and deficiency (such as plasma/serum and liver B_{12}) is indicated by comparative changes in the lactating ewe and her lamb [12]. MMA can rise in early lactation without change in ewe B_{12} concentrations in serum, simply because propionate turnover increases, and with it the load on the Adocbl-sensitive pathway. However, MMA in the sucking lamb can exceed concentrations that are harmful to weaned or adult sheep because the availability of an alternative glucose precursor, milk

lactose, reduces the importance of the Adocbl pathway. Response to Co therapy provides the surest diagnosis, but a flexible interpretation of data on vitamin B_{12} status is given in Table 54.1.

Prevention and treatment

As the capacity to store vitamin B_{12} in the body is limited, continuous rather than discontinuous methods of treatment are preferred. Several methods are available and selection will depend on economic, dietary and general husbandry factors.

Discontinuous methods

Vitamin B_{12} synthesis by rumen microflora increases dramatically when Co is given orally, but responses follow the law of diminishing returns [1] and the high frequency of treatment required is rarely practicable. Administration of Co at monthly intervals with an anthelmintic can be a satisfactory compromise, but large doses [1.0 mg Co/kg live weight (LW)] may be required in severe deficiencies, and these can present formulation problems. A better oral method is the administration of a Co-containing bolus of high specific gravity ('bullets'), which slowly releases Co in the rumen. Giving such bullets to the pregnant ewe has improved the vitamin B_{12} status of her lamb after weaning, long after beneficial effects on her own plasma B_{12} status had disappeared [14]. An insoluble coating of calcium phosphate can form on the bullet, preventing the release of Co in some circumstances. Soluble glass boluses, containing Cu and Se, as well as Co, have provided plentiful supplies of Co for long periods, although there is a risk of regurgitation. Intramuscular (i.m.) or subcutaneous (s.c.) injections of the hydroxy or cyanocbl analogues of vitamin B_{12} rapidly increase concentrations in plasma, but large monthly doses of 70 μg /kg LW are required and, in the longer term, therapy should be continued by another method. Slowly mobilized forms of vitamin B_{12}, given as depot injections, are more effective [13]. Toxicity due to overdosing with vitamin B_{12} injections has not been reported.

Continuous methods

Where sheep are being fed concentrates, mineral mixes containing as little as 40 mg Co/kg will provide an adequate Co intake when included at 25 kg/tonne in a concentrate, which forms 10 per cent of total food intake. Risks of overdosing with Co are minimal, since sheep tolerate levels of 1 g Co/kg DM. The Co content of herbage can be increased to adequate levels by a single application of 2–3 kg/ha of hydrated cobalt sulfate ($CoSO_4$) in a low-volume spray or incorporated in a granular fertilizer at the time of manufacture. Where set-stocking is practised, 6 kg $CoSO_4$/ha can be applied to one-third of the grazing area. The effect of Co fertilization often lasts for 4 years or more but is governed by soil conditions. Effects are short-lived on soils of extremely low (<5) or high (>6.5) pH and/or high Fe or Mn content, because the added Co becomes fixed in non-available forms. Co should not be applied too soon after liming and the Mn status of the soil should be checked first as Co fertilizers are expensive.

COPPER DEFICIENCY

Cause

Poor absorption

The unique susceptibility of ruminants to clinical Cu deficiency on natural diets is related to events in the rumen, which commonly leave less than 10 per cent of ingested Cu in an absorbable form. Scottish Blackface ewes on permanent pasture have been found to absorb only 2 per cent of the Cu in summer herbage, and the value can fall to 1 per cent in autumn. Ingestion of soil as a leaf contaminant may be partly responsible for the seasonal drop in Cu absorption, and for the high incidence of swayback and hyopcuprosis after mild winters with little snow cover, eliciting an antagonism between Fe and Cu [1]. Other important factors that lower Cu absorption still further are the small increases in herbage molybdenum (Mo) (up to 6 mg Mo/ kg DM) and S (up to 4 g S/kg DM), which can follow liming and fertilization of temperate pastures or irrigation of otherwise arid pastures. At these low levels of absorption, dietary concentrations of 10 mg Cu/kg DM or more are required and few pastures exceed this level. Grass conserved as hay yields twice as much of its Cu for absorption as the fresh green material, and dry summer grazings may resemble hay in this respect. With dry forages and other sources of highly absorbable Cu (including cereals and brassicas), a dietary concentration of

3–5 mg Cu/kg DM may be adequate. Since poor Cu absorption is caused by imbalance between Cu and its antagonists (Mo, S, Fe), dietary Cu concentrations alone do not provide a definitive guide to the cause of deficiency problems.

Enzyme dysfunction

The functional disorder that results from a lack of absorbable Cu in the diet is hard to define. Cu forms an essential part of at least ten major metalloenzymes, which perform quite distinct biological functions, and it is uncertain which becomes rate-limiting in the diverse pathological manifestations of Cu deficiency [1]. The development of swayback, for example, may be related to a deficiency of cytochrome oxidase, causing anoxia and chromatolysis in the neurones, or to impaired synthesis of phospholipids, and hence of myelin, or to a deficiency of dopamine-β-hydroxylase and the subsequent accumulation of catecholamines in the CNS. Abnormal bone matrix formation may be associated with a defect in cytochrome oxidase activity in osteoblasts or lysyl oxidase in chondroblasts. Growth retardation may be related to a localized depletion of Cu in the intestinal mucosa, which influences digestion, motility or inflammatory responses to gut parasites. Lowered resistance to infection may be explained by the fact that granulocytes from hypocupraemic ewes and lambs exhibit reduced activities of the Cu-dependent enzyme superoxide dismutase (SOD) coupled with impaired microbial killing capacity [16]. A cause and effect relationship is possible in that generation of the superoxide radical is involved in microbicidal activity. There is no evidence that natural exposure to Mo impairs health other than by lowering Cu status.

Breeding

Breeds, and individuals within breeds, differ in their susceptibility to Cu deficiency, partly as a result of genetic differences in the efficiency with which they absorb Cu from the diet. For example, the Scottish Blackface ewe absorbs Cu 50 per cent less efficiently than the Welsh Mountain ewe, and is more susceptible to swayback [17]. Texel and North Ronaldsay sheep absorb Cu more efficiently than other breeds studied and are unlikely to become deficient. Some control over Cu deficiency may therefore be obtained by appropriate selection of breeds and crosses, and by culling susceptible individuals and lines [17]. The susceptibility of breeds to Cu toxicity is, however, inversely related to susceptibility to deficiency and risk of toxicity would be increased by such policies. Breed effects further weaken the relationship between dietary Cu intake and disease incidence.

Clinical signs

The earliest sign of Cu deprivation is the loss of wool crimp ('steely wool') (Figure 54.4) and transient deficiencies show up as a band of uncrimped wool of low tensile strength. In breeds with coloured fleeces, the 'steely' band may lack pigmentation. Another early manifestation of Cu deficiency in sheep is swayback (enzootic ataxia) in lambs. Swayback takes two forms, a congenital one, which is apparent at birth, and a delayed one, which does not become apparent until lambs are several weeks old (see Chapter 38). Affected lambs are uncoordinated and have a tendency to sway on their hind legs. The congenital form is often more severe, and some newborn lambs may be so badly affected that they cannot get up. At the other extreme, the delayed form may not become apparent unless the flock is driven. Other signs of Cu deficiency, first reported in lambs on improved hill pastures in Scotland, can occur when the deficiency is imposed after birth: they include poor LW gain, anaemia and fragile bones, and are collectively described as 'hypocuprosis'. Susceptibility to infection by common bacterial pathogens is increased in hypocuprotic lambs but fertility is not compromised [17]. In the UK, there are some ten times more incidents of hypocuprosis than swayback every year.

Pathology

In lambs severely affected with congenital swayback, there is cavitation of the cerebral white matter and marked internal hydrocephalus. In less severe cases, brains and spinal cords may be grossly normal and characteristic lesions only detected histologically: these consist of chromatolysis in the brainstem and bilaterally symmetrical demyelination in the spinal cord. The critical site moves from the cerebrum in neonatal ataxia to the spinal cord in delayed swayback, and coincides with the peak periods of myelin formation in those parts of the CNS, suggesting that

Figure 54.4: Fleece of a normal Scottish Blackface lamb (left) compared with that of its twin (right) which shows the characteristic loss of wool crimp associated with copper deficiency [46].

impaired myelin synthesis is an important factor. Contrasts between CNS lesions in congenital and delayed swayback, and their absence in growth-retarded lambs, illustrate the importance of the stage of development at which Cu deficiency is imposed to the pathological outcome (see Chapter 38). In cases of ill-thrift referred to earlier, post-mortem lesions were non-specific and similar to any other 'pine' condition, namely emaciated carcasses, lack of body fat, and haemosiderin deposits in the spleen and liver. There were fractures of the long bones and osteoporosis was detected histologically. Hypochromic anaemia is a late consequence of deficiency: it is microcytic in lambs and macrocytic in ewes, and can be associated with the formation of Heinz bodies.

Diagnosis

The non-specific clinical signs of Cu deficiency make hypocuprosis hard to diagnose, and even swayback may be confused with several other conditions that produce ataxia (see Chapter 38). Swayback can be differentiated at autopsy, and confirmed by histopathological examination of the brain and spinal cord, and the detection of low Cu concentrations in these tissues (<78.5 μmol Cu/kg DM). Cu concentrations at other sites, such as the liver and bloodstream, may be particularly unhelpful in confirming delayed swayback, in which lesions can develop several months before clinical disease is detected. Whatever the clinical manifestation of deficiency, liver and blood Cu concentrations require careful interpretation. During depletion, liver values can fall to around 157 μmol Cu/kg DM before Cu reserves are exhausted, but this point can be reached rapidly in the newborn lamb in which the size of the liver and its Cu store are small. Plasma Cu concentrations below 9.4 μmol/l generally indicate a state of deficiency, but this may not have persisted for sufficient time or been present at the critical sites of development necessary for health to be impaired. A second diagnostic threshold of around 3 μmol Cu/l plasma may have to be crossed before hypocuprosis limits performance [1]. Serum should not be used because of variable entrapment of Cu in the blood clot. Cu concentrations may be 1.57–3.14 μmol/l higher in whole blood than in plasma, from the same animal, and diagnostic limits should be set correspondingly higher. Because they decline at a slower rate, low SOD activities in erythrocytes indicate a prolonged deficiency, and low values are more likely to be associated

with impaired function and growth. 'Normal' SOD levels of >0.4–0.5 IU/mg haemoglobin (Hb) for lambs and >0.3–0.5 IU/mg Hb for ewes have been recommended [1], but reference values may vary from laboratory to laboratory. Further analyses should be carried out on tissues such as kidney, in which Cu values are usually <100 μmol/kg DM in the hypocuprotic lamb [3, 17], to confirm diagnosis. An interpretive framework for the major biochemical indices of Cu status is given in Table 54.1.

Prevention and treatment

Since damage to the CNS and wool fibre in Cu deficiency is irreversible, infections sometimes fatal and compensatory growth in treated lambs may be incomplete, the emphasis must be on prevention: several methods are available. With all methods of prevention, maintaining the Cu status of the ewe does not necessarily protect her lamb from growth retardation for two reasons. First, Cu transfer via the placenta and milk in sheep is limited and, second, a dietary Cu antagonist may cause a localized depletion of Cu in the lamb's intestinal mucosa, which systemic reserves of Cu may not fully ameliorate. Treatment of the lamb before weaning will be inefficient if Cu deficiency has reduced milk yield in the dam. Weaned lambs can be given Cu, preferably orally, at rates (per kg LW) that are appropriate for adults. Because of the ever-present risk of toxicity, only one method of Cu supplementation should be applied at any time.

Fertilizers

It is impossible to generalize on the efficacy of this method of prevention. On sandy aeolian soils in Australia, a single application of 8.25 kg hydrated copper sulfate ($CuSO_4$)/ha in 1963 had doubled the Cu content of herbage sampled 13 years later, and responses in liver Cu were found after 17 years. By contrast, on peaty soils in New Zealand, yearly treatments with Cu are sometimes needed. Small annual foliar applications of 0.2 kg $CuSO_4$/ha increase herbage Cu concentrations, but the extent and persistence of the effect is dependent on subsequent rainfall and in drought conditions, Cu toxicosis is a hazard. Application of Cu-rich pig slurry and municipal sewage sludges increase soil Cu concentrations substantially, but effects on herbage Cu are small.

Oral dosing

Solutions containing 1 g Cu, given 8 and 4 weeks before lambing, have been used for many years to prevent swayback. Dosing newborn lambs to prevent delayed swayback may be effective but, since they absorb Cu up to 50 times more efficiently than the dam, the required dose is much reduced and 1.6 mg Cu/kg LW should suffice. Alternatively, 0.5 mg Cu/kg LW may be injected s.c. as copper heptonate. Provision of Cu orally in a single dose, slow release form is widely practised using particles of cupric oxide (CuOP), which lodge in the abomasum: they are effective in suckling, as well as in weaned lambs or adult sheep, at doses of 0.1 g/kg LW. Retention of particles in the alimentary tract, and hence efficacy, may be lowered by infectious diseases such as nematodiasis. The slow-release, glass bolus can provide sufficient absorbable Cu to prevent hypocuprosis, but the other elements present, Co and Se, may not be needed. Dosing is contraindicated in sheep due to be housed, since high availability of Cu in rations for housed sheep generally obviates the need to supplement with Cu.

Feed supplementation

Continuous Cu supplementation via compound feeds intended for sheep is forbidden in Europe, although risks of poisoning are insignificant for outwintered stock (the Texel and North Ronaldsay breeds being exceptions). Supplementation of homegrown feeds with 20 mg Cu/kg DM is an attractive alternative for hill breeds kept outside. Sheep indoors should never be supplemented unless hypocuprosis is present or imminent.

Injection

The subcutaneous (s.c.) or intramuscular (i.m.) injection of chelated Cu has been widely used but, although generally an effective preventive measure, this method is not without hazard. Methionates and glycinates are slowly translocated from injection sites, produce large local reactions, which can reduce carcass value, and they afford variable protection, albeit with virtually no risk of acute toxicity. At the other extreme, diethylamine oxyquinoline sulfonate is rapidly translocated and gives no local reactions, but its acute toxicity at 1–2 mg Cu/kg LW limited doses to amounts that were sometimes ineffective.

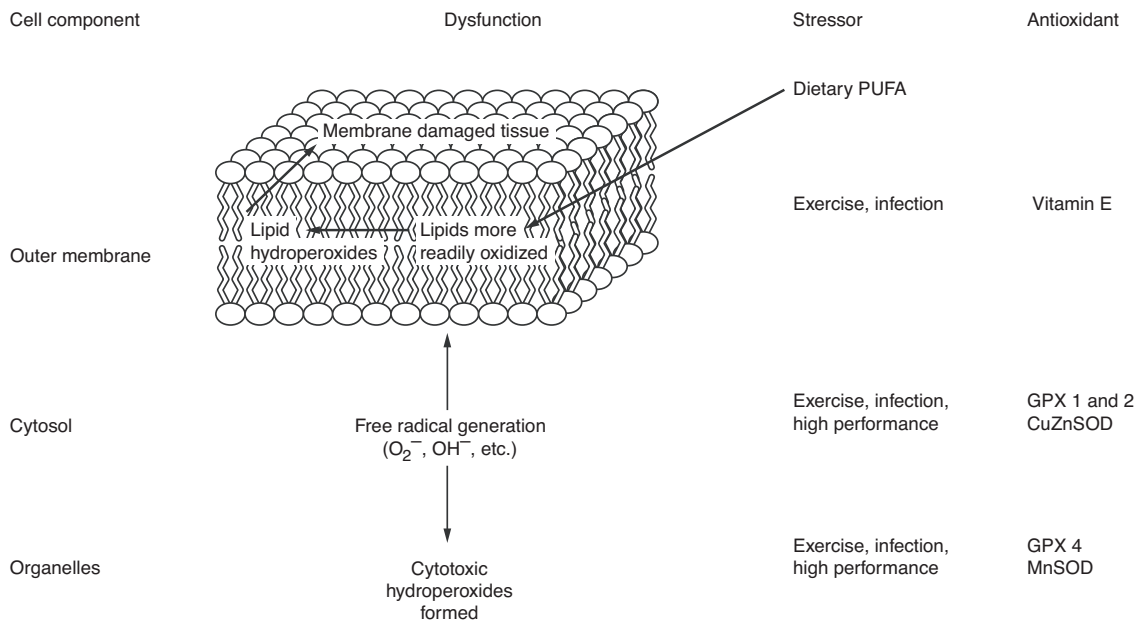


Figure 54.5: Schematic of cell membrane oxidative metabolism/antioxidant interactions, PUFA, polyunsaturated fatty acids; O_2^-, superoxide anion, OH, hydroxyl radical; GPX, glutathione peroxidase; CuZnSOD, copper/zinc-dependent superoxide dismutase; MnSOD, manganese-dependent superoxide dismutase.

The ethylene diamine tetra-acetate chelate (CuCa EDTA) is intermediate in all respects and acute toxicity is extremely rare at doses of 1 mg/kg LW. It should be noted that the aforementioned complexes have been withdrawn from sale for sheep in some countries. The Cu heptonate combines safety with efficacy at lower doses (0.5 mg/kg LW). For swayback prevention, the decision to inject should be based on the presence of hypocupraemia (Table 54.1) in mid or late pregnancy; one hypocupraemic ewe in a sample of 10 per cent of the flock may merit the injection of the whole flock.

SELENIUM AND VITAMIN E DEFICIENCY

Cause

In many circumstances, Se deficiency cannot be considered alone and must be addressed in concert with vitamin E deficiency. The characteristic clinical outcome of both is white muscle disease (WMD), and this reflects their complementary, although independent, roles as cellular antioxidants [18]. The major cause of Se and/or vitamin E deficiency is inadequate dietary supply: both single or dual deficiency states can occur in the field, and all lead to an imbalance between antioxidant defence and oxidant stress. While both Se and vitamin E help to protect cells against the injurious effects of lipid peroxides and free radicals produced during normal cellular oxidative metabolism, their modes of action differ (see Figure 54.5). Se exerts its influence intracellularly principally as a component of glutathione peroxidase (GPX), a family of at least four separate selenoenzymes, and thioredoxin reductase [1]. Vitamin E is an essential structural component of lipid biomembranes and provides protection against toxic peroxidation of polyunsaturated fatty acids (PUFAs). Dietary excess of PUFAs can often exacerbate the effects of antioxidant, particularly vitamin E, inadequacy. Failure to arrest free radical production and peroxidation due to antioxidant deficiency leads first to membrane damage and ultimately to tissue necrosis, such as that evident in WMD. Tissues or cells that undergo rapid

increases in oxidative metabolism such as muscle (skeletal, cardiac or respiratory) and blood cells (e.g. erythrocytes, phagocytes and lymphocytes) are particularly susceptible. Failure of antioxidant defences may underpin other manifestations of Se- and vitamin E-responsive disorders, such as growth retardation and infertility, although the involvement of Se in a series of deiodinase enzymes may also contribute. These directly affect thyroxine metabolism [19] and Se-deficient sheep have an increased T4:T3 ratio [20]. Se deficiency may, therefore, indirectly influence basal metabolic rate and this could ultimately give rise to adverse effects on production. Moreover, recent evidence suggests that there may be sensitive interactions between Se and iodine deficiencies in sheep thyroid and brain [21]. Although there has been speculation, mostly based on experimental evidence, that Se and/or vitamin E deficiency may impair immune function and disease resistance [16, 18], practical confirmation in the field has proved elusive [1].

Incidence/natural occurrence

Se deficiency occurs in many regions of the world where soils contain naturally low levels of the element. Examples are soils of granitic origin found in mountainous regions of northern Europe (e.g. Finland, Sweden, Scotland) and those of volcanic origin such as the pumice soils of the North Island of New Zealand (see Chapter 67) and the tablelands of New South Wales. However, many other areas, including most of the UK, large parts of the north east of the USA and several provinces of China are inherently low in Se. Crops and pastures grown on Se-deficient soils provide a poor supply of Se to grazing livestock, and deficiency is regarded as endemic in many of these areas. Effects of soil origin may be confounded with those of climate and altitude: the Se content of forages is reportedly less at high than low altitude, probably due to the influence of high rainfall [22]. Se deficiency is typically more prevalent in areas of high rainfall than drier regions, probably due to leaching of Se from the pastures. Se uptake by crops and forages is also influenced by soil pH, being higher on alkaline than acid soils. In addition, Se deficiency can be exacerbated by, or result from, continual and/or intensive farming practices. For example, productivity trials have demonstrated that marginal Se deficiency can become severe after superphosphate application (which reduces Se uptake by plants) and stocking rate increases.

Vitamin E deficiency is independent of soil type and more closely reflects forage/feed quality. In general terms, fresh legumes and pasture are good sources of vitamin E, whereas silage, oil seeds, root crops, cereal grains and dry hays are not. Vitamin E concentrations are naturally high in green swards but fall rapidly during dry periods, or in stored forages, hay and grain. Prolonged storage of feedstuffs can result in up to 50 per cent losses of vitamin E per month. In high rainfall areas such as New Zealand, Se is likely to be more limiting than vitamin E, whereas in areas with a Mediterranean climate and pronounced dry seasons, such as Western Australia, vitamin E is likely to be more limiting than Se. The vitamin E status of forages can be reduced by adverse harvesting and storage conditions. For example, concentrations of the vitamin in early-cut hay may decrease to less than one-third within a few months, and even more rapidly under damp harvesting conditions. Several methods used to treat and preserve grain (e.g. addition of alkali, propionic acid) and silage may also destroy vitamin E.

Effects of single or dual dietary insufficiencies may be compounded by the presence of high PUFA concentrations, such as those found in young rapidly growing pasture. This may be particularly important in young lambs weaned off ewes of marginal vitamin E status. Root crops such as turnips, should be used guardedly because not only do they have particularly low contents of Se and vitamin E, but they also contain sulfoxides which may present an oxidative stress.

Clinical signs

General unthriftiness may sometimes be the only clinical sign of Se and/or vitamin E deficiency. However, the most commonly recognized, and characteristic, 'Se/vitamin E- responsive' condition is the syndrome most often referred to as WMD, but also known as stiff lamb disease, or nutritional (enzootic) muscular dystrophy (NMD). Another term, weaner nutritional myopathy (WNM) has been used to describe a clinically similar condition which occurs in animals of adequate Se status but deficient in vitamin E. WMD, is in fact, a degenerative rather than dystrophic disease of skeletal and/or cardiac striated muscle, which occurs without neural involvement. In some parts of the world, incidence is low (<1 per

cent), seasonal and sporadic while in others, including parts of Turkey and New Zealand, 20–30 per cent of flocks can be affected consistently. WMD primarily affects young lambs and presents as two major clinical forms, which can develop *in* or *ex utero*. In the first ('congenital WMD'), lambs may be stillborn or born weakly and die within a few days, often from acute cardiac arrest after physical exertion. In the second form ('delayed or acquired WMD'), signs develop after birth, most frequently within 3–6 weeks but, on occasion, as late as 4 months. Mortality rates are usually higher in lambs with congenital WMD.

In both forms of WMD the general presenting symptoms are similar: weakness, stiffness, rapid breathing and deterioration of muscles. However, there is a need for caution in the congenital case, because lambs born stiff and weak lamb may have conditions, e.g. vibriosis, polyarthritis, enterotoxaemia, rickets, other than WMD. Leg muscles are usually affected first and overt signs can range from mild stiffness and discomfort (often mistaken for joint-ill) to recumbency, reluctance to move or even collapse when driven. Lambs often have swollen hindquarters, exhibit an unsteady gait and obvious pain when walking, and adopt a hunched appearance when standing. Older lambs may suffer respiratory distress (often with secondary pneumonia confusing diagnosis). Ultimately, lambs lose body weight and condition, become prostrate and usually die, although, occasionally, mildly affected animals can recover spontaneously. Additional stressors such as severe bad weather, transportation, handling, vaccination procedures and weaning can exacerbate the occurrence and/or severity of WMD.

A number of studies, particularly from Australia and New Zealand, have provided evidence that Se and vitamin E deficiencies may impair productivity through effects on growth and/or reproduction where there is no WMD. Se-responsive 'ill-thrift', ranging from subclinical growth deficit to visible unthriftiness, rapid weight loss and even some mortality, has been reported in lambs and hoggets at pasture. Heinz body anaemia has also been seen in Se-deficient lambs stressed by exercise. Se supplementation can, therefore, improve lamb growth, wool production and lamb survival [20]. In ewes, high embryonic mortality around implantation has been attributed to Se inadequacy, although Se-responsive ill-thrift can occur with no drop in ewe fecundity [23]. In economic terms, subclinical forms may be the most important manifestation of dysfunction but, at least in New Zealand [24], extremely low Se status is required (blood Se $< 0.13\,\mu$mol/l) for growth to be retarded. Although less well documented in sheep than in cattle, vitamin E supplementation can sometimes provide a similar range of subclinical benefits either alone or together with Se [25].

Pathology

Gross pathology is often hard to see in mild cases of WMD but, in severe (fatal) cases, affected skeletal and/or cardiac muscles appear pale with white plaque areas of calcification frequently evident (see Figure 54.6 in the colour plate section). Most muscles can be involved, but macroscopic lesions are most common in the heart, particularly in congenital WMD, or in the large muscles of the shoulder, girdle, back and thighs. However, those of the diaphragm and tongue can also be affected. Abnormalities in the electrocardiogram develop early in affected lambs and become more marked as death approaches. In older lambs, a bilaterally symmetric distribution of muscle lesions, particularly in the thigh and shoulder regions, is characteristic: the deep muscles overlying the cervical vertebrae also show white striations. The basic WMD lesion involves hyaline degeneration followed by coagulative necrosis and calcification. Indeed, the ultimate biochemical lesion may be mitochondrial Ca overload due to structural changes, with reduction in Ca-binding proteins leading to impaired Ca uptake by the sarcoplasmic reticulum [26]. Individual muscle fibres may be oedematous and swollen with loss of cross striations and sarcolemmal proliferation. Signs of haemorrhage are often present as a result of blood vessel degeneration.

Diagnosis

Overt signs may be non-specific (e.g. ill-thrift, poor growth, reproductive problems) and, even in the case of WMD, similarity to other conditions (e.g. joint-ill, swayback, spinal abcesses, vibriosis, enterotoxaemia, rickets, or even pneumonia when intercostal muscles are involved) demand careful differential diagnosis, and require specific biochemical measurements for confirmation. In advanced WMD, enzyme release from damaged musculature can result in plasma/serum creatine kinase (CK) activities reaching 5000–10 000 IU/l,

which is 10–20 times the upper reference level for healthy lambs. Less dramatic changes (500–1000 IU/l) occur in mild or early WMD, and these may be confused with transient increases associated with muscular stress during transportation, handling or exercise. Separation of different tissue-specific types (isoforms) of CK may be useful in pinpointing lesions to skeletal or cardiac muscle. In addition, serum aspartate aminotransferase (AST) activity may be elevated 3–10-fold (300–1000 IU/l) above normal for long periods in WMD. Alanine aminotransferase (ALT) activity may also be raised, and a combination of tests for plasma CK and ALT, along with plasma α-tocopherol (vitamin E) and whole blood GPX, have been recommended for the differential diagnosis and appropriate treatment of WMD [27].

Direct measurements of Se and vitamin E in the circulation are deceptively simple to perform, but equally easy to misinterpret unless their limitations are recognized. The use of a two-tier system of reference ranges/limits (see Table 54.1) for predicting and/or diagnosing Se deficiency is recommended as an aid to the interpretation of clinical chemistry data. Owing to the speed and simplicity of analysis, compared with elemental Se, most laboratories regard erythrocyte GPX(1) as the parameter of choice for assessing Se status. Although deficiency thresholds, reporting units (see Appendix B) and precise assay conditions are still not universally harmonized, GPX activities <20 IU/ml packed blood cells (assayed at 30°C) are often regarded as carrying increased risk of disorder. Diagnostic limits for elemental Se in serum have been determined in Australia, in relation to wool production, and have been suggested to be a sensitive index for Se adequacy in sheep. For barren Merino ewes, Se concentrations <0.25 and <0.51 μmol Se/l for plasma and blood, respectively, were associated with reduced wool yield, although corresponding values in pregnant and lactating ewes were approximately double [28, 29]. Other reports have suggested that minimum concentrations of <0.51 and 0.75 μmol/l for plasma and blood, respectively, should be maintained in all animals to avoid sub-clinical deficiency [30]. Serum Se responds more rapidly than blood Se or GPX to a sudden change in dietary Se supply. Laboratories should standardize GPX assays in terms of 'equivalent elemental Se'. Studies on different sample types indicate that the correlation between GPX and Se was highest (r = 0.97 in 90 sheep) in heparinized whole blood compared with serum, heparin and EDTA plasma, and EDTA whole blood [30]. For vitamin E, plasma α-tocopherol concentrations below 1 μmol/l are widely regarded as carrying increased risk of disorder, even when Se status is adequate. However, where Se status is marginal (GPX 20–30 IU/ml packed blood cells) α-tocopherol concentrations as high as 2 μmol/l may be insufficient. The converse relationship can also apply with higher blood or serum Se concentrations required when vitamin E status is low. For suspected cases of deficiency, for which the aetiology is not clear, it is recommended that the status of both nutrients should be evaluated.

Treatment

Field experience and factorial models estimate that the daily dietary Se requirement for sheep is around 0.025 and 0.035 mg Se/kg DM for diets of low and high digestibility, respectively [1]. A review of field problems (mostly WMD) [30] placed the requirement at 0.03 mg Se/kg DM for diets adequate in vitamin E. Requirements for vitamin E remain the subject of debate, with estimates generally ranging from 15–40 mg vitamin E/kg DM [25], although even higher levels may be needed to optimise performance where Se status is marginal. A number of supplementary approaches are possible [31], but dietary deficits are routinely treated by s.c. injection of Se/vitamin E mixtures. A range of commercial preparations is available, generally containing 0.5–1.5 mg Se and 68–150 mg vitamin E/ml. All registered products in use in the UK have recommended doses of 1–2 ml for adult sheep and 0.5–1 ml for lambs. The i.m. route is equally effective but not recommended because of potential adverse effects on carcass quality due to muscle damage [25]. Plasma vitamin E concentrations fall rapidly soon after treatment, and injections may have to be repeated weekly for several weeks in severe WMD: in such instances the risk of Se toxicity from Se/vitamin E mixtures should be considered. At least 2 months should elapse between the last injection and slaughter for human consumption for the most Se-rich formulations.

Prevention

Methods for supplying Se and vitamin E prophylactically have been comprehensively reviewed by others

[1, 25, 31]: they include periodic injection, supplementation of concentrate feeds, addition to rations of feedstuffs rich in Se and vitamin E, Se supplements in free-access minerals, oral administration of slow-release Se pellets/boluses, parenteral injection of Se in slow-release form (e.g. barium selenate), periodic oral drenching with Se salts, and application of Se salts directly to soils. The injectable preparations used for treatment of clinical disorder also are used prophylactically in ewes during late pregnancy (3 months onwards) and in newborn or weanling lambs. Maintenance of adequate vitamin E status is particularly important during early periods of pasture growth, when PUFA content in the green swards is exceptionally high. A formula for calculating vitamin E requirements on the basis of physiological demands has been suggested as follows: 1 mg/day/kg body weight plus 5 mg/kg milk production plus 3 mg vitamin E/g PUFA present in the diet [32]. No dietary regime should supply more than 2 mg Se/kg DM for any length of time to prevent risks of Se toxicosis. In the United States, Se supplementation is controlled by law and for sheep Se can be supplemented in a complete ration at a level up to 0.3 ppm in a feed supplement such that intake does not exceed 0.7 mg/head/day, and in salt/mineral mixes at 90 ppm as long as consumption does not exceed 0.7 mg/head/day. However, Se licks and free-choice minerals are not recommended because of variable intakes. Oral drenching, which can be combined with anthelmintic administration, is effective as is the use of slow-release methods based on soluble glass boluses or grits. A single 10 g pellet of 5 per cent w/w elemental Se in an iron grit was found to protect ewes in Se-deficient areas for up to 3 years and a major study in Western Australia [33, 34] showed that single applications of prilled selenate-based fertilizers at rates of 10–20 g Se/ha could also supply sufficient Se to grazing animals for 3 years. Combined Se/vitamin E supplements can sometimes be conveniently and efficiently given via the diet. Total dietary concentrations of 0.1 mg/kg DM for Se and 30–50 mg/kg DM for vitamin E should ensure adequate status of both nutrients. Vitamin E supplementation is particularly necessary for diets based on weathered hays, moist or propionic acid-preserved grain and root crops.

IODINE DEFICIENCY

Cause

Iodine (I) deficiency may arise from a simple lack in the diet which, in turn, may reflect a low I content in the soil. Soils in the interior regions of most continents are naturally low in I, whereas coastal areas receive I by deposition from oceanic sources. In coastal regions and islands, I deficiency is usually induced by exposure to goitrogens. These act by disrupting I metabolism, either impairing uptake of I by the thyroid gland (e.g. the thiocyanate or nitrate goitrogens found in brassicas or legume crops) or iodination of tyrosine residues within the gland (e.g. the thiouracil goitrogens found in brassica seeds). In New Zealand, cases occur in lambs born to ewes fed on brassica crops for long periods during pregnancy [35, 36]: other goitrogenic factors also may be present such as the cyanogenic glycosides of white clover. The problems of I deficiency in New Zealand are discussed in detail in Chapter 67. I is an essential constituent of tri-iodothyronine (T3) which controls oxidative phosphorylation and therefore basal metabolic rate (BMR) and protein synthesis. Factors that increase BMR, such as low winter temperatures, subsequently increase I requirements [1]. As mentioned earlier, Se deficiency can have a profound effect on thyroxine metabolism, owing to its essential role in deiodinase enzyme activity [19, 21], and links between Se deficiency and susceptibility to goitre have been reported.

Clinical signs

Sheep are more susceptible than cattle to I deficiency. Older animals are rarely affected clinically, although infertility, loss of libido in the male, poor wool growth, depressed milk yield and reduced weight gain have all been recorded. The common clinical manifestation of I deficiency is late abortion or the birth of weak lambs with visibly enlarged (up to 50 times normal) thyroid glands (i.e. goitre). Retarded development of the CNS has been observed in severely depleted fetal lambs, and behavioural abnormalities may ensue. Modest enlargement of the thyroid, without any apparent ill-effects on health and production, was a consistent finding during 4-year grazing trials on various brassicas, including kales, turnips and Chinese cabbage [36].

Table 54.2: Reference ranges for biochemical indices of micronutrient status for vitamin E, iron, iodine, manganese and zinc

	Diet (mg/kg DM)	Serum (μmol/l)	Tissue* (μmol/kg FW)	Conversion factor to (/) or from (×) SI units†	Other indices
Vitamin E	15–30	1–2	3–5 (L)	0.43	Serum CK > 1000 IU/l
Iron	30–50	20–30	400–600 (L)	0.056	Blood Hb 70–100 g/l
Manganese	8–20	1.8–2.0	0.6–0.7 (H)	0.055	
Zinc	10–20	6.1–9.2	<275 (P)	0.065	Wool 1.2–1.5 μmol/kg DM

*Values given for tissue of choice: L, liver; H, heart; P, pancreas.

†Reporting units vary between countries and laboratories. Diets are usually reported in mass units (e.g. g, mg, kg) while Systeme Internationale (SI) units (e.g. moles, μmoles, etc.) are mostly used for blood and tissues. The factors given will interconvert: mg/kg DM and μmol/kg DM; μmol/l and mg/l; μmol/kg FW and mg/kg (same as parts per million, ppm) FW.

DM, dry matter; Hb, haemoglobin; FW, fresh weight; CK, creatine kinase; T_4, tetraiodothyronine or thyroxine.

Pathology

Major changes are seen in thyroid structure of the I-deficient, newborn lamb. Colloid depletion, cell proliferation and cell enlargement occur sequentially and are followed by invagination, and finally collapse and infiltration of follicles.

Diagnosis

Thyroid gland to body weight ratios >0.9 g/kg are indicative of goitre, whilst values of 0.4–0.9 g/kg indicate marginal I deprivation. Total thyroid weight >2.8 g has been used as a diagnostic threshold for congenital goitre, but histological abnormalities have been found in smaller thyroids. I deficiency should be considered in the differential diagnosis of stillbirth and weak lamb syndromes. Values for normal serum thyroxine (as tetra-iodothyronine, T4) of >47 and >90 nmol/l have been used as thresholds of I sufficiency for ewes and lambs, respectively, while plasma T4 concentrations <20 nmol/l, and pasture I contents <0.09 mg/kg have been associated with high incidence of congenital goitre. Recent evidence that I supplementation of grazing ewes with 'normal' T4 concentrations improved lambing rates and reduced perinatal mortality [37] may indicate involvement of thiouracil goitrogens, which raise T4:T3 ratios: the responses were believed to have been initiated in early pregnancy, introducing a substantial lag between the relevant sampling time and manifestation of disorder. It should be noted that only the free form of tri-iodothyronine (T3) is physiologically active and, since it is by far the smallest fraction of total serum thyroxine and I, low total thyroxine (i.e. T3 plus T4) and I values are poor indices of functional I deficiency. Urinary and milk I concentrations are good markers of I intake [38]. Milk is an important route of I secretion and milk I concentrations below 0.55 μmol/l are indicative of dietary I deficiency. A full diagnostic framework is given in Table 54.2.

Treatment and prevention

All clinical and subclinical effects of simple I deficiency, including fetal brain retardation, respond to I administration. Injection i.m. of iodized poppy-seed oil is perhaps the most effective treatment, a single 1 ml injection can provide I lasting for up to 2 years. Estimates of dietary I requirement for sheep vary from 0.1 to 0.4 mg I/kg DM, with 0.4 mg I/kg DM recommended as a minimum during the winter if and where goitrogens are not a problem [1]. Cereals and root crops are a particularly poor source of I, containing only 0.04–0.09 mg I/kg, and when they, or goitrogenic crops such as leafy and root brassicas or subterranean clover, form an appreciable part of the ration, particularly in late pregnancy, I supplementation is needed. I is routinely incorporated into mineral supplements as iodide, but less volatile forms such as periodates and organo-iodine compounds are preferable in warm, dry climates. Iodine supplementation is effective against goitrogens

of the thiocyanate or nitrate type and, in the absence of quantitative information on their antagonistic effects, a fourfold increase in I requirements should be allowed [39]. Use of feeds containing thiouracil-type goitrogens should be avoided in late pregnancy because they cannot be countered by I supplementation.

OTHER TRACE ELEMENT DEFICIENCES

The rare instances in which iron (Fe), manganese (Mn) and zinc (Zn) influence sheep health are discussed briefly below. Guidelines for the assessment of their status are given in Table 54.2.

Iron

Anaemia has been induced by feeding diets very low in Fe (<14 mg Fe/kg DM) to lambs before and after weaning [1]. Although milk is not particularly rich in Fe, requirements for Fe diminish with age and initial hepatic reserves, coupled with adventitious sources, e.g. soil and faeces, almost invariably provide sufficient Fe to prevent deficiency. Exceptions can arise when young rapidly growing lambs are reared indoors. The rapid expansion of red cell mass in early life leads to a mild anaemia reminiscent of the more severe problem, which invariably affects piglets reared indoors. On one farm, lamb weaning weights were improved by administering 200 mg Fe as Fe dextran by i.m. injection [40]. Fe supplements are rarely needed and their unnecessary use may exacerbate Cu deficiency.

Manganese

The swollen joints and stiff gait that affect lambs on experimental diets of very low Mn content (0.8 mg Mn/kg DM) may result from impaired mucopolysaccharide synthesis due to low glycosyl transferase activity [1]. Mn is an essential constituent of this and several other metalloenzymes, including a superoxide dismutase (MnSOD). There may be partial substitution of MnSOD for CuZnSOD during Cu deprivation, and reciprocal increases in CuZnSOD during Mn deprivation, which provide cover against antioxidant stress. However, the lamb is very tolerant of Mn depletion. Mn concentrations in heart fall more than those in liver or bone, and there is an associated reduction in MnSOD in cardiac tissue. A diet containing 13 mg Mn/kg DM, a level well below that found in most forages and concentrates, was adequate for growth and wool production in ram lambs [41]. Mn supplementation has improved fertility in ewes on pastures containing 20–30 mg Mn/kg DM in two out of three seasons [1], but there is little evidence that Mn deficiency is an important condition in sheep. The congenital bone deformities seen in depleted calves have yet to be reported in lambs.

Zinc

Under experimental conditions, Zn deficiency causes growth retardation, parakeratosis, wool loss, excessive salivation and impaired spermatogenesis [39]. With the exception of male fertility, abnormalities have occurred only on diets of very low Zn content (7–18 mg Zn/kg DM). Pastures rarely contain <20 mg Zn/kg DM and sheep are able to absorb Zn very efficiently at low intakes. This may explain why only three reports of natural Zn deficiency were noted in a 1980 review [39], and few have been added since [42]. Although the young, rapidly growing lamb has a particularly high requirement for Zn, ewe's milk is rich in Zn (around 7 mg/l) and protection may thus be afforded at the most vulnerable period. The conclusion that supplementation with 'organic' as opposed to inorganic sources of Zn offered no consistent nutritional or health advantage for sheep [1] remains valid [43].

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55

Diseases of the urinary system

N.D. Sargison and K.W. Angus

The principal functions of the kidney are the regulation of salt and water, and acid and base balance; excretion of nitrogenous waste products and other toxic substances; metabolism of vitamin D; production of a variety of hormones including erythropoietin, renin and prostaglandins; and homeostasis of glucose and essential electrolytes. The basic requirements for normal kidney function are adequate blood perfusion, adequate function of renal tissue and normal elimination of urine from the urinary tract. Renal failure, whether associated with pre-renal (usually caused by hypoperfusion of the kidneys due to congestive heart failure, shock or haemorrhage), renal or post-renal disease, therefore results in an imbalance of salt, water, acids and bases, with retention of waste products and involvement of other organ systems. A variety of non-specific clinical signs are associated with renal failure and its effects are often fatal [1].

Sheep kidneys consist of thousands of functional units or nephrons arranged in parallel rows, blood vessels and interstitial tissue within a protective capsule. Each nephron possesses a filtration bed, the glomerulus, an elongated resorption module represented by the convoluted tubule and loop of Henle, and a drainage system of collecting tubules which open into the renal pelvis. These structures are richly endowed with vasculature, the blood flow rate within which is regulated partly by hormonal and partly by nervous mechanisms, to accommodate filtration requirements. Unfortunately, this superbly efficient afferent circulation can convey toxic substances to the delicate and highly vulnerable tubular epithelium and may act as a convenient route for pathogenic bacteria to gain access to the kidney tissues. Damage to the blood vessels themselves may have serious consequences for other structures; arterial branches may be occluded, causing infarction, while lesions affecting the interconnected arterial loops in the glomerular tufts, which are continuous with a network of peritubular capillaries, will result in tubular atrophy and loss of function. Serious renal disease, therefore, can have far-reaching, often fatal, effects on the well-being of the animal. The diseases that directly affect the kidneys of sheep are summarized in Table 55.1.

CONGENITAL MALFORMATIONS OF THE KIDNEYS

Congenital defects of the sheep urinary system are uncommon. Agenesis of one or both kidneys, congenital hydronephrosis, dilation of the renal pelvis and cystic or polycystic kidneys have been reported [2]. Where they occur, kidney defects are usually associated with malformations in other organ systems and affected lambs are usually stillborn.

NEPHROPATHIES ASSOCIATED WITH INFECTIOUS AGENTS

Suppurative nephritis occurs sporadically and usually involves only individual animals in a flock. Septic emboli lodge in the glomerular or peritubular capillaries, leading to formation of abscesses. Suppurative nephritis is usually associated with neonatal bacteraemia, involving pyogenic streptococci and staphylococci. Abscesses are occasionally formed in the kidneys as a component of caseous lymphadenitis (see Chapter 44). Salmonellae and *Escherichia coli* can also cause embolic renal lesions as part of more generalized infections and embolic lesions are occasionally found in the kidneys of sheep dying from systemic pasteurellosis (see Chapter 32). Renal abscesses occur in melioidosis caused by *Burkholderia pseudomallei*

Table 55.1: Classification of sheep renal diseases

1. Congenital malformations	Renal agenesis Hydronephrosis Cystic and polycystic kidneys
2. Nephropathies associated with infectious agents	(a) Bacterial infections: Pyaemia resulting from joint-ill, tick infestation, caseous lymphadenitis or melioidosis Other septicaemic infections, e.g. *Salmonella* spp. *Escherichia coli* or *Pasteurella trehalosi* Leptospirosis: *L. interrogans* var *hardjo* nephritis Infection with *Chlamydia* Miscellaneous ascending infections (b) Viral infections: Sheep pox Adenovirus infection Pestivirus of border disease
3. Toxic nephropathies	Enterotoxaemia caused by epsilon toxin of *Clostridium perfringens* type D Inorganic (metallic) poisons Antibiotics, chemotherapeutics Vegetable poisons Nephrosis
4. Immunologically mediated glomerular disease	Membranous glomerulonephritis Mesangiocapillary glomerulonephritis of the Finnish Landrace breed (MCGN) Amyloidosis
5. Obstructive nephropathy	Urolithiasis
6. Neoplasia	
7. Abnormal pigmentations	Copper toxicity, brassica poisoning, for example

infection, although this disease, which is endemic in northern Australia and South-east Asia, does not occur in northern Europe. Chronic interstitial nephritis in lambs has been associated with *Leptospira interrogans* serovar *hardjo* infection, with haemorrhage into renal tubules, haematuria and nephrosis, as well as septicaemia and hepatitis. However, while sheep are maintenance hosts for *L. hardjo*, clinical disease is only rarely seen (see Chapter 19). Fatal nephritis due to *Leptospira interrogans* serovar *pomona* has been described in New Zealand.

The diagnosis of bacterial nephritis is confirmed at post-mortem. The kidneys are usually enlarged and peppered with yellow, or sometimes haemorrhagic, spots of uniform appearance but varying size, both on the surface and deeply within the kidney when incised.

Viral infections only rarely involve the kidneys of UK sheep. However, in countries where sheep pox is endemic, lesions caused by cellular infiltration are often present in the renal cortex (see Chapter 43). Adenoviruses are usually associated with respiratory disease in sheep, although some strains have been shown to cause mild, multifocal suppurative interstitial nephritis. Renal lesions have been identified in lambs which are persistently infected with the pestivirus of border disease (see Chapter 18), characterized by focal, dense interstitial infiltrations of lymphoid cells in subcapsular regions of the cortex,

around vessels in the corticomedullary junction, or in the medullary calyces.

Kidneys may also be infected by ascending infection from the lower urinary tract, causing pyelonephritis. Pyelonephritis, characterized by inflammation and necrosis of the calyces in association with areas of tubulointerstitial inflammation and necrosis, is uncommon in sheep and is usually accompanied by urethritis and cystitis. In young lambs the most likely cause is spread from an infected umbilical cord or urachus, while in older sheep, ascending infection is sometimes associated with urolithiasis.

TOXIC NEPHROPATHIES: TUBULAR NECROSIS

Numerous substances, including heavy metals, antibiotics, bacterial toxins, toxic principles of poisonous plants and mycotoxins have the capacity to cause nephrotic tubular necrosis. The kidneys are particularly sensitive to toxic damage for various reasons: they acquire about 20 per cent of cardiac output exposing them to a high proportion of any blood-borne toxins that may be present; glomerular capillaries provide a large contact area for toxin interaction with epithelial cells; high metabolic rates and transport functions in the proximal tubules and loops of Henle make epithelial cells especially sensitive to toxins that disrupt their energy sources or membrane functions; and in the process of urine filtration, tubular epithelial cells may actively resorb toxins, which they can accumulate to dangerous intracellular levels. The usual effect of renal poisoning is selective necrosis of the tubular lining cells with functional consequences on a range of regulatory mechanisms. The basement membranes often survive and regeneration is possible if the toxic state is reversed. Tubular necrosis can also occur as a result of ischaemic damage to the capillary endothelium caused by Gram-negative bacterial endotoxin, resulting in total death of the tubules involved.

Post-mortem findings of discoloration of the kidneys do not necessarily indicate toxic damage. Kidneys may appear dark due to the accumulation of haemoglobin by-products in the tubules in cases of chronic copper poisoning or haemolytic anaemia caused by S-methylcysteine sulfoxide in brassica crops, or they may appear pale in amyloidosis. Kidneys tend to autolyse rapidly after death, so histological examination may be useful to confirm the presence of renal pathology.

Pulpy kidney

The epsilon toxin of *Clostridium perfringens* type D toxin is lethal to the renal tubules, completely destroying them within a matter of hours and resulting in rapid death. Enterotoxaemias are described in Chapter 23.

Nephrosis in lambs

Nephrosis is an imprecise term applied to non-inflammatory renal disease, particularly tubular degeneration [3, 4]. Nephrosis is a sporadic cause of death in 2–12-week-old lambs. In some parts of northern England, annual losses of up to 3 per cent have been reported. The initial clinical signs are dullness, diarrhoea and a staggering gait. Affected lambs stop sucking and lose weight, invariably leading to death after a period of 2–10 days. Such lambs are azotaemic, with increased total proteins and a decreased albumin to globulin ratio. Nephrotic lambs develop metabolic acidosis with exchange of intracellular potassium into the circulation. Death is often due to heart failure associated with hyperkalaemia.

On post-mortem examination kidneys are enlarged, soft and pale, with expanded cortices (see Figure 55.1 in the colour plate section). On histological examination, groups of cortical tubules are distended with casts of serum protein-like or fibrin-like material, and lined with undifferentiated, low cuboidal cells. Ultrastructural studies have shown evidence of severe toxic effects in proximal convoluted tubules and, to a lesser extent, in some glomeruli.

No putative nephrotoxic factor has been identified and the cause and risk factors for nephrosis are unknown. The disease invariably occurs in lambs at pasture, but no breed or sex predisposition, or association with management practices such as feeding or access to mineral supplements, has been identified. Concurrent cryptosporidiosis, nematodirosis or coccidiosis is often diagnosed, but nephrosis is also seen in flocks with no evidence of these diseases. The response to supportive therapy in affected lambs is poor and until the identity of the nephrotoxic factors is known, it is inappropriate to recommend any preventive strategy.

Toxic nephropathies

Plants such as rhubarb, sugar beet and sorrel contain high concentrations of oxalates, while oak leaves and unripe acorns brought down after storms contain hydrolysable tannins which are broken down to nephrotoxic metabolites in the digestive tract. Mouldy grain sometimes contains a variety of nephrotoxins, such as calcium oxalate, ochratoxin and citrinin. Numerous other plants, which are described in Chapter 56, contain active principles that can cause nephropathy [5].

Sheep are more susceptible to oxalate toxicity than other ruminant species, lambs being more susceptible than adults because most ingested oxalates are metabolized in the rumino-reticulum to innocuous carbonates and formates. Free circulating oxalate damages arterioles and capillaries causing pulmonary and cerebral oedema, while continuous ingestion of small amounts of soluble oxalates causes nephrosis due to precipitation of calcium oxalate crystals in the lumen of the renal tubules. The diagnosis of oxalate poisoning is based on the history of access to oxalate-rich plants and supported at necropsy by histological findings of calcium oxalate crystals in the renal tubules and rumen wall.

Tannins cause necrosis, primarily of the proximal renal tubules. Clinical signs of tannin poisoning usually appear several days after ingestion, initially with dullness, anorexia, constipation and cessation of urination. Later there is persistent diarrhoea and dysentery, with dark urine and serous ocular, nasal and oral discharges in some cases. The prognosis is aided by measurement of blood urea concentrations, those sheep with levels exceeding 50 mmol/l (normal 2–6 mmol/l) usually dying, while in surviving animals levels usually remain elevated between 10 and 20 mmol/l for several weeks. Post-mortem findings include a uraemic-smelling carcass, haemorrhagic abomasitis, subcutaneous haemorrhages and oedema, especially of the abomasum and perineum.

Iatrogenic renal toxicity was once a hazard associated with drenching with carbon tetrachloride or phenothiazine for control of gastrointestinal helminths. Renal tubular damage due to errors in incorporation of monensin into rations for control of cococcidiosis or toxoplasmosis was once commonplace, although monensin can no longer be used in food-producing animals in the UK. Overdosing with non-steroidal anti-inflammatory drugs, aminoglycoside, tetracycline and certain sulfonomide antibiotics can cause acute renal failure, especially in young lambs. The toxicity of these drugs is exacerbated by systemic states, such as dehydration or shock, which concomitantly impair renal function in the affected animal.

Heavy metals such as lead, copper and mercury cause direct renal tubular necrosis. These are described in Chapter 57.

IMMUNOLOGICALLY MEDIATED GLOMERULAR DISEASE

Glomerulonephritis refers to the deposition of immune-complexes along the basement membranes of the glomerular capillaries. Complexes consist of antigen, immunoglobulins and complement, with antigen in slight excess, so that complexes are continually being generated. These immune complexes are so soluble that they evade the phagocytic cells lining the liver sinusoids or in the splenic pulp. However, they become trapped in the glomerular capillaries, stimulating overgrowth of the basement membrane tissue which becomes thickened and forms spiky projections enclosing packets of the complexes. This form of deposition, designated membranous glomerulonephritis, is the most common form found in sheep. Subendothelial deposits occur in mesangiocapillary glomerulitis in which attempts by the mesangium to remove the accumulated complexes causes proliferation of the mesangial areas of the affected glomeruli.

Membranous glomerulonephritis

Membranous glomerulonephritis occurs sporadically in adult sheep, usually in association with chronic inflammatory disease. Affected sheep are ill-thrifty and may show clinical signs of ascites, hydrothorax and anasarca (subcutaneous oedema) (Figure 55.2), with azotaemia and proteinuria. On post-mortem examination the kidneys are pale, contracted and stippled with white specks. The diagnosis is confirmed by histology, revealing shrunken and fibrosed glomeruli, occluded capillaries, and atrophied, fibrous tubules and interstitium.

Figure 55.2: Anasarca involving the face of a Texel ram with membranous glomerulonephritis.

Mesangiocapillary glomerulonephritis of Finnish Landrace sheep

Mesangiocapillary glomerulitis is a very rare, genetically controlled, congenital disease which occurs primarily in purebred Finnish Landrace lambs less than 4 months old [6, 7], although there are reports of the disease in cross-bred lambs sired by Finish Landrace rams. Immunofluorescent studies demonstrate subendothelial deposits of immunoglobulins and complement and neutrophil infiltrates in glomerular capillary walls. Lesions develop *in utero* and are present at birth, although mesangial expansion is delayed until circulating IgG is incorporated into the complexes.

Lambs with mesangiocapillary glomerulitis are clinically normal at birth, but stop sucking after a few weeks. Affected lambs are anorexic, dull and afebrile, but appear blind, with fine muscle tremors due to secondary encephalopathy and show signs of crouching and tail swishing, indicative of abdominal pain. Kidneys are enlarged and tender on palpation of the abdomen. The diagnosis is supported by ultrasonographic identification of enlarged kidneys and determination of azotaemia and hypoalbuminaemia. Most affected lambs die within a few weeks, although some survive to adults.

On post-mortem examination the kidneys are enlarged to six times their normal size with red or yellow spots in the cortex, indicative of haemorrhagic glomeruli. On histological examination, the mesangial regions of all glomeruli are expanded and highly cellular, while peripheral capillary walls are swollen, refractile and infiltrated with neutrophils. Urinary spaces are distended with serum, protein, fibrin and blood, and proliferation of the capsular epithelium results in the formation of huge glomerular crescents. Focal haemorrhage and oedema are present in the cerebral cortex, probably caused by immune complex vasculitis. Immunoglobulins and complement can be identified in the capillary walls under electron microscopy.

Inheritance of mesangiocapillary glomerulitis is complex and probably polygenic and dominant, with incomplete penetrance. Defects may be latent in females and expressed only when carrier ewes are mated with a carrier ram. Both affected and unaffected lambs may be born together in a litter, while carrier ewes do not necessarily give birth to affected lambs after consecutive matings with a transmitting sire. Control is straightforward, involving culling the sires of the affected lambs and culling all ewes giving birth to affected lambs.

Amyloidosis

Reactive systemic amyloidosis occasionally occurs as a sequel to chronic inflammatory or neoplastic disease and has been reported in sheep used for antiserum production, or in cases of caseous lymphadenitis. Amyloidosis is a chronic wasting disease caused by deposition of fibrils formed by the polymerization of protein subunits in various tissues, in particular the intestine and kidneys. Clinical signs of renal amyloidosis include chronic weight loss, ventral oedema, ascites, pleural and pericardial oedema, hypoproteinaemia and proteinuria.

NEOPLASIA

Primary kidney tumours are uncommon, but renal involvement in multicentric and localized lymphosarcoma is diagnosed sporadically. Renal tumours are described in detail in Chapter 58.

POST-RENAL NEPHROPATHY

Urinary obstruction can lead to renal failure due to the development of hydroureter and hydronephrosis caused by back pressure and due to subsequent ascending infection. The principal cause of post-renal nephropathy in sheep is urolithiasis, although it is also occasionally associated with balanoposthitis.

Urolithiasis caused by struvite calculi

Urolithiasis is an important disease of concentrate-fed wether lambs, which can affect large numbers of animals, with high mortality rates and subsequent ill-thrift in recovered animals following treatment [8]. Individual cases are sometimes seen in pedigree rams which have been intensively fed, often in preparation for shows or sales. The disease is characterized by blockage of the urethra, usually by a sludge of calcium, magnesium, ammonium and phosphate solutes, combined with proteins and precipitated from urine. Blockage occurs in the urethral lumen at the ischial arch, at the sigmoid flexure, in the glans penis and at the vermiform appendage of the penis.

Wether lambs are most susceptible because their urethra is less developed and narrower than in ram lambs. The usual cause is feeding rations high in magnesium (over 0.2%) and phosphate (over 0.6%), and the calculi usually form in alkaline urine, which is the normal status in ruminants. A genetic predisposition to calculus formation has been suggested, related to the individual animal's capacity for urinary excretion of phosphate.

The clinical signs in mildly affected animals are separation from the flock, anorexia, intermittent straining, kicking at the abdomen and repeated tail swishing. Affected sheep sometimes dribble small amounts of blood-stained urine and small rice grain-like crystals are present on the preputial hairs (see Figure 55.3 in the colour plate section). In more severe cases the lower abdominal wall and prepuce become swollen due to leakage of urine from the urethra into subcutaneous tissues, which become infected and eventually slough. In some cases following leakage of urine from a distended bladder, the abdomen becomes bilaterally distended with an appreciable fluid wave on ballottement. Actual rupture of the renal pelvis has been demonstrated on post-mortem examination, with leakage of dark, blood-tinged urine into the perirenal fat. Calculi, swelling and pressure necrosis are sometimes be seen at the vermiform appendage of the penis (see Figure 55.4 in the colour plate section). In the terminal stages of the disease, sheep are anorexic and dehydrated due to uraemia, and azotaemic, with blood urea nitrogen and creatinine concentrations over 50 and 400 mmol/l, respectively.

The diagnosis of urolithiasis can be supported by real-time, transabdominal, B mode ultrasonography to determine the size of the bladder, examine the right kidney and determine the presence of uroperitoneum [9]. A 5 MHz sector or linear transducer is placed on the right inguinal area to examine the bladder and on the right paralumbar fossa (sector transducer only) to examine the right kidney. In cases of urolithiasis, the bladder is grossly enlarged, with a diameter greater than 16 cm (normal 6–8 cm) and extends up to 10 cm or more over the pelvic brim (Figure 55.5). Hydronephrosis of the right kidney is identified by the presence of a grossly distended renal pelvis and much reduced medulla.

Medical treatment using spasmolytic and anti-inflammatory drugs is usually ineffective. Urethral catheterization and retrograde flushing of calculi into the bladder is prevented by the presence of a urethral diverticulum at the level of the ischial arch. In the short term, affected sheep can be treated surgically. If the vermiform appendage is obstructed, then amputation at its base may clear the blockage. However, sludge is usually present proximal to the vermiform appendage, leading to obstruction at another site. Perineal urethrotomy, urethrostomy or penile amputation can be attempted (see Chapter 69), but the long-term prognosis is poor. A technique has been described for valuable breeding rams involving laparotomy followed by flushing of the bladder via a Foley catheter [10]. Intravenous fluid therapy is necessary to correct fluid and electrolyte deficits.

The most important consideration is the control of further cases. The level of concentrate feeding should be reduced if practical. The ration should be checked to ensure that it contains less than 0.2 per cent magnesium and 0.6 per cent phosphate and levels altered accordingly. Addition of calcium carbonate and increasing the roughage in the diet may help to correct a calcium to phosphate imbalance to achieve a ratio of 1.2:2.1. Adequate water intakes can be ensured by addition of 3–5 per cent salt to the ration or provision of salt licks. Acidification of the urine

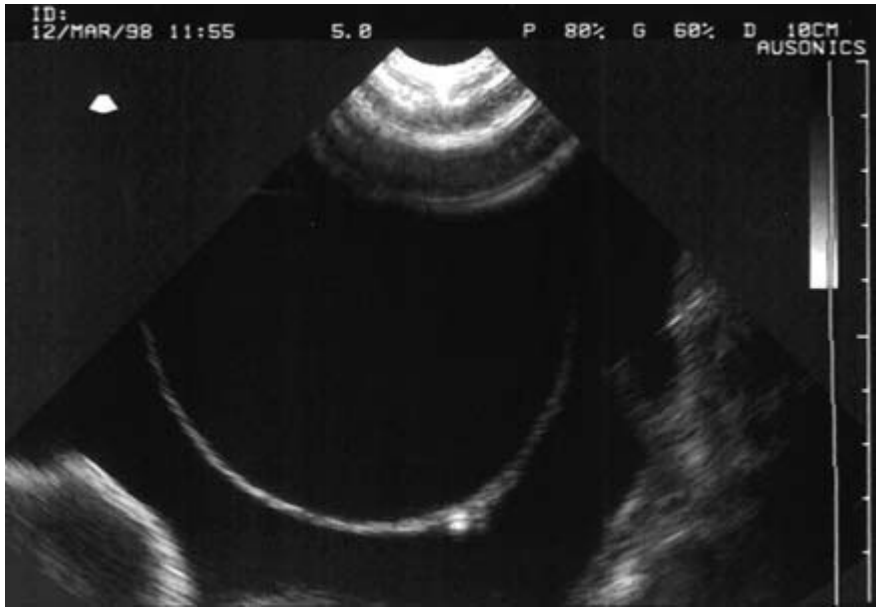

Figure 55.5: Considerable distension of the bladder with leakage of urine into the peritoneum (uroperitoneum). The bladder wall appears as a distinct hyperechoic (white) line; the urine appears anechoic (black) (5 MHz sector scanner). (Courtesy of Dr Phil Scott.)

reduces the likelihood of sludge precipitation. This can be achieved by feed withdrawal for 24 hours and dosing with 1 g of ammonium chloride in water solution three times daily. In the longer-term, addition of 2 per cent ammonium chloride to the diet can be useful, displacing magnesium and phosphate from centres of nucleation in the urine.

To prevent urolithiasis, concentrates should be introduced gradually and a source of roughage should be provided. It is also important to ensure that animals have unrestricted access to fresh water at all times and that the mineral content of the ration is correctly formulated. Ewe rations frequently contain high levels of magnesium, so should never be fed to male sheep. Similarly, high magnesium licks for the prevention of hypomagnesaemic tetany in ewes should not be available to male sheep.

Other causes of urolithiasis

Siliceous calculi are common in parts of North America and Australia, in sheep grazed on silica-rich pastures. Silica calculi, sometimes mixed with calcium oxalate or carbonate, are hard, white to dark coloured, laminated and smooth surfaced with a friable core.

Oxalate calculi are sometimes seen in sheep grazing on grain stubble, but their cause and the source of oxalate is not generally known. Oxalate calculi are hard, heavy, white or yellow and covered by jagged spines.

Soft, pulpy, yellow and scantily mineralized material referred to as clover stones sometimes develops in the pelvic urethra in up to 10 per cent of sheep grazing on subterranean clover. The material consists of desquamated cells and secretions of accessory glands, which probably accumulate under the influence of oestrogenic hormones from the crop. So-called clover stones can result in urethral obstruction in both male and female lambs.

Balanoposthitis

Balanoposthitis (pizzle rot) is a common finding in rams and wethers, which occasionally leads to urinary obstruction and post-renal nephropathy. Early and mild cases are characterized by small ulcers around the external orifice of the prepuce (posthitis).

Scabs develop over the ulcers, which become necrotic and slough (see Figure 55.6 in the colour plate section). The wool surrounding the prepuce becomes stained, smelly and sometimes flystruck. In severe cases the internal preputial membranes (balanitis) are also involved. Foul smelling exudate accumulates within the prepuce, which may become blocked by the presence of scabs at its opening. Animals may have difficulty urinating and the prepuce and ventral abdomen may become swollen due to the accumulation of urine and exudate. Animals may die from kidney failure. Most cases in rams involve only external lesions and the severe disease is usually limited to wethers.

The disease is caused by *Corynebacterium renale* which grows in alkaline urine produced by animals on a protein-rich diet. *C. renale* produces ammonia from urinary urea, which burns the prepuce and penis [11]. High levels of urea are present in the urine of animals which are fed on protein-rich diets such as improved pastures or barley and maize gluten concentrate rations. Balanoposthitis is most common in wethers because their penis is less developed than in rams, so they urinate into the prepuce.

Mildly affected animals usually respond to topical antiseptic and parenetral antibiotic treatment within a few days. Affected animals should be isolated to avoid further contamination of the environment by *C. renale*. The wool around the prepuce should be clipped to remove bacteria-contaminated wool and to enable wound irrigation and topical treatment with antiseptic ointment. Where possible, the amount of protein in the diet should be restricted and free access to fresh water provided. Oral administration of ammonium chloride solution may help by acidifying the urine.

Severely affected animals may require surgical drainage of the prepuce and the inclusion of sodium or ammonium chloride in the drinking water to promote diuresis. The longer-term prognosis is poor in these cases.

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Part XI
Poisons

56

Plant poisoning in Britain and Ireland

K.W. Angus

The taxonomy of plants has been reviewed since the publication of the third edition of *Diseases of Sheep*. In this chapter, plants are referred to by common name, Latin name in italics, current family name followed by former family name in square brackets. With the family Leguminosae it is considered correct at present to use the former family name for some species and the new family name, where appropriate, for others, and this system of nomenclature has been adopted accordingly.

The plant species which flourish in the temperate climate of Britain and Ireland are very numerous, and many contain poisonous principles – phytotoxins – capable of poisoning livestock, including sheep, if circumstances permit (Table 56.1). Figures 56.1–56.5 (all in the colour plate section) illustrate some of these poisonous plants.

Many phytotoxins have been identified chemically, but there are still a good few that are recognized only by their effects. Phytotoxins may act directly or they may be present as harmless pro-toxins requiring further elaboration; conversion to the toxin may take place during feed storage, in the feed or water trough, in the rumen or after absorption from the gut.

Plant poisoning is dependent upon many factors, between which there may be complex and infinitely variable interrelationships (Table 56.2).

Most of the plant factors are self-evident: plants need appropriate soil and climatic conditions in order to flourish. This can account for the many regional differences in the availability of poisonous species. Toxicity may be higher in young leaves or shoots than in mature plants; other plants are much more poisonous in the late summer and autumn than they are during early growth. Some plants are more poisonous in winter than they are in summer. Certain plants act as hosts for saprophytic or parasitic fungi or moulds, and in some plant species the presence of such organisms actually increases the concentrations of the native toxic principles. The application of artificial fertilizers can increase levels of toxicity, e.g. nitrate levels in fodder rape, while drying of poisonous plants, e.g. by haymaking, does not necessarily render them safe and may actually enhance their toxicity. Treatment with herbicides may make some plants not only more palatable but also more poisonous for animals. Conservation and biodiversity programmes may encourage the growth of noxious weeds as well as more attractive wild flowers.

Sheep generally are very conservative in their feeding habits. They are, in the main, selective close grazers, and unlike cattle, for example, they are not drawn to unfamiliar foliage out of a sense of curiosity. In extremes of cold or drought, however, they may turn for nourishment to plant species which normally are unattractive to them; similarly an increase in the density of undesirable plant species may follow overgrazing, encouraging sheep to feed on them out of

Table 56.1: Poisonous principles in plants that may poison sheep

Pyrrolizidine alkaloids	Miscellaneous glycosides	Saponins
Quinolizidine alkaloids	Haemolytic anaemia factors	Goitrogens
Miscellaneous alkaloids	Photosensitivity factors	Oestrogens
Cardiac glycosides	Nitrates	Abortifacients
Cyanogenic glycosides	Oxalates	Unclassified toxins

desperation. It is obvious that if a flock is enclosed in a field containing a crop composed of a single plant species which contains harmful toxins, poisoning almost inevitably occurs. Given these generalities, there is unfortunately no guarantee that sheep, especially growing lambs, will not eat even the most unpalatable plants in circumstances where there is plenty of good grazing available. Variations in breed and, or, individual susceptibility to poisoning by certain plants have been shown to exist, but the toxic principles in most poisonous plant species can overwhelm these supposed tolerances. Climatic conditions may influence the appearance of clinical signs, e.g. bright sunlight in poisoning by plants which contain photosensitivity factors. Some valuable fodder plants contain poisonous substances, e.g. rape and other brassicas, and strict limitations may have to be enforced on the amount of daily time sheep can be safely permitted for feeding on the crop.

Apart from the plants native to our islands, non-indigenous, often poisonous, species are constantly being introduced, and, with the climatic changes which arise in parallel with the over-production of greenhouse gases, these have the potential to proliferate locally. The current decline in the beef and dairy industries, with the increase in the number of set-aside hectares with poorly controlled weed populations capable of aerial spread, are recipes for greater exposure of sheep to phytotoxins.

Diagnosis of phytotoxicity is straightforward if a precise history of the circumstances is known, but more often it is hedged with difficulties. Certain forage crops may give rise to toxicity if fed to excess or at the wrong stage of their development. When this occurs, the history, clinical and necropsy findings are usually sufficient to establish a diagnosis. Similarly, where sheep stray into gardens or parks and consume herbaceous plants or shrubs, there is usually evidence of nibbled shoots or stems, with trampling and other obvious damage to growing plants, from which a provisional diagnosis of poisoning may be made. However, differential diagnosis of plant poisoning all too often is complicated by the fact that clinical signs of poisoning closely resemble those of some acute infectious diseases or metabolic upsets. Necropsy findings, unless unequivocal evidence of ingestion of a specific poisonous plant can be demonstrated by identifying leaves, stems or pods in rumen contents, are often non-specific and confusing. Careful examination of clinically affected animals and the grazing environment are essential. Recognition of plants known to contain toxic principles requires experience and a good illustrated reference manual [1, 2]. Sophisticated laboratory techniques, which may be costly, often are required for precise diagnosis.

To a certain extent plants can be grouped according to their toxic effects and the clinical signs that these elicit. Where grouping is possible, it often follows that a single approach to treatment and control can be applied to poisoning by any one of the group. Unfortunately, several quite important toxic plant species cannot be so grouped because they contain unique single or multiple toxic principles.

Prevention of plant poisoning is largely a matter of management; once poisonous plants have been identified it makes sense to exclude a flock from

Table 56.2: Factors influencing plant poisoning in sheep

Plant factors	*Host factors*
Prevalence	Appetite
Palatability	Age
Availability	Breed variation in susceptibility
Suitability of soil	Genetic variation in susceptibility
Stage of growth	Exposure to inappropriate crop management
Strain variation in concentration of toxic factors	Exposure to contaminated hay or silage
Competition with conventional feeds	Exposure to bright sunlight
Relationship with seasonality	Exposure to extreme weather conditions
Relationship with cultivation practices	
Relationship with climate	
Relationship with artificial fertilizers	
Relationship with biodiversity programmes	
Effects of herbicides	

contact with them. Similarly, when sheep are to be folded on a fodder crop with the potential for toxicity they should be introduced to the crop gradually, and restricted in their daily consumption. Treatment of plant poisoning in individual sheep is often disappointing; often there is no antidote to the toxic factor or factors involved, and symptomatic treatment is the only recourse. The use of laxatives and activated charcoal orally, which may be of benefit in simple-stomached animals, is of dubious value in ruminants. Should recourse to activated charcoal be considered, the dose is 1–4 gm/kg body weight (BW), given in 50–200 ml of water [2]. Where antidotes are available for specific forms of poisoning, these will be referred to in relation to particular plants. Finally, it may be possible to save valuable breeding animals by performing emergency rumenotomy.

POISONING BY ALKALOID-CONTAINING PLANTS

Many plant species contain alkaloids, i.e. alkali-like substances (Table 56.3).

Alkaloids contain at least one nitrogen atom that can act as a base in a heterocyclic ring structure. Implicit in the definition is that these substances have pharmacological activities [1]. They are immensely varied both in chemical structure and clinical effect, and can be found in roots, stems, leaves, berries or pods of plants. Fortunately, most plants that do contain alkaloids are bitter and are rarely eaten, although some, once tasted, are addictive. Plants containing alkaloids of similar chemical structure often induce similar clinical signs of toxicity and many alkaloids act by mimicking or blocking the actions of neurotransmitter substances.

Among the major groups of toxic alkaloids are the pyrrolizidine alkaloids. These are not toxic in themselves but are metabolized in the liver to highly toxic derivatives, which cause progressive liver damage. In Britain and Ireland, the most important plant in this group is ragwort (tansy, benweed), which is widespread throughout northern Europe. In Britain ragwort is found in neglected or set-aside fields, in poorly managed grazing land, and is now spreading alarmingly along the margins of motorways and bypasses. A single plant can produce from 80 000 to 150 000 highly dispersible seeds, which can be spread by wind over wide areas. Ragwort is a legally proscribed noxious biennial weed, and landowners have an obligation under

Table 56.3: Alkaloid-containing plants that may poison sheep

Toxic agent(s)	Plant	Latin name	Family
Pyrrolizidine alkaloids	Heliotrope	*Heliotropum europeum*	Boraginaceae
	Ploughman's spikenard	*Inula conyza*	Asteraceae [Compositae]
	Ragwort	*Senecio jacobea*	Asteraceae [Compositae]
	Viper's bugloss	*Echium vulgare*	Boraginaceae
Quinolizidine alkaloids	Lupins	*Lupinus* spp.	Leguminosae
Miscellaneous alkaloids	Autumn crocus	*Colchicum autumnale*	Liliaceae
	Black nightshade	*Solanum dulcamara*	Solanaceae
	Delphinium	*Delphinium* spp.	Ranunculaceae
	False hellebore	*Veratrum* spp.	Liliaceae
	Hemlock	*Conium maculatum*	Apiaceae [Umbelliferae]
	Lucerne	*Medicado sativa*	Fabaceae [Leguminosae]
	Monk's hood	*Aconitum napellis*	Ranunculaceae
	Perennial ryegrass	*Lolium perennae*	Poaceae [Graminae]
	Potato	*Solanum tuberosa*	Solanaceae
	Thornapple	*Datura stramonium*	Solanaceae
	Woody nightshade	*Solanum nigrum*	Solanaceae
	Yew	*Taxus baccata*	Taxaceae

the Weeds Act of 1959 to keep it under control. The palatability of ragwort is increased in the cut plant, and toxicity is not reduced by drying, hence the danger of feeding hay contaminated by the plant.

The alkaloids in ragwort are cyclic diesters; metabolism of these to pyrrolizidines renders them toxic, in that they undergo further breakdown into lipophilic pyrroles and water-soluble N-oxides, which cause severe gastroenteritis and cirrhosis of the liver. The plant is particularly dangerous if consumed by cattle or horses, but is somewhat less so for sheep. Despite the availability of ragwort in some pastures, poisoning of sheep by this group of alkaloids is fairly uncommon. Research has shown that a combination of four species of bacteria found in the rumen of sheep can degrade the toxic pyrrolizidines found in ragwort, and will protect cattle from its toxic effects. However, it must not be assumed that sheep are tolerant to ragwort. The mistaken belief that sheep can control the plant by being encouraged to feed on the young growing rosettes must be discouraged. Subclinical poisoning can leave sheep with permanent liver damage, which could leave them susceptible to copper poisoning in subsequent seasons.

Methods of control recommended by the Department for Environment, Food and Rural Affairs (Defra) include ploughing, and hand- or machine-pulling, with burning of the uprooted plants. Alternative strategies include the use of herbicides, either as spot-control or blanket spraying. The recommended herbicides include MCPA, 2,4-D (Headland Staff) or citronella oil products.

Viper's bugloss and species of heliotrope, which contain similar alkaloids, may occur locally or as garden escapes in Britain and have the potential to cause toxicity. Several of the giant perennial *Ligularia* spp. (Asteraceae), which are popular in herbaceous borders in parks and large gardens, have recently been shown to be toxic to herbivores by virtue of their content of pyrrolizidine alkaloids or their N-oxides, although there are no reports so far of poisoning of sheep by these species. By contrast ploughman's spikenard, which is commonly found on chalky soil in Britain, caused illness and deaths in sheep in Germany; clinical signs in affected animals included salivation, circulatory disturbances, blood-stained faeces and muscle tremors. Necropsy findings included haemorrhages in the gastrointestinal tract, and degenerative changes in the heart, liver and kidneys.

Sheep poisoned by ragwort or other plant species containing pyrrolizidine alkaloids gradually lose condition, become emaciated and die. Severe liver damage with cirrhosis is the main necropsy finding. Treatment of affected sheep is purely symptomatic and seldom of any value, although Australian workers have suggested that cobalt in the form of vitamin B_{12} is beneficial in heliotrope poisoning. Sheep at risk should be removed from the offending pasture.

A second important group of alkaloids is the quinolizidines, which are found, among others, in various species of lupin. Of approximately 100 existing lupin species, most are native to the western USA where the wild plants may be grazed by sheep on mountain pastures. In Britain, lupins as wild plants have a very restricted distribution, although they do flourish in the Channel Islands and parts of the west of Scotland. They have been spread around the world as garden flowers and crops. Lupins can be grown commercially for green manure or as animal feed. On certain poor soils lupins yield considerably more dry matter (DM), energy and protein than pasture grass. They are grown as forage mainly for sheep particularly in West Australia and South Africa, but are not cropped in Britain; here poisoning would occur mainly by fortuitous ingestion of garden lupins.

The term lupin poisoning is reserved for an acute disorder of the central nervous system (CNS) caused by the alkaloids contained in bitter lupins. These include lupinine, lupinane, anagyrine and sparteine, and the plants also contain trypsin inhibitors and an oestrogenic substance, biochanin A. However, by a programme of selective breeding, the alkaloid content has been reduced to negligible amounts, so that the plants now known as sweet lupins have, since 1934, been cultivated as animal feed. This programme has not eliminated the condition known as lupinosis, a disease characterized by hepatitis and its sequelae, caused by a mycotoxin produced by a contaminating fungus, *Diaporthe toxica* (see below).

In bitter lupins the quinolizidine alkaloids are concentrated mainly in the seeds and seed pods, with less in the leaves and still less in the stalks. The alkaloids first stimulate and then paralyse the nerve centres of the medulla and cord, causing initial excitement followed by prostration, lowered blood pressure and heart rate, and dilation of the pupils. Death results from asphyxia and associated convulsions. Experimental inoculation of the alkaloids into laboratory animals and sheep has shown that there is a critical level above which clinical signs and death occur, but below which the alkaloids are detoxified so that there is no

cumulative effect. In the field, the onset is quick and the course rapid, signs and death usually occurring within 7 hours of exposure but occasionally delayed until 24 hours after ingestion of the lupins. When recovery occurs it appears to be complete but recovered animals remain fully susceptible to further occasional exposures. However by gradually increasing the amount of bitter lupin seed offered, tolerance of up to 0.5 kg/day may be acquired.

Diagnosis depends on a history of bitter lupins at the pod stage having been recently available and the characteristic clinical signs. Necropsy findings are associated with convulsive struggling and asphyxia; fragments of lupin may be found among rumen contents. Laboratory confirmation is available only in certain specialized research centres. Unaffected sheep should be removed immediately from the lupin pastures, disturbing any prostrate survivors as little as possible, because stimulation of the survivors may precipitate death; if left alone they may recover.

Lupinosis may conveniently be discussed here, though it is not caused by alkaloid poisoning. Sweet lupins become toxic after summer rain if they become infected with the fungus *Diaporthe toxica* (synonyms *Phomopsis leptostromiformis*, *P. russiana, Cryptosporium leptostromiforme*) which in sheep causes progressive hepatitis. The fungus may parasitize the living plant, and can survive as a saprophyte in the dead straw or litter. The main toxic principle is the linear hexapeptide phomopsin A, although phomopsins B, C, D and E have recently been identified. High intakes of the fungus or its mycotoxin cause acute illness with anorexia, depression or stupor. Affected sheep stand with the head lowered and turned to the side or backwards. They tend to sway and if they fall over cannot regain their feet unassisted. Pulse and respiration are accelerated and temperature elevated (40–40.6°C). There are clinical signs of icterus, constipation, dark yellow urine and sometimes swelling of the ears. Anorexia becomes complete in 24–48 hours and appetite returns only very slowly. Severe acute cases die in 3–11 days and mortality may be high. Lower intakes cause partial anorexia with voluntary intake insufficient to sustain growth and body condition. Anaemia and slight icterus may develop. The cachexia of chronic lupinosis mimics that of some trace element diseases.

At necropsy in the acute disease there is generalized icterus with an enlarged, friable, bright yellow, fatty liver. Subacute and chronic cases have atrophy, fatty degeneration and cirrhosis of the liver, which may be shaped like a boxing glove. There may be enlargement of the gall bladder, splenomegaly, nephrosis, impaction of the large intestine, ascites, hydrothorax or hydropericardium. Histological examination of an acutely affected liver shows fat metamorphosis, eosinophilic globules in hepatocyte cytoplasm, karyorrhexis and vesiculation of the nuclei, and nuclear pleomorphism with accelerated mitosis. In subacute or chronic forms there is accumulation of pigment in hepatocytes, megalocytosis, bile duct proliferation and centrilobular fibrosis. Nuclear pleomorphism with bizarre nuclear shapes persists into the chronic stage. Haemosiderosis occurs in liver and other organs. In both forms of lupinosis there is spongy transformation of the brain commensurate with the degree of liver damage, and vacuolation of brainstem white matter but no malacia, neuronal damage or gliosis [3].

Evidence that lupins have been grazed, along with the appropriate clinical signs and necropsy findings, are sufficient to warrant immediate action. Blood samples may be taken for supportive laboratory evidence of liver dysfunction. Liver samples from dead sheep should be fixed and examined histologically. Sheep should be withdrawn from affected pastures, and clinically ill animals grouped for special care. Treatment is symptomatic. Ambulatory cases should be checked daily in case of misadventure.

Autumn crocus (meadow saffron) and hemlock are fairly common locally in Britain in damp meadows or open woodlands in southern regions. Autumn crocus contains coniine and colchicines; the former is the more toxic. These alkaloids affect the gastrointestinal tract, and block neuromuscular connections in peripheral nerves. As its name suggests, it flowers in the autumn and produces its leaves for a brief spell only in the spring and early summer, when they may be eaten. Sheep have been poisoned by hay containing the dried plants. Necropsy findings include gastroenteritis, enlarged liver and spleen, oedema of lymph nodes, and haemorrhages in lungs, kidneys and heart.

Hemlock contains a cluster of piperidine alkaloids; the most poisonous, γ-coniceine, being the precursor of at least four other toxic principles, which cause motor nerve-ending paralysis, depression of the central nervous system, and respiratory distress. However, the plant has an unpleasant mousey odour, which may deter animals from eating it, and sheep are believed to be fairly resistant to its effects. Hemlock and autumn crocus poisoning can cause similar clinical signs in sheep, namely apathy, excessive salivation

with drooling, abdominal pain with groaning or grinding of the teeth, rapid breathing, muscle tremors, incoordination or paralysis. Attempts at regurgitation and diarrhoea may be seen in hemlock poisoning, while sheep poisoned by autumn crocus develop coldness of the extremities and die of respiratory and circulatory failure.

Black nightshade and woody nightshade are very common closely related weeds. Black nightshade contains solanine and variable amounts of nitrates and nitrites, while woody nightshade contains a mixture of steroidal glycoalkaloids including solanine. Woody nightshade may be more poisonous than black nightshade, and sheep poisoned by the plant developed rapid breathing, fever, intermittent rapid pulse, dilated pupils, green diarrhoea, staggering, then falling followed by rapid death. Necropsy findings included tarry blood, contracted ventricles, and the presence of seeds and stems of the plant in the stomach. Potatoes also contain solanine, and are poisonous if they are greened by sunlight, or if shoots or peelings are fed to sheep. The leaves and vines are also poisonous. The clinical effects are similar to those exhibited in poisoning by the nightshades.

Thornapple (devil's trumpet), false hellebores and monk's hood (wolf's bane) are rarely seen in Britain outside gardens. Thornapple is not native to Britain, but grows wild in parts of southern England, mainly on rubbish tips and embankments, although it may infiltrate cultivated ground. It does best in hot summers. The plant may have been introduced in imported birdseed. Thornapple contains hyoscine and hyoscyamine, and is very poisonous but extremely unpalatable. Nevertheless, eight ewes died in Scotland after consuming kale contaminated by this plant. Clinical signs of poisoning included restlessness, incoordination, dilation of pupils, increased respiratory rate, convulsions and paralysis.

False hellebores contain up to 20 different steroidal alkaloids, including proveratrine, protoveratrine, veratramine and jervine. All these affect the heart and paralyse the nervous system; 400 g of fresh leaves are supposedly fatal for sheep, and would cause gastrointestinal disturbance, salivation, trembling and, eventually, depression of the central nervous system. If false hellebores are eaten by pregnant sheep, fetuses may be born deformed. Monk's hood is said to be the most poisonous plant in Britain, so much so that plants escaping from gardens are often destroyed for safety. All parts of the plant contain aconitine that when hydrolysed, splits into picraconitine and napelline; further hydrolysis yields the highly toxic aconine. Sheep poisoned by monk's hood show weakness, cardiac stimulation, slow breathing, incoordination, muscular spasms, recumbency and death in a few hours from respiratory paralysis. However, there are no reports so far of poisoning of British sheep by these two species. The closely related garden plant delphinium (larkspur) contains alkaloids with similar actions to aconitine, but there are no reports of poisoning of sheep in Britain by this plant. Calves poisoned experimentally with the active principle of larkspur responded to injections of physostigmine (0.08 mg/kg BW).

Grazing on perennial ryegrass, especially in August and September after rain or heavy dew precipitation, can cause an acute form of neurotoxicity in sheep and cattle known as 'ryegrass staggers'. The cause of the toxicity is a seed-borne endophytic fungus, *Neotyphodium* (*Acremonium*) *lolii,* which contains a tremorigenic alkaloid toxin, lolitrem B. Recently a precursor of lolitrem B, paxelline, has been demonstrated in *N. lolii* and some *Penicillium* species. Ergovaline, an ergopeptide alkaloid, has also been identified in endophyte-infected perennial ryegrass. This substance may constrict blood vessels, leading to heat stress in hot weather, and driving sheep to water, where they may collapse and drown due to the effects of lolitrems.

Over-grazing predisposes to ryegrass staggers since the highest level of toxins are found in the crowns and basal leaf-sheaths of the plant. Lolitrems have excitatory followed by inhibitory effects on smooth muscle, particularly in the reticulum and rumen. Affected animals hang back from the others and develop hindlimb ataxia, incoordination, a wide-based stance, a stiff-limbed action, tremors and collapse. A 'dog-sitting' posture may be adopted, or there may be spasmodic bouts of lateral recumbency with rigidity of the limbs. An outbreak may involve many sheep simultaneously, although clinical signs are often only evident when they are disturbed. Mortality is low, although lolitrems can damage cerebellar Purkinjé cell neurons. Animals may take up to a week to recover after removal from the affected grazing. Diagnosis can be established by microscopic examination of thin strips of plant epidermis taken from the base of the sward and stained with lactic acid–aniline blue or lactophenol cotton blue to demonstrate fungal hyphae.

Yew contains taxine, a complex mixture of at least 11 alkaloids, the major constituent being taxine A. The plant also contains an irritant volatile oil and traces of a heteroside, taxicatoside, and is extremely toxic for livestock. All species of yew are equally poisonous. Yew trees have been grown near places of worship since pre-Christian times and are commonly found in churchyards and burial grounds. All parts of the plant are highly poisonous except the berries, which seem to be relatively harmless. Yew is at its highest level of toxicity during the winter months, and toxicity is greater in dried clippings than in fresh leaves. The poisonous dose for sheep is 10–12 g/kg BW, showing that sheep are slightly more tolerant than other herbivores to yew poisoning. Taxine is thought to act directly on the heart, slowing its rate and causing cyanosis and heart failure. Death is sudden and may occur within minutes of ingesting the plant; the symptoms, which are rarely seen, are trembling, dyspnoea, incoordinated movements, coldness, rapid then weak pulse, sometimes excitability preceding stupor, collapse which may be accompanied by groaning, and rapid onset of death with no struggling. Fragments of yew may be found in the rumen at necropsy; other findings may be abomasitis and congestion of liver, spleen and lungs. The heart is reported to stop in diastole. Survivors should be removed from the vicinity of the trees immediately.

Lucerne (alfalfa) is grown as a forage crop in parts of Britain, often under-sown with a grass. The plant contains phytoestrogens in the form of the coumestrols, genistein and formonetin. These substances can mimic the oestrogenic effects of some clover species by causing reproductive failure in female lambs. Affected animals have enlarged vaginas and uteri, while their ovaries are correspondingly abnormally small.

POISONING BY PLANTS CONTAINING TOXIC GLYCOSIDES

Glycosides are organic substances in which one or more carbohydrate molecules are combined with a non-sugar entity, or aglycone, the pharmacological nature of which determines the toxicity of the glycoside. Aglycones are released on hydrolysis either by ingestion of the plant, or sometimes by plant cell damage before ingestion. Plants containing cardiac and cyanogenic glycosides are highly toxic for livestock (Table 56.4).

Cardiac glycosides increase the contractility of cardiac muscle. They bind to and inhibit the sodium–potassium–ATPase pump, preventing the extrusion of Na^+ ions and the intake of K^+ ions. This results in an increase in intracellular Na^+ and because cardiac muscle cells can exchange Ca^+ for Na^+ there is a net increase in myofibrillar Ca^+. This facilitates rapid contraction of myofibrils, causing heart arrythmias and a variety of clinical signs often terminating in death. The effects are enhanced by the sugar moiety of the aglycone by affecting its lipid solubility and cell permeability.

Foxgloves contain various cardiac glycosides, including digitoxin, digoxin, digitalin, digitalismin and

Table 56.4: Glycoside-containing plants that may poison sheep

Toxic agent(s)	Plant	Latin name	Family
Cardiac glycosides	Foxglove	*Digitalis purpurea*	Scrophulareaceae
	Hellebores	*Helleborus niger*	Ranunculaceae
		Helleborus viridis	Ranunculaceae
Cyanogenic glycosides	Birdsfoot trefoil	*Lotus corniculatis*	Fabaceae [Leguminosae]
	Cherry laurel	*Prunus laurocerasus*	Rosaceae
	Clovers	*Trifolium pratense*	Papilionaceae [Leguminosae]
		Trifolium repens	Papilionaceae
	Elder	*Sambuca nigra*	Caprifoliaceae
	Flax	*Linum usitatissimum*	Linaceae
	White mustard	*Sinapis alba*	Brassicaceae [Cruciferae]
Miscellaneous glycosides	Lesser celandine	*Ranunculus ficaria*	Ranunculaceae
	Lesser spearwort	*Ranunculus flammula*	Ranunculaceae

gitalonin, several of which are useful in human medicine. These have a direct effect on the heart. The plant is fairly unpalatable, but sheep in Scotland exhibited dullness, abdominal pain, frequent urination and diarrhoea; foxgloves growing on an adjacent bank showed evidence of having been eaten. Other signs of foxglove poisoning in sheep are believed to include tachycardia, cardiac arrythmia, rapid irregular pulse, colic, mild to moderate enteritis, albuminuria, haematuria, depression, drowsiness, tremor and possible cardiac arrest. Consumption of foxgloves may cause abortions in ewes.

Hellebores contain a volatile oily substance, protoanemonin, which can cause intense irritation of the mouth and gastrointestinal tract (see below under lesser celandine and lesser spearwort). The most common species are *Helleborus niger* (Christmas rose) and *H. viridis* (Lenten rose), and both contain in addition cardiac glycosides often referred to as bufadienolides, which include hellebrin, desglucohellebrin and Nα-hydroxydesglucohellebrin. Christmas rose is generally confined to gardens, but green hellebore can be found sometimes growing wild on chalky soils. Hellebores have an exceedingly bitter taste, yet sheep have been poisoned by the plant; clinical signs included bloody mucoid diarrhoea with violent straining alternating with quiet spells, and frequent urination. Necropsy findings were gastroenteritis and petechiation in the intestine and on the heart.

The flowering shrub oleander flourishes everywhere in Mediterranean countries, but fortunately does not grow wild in Britain, although it is perfectly capable of thriving in the south of the country. It contains various cardiac glycosides with actions similar to digoxin, and all parts of the plant are extremely toxic, even by contact or by inhalation of the smoke from burning wood. Sheep would only encounter the shrub if they gained access to parks or botanical collections. Poisoning would be lethal; one leaf is said to be toxic for sheep, the lethal dose being 0.5–1.0 g/kg BW. Necropsy findings are gastroenteritis with haemorrhages in the gastrointestinal tract and heart. Clinical signs in experimental poisoning of sheep were irregular heartbeat, difficulty in breathing and muscular tremors. Recommended treatment is to give sedatives or tranquillizers; calcium salts are contraindicated. Spindle (skewer tree) is a deciduous shrub or small tree commonly found in woods, scrub and hedges in Britain. All parts of the plant contain several toxic principles which almost certainly are cardiac glycosides. Sheep and goats have been poisoned by eating the twigs and leaves.

The important group of plants whose toxic principles are cyanogenic glycosides contain enzymes which can convert glycosides into sugar, hydrocyanic acid (prussic acid, HCN) and either an aldehyde or a ketone. In the intact plant, the enzymes are separate from the glycosides, but HCN is released on disruption of the plant cells by ingestion or trampling. Ruminant species are particularly vulnerable to the action of cyanogenic glycosides, partly because the neutral pH of the rumen favours the action of plant enzymes, but also because of a synergistic effect of enzymes in certain rumen bacteria in hydrolyzing the glycosides. When released, the HCN reacts with the trivalent iron of mitochondrial cytochrome oxidase, thus inducing histotoxic anoxia in the tissues and rapid onset of death.

Birdsfoot trefoil and flax contain the cyanogenic glycosides linamarin and lotaustralin. Birdsfoot trefoil is widespread in Britain on lowland meadows, downland and hill pastures, particularly where the soil is thin. In Wales, the plant grew in profusion when scrubland was cleared, while in Scotland the re-establishment of flower meadows from seed led to the introduction of non-native trefoil strains, thus the potential for poisoning is there. Sudden deaths from cyanide poisoning might be expected if sheep grazed on the young plants. Juice from freshly cut foliage caused the death of sheep when it was introduced experimentally into their stomachs. Toxic amounts of HCN can occur in uncooked linseed cake; it is recommended that sheep should not be given linseed cake.

Both red and white clovers indigenous to Britain and Ireland can cause HCN poisoning in ruminants. However, clovers also contain other toxic principles including goitrogens, oestrogens, nitrates, and substances which can cause bloat, blood coagulation disorders and photosensitivity in sheep. White clover contains the cyanogenic glycosides linamarin and lotaustralin (see birdsfoot trefoil and flax). Amounts are highest in young growing plants, and the application of nitrogenous fertilizers increases the level of toxicity of clovers, although this may be due to an increase in nitrate levels. Clinical signs reported include prostration, dullness, torpor, ataxia, bloat, recumbency, difficulty in breathing, congestion or cyanosis of visible mucous membranes and, occasionally, death due to asphyxia. Suggested treatments specific for clover poisoning are intravenous sodium hyposulfite (3 g/100 kg), intravenous dicobalt edetate

(20–25 mg/kg) or sodium nitrite (5 mg/kg in a 3 per cent solution); care must be taken with this last treatment that the clinical picture is not due to nitrates in the clover. Phytoestrogens in clovers can cause swelling of the genitalia of ewes and may contribute to the development of ringwomb, while goitrogens may cause thyroid enlargement in lambs.

The ornamental shrub cherry laurel is rich in the cyanogenic glycosides prunasin and amygdalin, and several incidents of acute poisoning have been described in sheep that had access to this shrub. In a recent report, sheep in Scotland died in a public park after eating from a hedge of cherry laurel. Clinical signs were dilated pupils, rapid breathing, jerky movements, staggering, falling and convulsions. Cherry laurel leaves were found in the rumen. In a different outbreak, sheep which had access to the shrub only consumed it during severe wintry conditions. The young green leaves, bark and berries of elder (bourtree) contain the highly toxic cyanogenic glycoside D-sambrunigrin. References to elder are numerous in folklore, and at one time the young bark was recommended as a cure for footrot. However, an outbreak of cyanide poisoning was recently suspected in two ewes which died in Scotland; pithy stems of the shrub were found in rumen contents.

There are no significant necropsy features in sheep which die suddenly of cyanogenic glycoside poisoning, but in those which had a brief illness the presence of cyanosis of organs and bright-red blood may arouse suspicions. In cherry laurel poisoning there may be a strong smell of almonds and the lungs may be congested. The lining of the mouth or vagina may be bright red, because the oxygen in the blood is not being transferred to the tissues. A simple test on rumen contents using picric acid paper may confirm the presence of cyanides; a colour change from yellow to red being positive. Treatment has to be initiated quickly if it is to be effective. Sodium thiosulfate (0.5 g/kg BW) should be given intravenously initially, then sodium nitrite (10–20 mg/kg BW) [1, 2].

Members of the Brassica family of plants, for example rape, contain glycosides which, when hydrolysed, yield aglycones with specific actions on the heart or with marked goitrogenic properties. However, other factors present in rape (see under 'Poisoning by plants containing haemolytic anaemia factors' later) are more usually the cause of problems in lambs grazed on rape.

The aglycone protoanemonin, an unstable irritant oil, is hydrolysed from the harmless glycoside ranunculin in lesser celandine and lesser spearwort. This substance causes intense salivation, ulceration of the mouth and damage to the gastrointestinal tract and urinary system, with consequent diarrhoea and passing of dark or blood-tinged urine. These symptoms may be followed by incoordination, convulsions and death. Treatment is symptomatic and should include administration of demulcents.

POISONING BY PLANTS THAT CONTAIN SAPONINS

Saponins are high molecular weight glycosides with distinctive foaming characteristics and a bitter taste. They consist of a polycyclic aglycone that is either a choline steroid or a triterpinoid attached to a sugar side chain. For convenience, poisoning by saponin-containing plants are grouped together in this section.

Cuckoo-pint (lords and ladies, *Arum maculatum,* Araceae) contains a saponin tentatively called aroin. The plant has a bitter taste, and generates a highly obnoxious, faecal smell due to the presence of dimethyl-oligosulfides, apparently to attract blowfly larvae. Suspected poisoning in a ewe caused intermittent muscular weakness and a profuse greenish-yellow diarrhoea. A related plant, bog arum, also grows wild in Britain, and contains a similar active principle.

Ivy (*Hedera helix,* Araliaceae) contains triterpenoid saponins that undergo hydrolysis to form α- and β-hederin, irritant substances likely to cause diarrhoea and muscle spasms leading to paralysis. However, sheep would eat ivy only if no other green bite was available. Scarlet pimpernel (poor man's weatherglass, *Anagallis arvensis*, Primulaceae) contains a toxic saponin. Ingestion of the young growing plants has caused anorexia, depression and diarrhoea in sheep. Necropsy findings included haemorrhages in the kidneys, heart and rumen, congestion of the lungs and a pale, friable liver.

The highly toxic plant pokeweed (American nightshade, *Phytolacca americana*, Phytolaccaceae) is not native to Britain but has occasionally escaped from gardens to flourish locally. Its active principles probably are triterpenoid saponins, phytolaccatocin and related terpinoids; an alkaloid, phytolaccine and oxalic acid. Between them these cause intense burning of the mouth, salivation, diarrhoea, muscle cramps, impaired

vision, weak respiration, coma and death in livestock. The plant has an acrid taste, which may deter sheep from eating it if other herbage is available.

No specific treatment is available for saponin poisoning, but supportive symptomatic therapy may be useful.

POISONING BY PLANTS CONTAINING HAEMOLYTIC ANAEMIA FACTORS

Brassicas, particularly rape, *Brassica napus*, kale, *B. olorea* variety *acephala*, and cabbage, *B. olorea* variety *capitata* (Brassicaceae) are important winter feeds for sheep and cattle. Brassicas provide soluble carbohydrate, good quality protein, vitamins and minerals in proportions that theoretically should give high yields in the animals to which they are fed. These are achieved when brassicas form part only of the ration but when they are fed exclusively the potential is seldom reached. The deficit almost certainly results from the subclinical effects of toxic substances in the brassicas, from suppression of appetite or from the effects of copper deficiency if the crop is fed for a long time.

Four types of disease have been identified in ruminants fed on rape: respiratory, urinary, nervous and digestive. An important constituent of these brassicas is the amino acid *S*-methyl cysteine disulfide (SCMO), which on hydrolysis to dimethyl sulfide causes irreversible changes in haemoglobin, part of which is precipitated as Heinz bodies (HBs). The affected cells then lyse and the HBs are removed from circulation by the spleen. The plants also contain sulfur-based heterosides, for example gluconapine in rape and progoitrine in both rape and kale, which yield thiocyanates on hydrolysis and may lead to goitre, and nitrates, which can give rise to toxic nitrites in the digestive tract (see under 'Poisoning by nitrate-containing plants' later).

Progressive anaemia is probably the most important manifestation of brassica poisoning. In the acute form this proceeds rapidly to give haemoglobinuria and death, but more commonly is characterized by lowered production. Sheep grazing brassica crops occasionally develop photosensitization, but the precipitating cause has not been identified.

The severity of the disease relates directly to the amount of SMCO ingested and analysis of the crop for SMCO gives a useful indication of the potential hazard. Daily intakes of 10–18 g SMCO/100 kg live weight (LW) produce low-grade anaemia and higher intakes acute haemolytic anaemia. A 33-kg weaned lamb ingesting 1 kg DM daily of a brassica crop containing 0.4 per cent of its DM as SMCO would be in the low-grade anaemia category (12 g SMCO/100 kg LW). A crop with 1.4 per cent of its DM as SMCO would provide the lamb with 42 g/100 kg LW, an amount which, even if appetite were drastically reduced, would still be dangerous.

The SMCO content increases with the age of the crop. In the hybrid Maris Kestrel kale it may rise from 0.4 per cent DM in August to 1.4 per cent DM in January. However, both the winter-hardy Maris Kestrel and Cauletta varieties are fairly low in SMCO when compared to traditional varieties. Brussels sprouts, cabbage and kale contain similar relatively high levels of SMCO, whereas rape has considerably less. SMCO has an appetite-limiting effect which also contributes to the lower than expected growth rate. The SMCO content of the crop may respond to manurial nitrogen depending on the initial size of the nitrogen pool in the soil. Additionally, nitrogen fertilization increases the risk of nitrate/nitrite poisoning, thus access to the crop should be strictly controlled, if not abandoned altogether. As during lactation the voluntary intake of leafy brassicas increases greatly, lactating ewes should be rationed to no more than 30 per cent of their diet in this form. The coincidence of lactation in sheep with the availability of brassica crops, however, is unusual in traditional breeding programmes, but would occur if sheep were being bred to lamb in the autumn.

Clinical signs usually are those of severe anaemia. Asymptomatic animals may have an undetectable shortening of the erythrocyte lifespan. Depression of growth or production may be a subclinical manifestation of toxicity. Lassitude, salivation, lack of rumination, respiratory difficulties, coughing, nasal discharge, congestion of mucous membranes, fever, dark-coloured urine and, occasionally, photosensitivity have all been reported in sheep fed continuously on rape.

Death may occur due to asphyxia, while bloat and constipation are signs of severe digestive upset. A nervous manifestation with transient blindness has been reported in British sheep; these recovered completely after being withdrawn from the crop.

Gross pathological features in acute toxicity include haemolysis with icterus, haemoglobinuria and anaemia.

There may be congestion of kidneys, spleen and liver, and the presence of active healthy red bone marrow. Histologically there are zones of necrosis in the liver and widespread haemosiderosis of liver, spleen and kidney. Haemorrhages are not a feature. It is important to differentiate acute haemolytic anaemia from nitrite poisoning so that immediate treatment of the latter may be given if necessary. If a freshly dead sheep is not available for necropsy, blood samples should be drawn from sick animals for assessment of colour and consistency. In haemolytic anaemia the blood is dark red and watery, whereas in nitrite poisoning it is brown and opaque due to the formation of methaemoglobin. Samples for laboratory confirmation should include the crop for SMCO and nitrate plus nitrite determination, and blood from sick sheep for haematology. An additional blood sample diluted with 20 volumes of phosphate buffer (pH 6.6) to stabilize methaemoglobin should be taken. Methaemoglobin seldom exceeds 12 per cent of the haemoglobin, while in nitrite poisoning it exceeds 20 per cent and may be as high as 80 per cent.

Sheep should in all circumstances be introduced gradually to the crop, and allowed only limited daily access. A companion crop such as hay or cereals should be used to make up the energy deficit. In the event of illness, affected sheep should be removed from the crop and carefully introduced to, and given, alternative supplementary feed. It is usually unnecessary to administer iron, as much of the iron lost in haemolysis is recycled. Prolonged brassica feeding may deplete copper reserves, as brassicas are relatively rich in sulfur and contain molybdenum, both of which impair absorption of copper (see Chapter 54). Diagnostic blood samples should be sent to a laboratory for copper determination if feeding on brassicas is to continue in sheep with unsatisfactory growth rates. If copper values are very low, appropriate supplementation must be supplied.

Goitre can occur in sheep fed continuously on kale, and is passed on to their lambs. This is due to secondary iodine deficiency, as the thiocyanates in the kale compete with the thyroid gland for uptake of iodine, thus preventing accumulation of iodine in the gland (see Chapter 54). The thiocyanate content of the crop doubles during September and October, the young leaves containing up to five times as much as older leaves. Lambs of affected ewes are born weakly and have enlarged thyroids. Oestrogenic substances present in traces in kale may give rise to reduced fertility in sheep.

Onions (*Allium cepa*, Alliaceae) contain *N*-propyl disulphide and a phenolic compound with haemolytic properties, while ramsons (*A. ursinum*, wild garlic) contain the haemolytic factor di-(2-propenyl) disulfide. *N*-propyl disulfide affects the enzyme glucose-6-phosphate dehydrogenase in red blood cells, and interferes with the hexose monophosphate pathway. This leads to an insufficiency of phosphate dehyrogenase or glutathione to protect the red cells from oxidative damage. Erythrocyte cell membranes are damaged, in addition to HB formation, and sheep fed on cull onions can suffer from haemolytic anaemia with, in severe cases, haemoglobinuria. Clinical signs include a rapid weak pulse and pale mucous membranes. Wild garlic may be more toxic although less palatable, however it has been suspected of causing the death of ewes in Britain. Necropsy findings included evidence of anaemia, haemoglobinuria, dark kidneys with tubular necrosis, mottled golden-brown liver and marked haemosiderosis of the spleen. The carcasses smelled strongly of garlic.

Dog's mercury (*Mercurialis perennis*, Euphorbiaceae) contains methylamine, trimethylamine, a poorly defined substance tentatively named hermidine, saponins and a volatile oil. The plant is unappetizing, and has a strong unpleasant smell and an acrid taste. The young leaves and shoots are highly poisonous, and have caused haemolytic anaemia and acute gastroenteritis in sheep. Clinical signs included diarrhoea, weakness, jaundice, pain on urination and haemoglobinuria. Necropsy findings included subcutaneous oedema, haemorrhages in the liver, kidneys and heart, dark kidneys and gastroenteritis. There is no specific antidote to dog's mercury poisoning, but symptomatic treatment may be beneficial.

POISONING BY NITRATE-CONTAINING PLANTS

Nitrates are absorbed from the soil and accumulate in certain plants; moreover, the application of nitrogenous fertilizers can increase the degree of accumulation. Although nitrates are relatively non-toxic, bacterial action mainly in the gastrointestinal tract may convert them into highly toxic nitrites. These are absorbed into the blood and combine with haemoglobin in the red cells to form methaemoglobin, a

compound which cannot take up oxygen. Symptoms of nitrite poisoning are therefore those of progressive oxygen deficiency and a cardinal sign of poisoning is a change in the colour of the blood from red to brown. Sheep tend not to develop methaemoglobin to the same extent as other herbivores, but they are far from being fully tolerant to the ingestion of nitrate-containing plants.

Many brassicas (Brassicaceae [Cruciferae]) accumulate nitrates, although some of these contain other important toxic principles (see under 'Poisoning by plants containing haemolytic anaemia factors' earlier). The roots of freshly harvested mangels (mangolds) or beets (*Beta* spp., Chenopodiaceae) may contain nitrates as well as oxalates (see under 'Poisoning by oxalate-containing plants') and it is thus doubly hazardous for these roots to be fed without a storage period of several months. Most livestock owners know that the leafy tops of turnips, for example swedes, are poisonous; these can contain from 5 to 9 per cent nitrates as well as oxalates. The topped roots also can give rise to severe lactic acidosis if sheep are introduced to them too abruptly.

The root nodules of some species of clover (*Trifolium* spp., Leguminosae) also contain substantial amounts, and under certain conditions both sheep's sorrel, *Rumex acetosella*, and common sorrel [sour dock, *Rumex acetosa* (Polygonaceae)], similarly can accumulate nitrates. All species of mallow (*Malva* spp., Malvaceae) are particularly toxic, particularly the marsh mallow, *Malva parviflora* (cheeseweed), which has recently been introduced to Britain and has become established locally, particularly on waste ground.

All these plant species cause laboured breathing and staggering, with eventual recumbency and death. Visible mucous membranes may be cyanotic. Incoordination, trembling, arched back and extended head have been described in marsh mallow poisoning – 'shivers' or 'staggers' – of lambs. Suckling lambs become poisoned through the milk. Affected animals tend to fall over and die on being driven. It is suggested that if animals are kept calm and removed circumspectly from the noxious plants they will recover.

The diphenylamine test can be used to detect the presence of nitrate/nitrite in the aqueous humour of dead animals. This test is based on the capacity of nitrites to degrade aromatic amines, a positive reaction being a change in colour from orange to red. Necropsy features include a dark-brown appearance of the blood and haemorrhages on many organs. Treatment is by intravenous injection of methylene blue at 10 mg/kg BW, with supportive therapy.

POISONING BY OXALATE-CONTAINING PLANTS

Accumulation of oxalates in plants depends on the type of soil and the age of the plant, among other factors. The highest concentrations can be found in the aerial parts of the plant. Ruminants tolerate quite large amounts of oxalates, due to conversion by ruminal flora into harmless carbonates or bicarbonates. However, when hungry animals graze on a heavy growth of oxalate-containing herbage, acute poisoning may result. Oxalates can combine with free calcium in the digestive tract, forming insoluble calcium oxalate. If oxalates are absorbed into the bloodstream, they deplete the blood of calcium and the intravascular calcium oxalate thus formed is deposited in the kidneys and other tissues. Acute poisoning generally induces symptoms of hypocalcaemia, while chronic poisoning causes progressive renal failure, with associated uraemia and interference with energy metabolism. Castrated male sheep may experience urethral obstruction due to precipitation of calcium oxalates.

The leaves and roots of fresh beets, mangolds or turnips can contain up to 12 per cent of soluble and insoluble oxalates, and indiscriminate feeding can result in hypocalcaemia, red cell breakdown, kidney damage and crystallization of oxalates in the brain, causing paralysis and other nervous signs. Another potent source of oxalates is the common weed, fat hen (*Chenopodium album*, Chenopodiaciae). Fat hen is the most common member of the Chenopodium family growing wild in Britain, and can be found in profusion on waste ground. Ingestion of this plant caused shallow respirations, weak heartbeat, muscular incoordination followed by recumbency and deaths in sheep in The Netherlands.

Similar symptoms can follow ingestion of common sorrel, sheep's sorrel, curled dock (*Rumex crispus*) and wood sorrel (sleeping beauty, *Oxalis acetosella*, Oxalidaceae), which are commonly found in Britain. Common sorrel in particular is known to contain potassium binoxalate and oxalic acid. A related plant, procumbent yellow sorrel, *Oxalis corniculatus*, is an introduced species also capable of causing oxalate

poisoning. These sorrels thrive on acid soil; their seeds germinate during the winter, thus the early growth may prove attractive to sheep in the spring. Acute illness and deaths have occurred in British sheep which had access to a dense crop of common sorrel. Clinical signs included loss of muscular coordination, frothing at the mouth, falling with inability to rise, dilated pupils and coma. Chronic poisoning of sheep with sorrels is reported to cause stiffness, paralysis, staggering with falling and recumbency with outstretched neck, muscular spasms, nasal discharge, excessive salivation, dilated pupils and, eventually, coma and death. Some of these symptoms are attributable to renal failure. However, recently it has been reported that the *Rumex* species of sorrel also contain anthraquinone glycosides, which may be important toxic factors. Cuckoo pint (*Arum maculatam*, Araceae) also contains oxalates and could cause poisoning if highly concentrated in an area grazed by sheep. However, the plant has a bitter taste and a most unpleasant odour (see 'Poisoning by plants that contain saponins' earlier) and would not be eaten unless there was no alternative green bite available.

Treatment of acute oxalate poisoning is by intravenous administration of calcium borogluconate (100 ml of a 20 per cent solution). This may be less effective in chronic cases due to permanent renal damage, and selective culling may be necessary. Sheep should be removed from the source of the offending plants.

POISONING BY PLANTS THAT CAUSE PHOTOSENSITIVITY

Photosensitivity or photosensitization is dealt with more fully elsewhere (see Chapter 49) and is discussed only briefly here. Two main forms of photosensitivity can be caused by plants. In primary photosensitivity, photodynamic substances are absorbed and transported by the blood to the skin after ingestion of particular plants. The most common form of photosensitivity in sheep, hepatogenous photosensitivity, occurs when a toxin produced by a plant, fungus or alga causes liver damage resulting in retention of the photosensitizing agent phylloerythrin in the blood and the skin. When bright sunlight strikes the sensitized skin, cells are damaged and the reaction of the body is manifested as itchiness, redness, heat, oedema and swelling of the skin, usually in lightly pigmented areas of the head or back. Sheeps' ears droop and the pinnae may eventually separate and drop off. Affected animals usually recover and thrive well in later life.

Perhaps the most important example of photosensitivity in sheep in Britain and Ireland is the condition variously named saut (Cumbria), heddles or hard lug (Co. Antrim, Northern Ireland), and yellowses, plochter or plochteach (Scotland). The term plochteach is derived from the Gaelic *plocach* (pronounced *plochgach* = having a swollen head). In a very similar condition in Norway called alveld (elf-fire), a definite association with ingestion of bog asphodel (*Nart hecium ossifragum,* Liliaceae) has been established, and there is also strong evidence for an association between this plant and the British and Irish conditions (see Chapter 49). Bog asphodel is found mainly in acid bogs and on moors, heaths and mountain areas, particularly in Scotland. A recent survey of sheep holdings in Perthshire and Argyll in Scotland showed that ploteach was widespread in the area studied, and that up to 10 per cent of the lamb crop could be affected, with mortality up to 50 per cent of clinically affected animals. Furthermore, bog asphodel was present in almost all grazed and ungrazed flush areas of the hill pasture, although up to 11 other major plant species also were identified in the grazings. Blood biochemistry of clinically affected lambs showed a highly significant correlation between hepatic damage and death. However, there was no evidence that the plant was preferentially grazed, though plants which had been eaten did not flower, and consequently were difficult to identify.

Bog asphodel contains steroidal saponins, and it is postulated that these are hydolysed by rumen bacteria into the free sapogenins, sarsapogenin and smilagenin, which are spirostanol or furostanol glycosides bearing one or more sugar chains. Metabolites of these sapogenins can cause liver damage with subsequent photosensitivity. The pathogenesis additionally may be complicated by mycotoxins. Observations in Norway have shown that certain species of microfungi can be found on plants growing in bog asphodel habitats. These include the saprophytic fungi *Pithomyces chartarum* and *Cladosporium magnusianum*, some strains of which produce the hepatotoxin sporodesmin, which also can cause liver damage in sheep, notably in the condition known as facial eczema in New Zealand (Chapter 67). However, recent research suggests that these fungi may act only indirectly by stimulating an

increase in the concentration of free sapinogens in the plant as a defence mechanism against infection by them [4]. Sapinogen concentrations also increase over the growing season.

Extensively grazed weaned lambs are most at risk; deaths result usually from misadventure resulting from obscured vision caused by the oedema of the head. Sheep have been excluded from certain moors because the prevalence of the disease was unacceptably high there. However, recent findings suggest that there are differences in breed susceptibility, and genetic differences in liver enzyme activity in sheep, which could influence the occurrence of outbreaks. There are, in addition, regional differences in the concentrations of smilagenin; for example, concentrations in the plant in Norway in areas where photosensitivity occur were two to four times those found in Scotland [4].

Diagnosis is usually made on the basis of the oedema and subsequent scab or scar formation of the skin of the head and possibly the back. Supporting evidence may be obtained by post-mortem demonstration of the incriminating plant in the rumen (in alveld, the entire ingesta may consist of the scimitar-shaped leaves of *N. ossifragum*) while the blood of clinically affected sheep may show increased amounts of aspartate aminotransferase, bilirubin and phylloerythrin, with prolonged retention of bromsulfthalein.

Several plants can cause primary photosensitivity when eaten by livestock. Buckwheat (*Fagopyrum esculeatum*, Polygonaceae) contains a pigment, fagopyrin, thought to be a naphthodianthrone derivative. Buckwheat is cultivated on a small scale particularly in East Anglia, where it also occurs as a weed. Common St John's wort (*Hypericum perforatum,* Hypericaceae) is widely distributed in Britain. It and other *Hypericum* species contain red fluorescent pigments, including hypericin and pseudohypericin. The plant also contains several flavones and flavenols including quercitrin, quericin, rutin, kaemferol and luteolin, all of which are quercetin glycosides. Common St John's wort, an upright perennial with characteristic bright yellow flowers, is native to Britain, growing freely on dry, calcareous soils, and extracts of it are favoured by herbalists. Sheep will eat it only at the young growing stage. Once sensitized to these pigments, an animal remains photosensitive so that further eating of the plant and exposure to sunlight produces photosensitization of increasing severity. Primary photosensitization also occurs occasionally in sheep fed on rape or exposed to blue–green algae (see later).

Control of photosensitivity requires that affected sheep should be removed indoors or to shade. The immediate decision whether to remove the entire flock depends on the proportion of sheep affected and the availability of alternative uncontaminated pasture. In Norway, elimination of *N. ossifragum* and of alveld was achieved after 3 years by applying phosphorus to the infested wet moorland each spring at 4 g/m^2 as superphosphate.

POISONING BY PLANTS WITH OTHER TOXIC PRINCIPLES

Several important poisonous plant species contain substances or complexes of substances with unique actions; these may cause quite characteristic syndromes on ingestion (Table 56.5).

Perhaps the most important of these is bracken fern. Bracken is distributed throughout the world, and in Britain is most abundant in the moister west, covering large areas of woodland, thickets and open hillsides. The success of bracken as a colonizer arises from a rhizome system with enormous reserves of energy and an ability to tolerate wide ranges of soil types and pH. All parts of the plant, fresh or air-dried, are poisonous.

Bracken has several toxic constituents and induces several different manifestations in animals [5]. The toxins so far identified are prunasin, a cyanogenic glycoside; a thiaminase which causes thiamine deficiency in horses and pigs but not in ruminants, and ptaquiloside, a water-soluble norsesquiterpene glycoside of the illudine type. Other compounds found in bracken include quercetin and shitimake, but their role in toxicity is unclear. Prunasin is a bitter substance, but some strains of bracken contain very little of it, and animals are readily prepared to eat the tempting young growing fronds.

Ptaquiloside is carcinogenic. As a dianone intermediate, it can react with DNA which has affinity with certain base sequences, especially those associated with adenine. The toxin mutates codons associated with known oncogenes. These in turn may interact with papillomaviruses to stimulate tumour formation. Ptaquiloside was once believed to be the cause of the haemorrhagic syndrome that is so prominent in cattle, but recent experimental studies have cast doubt on this. However, when given experimentally to a calf thrombocytopenia resulted.

Table 56.5: Plants containing miscellaneous active principles that may poison sheep

Plant	Latin name	Family
Azalea	*Azalea* spp.	Ericaceae
Bracken	*Pteridium aquilinum*	Dennstaedtiaceae
Charlock	*Sinapis arvensis*	Brassicaceae [Cruciferae]
Chickweed	*Stellaria media*	Caryophyllaceae
Field peas	*Pisum sativum*	Leguminosae
Goat's rue	*Galega officinalis*	Papilionaceae [Leguminosae]
Hard rush	*Juncus inflexus*	Junacaceae
Hemlock water dropwort	*Oenanthe crocata*	Apiaceae [Umbelliferae]
Kalmia	*Kalmia* spp.	Ericaceae
Oak	*Quercus* spp.	Fagaceae
Pieris	*Pieris japonica variegata*	Ericaceae
Privet	*Ligustrum vulgare*	Oleaceae
Rhododendron	*Rhododendron ponticum*	Ericaceae
Sun spurge	*Euphorbia helioscopia*	Euphorbiaceae
White mustard	*Sinapis alba*	Brassicaceae [Cruciferae]

The bone-marrow depression in bracken poisoning involves mainly megakaryocytes and results in extreme depletion of blood platelets. In sheep, a profound lymphocytopenia is also induced by prolonged consumption of bracken. However, because it is known that these effects take several weeks to reach their crisis points, it is generally accepted that sheep may be used to suppress the early growth of bracken.

The acute, haemorrhagic form of poisoning is much less commonly seen in sheep than in cattle, usually being confined to sheep introduced for the first time to grazings containing bracken. The term acute is really a misnomer arising from the briefness of the terminal phase of toxicity, which results from prolonged exposure of the red bone marrow to bracken-derived toxins. The body's defences are weakened and blood clotting time is increased, with potential consequences of microbial invasion, haemorrhage and anaemia. The clinical signs of the terminal crisis are well documented for cattle but not for sheep. Usually, the first sign in a group of sheep is the passage of blood in the urine and faeces: a most useful signal to this may be an individual being followed by the others, presumably being attracted to the blood by the smell. Other signs are high fever (rectal temperature 40.3°C and above), anaemia, epistaxis, melaena, haematuria, and very fast respiratory and heart rates. Necropsy findings include severe anaemia and pallor, watery blood and sparseness of blood clot in the heart chambers, blood clots in the bladder and gut lumens, mucosal haemorrhages and infarctions in the liver, kidney, lungs or rumen wall. Pasteurellosis may supervene terminally, thus the underlying salient features of bracken poisoning may be overlooked. The important primary histological feature is aplasia of red bone marrow.

A non-fatal effect in sheep of prolonged exposure to bracken is a progressive retinal atropy with increased reflectivity of the tapetum lucidum known as 'bright blindness'. In 1965, a blindness of ewes grazing the upland bracken-infested pastures of west Yorkshire was described, which was known to local farmers for a long time but had become commoner [6]. The condition was reproduced experimentally by feeding ewes for many months 1 kg/day of pelleted ration containing 50 per cent bracken. Clinical signs are progressive bilateral blindness in sheep over 2 years old, identified usually in the autumn by inadvertent separation of individuals from the flock, with the high-stepping gait and alert high-held head characteristic of blind sheep. The eyes shine abnormally brightly in semi-darkness, and the pupils become circular and react poorly to light. There is no inflammation and no opacity of the lens. Through an opthalmoscope the arteries and veins appear narrower and the main vessels more widely separated than normal; subclinical cases may be identified by such examination. Pathologically, there is degeneration of the neuroepithelium of the retina. Early on, the rods and cones become fragmented and later completely destroyed, together with the outer, and portions of the inner, nuclear layers.

Bracken has also been incriminated as a cause of certain cancers in sheep. Fibrosarcomas of the mandible and maxilla were found in mature sheep which had grazed bracken-infested moors in north Yorkshire for two to three summers (see Chapter 58). Bracken has been cited as a possible cause of mixed bladder tumours and rumen papillomas in sheep, and continuous feeding of bracken experimentally has caused bladder cancer, although bladder cancer has not been found in naturally poisoned sheep in Britain. There is no evidence for any association in sheep between grazing of bracken, intestinal and bladder carcinoma, and papillomavirus infection, as has been demonstrated in cattle.

For diagnosis, evidence should be sought that bracken was available for long periods and appeared to have been grazed. The rumens of dead sheep may contain fronds of the plant. Ill or dead sheep should have the appropriate clinical or post-mortem features of the disease. Sick animals should be separated for treatment; they also provide material for laboratory confirmation. Blood in anticoagulant should be examined for differential white cell and thrombocyte counts, which will show leucocytopaenia, thrombocytopenia and severe anaemia. Faeces should be taken for detection of blood if clots are not obvious, and urine to confirm haematuria. Red marrow smears may be taken using anaesthesia and biopsy equipment but only if necessary and by special arrangement with the laboratory. The appropriate post-mortem specimens are rumen contents for microscopical identification of bracken if necessary, red bone marrow fixed immediately after death and lesions (eyes, tumours) fixed for histology.

To control an 'acute' outbreak, the flock should be excluded from bracken-infested pastures for as long as possible and inspected immediately and daily thereafter for new cases. In the unlikely event of a live sheep being diagnosed with the acute haemorrhagic form of bracken poisoning, empirical treatment, based on recommendations for cattle, should include parenteral administration of prednisolone and broad-spectrum antibiotics, and drenching with magnesium trisilicate. Sheep with obvious leucopenia should be provided with shelter and treated symptomatically with vitamin K and iron, and introduced gradually to a highly nutritious diet based, during convalescence, on the lactation requirements of ewes. In the long term, it is important to reduce as much as possible the exposure of the flock to bracken.

Control of bracken by herbicides is feasible but very costly. Herbicides which might be considered include glyphosate, a solution of asulam (methyl 4-aminobenzene sulfonyl carbanate) in water or metsulfuron. It is worth bearing in mind that the wilted, dried or grubbed up plant may be palatable and toxic.

Rhododendron, an attractive evergreen shrub, flourishes particularly in the milder regions of western Scotland and also in Wales, where it is becoming a serious pest. The leaves contain acetylandromedol (synonyms: andromedotoxin, grayanotoxin 1), a nitrogen-free non-glycosidic polycyclic diterpene, along with several other unspecified grayanotoxins. Azalea and kalmia species and *Pieris japonica variegata* are now classed as rhododendrons, and contain similar active principles. Poisoning of sheep by *Rhododendron ponticum* is rare, but when it occurs many may be affected and mortality can be high. A common history is that sheep have been drawn to eat the green leaves as the only source of green food during severe wintry weather with snow. Carelessly discarded garden clippings of rhododendron species are another source of poisoning. Weaned lambs admitted for the first time to a field of leafy brassicas which bordered grounds containing rhododendron chose first to sample the overhanging rhododendron leaves and so were poisoned. Rams are said to be less tolerant than female sheep. Azaleas, kalmia species and *Pieris japonica variegata* are ornamental shrubs that tend to be confined to gardens. Clinical signs of poisoning by all these species in sheep include drooling, distressing attempts to vomit, abdominal pain, diarrhoea, respiratory distress, staggering and collapse into lateral recumbency with paddling movements leading to death. Necropsy features include the presence of the leaves in the rumen and congestion of the lungs.

For treatment, individual sheep should be selected according to their clinical condition for stomach emptying by rumenotomy. Morphine (60–200 mg depending on BW) is said to be effective in goats. Antibiotic cover may guard against secondary pasteurellosis. Many affected sheep recover with good nursing.

MISCELLANEOUS PLANT POISONING

Privet, which grows as a native shrub in southern England and is cultivated throughout Britain and

Ireland as a hedging plant, contains several glycosides, including syringin (ligustrine), various secoiridoid glycosides (nuzhenids) and possibly saponins, concentrated in the leaves and particularly in the berries. These toxic principles are poorly categorized and their actions poorly understood, thus poisoning by privet has been included under 'Miscellaneous poisoning' rather than 'Poisoning by glycosides'. Sheep sometimes nibble at enclosing hedges of privet or are attracted to clippings, resulting in gastroenteritis, incoordination and other nervous signs, leading to rapid death. Privet leaves are often found in the rumens of sheep which have died after ingesting the shrub, accompanied by an intense gastroenteritis, hepatitis and blood splashing in the thorax. Axonal degeneration has been found in the spinal cord. Treatment is symptomatic.

White mustard has been grown as a forage crop for sheep. However, once the pods have formed it becomes increasingly toxic. The seeds in particular contain sinalbin, which is *p*-hydroxybenzyl-glucosinolate. Crushing or mastication releases enzymes in the plant, which cause this compound to undergo hydrolysis to acrinyl isothiocyanate, or mustard oil, a highly irritant substance, and *p*-hydroxy-benzyl-alcohol. Lambs in Britain which grazed for 2 days and nights on white mustard in which the pods had formed became acutely ill with frothy salivation and diarrhoea; five died and 40 were ill and unable to stand. The seeds of the closely related charlock, a common weed with a yellow flower, contain the glucosinolate sinigrin, from which is released allyl isothiocyanate, another volatile, highly irritant mustard oil, by enzymic hydrolysis. Charlock can grow luxuriantly in aftermath grazings unless controlled by herbicides. Like white mustard, the plant is poisonous only when the pods appear. Once the plants reach a height of 5–10 cm it is unsafe for sheep to eat them. However, they are not likely to be eaten unless they contaminate conventional fodder crops. For example, lambs folded on a field of rape in which charlock with well-formed pods was rife developed acute gastroenteritis with frothy salivation, diarrhoea, abdominal pain with grunting, and some died. Necropsy findings included acute rumenitis, enteritis and nephritis. Compounds with goitrogenic properties and variable amounts of nitrates may also be found in these species.

Similar symptoms are seen in sheep poisoned by certain spurges (*Euphorbia* spp., Euphorbiaceae). These plants exude a milky latex containing polyhydric diterpene esters, among other principles. For example sun spurge (mad-woman's milk) contains four esters of 12-deoxyphorbol, the most toxic of which is 12-deoxyphenol-13-phenylacetate-20-acetate, as well as an alkaloid, euphorbin; also a glycoside, a dihydroxycoumarin and a complex substance named euphorbiosteroid. Like all spurges, sun spurge is bitter and unpalatable. However, sheep which ate kale extensively contaminated with the plant suffered severe swelling and inflammation of the mouth, with profuse salivation and diarrhoea, although they all recovered.

Goat's rue, sometimes known as French honeysuckle or Italian fitch, is not native to Britain but has become established locally, particularly on roadsides, waste ground, rubbish tips and railway banks. In France and several other countries severe outbreaks of poisoning occurred in sheep when the plant was eaten at pasture or in hay or forage. The plant contains galegine (isoamyleneguanidine) and hydroxygalegine, which are the main toxins, as well as saponins, flavenoids and peganin. Symptoms of poisoning are laboured breathing, oedema of the neck, frothy nasal discharge, loss of balance, muscle spasms with the head thrown back, and convulsions often leading to death. Necropsy findings in sheep in Essex which died after apparently consuming goat's rue growing on a road embankment consisted of marked subcutaneous oedema, pulmonary congestion, hydrothorax, renal congestion and myocardial haemorrhage [7].

Another highly toxic plant is hemlock water dropwort, colloquially known as dead man's fingers or devil's parsnip due to the appearance of the roots. The tall, hollow-stemmed plants, which have an aniseed-like smell, grow along the banks of streams, and the roots, which are particularly poisonous, may be exposed after flooding or ditching, attracting ruminants. The active principle, oenanthetoxin, is a polyunsaturated alcohol which acts as a convulsant poison. Sheep are said to be relatively resistant to poisoning with this plant, although they can succumb: 2 g/kg of the fresh root per kg BW are said to be toxic for sheep. Clinical signs of poisoning include salivation, dilated pupils, possibly abdominal pain, respiratory distress, clenched jaws, ataxia, falling, convulsions, recumbency and/or coma, and, later, diarrhoea; sheep often recover when moved out of danger. No treatment is available.

Sheep can be poisoned, sometimes fatally, by eating the young leaves or shoots of oak but more rarely by acorns, which they are less inclined to eat. The

astringent tannins in all species of oak are hydrolysed in the digestive tract to phenolic compounds such as gallic acid or pyrogallol. Gallic acid on absorption can damage the liver and kidneys, while pyrogallol can oxidize haemoglobin to form methaemoglobin. Acorn poisoning in sheep is said to cause haemorrhagic gastroenteritis, bloat, constipation and renal failure. In severe cases, rumenotomy and emptying of the rumen is the only rational treatment; prognosis is poor.

Horsetail contains thiaminase, which does not affect sheep. However, the plants additionally can concentrate silicates in their foliage, which may cause gastrointestinal irritation. Sheep are only rarely affected by eating the plant, but may show muscular weakness and diarrhoea. Necropsy findings include slight jaundice, degenerative changes in the liver, kidney and possibly in the brain, with catarrhal inflammation of the gastrointestinal tract.

Sheep have been poisoned by hard rush, which is prolific in acidic wetlands. This plant contains an unidentified toxin or toxins, and the clinical signs are said to be gastritis, nervousness and diarrhoea. Blindness has been reported in calves which ate the plant, though they later recovered. Ruminants are believed to develop a taste for hard rush, but it is not certain whether this includes sheep. Chickweed, a very common weed, contains saponins which are readily metabolized in the digestive tract and are relatively harmless. However, an old report blamed chickweed for causing digestive disturbances and deaths in lambs due to the formation of indigestible masses in the abomasum.

The haulms, pods and seeds of garden or field peas (canning peas) are normally regarded as safe for feeding to sheep, but in one report [8] the lambs of ewes fed pea silage and pods from canning peas suffered incoordination. The lambs were tense, ran backwards and fell over when disturbed.

POISONING BY BLUE–GREEN ALGAE AND OTHER FUNGI

Growth of algae can be found in many inland waters in Britain. The most important of these probably is *Microcystis aeruginosae,* but other toxic algae found in Britain include species of *Anabaena, Aphanizomenon* and *Oscillatoria*. *M. aeruginosae* forms blue or blue–green floating colonies consisting of individual cells the size of grains of sand embedded in a mucilaginous matrix. Their growth is especially profuse where there is a run-off of fertilizer from adjacent fields. Ponds enriched with phosphate fertilizers are particularly vulnerable to algal growth.

M. aeroginosae and *Anabaena* spp. produce groups of toxins, cyclic hepatopeptides or microcystins, which have extremely complex molecular structures and collectively are ten times as toxic as strychnine, as well as various alkaloids and lipopolysaccharides. Microcystins gain entry to liver cells by utilizing membrane transporters which normally are used to transport essential chemicals and nutrients [9]. The toxins damage hepatocytes by inhibiting protein phosphatases 1 and 2A, resulting in excessive phosphorylation of specific intracellular proteins and cell death. Damage can be widespread enough to cause focal necrosis and intrahepatic haemorrhage, with associated hypoglycaemia and circulatory shock.

Sheep were poisoned by blue–green algae at Rutland Water in Lincolnshire, with numerous deaths, often sudden with no warning. Necropsy findings in this outbreak were widespread haemorrhages, congestion of the liver and toxic tubular nephritis. Clinical signs of algal poisoning usually are gastrointestinal disturbances, abdominal pain, bloody diarrhoea, weakness, with reduced activity and responsiveness leading to death, while necropsy findings may include liver swelling, haemorrhage and necrosis, with oedema round the wall of the gall-bladder. Suggested emergency treatment is oral dosing with activated charcoal. A form of hepatogenous photosensitivity has occurred in sheep which ventured to drink water contaminated with blue–green algae in Britain. Serum bilirubin and a range of liver enzymes may be elevated in chronically ill sheep, and normal liver function may not be restored for months after exposure.

Aflatoxicosis, a collective term for poisoning by toxic metabolites from moulds that can accumulate on stored feed, is not a problem in sheep as a rule. An exception is malt culm poisoning, caused by contamination of stored culms with the saprophytic fungus *Aspergillus clavatus.* Poisoning causes brain damage with ataxia, paralysis and death in sheep. Histological findings include chromatolysis and neuron necrosis in the red nucleus and other motor centres, and in the dorsolateral horns of the spinal cord, with associated axonal degeneration. Stored cereals contaminated by *Penicillium* species and other moulds apparently

caused oxalate poisoning in lambs in Scotland; five of 60 pure-bred Suffolk lambs died. Affected lambs had high blood urea levels and numerous crystals of calcium oxalate were found in the kidneys of dead lambs. Utilization of calcium in the feed by *Penicillium* moulds was believed to have been the source of oxalates in the outbreak. Cocksfoot (*Dactylis glomerata,* Poaceae [Graminaceae]) contains a metabolic inhibitor which affects the growth of lambs by interfering with cellulose digestion. The plant additionally can be infested by the sporodesmin-containing fungus *Pithomyces chartarum*, which has caused so-called facial eczema in sheep in south-east England after ingestion of cocksfoot. Treatment of all these poisonings is essentially symptomatic and supportive.

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APPENDIX

The UK Veterinary Poisons Information Service (VPIS)

Contact addresses and telephone numbers for information on poisonous plants and other poisons that are hazardous for animals.

VPIS (London)
Medical Toxicology Unit
Avonley Road
London SE14 5ER
Tel. 020 7635 9195
www.medtox.org

VPIS (Leeds)
The General Hospital
Great George Street
Leeds L51 3EX
Tel. 0113 245 0530
email
medicines.information@
Leedsth.nhs.uk

VPIS is a subscription-only service to UK veterinary practices.

Full imprint of *Poisonous Plants and Relateds Toxins*: Acamovic, T., Stewart, C.S. and Pennycott, T. (eds), 2003. (Collected papers from the 6th International Symposium on Poisonous Plants, 2001, Glasgow, UK.)

CABI Publishing
CAB International
Wallingford
Oxon OX10 8DE
UK

CAB Publishing
7th Floor, 875
Massachusetts Avenue
Cambridge, MA 02139
USA

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Inorganic and organic poisons

W.J. McCaughey

Currently, reports of inorganic poisonings of sheep originate most frequently from countries with rapidly developing industries. Case reports of poisoning in sheep in the UK are rare. However, information on cases diagnostically confirmed by the Veterinary Laboratories Agency indicates the relative importance of some heavy metals. For the period from 1995 to 2005 these are summarized in Table 57.1. The results show that copper is the most common laboratory-confirmed cause of poisoning in sheep in the UK followed by lead and indicate that controls of other inorganic poisons are currently effective.

A second source of analyses showing exposure of sheep to heavy metals are the annual reports of the National Statutory Surveillance Survey undertaken to satisfy European Union (EU) requirements for residue testing outlined in EU Directive 96/23/EC. These are published in annual reports of the UK Veterinary Medicines Directorate. The samples taken for these analyses are collected from animals that have been slaughtered for the human food chain and that have passed ante- and post-mortem meat inspection procedures. The results are therefore not associated with clinical abnormality but may be taken as indicators of exposure. During the decade 1991–2000 these results show evidence of very low level exposure to cadmium, lead, arsenic and mercury. The concentrations found have been examined by toxicologists and are considered harmless for consumers.

ALUMINIUM

Although the third most abundant element, aluminium (Al) is found only in trace amounts in animals and plants and is not essential [1]. Grazing animals consume soil attached to the herbage and are therefore exposed to Al. Additional intakes have occurred when certain clays, such as kaolin and bentonite, have been

Table 57.1: UK sheep poisonings (farm incidents) confirmed by the Veterinary Laboratories Agency

Year	Copper	Lead	OC/OP*	Basic slag	Total
1995	86	5			91
1996	76	4			80
1997	89	5			94
1998	99	4			103
1999	25	2			27
2000	44		1	1	46
2001	38				38
2002	48	9			57
2003	77	1			78
2004	71	6			77
2005 (to June)	38	1			39
Total	691	37	1	1	730

*OC/OP, organochlorine and organophosphate.

added to animal diets as binders in commercial feeds, to prevent clumping of pellets or for beneficial gastrointestinal effects. It has been reported that Al binds phosphorus in a non-absorbable complex resulting in clinical phosphorus deficiency when 2200 ppm was fed for 77 days [1]. However, these effects are moderated by the solubility of the Al source and the concentration of phosphorus in the diet. In basal ruminant diets, concentrations ranging from 147 to 210 ppm have been reported. In sheep, adverse effects were not noted from dietary levels of 1215 ppm [2] and in beef type steers levels up to 1200 ppm did not influence performance [3]. Based on these relative intakes poisoning due to Al is unlikely to occur in ruminants.

ANTIMONY

Very little antimony (Sb) occurs free in nature but exposure can occur through environmental contamination during industrial refining of ore or directly from ingestion of discarded alloys. Poisoning cases are rare. Ruminants are able to withstand large doses of Sb, although repeated administration may cause fatty degeneration of the liver; underfed animals are particularly susceptible to this effect [4]. The symptoms of acute poisoning are abomasitis, enteritis and scours. Chronic poisoning induces fatty degeneration of the liver. The condition is diagnosed by chemical confirmation of Sb in the faeces, gut contents or liver. Surviving animals should be removed from the potential source, may be treated by drenching with calcium hydroxide or magnesium oxide (to precipitate the Sb) and given increased fluid intakes.

ARSENIC

Arsenic (As)-containing compounds occur widely as inorganic and organic forms both of which are highly poisonous but distinction must be made between their respective toxic effects. Inorganic compounds, mainly the arsenites and arsenates, possess water-soluble anions that bind to, and interfere with, important enzyme systems, particularly the keto-acid hydrogenase system, where keto-acid oxidation is blocked. Organic arsenicals have a complex mode of action that is not clearly understood, but some bind to the important sulfhydryl groups of enzymes and amino acids, blocking the metabolic pathways that they serve [5].

Most metal ores contain small quantities of As that can be distributed on pasture neighbouring processing plants. Run-off water from these areas may concentrate the contamination, leading to poisoning when sheep drink from this water. As-containing industrial effluents or gases from smelting plants or other industrial processes may also contaminate herbage with similar effects.

Historically, arsenical poisoning from on-farm sources was not uncommon, owing to the widespread use of acaricidal dips or foot-rot preparations containing As compounds. Sheep have been poisoned from accidental intakes during dipping, access to foot-rot preparations or improperly disposed containers. Long-woolled sheep may also be poisoned by absorption through the skin. Undiscarded dips or footbaths containing As compounds were often a temptation for sheep being held in dry areas for prolonged times after treatments. As these were replaced, the main sources of As became arsenical herbicides, fungicides or insecticides, or occasionally rodenticides, which accidentally contaminate feeds, forage or drinking water [5]. Arsanilic acid (4-aminobenzarsenic acid) has been incorporated into pig and poultry feeds as a growth promoter, and can cause poisoning when incorrectly formulated or when accidentally fed to the wrong species [1].

Chronic poisoning may result either from industrial pollution of the herbage or from exposure to organic compounds of As used as herbicides or insecticides. Although most forms of As are readily absorbed and rapidly excreted, constant intakes result in accumulation of As in the liver, kidneys, spleen, bones and wool. Symptoms may take months to develop and include digestive disorders, inappetence, respiratory problems and unthriftiness. In suspect chronic cases animals constantly exposed to very small amounts in the herbage may develop a form of tolerance. Since As accumulates in the wool, analysis of wool fibres will give a measure of the level of exposure, the average normal value being less than 134 μmol/kg (10 ppm) [5].

Acute poisoning generally results from the ingestion of water-soluble inorganic salts. The interval between ingestion and illness is variable. The major action is on the gastrointestinal tract and the cardinal signs are acute gastroenteritis, with profuse, watery diarrhoea, sometimes tinged with blood. This is accompanied by acute colicky pain followed by dehydration, weakness

and depression. Some animals may be found dead. As directly affects the vascular system causing dilatation of vessels and a rapid drop in blood pressure resulting in cardiac collapse. Animals develop muscular twitching and convulsions, followed by coma and death after only a matter of hours, but milder cases may live for up to a week. In these, an acute haemorrhagic gastroenteritis with pseudomembrane formation is the main finding at necropsy. Diagnosis is based on the history, clinical and necropsy findings with confirmation of high levels of As in suspected feed, water or herbage, stomach contents, liver, urine, spleen and other organs obviously affected at necropsy [5]. Figures quoted for toxic concentrations of As include more than 134 μmol/kg (10 ppm) in liver, 13.4 μmol/kg (1 ppm) in urine and 67 μmol/kg (5 ppm) in faeces.

Various treatments have been proposed for As poisoning, but acutely affected sheep should be slaughtered on humane grounds, as the chance of recovery in negligible. The effectiveness of ferric hydroxide, although it may reduce immediate fatalities, has been questioned [4]. Saline purgatives with animal charcoal powder are recommended for mild cases, and sodium thiosulfate in a 20 per cent solution may be given intravenously initially, repeated every 12 hours until symptoms abate. The dose for an adult sheep is 5 g [5]. Adrenergics to support the circulation may be combined with rehydration by the use of dextrose saline. Theoretically, 2,3-dimercaptopropanol, which binds As to its thiol groups, ought to be effective, the suggested treatment regime being 2–3 mg/kg every 4 hours for 2 days, then every 6 hours for 2 days and finally every 12 hours for 2 days [5]. As the substance is itself toxic, these guidelines should not be exceeded.

CADMIUM

Cadmium (Cd) is a hazardous heavy metal and is a risk to sheep grazing pasture to which sewage sludge has been applied [6] or in the vicinity of industries using zinc as Cd is a constituent of zinc ores [4]. There have not been any recent reported cases of poisoning due to Cd intakes. However, randomly collected samples of sheep liver are regularly analysed for Cd concentration as part of the National Surveillance Scheme of residue testing. During the period 1990–2003 concentrations of the metal have been identified in a small proportion of randomly collected samples. Toxicologists have concluded that these concentrations do not present a hazard to consumers. However, the findings do indicate that exposure has occurred during the sheep's lifetime. Follow-up investigations have not yet identified a definitive source for this exposure.

Cd is readily absorbed from the gut (80–90 per cent is excreted in 24 hours in the faeces) and fixed in tissues particularly the kidneys, liver and spleen [7] and is nephrotoxic. Toxicity depends on the ability of sheep to sequester Cd with metallothioneins [8]. As there may be a competitive inhibition with zinc the zinc status of sheep is likely to be a significant factor in determining whether Cd intakes are toxic [9].

Acute poisoning of farm animals does not occur under normal conditions [5]. Studies to determine the toxic effects of Cd have frequently used high doses administered by injection but these may not reflect the exposure levels that may occur in farm situations nor establish the effects on sheep production or wool quality of moderate pasture contamination [9]. Cd fed for 20 days at a level [286 μg/kg of feed dry matter (DM)] typical of polluted regions increased water retention but did not affect intakes or digestibility. These observations indicate that, in sheep, dietary Cd has little effect on the fermentation functions of rumen bacteria, although adverse effects on protozoan populations have been reported [10]. Increases in weight during the experimental period were suggested to be due to increased organ weights, water retention, reduced throughput of digesta and/or decreased faecal output. Changes in water retention were accompanied by increased urinary concentrations of potassium, iron, molybdenum, chromium, bromine and calcium. It was concluded that the decreased sodium balance was typical of renal tubular disorders.

Raised Cd concentrations have been recorded in the wool of sheep grazing polluted areas, such as close to major roadways [11] or in industrial areas [12]. However, in feeding experiments [9, 10] no increased incorporation of Cd into wool was noted, so the elevated concentrations may result from surface contamination. As Cd intakes increased wool potassium content, and may decrease the natural protection against yellowing, there may be commercial implications from low-level exposure [9]. Cd also has an affinity for interrupting testicular function causing necrosis and interruption of spermatogenesis [5], effects partly reversible by the administration of zinc and selenium. However, when rams are fed 7.5 mg Cd daily for 60 days there were no differences

in sperm concentration or sperm motility in ejaculates [13]. Treatment reduced the number of flehmen responses and altered feeding behaviour by increasing grazing time at the expense of rumination.

CAESIUM

The nuclear reactor accident at Chernobyl in the former USSR on 26 April 1986 released radionuclides into the atmosphere. Caesium (Cs) is one of the group of alkali metals. The isotopes most significant for human health are Cs-134 (half-life 2.06 years) and Cs-137 (half-life 30.2 years). They are formed during nuclear fission of uranium-235 and emit both beta and gamma rays. Very large amounts of these isotopes formed part of the nuclear cloud that swept northern Europe. Contamination by this radioactive fallout was deposited in rainfall in various parts of the UK in early May.

By June 1986 sheep in certain upland areas of Wales, Cumbria, and south-west Scotland were found to have accumulated between 1000 and 4000 becquerels (Bq) (this was the level set as an acceptable maximum in the UK). Similar levels were identified in areas of Northern Ireland. Although there was no related clinical illness in exposed sheep, the element is likely to be concentrated in muscle and therefore it was considered that meat from sheep with those levels of radioactivity should not be eaten, in view of the possible relationship between consumption and cancer. As a result, statutory controls of the movement, slaughter and sale of dressed carcasses of sheep in those areas was introduced, monitored by repeated testing and remained in force for more than a decade.

The areas most affected by the fall-out in Northern Ireland had a deep acidic soil with little mineral content. As a result, the radiocaesium remained in the topsoil and was available for uptake by plants grazed by sheep. Countermeasures to prevent or to reduce the accumulation of radiocaesium in animal tissues were studied as part of the control programme. Earlier studies in relation to atmospheric weapons testing in the 1960s had shown that Prussian blue and the clay minerals, bentonite and clinoptilolite, fed as dietary supplements, are effective in reducing the transfer of radiocaesium to milk. This approach was not considered a practical means of control in grazing animals. However, an indwelling rumen bolus containing Prussian blue persisted for 12 weeks and reduced radiocaesium levels by approximately 80–90 per cent without any detrimental effects on the animals [14]. Simultaneous studies on plant growth, plant biodiversity, soil microbial activity and carabid beetles failed to show significant impacts. These results indicate that Prussian blue boli may be considered if a similar nuclear accident occurs in future.

COPPER

Copper (Cu) is an essential nutrient, but is also toxic for farm animals. Sheep are particularly susceptible to the toxic effects possibly because they do not appear to be able to increase biliary excretion in response to increased intakes [15]. Cu poisoning, acute or chronic, has been reported from most sheep husbandry areas of the world. A gradual upward trend in the incidence of chronic Cu poisoning in sheep may be associated with a waning antagonism from pasture sulfur [16]. It is the most common poisoning in sheep in the UK as diagnosed by the Veterinary Laboratories Agency. During the decade 1995–2004 the annual frequency ranged from 25 to 99 and the total was 653 recorded cases (Table 57.1).

Cu metabolism in sheep is complex and is influenced by various factors, including variable breed tolerance and the presence of antagonists, e.g. sulfate, molybdenum and iron in the diet (Chapter 54). All breeds of sheep are highly susceptible to Cu toxicity. North Ronaldsay, Charollais, Texel and Suffolk breeds are much more vulnerable than the Scottish Blackface. Genetic variation in susceptibility is greater than in other species and selective breeding has been suggested as a means of reducing susceptibility to hypocuprosis or poisoning [17].

Two important sources of Cu for sheep are compound feeds and mineral supplements. Although feeds specifically for sheep are compounded to contain low levels of Cu, it is difficult to achieve sufficiently low levels for absolute safety, even with the inclusion of Cu inhibitors, especially where housed sheep are fed intensively for prolonged periods. The current EU limit for Cu in sheep diets is considered too high for pure-bred Charollais, Suffolk and Texel lambs housed for 6–14 weeks fattening pre-slaughter [17]. Suffolk and Texel-cross lambs absorb Cu twice as efficiently as Blackface lambs: subclinical toxicity has

been recorded in such lambs on diets containing 12 ppm Cu/kg DM; a level often exceeded in commercial foodstuffs. Cattle and pig feeds contain higher concentrations and are unsuitable for feeding sheep. However, shared production lines within feed mills may result in cross-contamination between feed batches resulting in higher concentrations than intended in sheep feed.

Overdosing with Cu supplements to control deficiency can also cause poisoning. The increasing availability and use of sustained release products increases this risk if preventive treatments are given to recently purchased stock without information on previous treatments or a laboratory check on their status.

Additional Cu may enter the environment from a number of sources. For example, it is unsafe to graze sheep on pastures dressed with pig manure, which often contains concentrations of Cu dangerous for sheep, and Cu-based fungicidal or molluscicidal sprays applied to forage or pasture occasionally cause episodes of acute toxicity.

Cu poisoning can be acute, but more commonly presents in its chronic form.

Chronic poisoning is a constant risk in intensive sheep-rearing involving feeding concentrates. The risk is not directly in proportion to the dietary Cu concentration because, at high Cu levels, a smaller proportion is absorbed and more is excreted via the faeces. Chronic poisoning is usually associated with prolonged ingestion of diets from which Cu is efficiently absorbed (around 10 per cent) rather than dietary Cu concentrations. Complex interactions between Cu, iron and molybdenum have been recorded [18]. Cu toxicity has been reported in Texel sheep grazing on silage aftermath that had normal Cu, but extremely low molybdenum, concentrations. The reduced molybdenum safety-net allowed the Cu to accumulate to toxic levels. Pastures with a Cu:Mo ratio of 20:1 or greater may thus encourage Cu toxicity to develop, especially in susceptible breeds. Unusual diets that induce Cu poisoning have been recorded [19].

Although sheep have a capacity to store Cu in the liver, centrilobular necrosis with increased plasma aspartate transaminase (AST) concentration develops when liver Cu concentrations exceed 750 ppm DM. At higher concentrations a sudden release of Cu into the bloodstream occurs precipitating a haemolytic crisis. As a result kidney function declines dramatically causing the accumulation of metabolites such as urea in the blood, while spongiform degeneration may occur in the brain. Characteristic post-mortem features are widespread jaundice, a discoloured (yellow/orange) liver distended, and discoloured (bronze/black) kidneys and haemoglobinuria. Plasma Cu concentrations rise only shortly before the haemolytic crisis. A number of laboratory analyses have been suggested for monitoring progression and γ-glutamyltransferase (GGT) followed by AST have been recommended following an experimental study of Cu loading in Suffolk lambs [20].

Ingestion of plants containing hepatotoxic alkaloids, e.g. pyrrolizidines, can precipitate Cu toxicity, presumably by impairing the ability to excrete excess Cu. Other hepatotoxic agents such as carbon tetrachloride produce liver lesions similar to those in chronic Cu toxicity and other haemolytic diseases e.g. rape/kale anaemia may also give rise to some of the necropsy features characteristic of Cu toxicity. The full combination of features with elevated concentration of Cu in the liver (1000 ppm DM) and kidney (50 ppm DM) will confirm the diagnosis.

Acute poisoning with rapid death may occur within 2–3 days of the subcutaneous administration of Cu for prevention of Cu deficiency or access to Cu-containing materials. Accidental water deprivation (>17 hours) resulted in 27 sheep drinking a 5 per cent solution prepared as a footbath; ten died, six were hospitalized and the remainder had mild symptoms and recovered [20]. In acute cases clinical signs include depression, anorexia, abdominal pain, diarrhoea, dehydration and shock. Necropsy findings include acute inflammation of the abomasal mucosa with ulcerations and haemorrhagic areas are often present. The gut contents may be stained blue–green. Signs of jaundice and haemolysis may be absent. Tissue Cu concentrations are lower than those found in chronic poisoning, particularly liver Cu, where concentrations may not exceed 300 ppm DM. Analytical confirmation should be directed towards the gut contents.

A specific form of acute poisoning involving ataxia, paresis and recumbency 2–3 days after intramuscular injections of Cu preparations into the neck have been reported in ten UK sheep flocks. It has been suggested that these resulted from local nerve damage and/or diffusion following the injections and the need for careful administration is stressed [21].

Where Cu poisoning is a potential risk, top-dressing grazing paddocks with molybdenum (1 oz/acre: 70 g/ha) as molybdenized superphosphate may be used as a preventive [22]. Housed animals may have 7 mg/kg molybdenum incorporated into the feed. The

efficacy of a molybdate formulation and of a zinc oxide bolus in the prevention of Cu accumulation have been evaluated but neither proved successful [23]. When poisoning is suspected, stress factors, for example withdrawal of food and simultaneous administration of anthelmintics, may render some sheep more susceptible than others to acute Cu toxicity and should be avoided. In a Brazilian study management changes and transport associated with an international show, together with high concentrate intakes, have been cited as precipitating factors for the haemolytic crisis [24].

In chronic poisoning when the haemolytic crisis has supervened, treatment is unlikely to be successful. In less severely affected animals with high blood Cu, ammonium tetrathiomolybdate, 1.68 mg/kg given intravenously three times over a period of 5 days or 3.4 mg/kg subcutaneously on 3 alternate days may reverse the toxicity [25, 26]. Daily oral administration of 100 mg ammonium molybate alone and/or 1 g sodium sulfate may reduce further clinical cases and mortalities. It is important to alter the diet by excluding concentrates, the most likely source of Cu and, where available, to offer silage with a satisfactory Cu:Mo ratio. The surviving sheep should be offered, as their sole source of fluid, drinking water medicated with 20 mg/l ammonium molybdate. These treatments increase faecal excretion of Cu but do not influence urinary excretion. However, it has been reported that ammonium tetrathiomolybdate adversely affects the hypothalamo-adrenohypophyseal axis leading to the cessation of reproductive activity [27].

In acute cases palliative therapies to counter acute gastrointestinal inflammation and shock may be given. Repeated administrations of D-pencillamine increase excretion, but overall urinary excretion induced by a mean dosage 28 mg/kg body weight was considered small relative to liver Cu content [28]. An oral dose based on 50 mg/kg body weight/day maintains limited control of Cu elimination. This may be the safest option, but its high cost limits its use to valuable individuals.

FLUORINE

Chronic fluorine (F) poisoning of livestock, or fluorosis, occurs when animals continually graze on pastures or are exposed to feedstuffs contaminated by industrial fumes containing fluoride. Until means were found, and legislation was enacted, to prevent such emissions, fluorosis occurred regularly downwind of aluminium factories. Factories producing acid phosphates from rock phosphates or bricks from F-bearing clays are also potentially hazardous when they fail to prevent F-contaminated emissions. In Australia, poisoning has been reported where bore water or rock phosphates have high concentrations of F [29].

Chronic F poisoning occurs when excess is ingested over a period. Urinary excretion rises rapidly to a maximum, after which F accumulates slowly in bones and teeth to approximately 40 times the normal level, finally 'spilling over' into the soft tissues, causing a rise in plasma F concentration. During the cumulative stage, mottling, dark staining and excessive wear of the permanent teeth formed during the period of exposure to F are found consistently. Exostoses may develop on long bones and on the mandible. Stiffness, lameness, depression and inappetence become apparent. Acute F poisoning occurs when sodium fluoroacetate or sodium fluoracetamide used widely in many parts of the world to control vermin are ingested. These fluoroacetates are converted in the body to fluorocitrate, which blocks the citric acid cycle. There is a lag phase of at least half an hour after consumption of the poison while this conversion takes place, then incoordination, weakness, depression and death follow rapidly [5].

Differential diagnosis of chronic poisoning requires confirmatory fluoride determinations to differentiate from osteoporosis and deficiencies of vitamin D, Ca or P. Urinary F levels increase above the normal range of 10 and 15 ppm indicates abnormal F intakes. As this is likely to be a flock problem, it is considered advisable to take samples from a number of animals [4]. Cortical bone taken from affected animals may contain up more than 5000 ppm. Acute poisoning is confirmed by analysis of F content of the rumen, blood and urine.

Livestock should be removed from the contaminated area and offered uncontaminated food. Ca and Al compounds may be added to the diet to combine with F and reduce its solubility and slow progression but do not prevent the development of toxicity. In acute cases the antidote is glycerol monoacetate (monoacetin), which is a competitive antagonist of fluoroacetate, given at 0.55 ml/kg body weight intramuscularly every half hour for several hours. Monoacetin may also be given intravenously, diluted in five parts of sterile isotonic saline. Symptomatic treatments

include demulcents and aluminium sulfate orally to convert the soluble fluorides and calcium borogluconate and glucose saline intravenously [29].

IMIDAZOTHIOLES

Levamisole has a relatively narrower margin of safety than benzimidazoles therefore overdosing occasionally causes poisoning in sheep. These compounds act by inducing neuromuscular paralysis in nematodes, and their effects in sheep are an extension of this action. Overdosing can occur if weights are not estimated accurately. Slight overdosing results in salivation and slight muscle tremors. Severely affected sheep salivate profusely; have marked muscle tremors, ataxia, urination, defecation and collapse [22]. Death results from respiratory failure. Atropine sulfate can reduce the severity of symptoms and betamethazone was found useful in a goat poisoned with levamisole, but is currently untried in sheep.

IONOPHORES

The ionophores are a group of antibiotics produced by the fermentation of fungal *Streptomyces* spp., which have activity against some Gram-positive bacteria, coccidia, neospora and toxoplasma. The group includes monensin, narasin, lasalocid, salinomycin and maduramicin. They are toxic when administered at an excessive dosage, to the wrong species, or combined with drugs with known potential hepatotoxic properties or with nitrates [30]. In sheep, the most important ionophore was monensin, which is no longer authorized for use in sheep. When used previously at therapeutic dosage to control toxoplasmosis, monensin was virtually non-toxic, but accidental overdosing, or dosing where there was exposure to nitrogen-containing or hepatotoxic plants or pasture top-dressed with nitrogenous fertilizers, could cause toxicity leading to deaths. Toxicity in sheep may occur at doses of 12 mg/kg and is associated with the active exchange of sodium and the passive exchange of calcium for potassium; consequently the main effects are on muscle, including the pericardium. Monensin may be incorporated into poultry or beef cattle rations therefore cross-contamination in sheep concentrate rations may occur. Mild toxicity causes irregularity in the cardiac rhythm; more severe effects include lethargy, diarrhoea, muscle weakness, stiffness, posterior incoordination and myoglobinuria [31]. In sheep muscle, lesions appear as pale areas that are visually and histologically similar to those of nutritional dystrophy. Diagnosis is usually clear from the history and/or feed analysis. Clinical chemistry may show marked elevation of creatine phosphokinase in the early stages. Other enzymes that become progressively elevated include lactic dehydrogenase isoenzymes, reflecting damage to red cells and cardiac myocytes, while elevated AST concentrations indicate progressive damage to the myocardium and replacement fibrosis, pulmonary oedema and hind limb muscle wasting. Differential diagnosis would include selenium deficiency. As there is no specific antidote for monensin, treatment is symptomatic.

LEAD

Sporadic cases of acute lead (Pb) poisoning occur in sheep. Thirty-seven cases have been recorded by the Veterinary Laboratories Agency from 1995 to June 2005 making it the second most commonly confirmed cause of poisoning (Table 57.1). As sporadic deaths in sheep are often not investigated, the existence of the problem may not be suspected until several animals have died.

The most common sources of acute poisoning are flakes of old lead paint, red lead, putty, car batteries, linoleum, roofing felt or other Pb-containing materials. These can get directly on to pasture by negligent or illegal dumping or indirectly by flooding. Some motor oils and leaded petrol are also toxic, as are effluents from Pb-smelting plants. The forms usually associated with livestock poisoning are oxide, sulfate, sulfide, carbonate or acetate. Swallowed metallic Pb is retained indefinitely in the reticulum, small amounts are being constantly transferred to the digesta and absorbed.

The clinical signs can include severe abdominal pain, hyperexcitability, blindness, convulsions and death. Chronic poisoning may be commoner than generally accepted, but is asymptomatic during a cumulative phase, before showing similar clinical signs to acute cases followed by death. The cause may be diagnosed readily if a source can be confirmed.

Acutely affected animals show colicky pain, anorexia and ruminal atony within a day or two of ingesting a fatal dose. They become very excited, stagger in distress, show intermittent convulsive seizures, paralysis and collapse before death within a few days. Those that survive for longer appear blind and, between fits, are unresponsive to external stimuli, often standing with the head pressed against a wall. Necropsy findings include a dirty grey appearance of musculature, severe gastroenteritis and liver degeneration, the kidneys may be pale with petechial haemorrhages on the surface. Suspect Pb-containing materials may be found in the gut contents.

Chronic poisoning results from continuous ingestion of Pb or persistent release of small amounts from metallic lead retained within the reticulum. Clinical signs develop over months, and may include depression, anorexia, weight loss, constipation, pulmonary oedema, muscular weakness, stiffness and recumbency leading to death in weeks. Initially, these signs may occur without the owner appreciating their significance. Pregnant ewes may abort. Stress factors, such as virus infections, are thought in some cases to precipitate the acute syndrome during the asymptomatic cumulative phase. At post-mortem examination, there is emaciation and other features that resemble those of acute poisoning, but gastroenteritis may be absent.

A third form of toxicity has been noted in localized areas where naturally high levels of environmental Pb are associated with osteoporosis. The signs include stiff gait, lameness and posterior paralysis that are associated with high levels of Pb in tissues, soil and pasture. The condition was investigated in the vicinity of disused mines in south Scotland, the northern Pennines and North Derbyshire [32]. Affected lambs developed osteoporosis with excessively fragile bones, resulting in fractures and bone malformations. These lambs (ranging from 5 to 18 per cent of the flock) took short steps, walked slowly on their toes or became paraplegic. 'Cappi', a term which refers to abnormally thin frontal bones, occurring in the same flocks is considered to result from concomitant excess or deficiency of other elements, e.g. Ca or P. These changes were also noted in 3 per cent of clinically normal lambs. In free-ranging lambs in these plumbiferous areas, it appears that Pb, acquired *in utero,* in the milk and possibly also directly ingested from the pasture or soil, causes failure of osteoid synthesis. At necropsy the essential feature was rarefaction of cancellous bone, fractures and their sequelae.

However, the effects of dietary Pb intakes depend on a number of factors other than the concentration in the diet. Lambs fed 400 mg Pb per kg experienced anorexia, weight loss and death within 5 weeks when the dietary Ca, P and S content was low but when these elements were supplemented lambs survived up to 10 months despite anorexia and weight loss [33].

Early studies of the administration of Pb compounds to ruminants provided invaluable information on Pb metabolism and yielded diagnostic criteria for Pb levels. Only 1–2 per cent of ingested Pb is absorbed, most being eliminated in the faeces whether given as the water-soluble lead acetate or the insoluble carbonate incorporated in paint. Most absorbed Pb is excreted in bile and a small amount appears in urine.

In acute poisoning highest values are found in kidneys. The lethal dose for young ruminants is 0.2–0.4 ppm live weight and is somewhat higher for the mature animal. After continuous oral administration the largest amounts are found in the bones. Following a single oral administration, the blood Pb level may rise from a normal of less than 1.2 μmol (0.25 mg)/l to 19 μmol (4 mg)/l and death may supervene. If the animal survives, the level declines very slowly and even 6 months later may still be elevated. The faecal Pb level, on the other hand, rises steeply to 1000 ppm DM but declines to normal 30 ppm in a few weeks. This is of considerable value diagnostically: if both blood and faecal levels are high, it can be assumed that ingestion occurred recently. If blood is high but faecal lead is normal, ingestion occurred some time before and the animal is likely to survive.

The pathogenesis of Pb poisoning is not fully understood. Accumulation of Pb in kidneys, liver or brain gives rise to cellular degeneration typified sometimes by intranuclear inclusions and glomerular lesions in the kidneys. To confirm a diagnosis, consideration must be given to the clinical signs, the availability of Pb, levels in blood and faeces of survivors, and the findings at post-mortem. Kidney cortex should be sent for Pb analysis. A value for fresh tissue of 40 ppm is definitely positive; 10 ppm can be regarded as positive with collateral evidence and highly suggestive in the absence of such evidence. Animals with blood levels above and below 1 ppm have unfavourable and favourable outlooks, respectively.

The prognosis for sheep showing acute clinical signs is poor. Removal from any grazing area that might be associated with discarded Pb-containing materials or high soil Pb levels is an obvious first step in control.

Feeding a diet in which the concentration of major minerals is enhanced may further reduce the accumulation of lead in tissues. Emptying the alimentary tract and precipitating Pb by a magnesium sulfate purge are secondary steps that may be taken.

For immediate treatment the agent of choice is sodium calcium edentate (CaEDTA: calcium disodium versenate) which is often dramatically successful [47]. It is given at the rate 75 mg/kg daily by slow intravenous injection, preferably during the first 48 hours as a divided dose at intervals of a few hours. CaEDTA is a chelating agent that combines preferentially with Pb forming a soluble complex and is excreted in the urine and bile. It should be used sparingly as it may cause toxic nephrosis or death from too rapid mobilization of Pb.

MERCURY

The main sources of mercurial compounds that cause poisoning are pesticides or fungicides, but fumes from industrial processes may contaminate herbage and mercury (Hg) salts in their effluents may pollute watercourses. Poisoning is usually by ingestion, rarely by inhalation of toxic fumes. Previously, the most common source was organic mercurial seed dressing, either fed accidentally to sheep or eaten when sheep gained access to the treated whole grain or occasionally after a build-up in the ground or by spillage from containers. Modern seed dressings tend not to contain mercurial salts. Mercuric salts involved in poisoning include methylmercury chloride or hydroxide, ethylmercury chloride, 2-methoxyethylmercury chloride or silicate, and several more complex substances [5]. Mercuric thiocyanate, a wood preservative, may cause poisoning if splashed near animals in small or enclosed spaces.

Several inorganic salts are extremely poisonous, are highly corrosive and severely damage the gastrointestinal tract mucosa. They may also accumulate in the colonic mucosa, liver and kidneys, causing severe toxic liver damage, nephritis and corrosive lesions in the urinary tract. However, apart from pollution by industrial effluents, poisoning by these is unlikely. Organic salts, used as antiseptics and diuretics, are not corrosive but are lipophilic and may penetrate many biological membranes, including the blood-brain barrier. They interfere with a wide range of cellular activities, including the inhibition of many enzyme systems. After absorption they become widely distributed throughout the body, but are most concentrated in the brain, kidneys, liver and muscles. They can also pass the placenta and poison the fetus. Their main effects are manifested by overt neurotoxicity, enzyme inhibition and retardation of protein synthesis [5].

The clinical course depends on the nature of the poisonous compound, the route of exposure and the amount ingested. Acute Hg poisoning causes a corrosive gastroenteritis, colic and scour followed by kidney failure. Death occurs within 24–48 hours, and the main necropsy findings are necrotizing pharngitis, oesophagitis, acute ulceration of the abomasum and large bowel and congestion of the lungs and kidneys. Chronic poisoning causes anorexia, ataxia, blindness, convulsions, paresis, coma and death. Very long-standing chronic cases may have anaemia, loose teeth and a black band of mercuric sulfide on the gums [5].

Laboratory confirmation may not be necessary if there is a clear history, but to confirm the diagnosis, grain treated with a seed dressing, or suspected herbage or water supply and liver, muscle, kidney and brain samples should be submitted for analysis. Poisoning is confirmed when abnormally high concentrations are found in the gut contents, the liver or kidneys. Mercury may be excreted for months in the urine of survivors. In chronic poisoning, Hg also accumulates in wool therefore samples of wool from suspects should always be included for analysis. The concentration of Hg in the liver is usually less than 0.3 ppm [5]. Following intravenous administration of mercuric chloride increased urinary excretion of alkaline phosphatase, leucine aminopeptidase and aspartate aminotransferase have been noted [34]. However, serum levels of the enzymes were not increased. Urinary alkaline phosphatase and γ-glutamyl transpeptidase activity are considered sensitive indicators of the renal damage following experimental mercuric chloride poisoning in sheep [35, 36].

There is little justification for attempting treatment of sheep acutely poisoned by Hg [4, 5]. In these cases rapid initiation is essential and drenching with demulcents, e.g. milk or egg white to bind Hg remaining in the gut, has been recommended [4, 5]. For cattle, drenching with sodium thiosulfate has been suggested as the mercurials are complexed. The use of 2,3-dimercaptopropanol and penicillamine have been suggested as possible antidotes. These chelate the mercury and aid excretion but neither is very effective

after clinical signs have developed [37]. In surviving cases dehydration should be relieved by normal saline solution injections.

NITRATE/NITRITE POISONING

The main source of nitrates on the farm are nitrogenous fertilizers and nitrate-containing plants. Poor application techniques and careless disposal of small residual quantities can expose sheep to small areas of high concentration. Fertilizers can also pollute watercourses if washed off treated pastures by heavy rain. Nitrates can also occur in sewage sludge spread on the land. Drought will prevent leaching of top-dressings while molybdenum deficiency in the soil can lead to enhanced uptake of nitrates by plants. Concentrations are highest in roots and stems, lower in leaves and lowest in seeds [37]. Nitrites have also been used as preservatives for certain foods; in Norway, nitrite-treated herring meal poisoned livestock, including sheep, owing to the development of toxic nitrosamines during storage. Sodium nitrite can be used as a rust inhibitor and cattle have been poisoned by effluent from a garage where sodium nitrite was used in a machine for wet-blasting car components.

Forage nitrates are reasonably harmless but are reduced by the microflora of the alimentary tract, particularly the rumen, to harmful nitrites, hydroxylamine and finally ammonia. Nitrites are absorbed and interact with ferrous iron in haemoglobin producing methaemoglobin thus reducing oxygen-carrying capability. Development of clinical nitrite toxicity depends on the quantity of nitrite ingested and the speed at which it is reduced. The signs are those of anoxia, namely weakness, rapid weak pulse, fall in blood pressure and fast laboured breathing. The linings of the vagina and mouth become brown then, in the terminal stages of poisoning, cyanotic. If, when withdrawn for confirmatory diagnosis, the blood is chocolate coloured and opaque, action to combat nitrite poisoning should be taken at once. Blood for methaemoglobin determination should be diluted with 20 volumes of phosphate buffer at pH 6.6. In nitrite poisoning values exceed 20 per cent and may reach 80 per cent. At the latter ratio death occurs. Nitrites can be detected chemically in urine.

Treatment is by intravenous infusion of a 4 per cent solution of methylene blue at 10 mg/kg body weight and may be repeated as necessary; prompt action will facilitate full recovery. Preventive measures include avoiding stemmy forages, removing suspect sources and preparing a composite diet that dilutes high nitrate forages [37].

ORGANOCHLORINES (CHLORINATED HYDROCARBONS)

These compounds, particularly DDT and dieldrin, were the first highly effective therapies for control of external parasites in food-producing animals and were frequently used in the 1950s and 1960s. However, as they are chemically stable, slow to degrade in the environment and accumulated as residues, their use was subsequently reduced drastically.

They can cause acute toxicity and, because of their accumulation in fat, also chronic poisoning. Today, accounts of poisoning are largely historical. Clinical signs are similar for both acute and chronic poisoning; central nervous system overstimulation, progressing from alertness and overexcitement to fasiculations of facial muscles and later all musculature, then shivering, convulsions and death. Necropsy findings are non-specific and include oedema and congestion of the lungs, widespread haemorrhages particularly involving heart muscle, increased volume of cerebrospinal fluid, and congestion of the brain and spinal cord. Chlorinated hydrocarbons are detectable in tissues, blood, brain, and fat being the most useful for interpretation. Treatment is essentially symptomatic and consists of controlling the convulsions with barbiturates such as pentobarbitone sodium administered intravenously or intraperitoneally at 10–30 mg/kg body weight or, for more prolonged activity, chloral hydrate. It has also been suggested that intravenous calcium borogluconate may be useful to neutralize any rise in serum potassium and reduce the severity of convulsions [4].

ORGANOPHOSPHORUS COMPOUNDS

Organophosphorus compounds (OPs) which were derived from the so-called 'nerve gases' developed for chemical warfare are marketed as aphid sprays,

molluscicides, rodenticides, anthelmintics and, most significantly, sheep dips. They appeared more acceptable due to a shorter persistence in the environment but reports of delayed adverse effects on users of sheep dips have occurred. On farms, other sources include hydraulic oil, flame retardants, coolants and petrol additives. OPs act by inhibiting the action of cholinesterase leading to an excess of acetyl cholinesterase at nerve endings. Clinical signs include hypersalivation, hyperpnoea, depression, incoordination, collapse, coma and death. In sheep, the most prominent are reported as profuse salivation and laboured breathing followed by neuromuscular disturbances and cyanosis, diarrhoea and miosis [38]. Lesions include pulmonary oedema and emphysema, petechial haemorrhages on the myocardium, ecchymosis on the omasal mucosa, and congestion of the abomasum, liver, kidney and bladder.

Laboratory confirmations of clinical diagnoses by blood or analysis at post-mortem have been inconclusive. Organophosphates lower the concentration of cholinesterase in tissues and blood. However, the depression may not indicate directly the severity of the poisoning but taken in combination with history and clinical signs depressed blood cholinesterase level is a useful aid [4].

The detection of OP residues in tissues is highly diagnostic but these disappear quickly. However, the greatest amounts are retained in the liver and fat depots. Residues may also be detected in milk, urine and ruminal contents [38].

Suspect cases are injected intravenously with atropine to reduce the clinical signs. The injections are repeated as necessary as the signs recur. The effectiveness of this therapy is illustrated by a report of 21 mule ewes that had consumed approximately 600 g of 4 per cent methiocarb pellets, designed for the control of leatherjackets and slugs. Three hours later, 11 had died, four were severely affected and six had milder signs [39]. Atropine sulfate (0.6 mg/ml), diluted with an equal volume of normal saline, was given by slow intravenous injection of 20 ml, followed by additional subcutaneous doses to the severely affected. Three milder cases were treated subcutaneously with the same dose. The clinical response was rapid. Within 5 min the saliva flow at the lips had ceased, the ewes became brighter and normal respirations were restored in an hour.

The safety of users must also be considered. Because of their inherent toxicity, OP sheep dips, especially in concentrate form, must be handled with care and the sale and supply of dips is restricted, by legislation, in the UK to persons trained in their safe use. To minimize risk to operators detailed instructions in safe handling procedures are included with these products and must be followed. Spent dip is an environmental hazard. Recently dipped sheep must be managed carefully, allowed free access to clean drinking water from an unpolluted source and denied access to natural water courses, that might become contaminated, until the fleece is thoroughly dry. Disposals of waste from handling areas and spent dips require regulatory consents.

PARAQUAT

Paraquat is a non-selective, non-residual, herbicide commonly used on stock farms for the pre-emergent control of weeds in green crops. It is marketed as paraquat or in combination with diquat or diuron. The latter combination has some residual effect but these products are rapidly deactivated on contact with soil. Skin and eye irritation has been reported following external exposure. Oral poisoning has been reported in several farm species, including sheep, and in humans. In sheep, most poisonings have resulted from accidental contamination of feed or grazing recently treated pasture.

Poisoning of a flock of 98 ewes from drinking contaminated water resulted in 48 deaths in Australia [40]. Affected ewes were depressed, incoordinated and anorexic. The interval from first clinical signs to death was approximately 1 week. At necropsy, the findings included pulmonary congestion, multifocal petechial haemorrhages, atelectasis and necrosis of the alveolar walls. The livers were congested and the kidney tubules showed dilatation and cellular vacuolation. Circumstantial evidence based on confirming paraquat in several water sources led to the diagnosis. As the water in the troughs was automatically replenished following drinking the toxic dose could not be estimated but experimentally the LD_{50} has been estimated to be 50–75 mg/kg. Recommended treatments include oral drenching with adsorbants such as bentonite and administration of diuretics.

PHENOLIC COMPOUNDS

Sources of phenolic poisoning include farm or industrial disinfectants or wood preservatives containing

phenols or cresols, phenolic industrial gases and effluents that may contaminate the herbage in the vicinity of chemical works or asphalt boiling plants and phenol-containing navel dressing fluids. Production of carbolic bloom dips for show sheep has been largely discontinued.

Phenolic compounds are poisonous not only when ingested but also by absorption through the intact skin when presented as topical applications, thus great care must be taken when using these preparations [41].

Disinfectants such as Lysol or Jeyes Fluid contain coal tar derivatives including phenols; their accidental ingestion is usually self-evident. Creosote, which contains liquid and solid aromatic hydrocarbons with appreciable amounts of tar acids and tar bases, is widely used on farms to preserve fences and other woodwork. Careless application can contaminate herbage accessible to sheep, or spilt amounts can be licked up. Sawdust from creosote-impregnated wood is also toxic by skin absorption if used as bedding. Sheep have been poisoned when rain washed creosote from a newly dressed roof into their lying area. An important cause of poisoning has been the use of carbolic dips to improve the bloom on the fleece of show sheep, particularly Suffolks, which seemed to have a particular susceptibility. These dips were impossible to standardize owing to variation in the sources of coal for the initial low-temperature carbonization process [42], thus some batches of dip could be particularly toxic. Such dips had very narrow safety margins, thus inaccurate dilution based on lack of appreciation of the exact capacity of the dipper could cause problems. Farmers were inclined to err on the side of greater, rather than lesser concentrations, and some used predipping washes containing carbolic compounds exacerbating the effect of the dip.

Sudden death is often the first warning of an episode of phenol poisoning. Clinical signs in live animals include muscle twitching or convulsions, nervous system depression, diarrhoea and poor thermoregulatory ability [41]. The breath may have a strong carbolic odour. Experimentally, creosote poisoning (6 g/kg) caused dullness, lassitude, scouring and passage of dark urine with a tarry odour, followed by death in a few days [43]. Inappetence, loss of condition, reluctance to move and chronic diarrhoea were seen in chronic cases. Carbolic dip poisoning was characterized by acute pneumonia with severe dyspnoea and marked grunting respirations, usually 1–3 days after dipping. Affected sheep stood away from the rest of the flock, and usually died within 12 hours of the onset of clinical signs. One survey of dip poisoning found that the visible membranes were cyanotic, there was a brownish nasal discharge and the breath smelled strongly of phenol. The rectal temperature often rose initially, but soon became subnormal as prostration supervened. An initial strong rapid pulse soon became weak and almost imperceptible. Excessive thirst, clonic spasms and opisthotonos were seen occasionally [42].

Necropsy findings in most forms of phenol poisoning include rumenitis, abomasitis and patchy enteritis. An index sign is that the carcass smells strongly of phenol. Less commonly, there is liver enlargement, cystitis and petechiation of renal cortex or along the coronary grooves. Lungs may be congested. Histological examination does not provide additional information in these circumstances. In carbolic dip poisoning the main feature is pneumonia with severe consolidation of both lungs which usually are dark red or purple and have a rubbery consistency. Cervical, pharyngeal, mediastinal and prescapular lymph nodes are usually enlarged and oedematous. Many other tissues are petechiated. The microscopic changes are characteristic and do not resemble other sheep pneumonias. All vasculature, but particularly alveolar capillaries, are engorged and alveolar walls are swollen by fluid leakage and cell infiltrates. Alveolar spaces are clogged by serofibrinous exudates, neutrophils, macrophages and necrotic alveolar lining cells. The most striking feature is diffuse lining of alveolar spaces by sheets of cuboidal cells (type II pneumoncytes) [42].

Diagnosis of phenol poisoning can usually be established from the history. In suspected carbolic dip poisoning, lung specimens should be taken for histology and culture. Where phenolic compounds have been absorbed through the skin, only minute residues remain by the time the dead animal is examined, thus histological examination may be the only diagnostic recourse available. Failure to isolate common lung pathogens, particularly *Mannheimia haemolytica*, together with the characteristic histological features described above, are diagnostic of dip pneumonia. If ingestion of phenolic compounds is suspected, samples for laboratory analysis should include stomach contents, lung, liver, kidneys and, if possible, source material. Samples should be frozen at −20°C and transported to an analytical laboratory.

Treatment of phenol poisoning is symptomatic. Demulcents should be tried for ingested phenols, with anti-diarrhoeal medicines and parenteral administration of electrolytes, amino acids and dextrose as fluid support therapy in cases of prostration. Of possible

antibiotics to prevent secondary infection, only tylosin can be claimed to cause improvement. In dip poisoning, excess dip should be washed from the fleece with soap and warm water. Control measures are largely a matter of commonsense once the source of poisoning has been identified.

SELENIUM

Deficiency of this essential trace element is widespread in the UK as herbage contains less that 0.1 μg selenium (Se)/kg DM. However, the margin of safety between dietary requirements of around 1.27 μmol/kg (0.1 ppm DM) and the maximum tolerable level [25.3 μmol/kg (2 ppm DM)] is small, thus there is a significant possibility of errors in compounding diets or incorporating supplements. Accidental acute poisoning with deaths has occurred in lambs following administration of a trace element supplement containing Se and Co mixed with an anthelmintic. Inefficient mixing may have resulted in some individuals receiving too much Se. Slight distress was the only premonitory sign observed [44, 45].

Poisonings can also occur when Se treatments are administered to correct a deficiency that does not exist, when more than one additional treatment is given with a short intervening interval or when overdosing occurs. In one serious episode of Se poisoning [46] 128 of 142 sheep died after injection of a supplement containing sodium selenite, which contains 41 per cent available Se, instead of the recommended sodium selenate, which contains 21 per cent available Se. Additionally, in this episode, the sheep were dosed at the rate of 5 ml/cwt (51 kg) instead of the recommended 5 ml/100 kg, thus the sheep received twice the recommended dose. Some sheep died in under 5 hours, others were staggering but showed no respiratory or other distress. However, when these sheep became recumbent, their breathing slowed and they died in minutes, with no struggling.

Raised temperature, rapid weak pulse, dyspnoea, bloating, colic, polyuria, watery diarrhoea, cyanosis, prostration and death have been recorded as signs of poisoning. Experimentally, 5 mg/kg Se given as sodium selenite orally or intraperitoneally to sheep caused severe damage to the cardiovascular, respiratory and urinary systems, with damage to secondary lymphoid tissue in various organs [47].

Necropsy findings (10 min after death) have included extreme congestion of the intestines and presence of straw-coloured fluid in the pericardium [46]. In other reports [44, 45] subcutaneous haemorrhages, pulmonary oedema with hydrothorax and destruction of the renal cortices were noted. Some sheep also had abomasitis, hepatic congestion and haemorrhages around the brainstem. Histopathological examination revealed degeneration of the kidneys and, in some, tubular cast formation. Elevated values for liver, kidney and heart Se are the most useful test criteria; toxic values range up to 62.8 ppm DM. Values for normal sheep seldom exceed 1 ppm DM.

Chronic Se poisoning (alkali disease; blind staggers) occurs mainly in semi-arid areas, with rainfall less than 50 cm annually, on seleniferous soils usually derived from shales. Such are found in the midwest and west of the USA, Canada and Mexico. Seleniferous areas have been identified also in Colombia, Israel, Australia and Ireland. The presence of indicator plants such as milk vetch, *Astragalus bisulcatus* (Leguminosae), can alert flock-keepers to the potential danger of Se poisoning. In Ireland, where rainfall is relatively high, Se concentrations of up to 60 ppm have been found in low-lying poorly drained soils of high organic matter. Alkali disease occurs after consumption of moderately toxic amounts of seleniferous plants containing 10–40 ppm Se over long periods of time. The clinical signs in sheep are dullness, emaciation, anaemia, stiffness of the joints and brittleness of hoof horn. The gross pathology includes atrophy of the myocardium and toxic change in the liver. Se accumulates in hair, values greater than 4 ppm indicating a high probability of toxicity.

There is no specific treatment for Se toxicity. Any Se-containing mineral supplements should be removed and sheep should be given access to a high protein ration. While non-clinical and mild cases may respond, those with severe signs are unlikely to survive.

SUPERPHOSPHATES

Heavy top-dressing of pastures with basic slag can cause oesophagitis and gastroenteritis with diarrhoea in sheep. Younger animals are more susceptible. The probability of poisoning on these pastures is increased during periods of drought. The usual signs include

colic, diarrhoea, reluctance to move and weakness. Deaths are rare, but fertilizer can be found encrusting the rumen, and kidneys may be discoloured owing to tubular necrosis with hyaline cast formation. There is no specific treatment. Sheep should not be put onto heavily fertilized pasture, particularly if these are relatively bare, until the dressing has been washed into the soil.

UREA

Feed supplements containing urea as a source of nitrogen or accidental feed or water contamination with grassland fertilizer occasionally cause poisoning in sheep. Gradual introduction of urea supplements into the ration may induce limited tolerance. Fasting or low-quality feed increases susceptibility. Urea is broken down by bacterial action in the rumen with ammonia as a by-product. Affected animals salivate profusely altering ruminal pH and promoting absorption of ammonia. Clinical signs include bloating, weakness, staggering, muscle twitching, other nervous signs and convulsions before animals become comatose. Blood pH and urea nitrogen concentrations are elevated. Necropsy findings include mild toxic hepatitis and nephritis. Treatment with weak acid solutions, e.g. 5 per cent acetic acid (120 ml per head) or vinegar (120 ml per head) diluted in water and given orally, has been recommended. Repeated dosing at 2–3-hour intervals is likely to be necessary.

WARFARIN

Poisoning by warfarin or other coumarin-based rodenticides occasionally occurs in sheep if they accidentally eat oatmeal mixed with poison for killing rats around farm buildings. Initial signs include lameness and stiff gait due to bleeding into joints. The bleeding time is prolonged, and sheep may develop haematomas in areas that get jostled, e.g. at trough feeding. Dead sheep have haematomas into joints or over bony prominences and in internal organs. Laboratory diagnoses include identification of warfarin in gut contents or suspect feed and prolongation of coagulation time. In surviving animals warfarin may be detected in urine for a time after exposure. To reduce losses when sheep are suspected of having been exposed they should be removed to areas where forceful contacts are minimized. Phytomenadione (vitamin K_1), blood coagulants and iron preparations are useful adjuncts to recovery [48].

ZINC

Poisoning of sheep with zinc (Zn) has been reported following the increased popularity of foot-bathing with zinc sulfate for the control of foot-rot, usually made up as a 10 per cent solution. Zn salts also have been used to treat or prevent the severity of facial eczema in sheep, and the lesions of lupinosis [49].

In the USA, deaths from acute intoxication occurred in sheep required to pass through a 20 per cent solution to obtain drinking water. Experimentally it was shown that 227 ml of the solution given orally caused death within 4 hours. Clinically, chronic Zn poisoning results in diarrhoea, weight loss, anaemia and deaths. A necropsy feature reported from Australia in sheep experimentally poisoned with oral doses of 3 g zinc sulfate in 20 ml water was green coloration and necrosis of the abomasal and duodenal mucosae. Histologically, crystals of Zn were observed in these areas. Animals that did not die in the acute stages of toxicity did not thrive and consumed significantly less feed than control sheep. At necropsy, sheep acutely poisoned by Zn have abomasal ulceration and renal damage, while chronic poisoning causes severe fibrosing pancreatitis [49]. Laboratory confirmation includes analysis of tissues and body fluids. There is no specific recommended treatment. Remove the sheep from any potential source. If the intake is recent promote emptying of the gut and reduce irritation by giving demulcents, egg white or milk.

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APPENDIX

The UK Veterinary Poisons Information Service (VPIS)

Contact addresses and telephone numbers for information on poisonous plants and other poisons that are hazardous for animals.

VPIS (London)
Medical Toxicology Unit
Avonley Road
London SE14 5ER
Tel: 020 7635 9195
www.medtox.org

VPIS (Leeds)
The General Hospital
Great George Street
Leeds L51 3EX
Tel: 0113 245 0530
email
medicines.information@
Leedsth.nhs.uk

VPIS is a subscription-only service to UK veterinary practices.

Part XII
Tumours

58

Tumours

R.W. Else

In general terms, the incidence of tumours in mammals increases with age. However, in Europe, production veterinary species, unlike the companion animals such as the dog, cat and horse, have a shortened lifespan as a result of agricultural management. Sheep, particularly, normally have a short life since they are part of the economic meat production cycle. Relatively few sheep in the UK are kept for significant wool production and the average age of culling for ewes is 5–7 years. Consequently, the occurrence of neoplasias in this species appears to be low. However, the natural lifespan of the sheep is greater than 6 years and, where breeding animals are retained for longer, as in Australia, the occurrence of tumours is similar to that seen in other farmed animals kept for longer periods and in companion species.

Obtaining accurate figures on the prevalence and distribution of tumours in sheep is difficult due to differences in individual sheep breeds within and between countries, geographical location (northern versus southern hemispheres) and the management systems involved. Furthermore, published surveys often have had biases depending on whether they have been purely abattoir-based [1, 2] or academic-based retrospective studies [3, 4]. In the past, abattoir surveys in many countries were unable to yield accurate information on the age distribution of tumours. In the UK, subsequent to the bovine spongiform encephalopathy regulations and the 2001 foot-and-mouth disease outbreak, requirements to identify slaughter stock more accurately may mean that future surveys in abattoir populations will be more accurate and meaningful. Clearly, some types of ovine tumours occur more frequently than others, and studies on individual flocks may reveal localized clusters of neoplasia not revealed by abattoir studies.

The continuing escalation of scientific publications means that there are more reports of well-recognized tumours in sheep and also evidence of more unusual types of neoplasia. Theoretically, all cell types can give rise to tumours but some neoplasias are more common than others and it is possible that commonly occurring tumours could be related to management practices and, hence, avoidable. Many tumours are thought to arise as a result of prolonged exposure to often unidentified carcinogens. Because in many farming systems sheep are culled well before the natural lifespan has elapsed, it is probable that the full effects of such carcinogens are not manifested. Most tumours develop post-natally but some neoplasias occur in young lambs and are most likely the result of intra-uterine malformations occasioned by carcinogens (such as viruses) acting *in utero*.

TUMOURS IN LAMBS AND YOUNG ADULT SHEEP

Most tumours seen in lambs (under a year old) are single cases and often of blastoid cell type, suggesting that they may be the result of intra-uterine carcinogenic effects. Care must be taken to differentiate other congenital abnormalities such as polycystic formations of biliary origin in the liver or melanosis (cf. melanoma). Adrenal neural tumours (ganglioneuroblastoma) have been recorded [5] and congenital tumours of the heart of lambs such as rhabdomyoma and neurofibroma are not that rare [4]. Neurofibromas appearing as multiple small nodular growths on nerves or in connective tissue are regarded as of no clinical importance. Some blastoid cell brain tumours occur in young lambs but care should be taken to differentiate cystic formations associated with neoplasia from parasitic cysts (coenurosis). Nephroblastomas

are not as common as in pigs. They are usually unilateral and slow-growing but can become quite large before causing clinical signs. Unilateralism often results in these tumours being discovered only incidentally at slaughter. Lung tumours are not uncommon in young sheep over one year old but a primary bronchioalveolar carcinoma has been reported in a suckling lamb [6].

Experimentally, betaretroviruses (jaagsiekte retrovirus, JSRV; enzootic nasal tumour virus, ENTV-1) have induced tumours that mimic naturally occurring neoplasias [7] and there is evidence of synergy between these viruses in the genesis of ovine pulmonary adenocarcinoma (OPA) [8] (see Figure 58.1 in the colour plate section). Intranasal adenocarcinoma has been reported in clusters of related flocks in mainland Europe, USA, Nigeria, Senegal and Japan. Recently, infection with enzootic intranasal tumour virus was reported in healthy sheep [9]. Enzootic intranasal tumours of sheep and goats (EIT) and OPA can be transmitted to neonatal lambs and kids, the tumours appearing in 12–24 months with EIT and in a shorter time with OPA. More recent evidence indicates that endogenous betaretroviruses may be important in modulating the development of OPA [10]. It is clear that individual flocks, management factors and geographical location, are modulating influences on the overall occurrence of EIT and OPA. The presence in the local environment of natural mutagens, carcinogens and immunosuppressive agents, known to exist in aflatoxin and bracken fern, may influence the spread of an infectious viral agent. The time needed to promote tumour growth is probably important and in this respect the age at which the lambs are first exposed to the viruses and other carcinogenic agents is important. Interestingly, the first cloned sheep, Dolly, was found to have OPA lesions at necropsy examination [11]. A fuller consideration of the EIT and OPA diseases is to be found in Chapter 30.

One of the most common tumours of sheep is lymphosarcoma. Lymphoid neoplasia can be identified in neonatal lambs (2 weeks of age) and all older sheep of both sexes; there is no evidence of preferential sexual distribution. Neoplasia of the solid lymphoid organs (lymphosarcoma) can appear as one of several anatomical forms: generalized or multicentric, localized and, less commonly, alimentary. The multicentric form is the most common and occurs globally. In the UK, the disease is sporadic but in many other countries multiple cases occur in individual flocks, mostly in sheep over 2 years old. Affected sheep are often unthrifty with palpably bilaterally enlarged superficial lymph nodes. At necropsy, all lymph nodes, spleen and, sometimes, thymus are enlarged, and non-lymphoid organs such as liver, kidney, gut and heart are infiltrated, with nodular or diffuse deposits of neoplastic cells. The skin and subcutis may be involved (one-third of cases) as part of the generalized form [4]. Some sheep may exhibit lymphatic leukaemia with very high circulating total white cell counts (20 000–4 000 000 per ml) with up to 100 per cent lymphocytes present; mitotic figures are not uncommon in circulating lymphoid cells.

Localized lymphosarcoma involves the liver, kidney or spleen, or combinations of these organs in diffuse or nodular tumour infiltrations. Thymic lymphosarcoma with limited spread to adjacent local nodes is less common. A small proportion of localized lymphosarcomas, often affecting the liver, prove to be mast cell type [4]. The localized form of lymphosarcoma tends to occur in older sheep. The purely alimentary form also affects older sheep but is the least common manifestation of this type of neoplasia.

Most of the alimentary or localized forms of lymphoid tumours have been shown by immunohistochemical methods to be of B-cell origin, whilst the majority of T-cell-derived tumours are multicentric. A B-cell lymphoma in the brain of a 6-year-old Texel ram has been reported [12], but the information on cell types in lymphoid neoplasia in the sheep is scant, compared to cat and dog.

In the wake of the discovery of feline leukaemia virus and the knowledge that multiple cases of lymphosarcoma and lymphocytosis occur in single flocks in many parts of mainland Europe, searches for the presence of causal viral agents have been made. Claims have been made of viral-like particles in New Zealand sheep inoculated with cell-free extracts derived from ovine lymphosarcoma cells [13]. Furthermore, lymphocytosis and lymphosarcoma develop in sheep inoculated experimentally with bovine leukosis virus [14]. Approximately 40 per cent of sheep experimentally infected develop multicentric lymphosarcoma within 3 years [15], but natural infection of sheep with bovine leukosis virus (EBL) is considered uncommon. Sporadic lymphosarcoma and multiple incidence lymphocytosis occur in the

UK, but the relationship to EBL and possible transfer of infection from BLV-positive cattle are unknown.

Skin tumours may occur in sheep of all ages but tend to arise at a relatively early age. Melanomas in black pigmented skin sites occur in Suffolk sheep. These tumours are usually highly malignant and metastasize widely [4]. Skin papillomas induced by papovaviruses are rare in the UK compared with Asian countries but have been reported in yearling and older sheep on the limbs and muzzle [16]. True viral warts regress and slough off as immunity to the virus becomes established. Subcutaneous fibromas and non-metastasizing fibrosarcomas have been described in sheep from 6 weeks to 7 years of age [4].

TUMOURS IN ADULT AND AGED SHEEP

In general, apart from lymphosarcomas, OPA and the more sporadic types of neoplasia mentioned above, the occurrence of neoplasia in adult sheep is closely linked to age, and where management practices allow late culling it is more likely that neoplasms will be seen as clinically apparent problems or discovered incidentally at slaughter.

Liver tumours in British sheep are not uncommon. They are derived from either hepatic parenchymal cells (hepatocellular) or, less commonly, from the biliary epithelium (cholangiocellular). Melanosis of the liver, especially where there is also pulmonary melanosis, is not unusual, but true primary melanotic hepatic tumours are rare. Similarly, secondary (i.e. metastatic) liver tumours seem to be rare.

Hepatocellular tumours are nearly always benign and often detected only at necropsy, usually in sheep over 3 years of age. They are solitary masses, up to approximately 15 cm in diameter, usually in the dorsal lobe, with a well-developed compression capsule sharply defining the neoplasia from the normal liver (see Figure 58.2 in the colour plate section). The cut surface has a pale cream or fawn lobular or more homogeneous appearance; large tumours have foci of necrosis or haemorrhage or green discoloration due to presence of trapped bile pigment. Histologically, the cells resemble disordered hepatic cords, or are arranged as irregular acini or festoons; portal tracts are not a feature. Foci of extra-medullary erythropoiesis may be seen. Similar tumours in lambs and sheep under a year old may be hepatoblastomas [4].

Bile duct tumours are less common and are usually cholangiocarcinomas. Tumours are multiple, firm, white nodules, and subcapsular formations are umbilicated. On gross sectioning, the tumours may be firm (cf. hepatocellular tumours) and often finely cystic. Affected sheep may have ascites and are usually cachectic. There is no evidence of a causal relationship with fascioliasis but long-term/low concentration intake of mycotoxins has been mooted as a cause [17]. It is important not to confuse benign congenital polycystic disease in young lambs, or benign cystic adenomas of the liver of older sheep with cholangiocarcinomas.

Intestinal adenocarcinoma has been reported as fairly common in New Zealand, Australia, the UK, Iceland and Norway, and occasionally elsewhere. In the UK and Australia there is an association between adenocarcinomas of the small intestine and bracken grazing but no specific carcinogenic agent has been identified. Similar intestinal carcinomas are seen in sheep in New Zealand and Iceland where bracken is not in the diet; in these cases exposure to phenoxy and picolinic acid herbicides have been implicated [18]. An association between bracken and alimentary papillomatosis and carcinomatosis has been established in cattle [19] but no links have been demonstrated for sheep.

Diagnosis of intestinal adenocarcinoma is usually made when the disease is advanced and the affected sheep are cachectic with obvious ascites (up to 35 litres). The tumours usually affect the jejunum, rarely the duodenum or colon. They are usually solitary and small annular stenosing thickenings 1–3 cm long; some are polypoid, extending into the gut lumen (see Figure 58.3 in the colour plate section). Occasional multiple primary tumours may occur. The intestine cranial to the tumour is dilated and the serosal wall thickened by white fibrosis as a result of reaction to lymphatic permeation and plugging by carcinoma cells. Loops of fibrosed bowel are often adhesed. Transcoelomic tumour deposits on the serosa of the omentum, abdominal wall diaphragm and pleura mimic chronic peritonitis/pleuritis. Lymphogenous metastases to the mesenteric and

posterior mediastinal lymph nodes are common but involvement of liver and lungs is rare.

Rumen papillomas are not uncommon as incidental post-mortem findings in sheep over 3 years old [4]. The lesions are usually sessile or pedunculate fibropapillomas and can be solitary or multiple (up to 30), 2–20 mm in size, on the ruminal pillars. A few lesions are histologically squamous cell carcinomas. Although a papovavirus has been demonstrated in ovine ruminal papillomas [20] very few viral particles were present, unlike sheep skin papillomas. Cattle grazing bracken have a higher incidence of oesophageal papillomas and squamous cell carcinomas, and intestinal adenocarcinomas [19], but no such relationship has been demonstrated for sheep. It has been suggested that the ruminal and dermal papillomas in sheep might be caused by different ovine papovaviruses that could normally be present in a latent form [21]. Latent virus might be triggered by ultraviolet light or a dietary carcinogen such as bracken, and then promoted to full carcinogenicity by similar or other environmental factors or immunosuppression in susceptible sheep.

Additional circumstantial evidence for the possible carcinogenic effect of bracken ingestion in sheep has been provided by the occurrence of fibrosarcomas of the jaw in some flocks of sheep in north Yorkshire [22]. In these adult sheep, prolonged grazing of bracken was associated with oral and ruminal squamous cell carcinomas and intestinal carcinomas, as well as jaw sarcomas. Experimental feeding of bracken to sheep from this locality induced a single jaw tumour and bladder tumours [23]. However, spontaneous bladder tumours are not reported in sheep grazing bracken naturally in the UK and, furthermore, late culling (up to 8 years of age) was a feature of the flocks investigated so additional management and age-related factors may have been significant.

Other ovine tumours in the UK tend to be more sporadic. Chondrosarcomas are relatively common in sheep throughout the world. Cartilage tumours may occur in lambs but chondrosarcomas are usually seen in sheep over 3 years old. Many tumours arise in one or more ribs from just above the costochondral junction; they may be solitary, nodules on several ribs of the same side of the thorax, or a large mass covering up to four ribs. The scapulae and sternum are favoured sites, but long bones such as humerus (Figure 58.4), ulna, femur and tibia can be affected

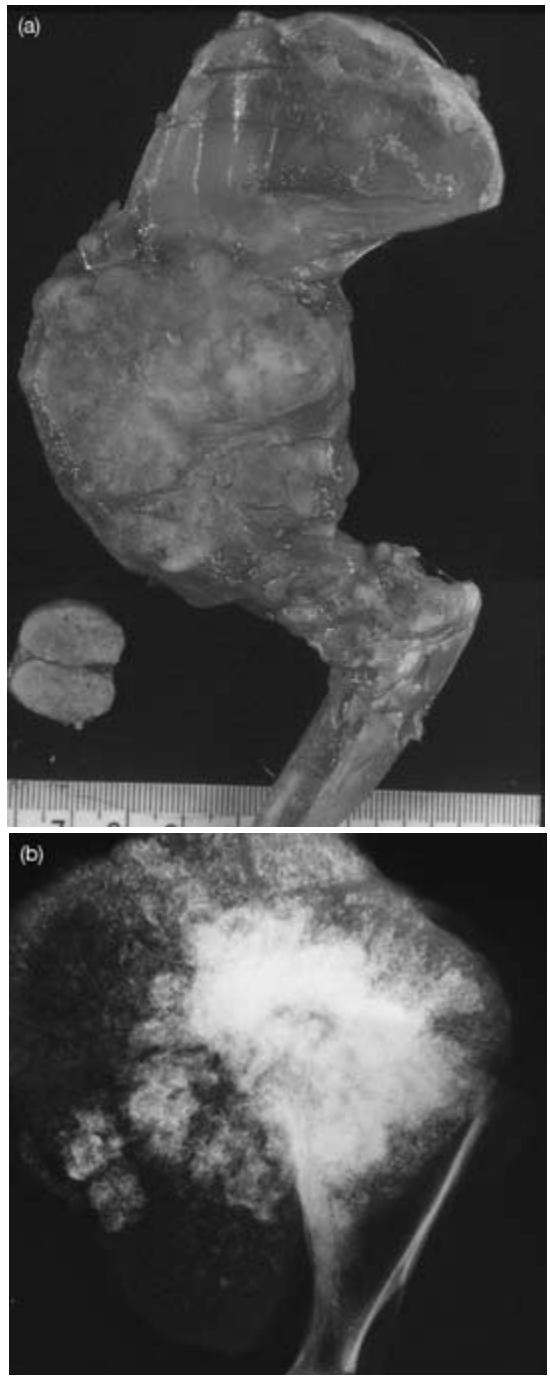

Figure 58.4: (a) Chondrosarcoma of the forelimb in a 5-year-old ewe. (b) Radiographic appearance of a chondrosarcoma of the scapula in a 6-year-old ram.

as well as vertebrae. Macroscopically, the tumours are a mixture of grey cartilaginous stroma with varying ossification giving some a gritty and granular texture. Metastasis seems to be confined to regional lymph nodes but lung involvement is also seen. True bone tumours appear to be rare and those recorded in the literature are skull osteomas [24].

Neoplasia of other body systems in British sheep are even more sporadic and are often incidental findings in abattoir or casualty slaughtered individuals rather than seen as clinical presentations. Tumours of the genital system are unusual despite the preponderance of female breeding stock but this is clearly related to management practices. Ovarian tumours, usually in elderly ewes, are mostly adenocarcinomas with transcoelomic metastasis, although a granulosa cell tumour has been reported in a 20-month-old ewe [25]. Adrenal cortical adenomas or, rarely, adenocarcinomas or medullary tumours, the latter invariably phaeochromocytomas, are not common [4] but may be seen as incidental findings in abattoir-slaughtered aged sheep. In UK sheep, unlike pigs, renal tumours seem to be rather uncommon; ovine nephritis and hydronephrosis are more common. Nephroblastomas occur in lambs and young sheep and renal tubular adenocarcinomas in aged ewes; both are usually abattoir findings.

Bladder tumours in sheep seem to be genuinely rare worldwide, which is interesting given the involvement of bracken in the diet of both cattle and sheep and in the greater incidence of bladder lesions in cattle.

Tumours of the central nervous system of sheep are rare; parasitic cysts (coenurosis) and septicaemic-origin abscesses are more frequent causes of lamb mortality. Gliomas can occur in lambs aged 1–9 months. Pituitary tumours, both functional adenoma [26] and carcinoma [27] have been reported in adult sheep.

Ovine squamous cell carcinomas (SCC) affecting integument and external mucous membranes are rare in northern Europe but common in arid subtropical parts of the world (Brazil, Egypt, Morocco, Saudi Arabia, South Africa, southern France, Spain, southern USA, Turkey), and are of economic importance in Australia. Tumours ranging from 1 to 4 cm or more in size may affect dorsal aspects of ears, eyelids, muzzle and mucocutaneous junctions of vulva and anus [16, 28]. The lesions are associated with sites susceptible to solar-induced keratosis and progress to less differentiated dermally invasive carcinomas. Metastasis to drainage lymph nodes and lungs occurs from the head lesions but spread from perineal lesions has not been reported. Most affected sheep are 3 years of age and older. The exact aetiology is multifactorial, but ultraviolet solar irradiation is a major factor and lesions preferentially occur on glabrous skin, eyelids and ears, and on scarred skin [29, 30]. Some Merino sheep are more susceptible since SCC in strains of this breed have been considered to be associated with epidermal cysts induced by penetrating grass awns [31]. Other work, however, has suggested that papovirus infection may be a predisposing factor to ovine SCC [21].

Whilst the exact aetiology of the major ovine neoplasias remains unidentified, there is strong circumstantial evidence that a combination of management and environmental factors, coupled in specific instances with infectious viral involvement, superimposed on genetic and age-related factors, is the underlying cause of spontaneous, naturally occurring ovine neoplasia.

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Part XIII
Other important diseases

59

Sarcocystiosis

A. Uggla and D. Buxton

Sarcocystis species are cyst-forming coccidia that are probably the most common and geographically widespread of the protozan parasites of sheep and other ruminants. Normally, they do not cause clinical signs but in some circumstances they are pathogenic and cause severe disease and even mortality in sheep. However, the effect of subclinical infection on the productivity and welfare of sheep has received very little attention. The term sarcocystiosis is used generally to describe the proliferative phase of a *Sarcocystis* infection in an intermediate host, while the chronic phase of infection, characterized by the persistence of sarcocysts in nervous or muscular tissues, traditionally has been called sarcosporidiosis.

CAUSE

The *Sarcocystis* species have obligatory two-host life cycles in which carnivores are the definitive hosts and herbivores or omnivores the intermediate hosts. The species can be distinguished by the size of the mature sarcocysts, the ultrastructure of their cyst walls and by their host range. The sheep is the intermediate host of five species of *Sarcocystis* [1, 2]. *S. tenella* (syn. *S. ovicanis*) and *S. arieticanis* are 'microcyst species' that form microscopic cysts (less than 1 mm in length) in ovine muscle and have canids (dogs and foxes) as definitive hosts. A further dog-borne species is *S. mihoensis* which forms sarcocysts that may be up to 2 mm in length. *S. gigantea* and *S. medusiformis* are 'macrocyst species' that form macroscopically visible cysts (up to 10 mm in length) and have felids as definitive hosts.

Mature cysts of *S. arieticanis* occur predominantly in skeletal muscles, tongue and oesophagus, and have a thin, 0.5–1.0 μm, non-striated cyst wall with delicate, 5–10 μm, hair-like protrusions. *S. tenella* occurs in similar tissues as well as in cardiac muscle and the central nervous system (CNS), and has a 1.5–2.5 μm thick radially striated cyst wall. *S. mihoensis* occurs in skeletal muscles, especially the diaphragm, and has a thick, 10–12 μm cyst wall with finger-like protrusions.

The macrocyst species *S. gigantea* (syn. *S. ovifelis*) forms 'thick' cysts (length no more than five times width) mainly in the tongue and oesophagus (see example in Figure 71.13 in the colour plate section), and *S. medusiformis* forms 'slender' cysts (length at least ten times width) mainly in striated, abdominal muscles. In addition to these five species there have been occasional reports of ovine protozoal myeloencephalitis caused by as yet unidentified *Sarcocystis*-like organisms [3, 4]. Some of these parasites may represent non-ovine *Sarcocystis* species for which the sheep is an aberrant host.

Life cycle

All species of *Sarcocystis* which infect sheep have principally similar life cycles [1]. The definitive canine and feline hosts become infected by ingesting ovine tissues containing mature cysts of the appropriate species. In the stomach the tissue is digested and the cyst wall dissolved to release thousands of individual parasites (bradyzoites). The bradyzoites infect the small intestine where they undergo a typical coccidian cycle of development culminating in the production of sexual stages in the lamina propria and the formation of coccidian oocysts. After a prepatent period of 10–14 days sporulated isosporan oocysts (each containing two sporocysts) or, more commonly, free sporocysts (each containing four sporozoites) can be found in the faeces. Oocyst or sporocyst shedding may continue for several weeks and since the definitive hosts do not become immune to reinfection,

episodes of oocyst or sporocyst shedding can recur. The sporocysts are very resistant and may survive in the environment for many months. Sporocysts of *S. gigantea* and *S. medusiformis* measure approximately $8 \times 12\,\mu m$, and those of *S. tenella*, *S. arieticanis* and *S. mihoensis* approximately $9 \times 15\,\mu m$.

Sheep become infected by ingesting sporocysts contaminating their feed or water. The sporocysts excyst in the gut and the released sporozoites penetrate the gut wall and establish infection in the endothelial cells of small arteries and capillaries. In these sites the parasites transform to merozoites and undergo two generations of asexual reproduction (merogony), during which the individual parasite enters an endothelial cell, divides repeatedly to form a meront (see Figure 59.1 in the colour plate section) that then lyses releasing a large number of organisms (merozoites) which then repeat the process. After the second merogonous generation the merozoites invade striated muscle cells (as well as cells of the nervous system in the case of *S. tenella)* and initiate the formation of sarcocysts where they multiply so that the sarcocyst gradually matures and increases in size to contain eventually, many thousands of slender, crescentic bradyzoites. Only mature sarcocysts are infective for the final host. Maturation of microcyst species takes 2–3 months whereas macrocysts require at least 6 months.

CLINICAL SIGNS

While clinical sarcocystiosis is rarely observed, *S. tenella* is potentially the most likely to cause disease with the macrocyst species being essentially non-pathogenic. Since relatively few clinical reports of outbreaks of ovine sarcocystiosis are available, most descriptions are derived from experimental infections with *S. tenella.* In these, the severity of clinical signs is largely dependent on the size of the infective dose administered. The most consistent signs described are fever, inappetence, and a normochromic and normocytic anaemia coinciding with the second merogonous cycle of the parasite's development, 3–5 weeks after infection [5, 6]. Fever has also been observed 10–15 days after infection during the first merogonous cycle. Massive infective doses (in the order of a million sporocysts) can cause an acute, fatal disease during the second merogonous cycle and pregnant ewes may abort [7]. In these circumstances, death is preceded by a brief period of inappetence, weakness, nervous disorders, recumbency and coma.

Low infective doses tend to induce subclinical infections characterized by anaemia, reduced levels of serum proteins and reduced weight gains in lambs [5]. These events may be widespread in the sheep population but naturally acquired clinical sarcocystiosis is rarely diagnosed. In the comparatively few clinical cases that have been described, findings such as anorexia, weight loss, generalized weakness, trembling, incoordination and other CNS signs have been observed [8]. Cases of idiopathic ovine protozoan myeloencephalitis usually present with predominantly neurological symptoms, particularly paresis or paralysis of the hind limbs [3, 4].

PATHOLOGY

Most knowledge of the pathology of severe ovine sarcocystiosis is gained from experimental infections. In the acute disease there is a haemorrhagic diathesis with petechial and ecchymotic haemorrhages in cardiac and skeletal muscles, as well as in other organs and tissues [6]. Pronounced oedema may be present in muscles and other tissues, and lymph nodes may be enlarged and oedematous. However, gross lesions are not always present.

Histopathological changes include haemorrhages and focal inflammation, mainly as perivascular infiltrations of mononuclear cells, in tissues throughout the body. Inflammation can sometimes be seen surrounding a focus of necrosis or a sarcocystis meront in an endothelial cell of a small artery or capillary. Meronts and inflammation are most commonly found in the brain, kidney, myocardium, tongue and skeletal muscles. The non-suppurative encephalitis is typically composed of foci of necrosis associated with surrounding microglial inflammation, mononuclear perivascular inflammation and protozoal meronts in vascular endothelium. In myositis and myocarditis there may also be multifocal myodegeneration associated with mononuclear cell infiltrations [6, 9].

Fetuses and placentas aborted by ewes with acute sarcocystiosis show a non-specific post-mortem picture and organisms are rarely found in the fetal or placental tissues [7].

Mature sarcocysts typically do not provoke an inflammatory response in the surrounding tissues. However, following degeneration of a mature cyst, focal infiltrations of mononuclear cells, eosinophils and neutrophils infiltrate a zone including the degenerating cyst. The so-called eosinophilic myositis characterized by multifocal eosinophilic granulomas in skeletal and cardiac muscles is considered to be triggered by significant degeneration of sarcocysts [10].

DIAGNOSIS

Only the *Sarcocystis* species transmitted by dogs are likely to be involved in clinical sarcocystiosis in sheep. Because of the non-specific clinical signs, a diagnosis of sarcocystiosis must be supported by laboratory investigations including clinical pathology, serology, histopathology, immunohistochemistry and/or molecular techniques.

Levels of antibody to *Sarcocystis* can be assayed using the indirect fluorescent antibody test (IFAT) or the enzyme-linked immunosorbent assay (ELISA), but the tests are not specific for one species of *Sarcocystis* [11]. Antibody levels rise during the second merogonous cycle and early sarcocyst formation (the time at which clinical signs are most likely to occur), and may be elevated during periods of excessive cyst degeneration [12]. Owing to difficulties in the interpretation of serological test results, particularly if paired samples are not available, *Sarcocystis* serology is not commonly applied and the tests are not generally accessible at veterinary diagnostic laboratories.

Reduced red cell counts, packed cell volumes and haemoglobin values are a consistent feature in experimental sarcocystiosis, as are reduced serum protein concentrations and elevated levels of enzymes reflecting muscle damage such as creatine kinase and aspartate aminotransferase [5, 13].

Post-mortem material should be examined histologically and immunohistochemically for the presence of organisms, either the merogonous stages or degenerating cysts. It must also be noted that cyst-forming coccidia, other than *Sarcocystis* species, may be present in the tissues examined. The identity of a given parasite should be confirmed using immunohistochemistry [11] or specific molecular techniques [14].

EPIDEMIOLOGY AND TRANSMISSION

The five ovine *Sarcocystis* species vary in their prevalence rates and distribution. S. *tenella* is extremely common and has prevalence rates reaching 80–100 per cent in sheep in many parts of the world, while *S. arieticanis* is less prevalent. *S. gigantea* is occasionally found in older sheep, while *S. medusiformis* has been recorded only in New Zealand and Australia. *S. mihoensis* has been described only from Japan. Mixed infections with any of the prevailing species are common.

Compared to the high prevalence rates of *Sarcocystis* infections in sheep, morbidity and mortality rates appear to be extremely low. Partly, this may be due to difficulties in diagnosis. However, because of the apparently low weight of infection in normal sheep breeding environments, non-overt infections and a gradual build-up of immunity appears to be the usual situation.

Anecdotal accounts suggest that clinical sarcocystiosis may occur when sheep are reared from birth in extensive hill conditions (relatively free from sarcocystis infection) and then moved to more intensive grazing areas nearer to the farm steading (where infected dog and fox faeces may commonly contaminate pastures), with the result that the naïve lambs may meet a considerable infective challenge and so develop clinical disease. The higher prevalence of the microcyst species compared to the macrocyst species may be because dogs and foxes are more commonly associated with sheep and are more likely to eat ovine tissue, either by scavenging or by predation, than cats.

TREATMENT AND CONTROL

There are insufficient data on the prevalence of clinical ovine sarcocystiosis, or on the impact of subclinical sarcocystiosis in sheep, to draw firm conclusions on the need for treatment and control. Certain anticoccidial drugs are effective against *Sarcocystis* infections, but while chemotherapy may be an option available to the practitioner faced with a verified outbreak of clinical sarcocystiosis, it would be difficult to justify the use of chemoprophylaxis against ovine *Sarcocystis* infections. Similarly, there seems to be no great impetus for the development of a *Sarcocystis* vaccine for use in sheep [11].

Preventing the spread of infection to sheep is probably impossible, but since clinical sarcocystiosis is likely to result from a heavy initial infection, any action that might reduce the weight of sporocyst contamination in the environment is probably beneficial. Dogs and cats should not be fed raw sheep meat or offal, sheep carcasses should be disposed of promptly to prevent scavenging by dogs, foxes or cats, and contamination of sheep feed or water supplies with dog or cat faeces should be avoided. These measures are thus in common with recommendations to control toxoplasmosis, taeniosis (cysticercosis) and echinococcosis in sheep.

ZOONOTIC IMPLICATIONS

None of the sheep-associated *Sarcocystis* species has any known zoonotic implications.

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60

Bluetongue

B.I. Osburn

Synonyms: sore mouth, ovine catarrhal fever, sore muzzle

Bluetongue (BT) is an arthropod-borne viral disease of ruminants, characterized by congestion of the buccal and nasal mucosae and the coronary bands. Although all ruminants can be affected, sheep appear to be the most susceptible of the domestic species. Even in domestic sheep, the clinical picture varies from inapparent or mild to severe, depending on the virus strain and the breed of sheep. Economic losses from bluetongue result from mortality and loss of meat and wool production. Surviving animals lose condition, and wool breaks may occur. Loss may result from the ban on export of live animals and germ plasm. BT infections have been reported in free-ranging carnivores and in pregnant bitches infected with bluetongue-contaminated modified live canine virus vaccines [1].

CAUSE

BT is caused by viruses within the *Orbivirus* genus in the family Reoviridae, of which bluetongue (BTV) is the prototype RNA virus for the genus. Globally, there are 24 distinct BTV serotypes based on virus neutralization tests. Other orbiviruses closely related to BTV include the epizootic haemorrhagic disease virus (EHDV) of deer, Ibaraki, Chusan, Eubenangee, Pata and Tilligerry viruses. EHDV, Ibaraki and Chusan viruses have been associated with a BT-like disease in cattle. EHDV is more often associated with a haemorrhagic disease syndrome in deer, antelope and other wild ruminants. The orbiviruses are related through group antigen, morphological structure and the ten RNA segmented genomes. The group antigen has been demonstrated by complement fixation, agar gel immunodiffusion and fluorescent antibody tests. Specificity can be demonstrated with neutralization tests and cross-protection studies.

BTV is 69 nm in diameter and has an icosahedral structure. The genome consists of ten double-stranded RNA segments that can be separated on polyacrylamide and agarose gels. Seven of the genes code for viral structural proteins and three gene segments codes for non-structural proteins. The genome is surrounded by 32 capsomeres in the nucleocapsid. A diffuse outer layer of proteins consisting of viral proteins (VP) 2 and 5 make up the outer coat of the virus. Viral attachment and neutralization is associated with VP2 in the mammalian host. VP3 and VP7 are the major internal core proteins. VP7 is important for viral attachment in the insect vector and therefore plays an important role in the ecological distribution of the viruses. This viral protein is often used as the group antigen for serological testing of BT viruses [2].

BTV is readily cultured in 10 and 11 day embryonated chicken eggs. The best and most widely used means of inoculation is by the intravenous route in chorioallantoic vessels. BTV usually kills the embryo 3–5 days following inoculation. Other methods that have been used include inoculation of susceptible sheep, intracranial inoculation of the newborn mouse, or use of either mammalian or insect cell cultures such as BHK 21, Vero, L cells or the C6-36 insect cell line. Confirmation of isolation is made by fluorescent antibody specific to BTV antigens or polymerase chain reaction (PCR) with identical oligonucleotide sequences.

CLINICAL SIGNS

BTV replicates in macrophages and lymphocytes, particularly those activated for cell division. The

virus has also been shown to replicate in endothelial cells, and this appears to be the basis for pathology and clinical signs. Clinical signs in sheep are associated with damage to the endothelial cells, either by direct cytolytic damage or possibly by the release of local vasoactive mediators [3]. The resulting lesions are caused by local thrombi with infarction or by leakage of fluid from vessels causing oedema [4]. Creatinine phosphokinase (CPK) values are elevated in affected sheep, indicating striated muscle necrosis. The incubation period in sheep is 4–12 days.

BT is characterized by fever reaching 42°C, which may last for several days. Animals are usually stiff, lame and reluctant to move. They often stand with an arched back, the head and ears are lowered, and the ears droop because of oedema. Increased respiration may be observed. The lips may be oedematous and hyperaemic and the buccal mucosae reddened. Erosions that may progress to ulcers may be found on the labia, and buccal haemorrhages are evident in the mucosae. On occasion, the tongue may be cyanotic, hence the name bluetongue applied to this disease. There is often excess salivation and even foaming or frothing from the mouth. Serous-to-mucopurulent exudation from the nostrils is often observed. Close examination of the coronary band often reveals a reddening or hyperaemia. Following the elevated temperature and oedema, there may be 'wool breaks', indicating temporary damage to the follicles. Those animals that develop the disease often lose condition, and 30 per cent may die. Clinical signs of disease occur in only a relatively small percentage of the viraemic sheep. Many sheep may be infected, but the appearance of clinical disease depends on the strain of virus and the breed of sheep. It is rare to find cattle or goats with clinical disease. Cattle seem to be commonly infected in certain areas of the world, but clinical expression of disease is seldom manifested. When expression of disease occurs in cattle, it is the result of an IgE immediate hypersensitivity [5].

Reproductive problems have been associated with BTV infection in sheep and cattle. The first report of the association of BTV with reproductive losses occurred following vaccination of pregnant ewes with a modified live BTV vaccine in the USA. The manifestations included birth of 'dummy' lambs, which were unable to stand, and many were blind. The lesions resulted from destruction of developing neurological tissue by the BTV leading to the fluid-filled calvarium, hydranencephaly or porencephaly. Subsequent reports have indicated that early embryonic deaths and abortions may occur also. Recent evidence suggests that modification of the virus for live virus vaccines selects for the teratogenic properties of the virus. In rare cases, BTV has been associated with hydranencephaly in calves.

Some clinical features of BT are illustrated in Chapter 64.

PATHOLOGY

The pathological lesions of BTV infection in adult sheep are associated with primary vascular damage. Haemorrhage, vascular leakage and thrombosis are the hallmark lesions leading to necrosis of cardiac as well as skeletal muscles and pulmonary and subcutaneous oedema. At necropsy, the erosions of the oral mucosa, oedema of the head and focal-to-scattered haemorrhages in the subcutis or muscles are often observed. Intrafascicular, yellow, gelatinous oedema is bound between muscle groups, and dry-to-pale skeletal and cardiac muscle is often apparent. A regular and pathognomic lesion is haemorrhage of the pulmonary artery (see the illustration in Chapter 64). Histological changes vary from local oedema to vascular thrombosis with a focal vasculitis and adjacent necrosis of striated muscle. Cardiac muscle may, on occasion, show evidence of mineralization. Pulmonary oedema is often present and, in some instances, secondary bronchopneumonia may be present. The epithelial mucosa of the oral cavity is often eroded and a mild vasculitis is associated with the lesion. The oesophagus and rumen papillae often show erosions or petechial or ecchymotic haemorrhages.

DIAGNOSIS

Diagnosis is based on clinical signs, pathological lesions, serology and, finally, by confirmatory virus isolation or identification. For those in BTV-endemic areas, the disease is relatively easy to diagnose clinically. The major diagnostic factors include the season, clinical signs and pathological lesions. Other diseases to consider include foot-and-mouth disease, orf, photosensitization, foot-rot, peste des petitis ruminants and pneumonia.

Clinical diagnosis in cattle is rare and, when it occurs, difficult because of similarities to foot-and-mouth disease, bovine virus diarrhea, epizootic haemorrhagic disease, vesicular stomatitis, mycotic stomatitis or rinderpest. Because of the similarities to these other diseases, virus isolation and identification of the particular serotype of virus causing infections is of utmost importance.

Serological tests include the enzyme linked immunosorbent assay (ELISA) with monoclonal antibody specific for VP7 antigen. These tests are available commercially and are recognized officially for regulatory purposes. The ELISA tests are indicators of exposure to BT viruses and, as such, are not the single diagnostic test of importance for field diagnosis. They serve as a complementary test to clinical signs and pathology.

Virus isolation is the most important test to confirm diagnosis. The best time to collect samples is during the height of viraemia, which occurs during the pyrexia. The best sample to obtain from the live animal is blood collected in heparin or ethylene-diamine tetra-acetic acid EDTA and then refrigerated at 4°C. Freezing the blood will kill the virus. Virus isolation is carried out by washing the red blood cells in sterile saline and then sonicating or treating them with hypotonic solution to rupture the cells.

Samples are inoculated into the chorioallantoic vessels of 11-day embryonated chicken eggs. Those embryos dying 3–6 days later are harvested and the samples placed on BHK21 cells for final identification by fluorescent antibody with BTV-specific antibodies. PCR technology also has been used to identify BTV-specific oligonucleotide sequences in the blood samples or cell culture [6, 7] and has proved to be a very accurate, specific and time-saving procedure for diagnosing this important disease [8]. PCR is a prescribed test for international trade and is of particular value in screening antibody-positive animals for BTV nucleic acid [9].

A confirmatory diagnosis can be made within 24 hours rather than 10 days, as with egg inoculations. Other tissues that may be used for virus isolations include the spleen and lymph nodes. Both viral isolates and PCR technology can be used to serotype the viruses. Serotype-specific antiserum is used on viral isolates and oligonucleotide probes to characterize gene segment 2. This information is important for the molecular epidemiology of the viruses and for the use of appropriate vaccines.

EPIDEMIOLOGY AND TRANSMISSION

Geographic distribution of the disease

The geographic distribution of the disease and infections depends solely on the distribution of the *Culicoides* (midge) vectors. The distribution of the vectors is dictated by ecological and climatic conditions. The viruses, evolved in the tropical and subtropical regions of the world, are particularly well adapted to various species and subspecies of the *Culicoides* genus. Most ruminants and many free-ranging carnivores are exposed to the viruses through the feeding of the *Culicoides* vectors [10]. Clinical disease is rare in free-ranging wildlife, as well as the indigenous domestic stock in the tropical and subtropical areas. BT was first recognized in South Africa and has now been seen on all continents where livestock are raised.

BTV has been sporadic in Europe until recently. The recent incursion of BTV into Mediterranean and eastern Europe appears to be endemic as several viral serotypes (BTV serotypes 1, 2, 4, 9 and 16) have been isolated [11]. The spread of BTV outside the tropical and subtropical areas appears to be associated with climatic changes (global warming) that permit extended distribution and establishment of the BTV-carrying vectors [12].

Climatic changes which do not favour the vectors lead to the elimination of the spread of infection from one mammalian host to the other.

Transmission and maintenance

Transmission of BT viruses is almost entirely by *Culicoides* vectors, although the virus can be spread by needles during vaccination of multiple animals. It has also been spread by a contaminated modified live virus vaccine for dogs [1]. Congenital and transplacental spread of infection may occur, leading to infected fetuses. It is unusual for this to be a significant means of spread or perpetuation of the virus in nature. Although *Culicoides* are the most important biological vectors of BT viruses, only a small percentage of the *Culicoides* spp. are involved in the spread of virus in nature. This is undoubtedly related to the ability of the products of gene segment 7 to permit infection of the *Culicoides*.

The major vectors include *C. imicola* in Africa and the Middle East; *C. variipennis*, *C. insignis* in south-eastern USA and subspecies in North America; *C. brevitaris*, *C. fulvus*, *C. actoni* and *C. wadai* in Australia; and *C. imicola* and, possibly, *C. obsoletus* and *C. pulicaris* in southern Europe [11, 13–15]. Different subspecies are being identified in Asia. Mechanical transmission occurs with blood-contaminated needles, *Melophagus ovinus*, *Aedes lineatopennis*, *Tabanus* spp. and *Stomoxyx* spp. The most important factors for vector transmission include temperature, moisture and wind. Relatively high ambient temperatures are favourable for *Culicoides*, and most favour manure-contaminated standing water. Some subspecies breed in animal dung. Cyclonic winds have been shown to spread *Culicoides* over large stretches of water to new locations [16]. The *Culicoides* survive only in climates that are temperate to subtropical or tropical, although larvae may overwinter in colder climates in manure-contaminated water.

Note added in proof: The first outbreaks of bluetongue in northern Europe were reported in the Netherlands and bordering EU states in summer 2006. BTV serotype 8 was isolated and a European midge, *C. dewulfi*, identified as the vector (*Veterinary Record*, 2006, **159**, 294 and 575).

The most important mammalian reservoir of BTV appears to be cattle. Because of the prolonged viraemia (up to 50–60 days) cattle are a very important source to other susceptible animals within 5–7 days after feeding on viraemic hosts. Sheep appear to be viraemic for 3–20 days.

TREATMENT, PREVENTION AND CONTROL

BT is not easy to control because of the ecology of the virus. The vectors and the variety of mammals that can be infected, all serving as a source of infectious virus, make control very difficult. In domestic animals, particularly sheep, control is best brought about through a combination of vaccination, vector control and limits on animal movement.

Probably the best means of control is to limit animal movement during the active vector season, thereby reducing the possibility of introducing new strains of virus into an areas where there are susceptible vectors. Another approach is to reduce vectors in the area by varying the water levels every 3–5 days where the *Culicoides* breed, to prevent the larvae maturing into adults. Use of pesticides, either on ear-tags or as pour-on insecticides, will reduce the number of vectors on animals. Larvicides may be applied to water to kill the immature *Culicoides*. Moving animals indoors during the active feeding times, either early morning or late afternoon will also reduce the chance of exposure to the *Culicoides*, which do not readily fly under roofs.

Currently, most vaccines are modified live virus vaccines, which produce good immunity to specific serotypes. On the other hand, there are problems associated with the use of these products. They will produce viraemia, which the *Culicoides* may pick up [17]. With this in mind, vaccination should be done during the non-vector season. Most BT vaccines are serotype-specific, so it is important that they contain the virus serotype causing infection in the area. Another potential problem, particularly if more than one serotype of vaccine is included at the time of vaccination, is that the viruses may reassort in the animal, yielding new strains of viruses, some of which may be more virulent and cause disease. A similar situation may occur if the animals that receive the vaccine are viraemic at the time of vaccination, when new strains of the virus may arise through reassortment of the vaccine with wild viruses [18].

New concepts and recombinant viruses are being developed. To date none is in use in the field. Some of these experimental vaccines show promise because they will be safer and, hopefully, more efficacious.

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61

Rinderpest and peste des petits ruminants

W.P. Taylor and T. Barrett

In the past, Rinderpest virus (RPV) may have affected sheep, but in modern times it has been in cattle and buffalo that its havoc has been wrought. Conversely, the niche occupied by its close relative, peste des petits ruminants virus (PPRV), is that of an agent causing severe disease in sheep and goats but showing almost no pathogenicity for cattle and buffaloes. Although both viruses are discussed as if extant, through the Food and Agriculture Organization's coordinated Global Rinderpest Eradication Programme (GREP) rinderpest (RP) is on the verge of extinction and of the two viruses, the only certainty is that PPRV remains.

CAUSE

Both viruses are negative sense, single-stranded RNA viruses classified in the *Morbillivirus* genus of the Paramyxoviridae, other members of the genus being measles and canine distemper viruses. Lipid envelopes surround the virus genome which is encapsidated with protein to form the ribonucleoprotein (RNP) core. After fusion with the cell membrane the nucleocapsid is released from the envelope and virus-encoded RNA polymerase initiates transcription of the positive sense mRNAs. Promoters at the 3′ ends of both the genome (GP) and antigenome (AGP) sense RNA direct transcription and replication of the RNA. All morbilliviruses share the same genome organization, although their RNA lengths differ slightly, each being just under 16 kb in length.

Morbilliviruses are not robust. Outside the host they are readily destroyed at 50–60 per cent relative humidity, and are sensitive to lipid solvents, heat, light and ultrasonic waves.

Both wild type and vaccine strains of RPV and PPRV have been completely sequenced, as have those of measles and canine distemper viruses. There are six contiguous, non-overlapping, transcriptional units which encode mRNAs for the six structural proteins, namely the nucleocapsid (N), the phospho (P), the matrix (M), the fusion (F), the haemagglutinin (H) and the large (L) proteins, the latter being the viral RNA polymerase. In addition, all morbilliviruses encode two non-structural proteins, V and C, using alternative expression strategies from the P gene transcription unit. The non-structural proteins are required for efficient replication and probably play a role in overcoming the host's innate response to infection determined by interferon production.

At some point after infection the viral polymerase stops transcribing mRNAs and generates a full-length positive sense antigenome RNA which, like the negative sense genome RNA, is always encapsidated by the N protein since both the GP and AGP sequences contain encapsidation signals. As a consequence, the synthesis of full-length antigenome RNA and N protein production must be linked. Although the precise trigger for the switch from the transcription to replication is not fully understood it is becoming clear that different accessory proteins of viral, and possible cellular, origin are associated with the polymerase that determine which function it performs.

Gene sequencing divides RPV into three phylogenetic lineages and PPRV into four. RPV lineages 1 and 2 have only ever been found in Africa while lineage 3 has only ever been found in Asia. With PPRV, lineages 1–3 are essentially African (except for a small domain in Oman where lineage 3 has been detected) but lineage 4 is distributed across a tract stretching from the Mediterranean to Bangladesh (Figure 61.1).

The historical introduction of RPV into Africa from Asia and the commonality of their Asian–African distribution can be used to suggest that, not

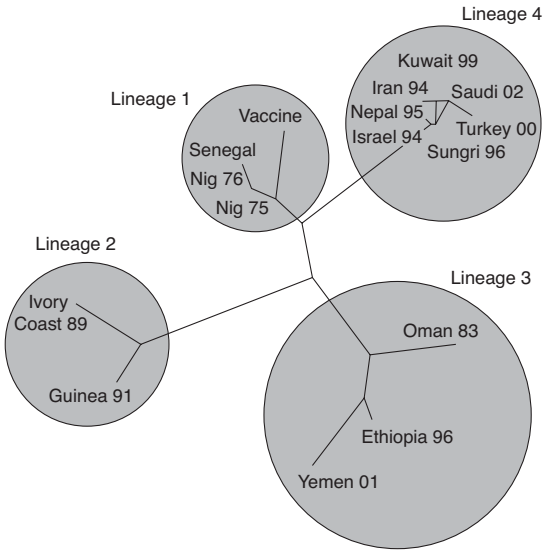

Figure 61.1 The four phylogenetic lineages of peste des petits ruminants virus.

withstanding its discovery in west Africa in 1940 [1], PPRV probably also came to Africa from Asia.

It is extremely doubtful if RP will ever resurface as a disease of sheep or goats. However, PPRV is far from extinct and its continuing spread represents an emerging animal health problem. Perversely then, as one morbillivirus of farm animals ceases to be important, another moves centre stage. Deaths among small ruminants due to PPRV continue to mount in the form of rolling epidemics moving across international borders to the extent that PPR is now regarded as the most constraining disease of small ruminant production across sub-Saharan Africa and the Indian subcontinent.

RINDERPEST

Epidemiology

Along with cattle, domestic buffaloes, yaks and numerous wildlife species, sheep and goats are susceptible to RPV. Several nineteenth-century authors described ovine and caprine RP disease in Europe and Russia [2], but later accounts suggest that recent African and Indian strains were less assertive among small ruminants.

RP invaded Africa only at the end of the nineteenth century disastrously affecting cattle in the following century, but there is scant evidence that it ever seriously affected small ruminants. In Uganda, RP was diagnosed in goats that had been in contact with clinically ill cattle [3] while in Kenya, RPV-neutralizing antibodies were detected in small ruminants that had been in contact with RP-affected cattle [4]. The minimal clinical effect (transient pyrexia) of experimental infection of sheep and goats with RPV was demonstrated in several studies in Africa [5–7] that also noted the low and irregular rate at which RPV is transmitted between infected cattle and small ruminants. It seems reasonable to conclude that in field situations African lineages 1 and 2 of RPV transmit only occasionally to small ruminants which in effect act as dead-end hosts.

With the benefit of hindsight there is every reason to think that the Asiatic lineage of RPV behaved in a similar way. However, in the late 1930s and again in 1950, epidemics of severe RP-like disease with high mortalities occurred in sheep and goats in India without concomitant disease in cattle. Originally reported as RP these outbreaks were probably the first recorded cases of PPR in India, although it was a further 50 years before laboratory confirmation was possible. Over that period several epidemics of PPR – always assumed to be RP – erupted in Indian small ruminants with a periodicity of about 10 years. Between times, the disease died down either through depletion of available hosts or through evolution of less virulent strains of virus.

While field infection with Asiatic RPV can be pathogenic for sheep [8], experimental passage of field virus in that species elicits more muted signs. This may explain why, in a major outbreak of RP in cattle and yak in Pakistan, only two mild cases were noted in small ruminants [9]. However, field and experimental evidence indicates that subclinical RP in small ruminants can transmit virus to large ruminants and also allow long distance spread of the virus during transport of apparently healthy sheep and goats [10, 11].

In the field then (and in contrast to the situation in Africa), Asiatic lineage RPV strains appear to be able to cause some degree of overt clinical disease amongst small ruminants and, while essentially showing only a limited capacity to spread, retain the ability for

occasional re-transmission to large ruminants. Whether this is due to a higher level of viral virulence or to a lower level of host innate resistance is unclear.

Clinical signs, diagnosis and control

There is good evidence that the Asiatic lineage of RPV is more pathogenic for Indian sheep than the African lineages for African sheep. From a confirmed RP outbreak in which there was no mortality, only 12 of 20 sheep examined showed clinical signs including fever, erosive stomatitis on labial and buccal mucosae, ocular and nasal discharges, and diarrhoea [8]. When the same virus was reintroduced to sheep and yearling cattle, the sheep developed no more than a low-grade pyrexia while the cattle developed typical signs of RP. On further passage sheep developed diarrhoea, anorexia and depression but no pyrexia or mouth lesions.

In large ruminants, it has been common practice to base a RP diagnosis on the presence of appropriate clinical signs and a positive reaction in a group reactive (RPV and PPRV) agar gel immunodiffusion test. For small ruminants, and based on the possible presence of a necrotic stomatitis with either RP or PPR, this is not possible. Therefore, during the final stages of the GREP, the differential immunocapture enzyme-linked immunosorbent assay (ELISA) test [11] became the accepted tool in the diagnosis of small ruminant RP and PPR.

Sheep and goats can be protected against RP using the classic vaccine for use in cattle and buffaloes, and at times the vaccine has been used extensively in sheep in southern India. In these hosts it is efficacious, innocuous and induces a durable immunity. Although sheep may have occasionally spread RP between the village populations of bovines, it is not recorded that this was a regular event. Accordingly, in the final stages of RP eradication in India and Pakistan, small ruminants were not included in mass vaccination campaigns.

PESTE DES PETITS RUMINANTS (PPR)

The name peste des petits ruminants (plague of small ruminants) reflects two things about this disease. First, that it was initially described from Francophone west Africa and second, that it is a disease that kills large numbers of sheep and goats.

Host range

Among domestic livestock, PPR is essentially a disease of sheep and goats. While both species can be affected clinically, the infection rate in sheep appears to be higher than that in goats even though the severity of individual cases appears to be greater in goats. The original description of PPR [1] stated that the number of sheep affected was less than the number of goats and that the recovery of sheep was more frequent, but a Nigerian field survey [12] found antibody prevalences of 19 per cent in sheep and 15 per cent in goats. Thirteen years later higher antibody prevalences were found in both species [13], but still higher in sheep (57 per cent) than goats (44 per cent). The extent to which host adaptation may play a part in the appearance of clinical signs remains unclear but that it exists is illustrated by a recently described outbreak in Nigeria which spread among sheep but failed to cause disease in companion goats. In Andhra Pradesh a major epidemic mostly affected sheep [14]. Conversely, in an Ethiopian outbreak within a mixed flock, only goats were clinically involved [15].

Serological evidence suggests that the virus can spread from sheep and goats to domestic cattle and buffalo. Although experiments suggest that the infection in cattle is subclinical, there may be circumstances when this is not the case. In the original study, when PPRV was inoculated into a 15-month-old calf, it induced a pyrexia of 42°C for 48 hours [1]. During a field outbreak of PPR in small ruminants in Sudan (an outbreak originally attributed to RP [16]), it was apparent that a degree of pathogenicity for cattle could exist [17]. More recently, pyrexia and mouth lesions have been recorded in Iranian calves in contact with PPR-affected sheep (Golam Kiani, personal communication to P.L. Roeder). Meanwhile, the only report of a disease of domestic buffaloes attributable to PPR remains just that [18]. Domestic pigs are dead-end hosts [19]. Camels appear to be susceptible [20, 21]. The status of the yak has still to be defined but in the mountain regions of the Indian subcontinent where there is an increasing likelihood of them coming into contact with PPR-infected small

ruminants, it would be useful to know their level of susceptibility, and that of their hybrids with cattle.

There are no records of PPR outbreaks in free-living wildlife species although a number would probably exhibit severe clinical signs if affected. In the course of an outbreak of PPR in a United Arab Emirates zoo [22] the disease clinically affected Dorcas gazelles (*Gazella dorcas*), Nubian ibex (*Capra ibex nubiana*), Laristan sheep (*Ovis orientalis laristanica*) and Gemsbok (*Oryx gazella*) and, subclinically, Nilgai (*Bosephalus tragocamelus*). More recently, semi free-living Thomson's (*Gazella thomsoni*) and Dorcas gazelles were involved in an outbreak in the east of Saudi Arabia [23].

Serological evidence of transmission to the African grey duiker (*Sylvicarpa gimmia*) has been obtained [24]. In other African species, neutralizing antibodies have been found in Buffon's kob, waterbuck, bushbuck, cape buffalo and roan antelope (Genevivè Libeau, personal communication). Collectively, these reports indicate a high degree of susceptibility in members of the family *Bovidae*. The situation among *Cervidae* remains uncertain as red deer are possibly insusceptible [22] but American white-tailed deer developed clinical disease following experimental infection [25].

Clinical signs

Following an incubation period of 3–5 days, the onset of PPR is characterized by a well-marked fever reaching 40–41°C by the second or third day of illness and remaining high for another 4–5 days. During this period the animal becomes progressively less interested in food and increasingly depressed.

Corresponding with the onset of pyrexia, most animals develop a watery nasal exudate that may remain light or progress to a heavy catarrhal outpouring. Eventually, this may form a dried crust blocking the nostrils, causing considerable distress as well as sneezing in an attempt to clear the blockage. In such cases a small area of superficial necrosis may be detected on mucosa of the nasal vestibule. The conjunctival mucosa becomes increasingly congested accompanied by an overflowing, serous discharge that wets the hair of the cheeks. Subsequently, the discharge may become catarrhal and fill the eye with a thin yellowish fluid. Later this thickens and can cause the eyelids to mat together blinding the animal, which then cannot eat or drink. There is also increased salivation with wetting of the hair on the chin.

Two or three days from the onset of fever, when serous nasal and ocular discharges are already apparent, a necrotic stomatitis begins to develop. This highly characteristic lesion usually commences as small roughened areas of epithelial necrosis on the lower gum below the insertion of the incisor teeth (see Figure 61.2 in the colour plate section) but in the next 3–4 days other areas of oral epithelia may become involved. In severe cases the epithelia of the cheeks, cheek papillae, dental pad, hard palate and the dorsum of the tongue may be affected. The basic lesion is a shallow erosion in which ulceration and bleeding do not feature. Severely affected animals stop eating and, as a consequence, the necrotic material may remain *in situ* and form a layer of caseous debris coating most of the inside of the mouth. The breath of such animals is highly unpleasant. The lips become swollen while a thin line of necrosis develops along the mucocutaneous junction giving rise to a series of tightly adherent scabs (see Figure 61.3 in the colour plate section).

A number of affected animals develop a severe watery, foul-smelling diarrhoea. They also suffer from viral and bacterial pneumonia, and severe cases show rapid, difficult breathing with marked extension of the head and neck, dilated nostrils, protrusion of the tongue and soft painful coughs. Such animals are profoundly depressed, dehydrated, anorexic and sunken-eyed, and have a poor prognosis. Most fatalities occur 7–10 days from the onset of fever after which, with good nursing, surviving animals recover after a short convalescence. Recovered animals do not carry the virus. They develop a lifelong immunity and cannot be re-infected by PPRV.

With RP, strains of the virus have evolved which give rise to mild clinical signs in cattle or none at all. Whether a similar situation holds true in the case of PPR is not known but it may well do so as both epidemic and endemic situations characterize countries where the virus is well established. There are also indications of different breed susceptibilities among goats. Thus, not all affected animals will show all possible clinical signs to the most severe degree possible and accordingly, less acute, more short-lived cases, without the accumulation of necrotic debris on the epithelial surfaces, are common.

In Pakistan, a recent analysis has shown that outbreaks occur throughout the year but at a greatly

increased level in the winter and spring months (December to April/May). In India, a different picture emerges [26], with the highest frequency of outbreaks during the summer months (March to June). In Nigeria, opinions differ as to the period of peak incidence, said to be either the cold winter months or the mid-year rainy season. These seasonal variations may, of course, be due to changes in husbandry practices but might also reflect seasonal variation in either host susceptibility due to low nutritional status and/or viral virulence.

Pathology

In external appearance the carcass is typically dehydrated, emaciated and soiled. The nose and cheeks probably bear evidence of mucopurulent discharges, the eye is sunken and the conjunctiva congested. The lips are swollen and may carry sit-fast scabs at the mucocutaneous junction. The nasal mucosa is congested.

In the oral cavity there are often extensive erosions of the gums, hard and soft palate, cheeks and tongue, extending through the pharynx into the oesophagus.

Pneumonic lesions are common.

In the alimentary tract, the ruminal pillars may show a mild engorgement but the reticulum and omasum appear normal. There is a severe engorgement or heavy discoloration of the abomasum (see Figure 61.4 in the colour plate section). There may be a variable degree of engorgement of the mucosa of the small intestine and darkening of the Peyer's patches. In the large intestines the mucosa is heavily engorged and discoloured, especially along the folds of the colon. These changes continue into the rectum. Mesenteric lymph nodes are enlarged.

Diagnosis

At the clinical level, and in the new-found situation where RP is no longer a differential diagnosis, it is possible to diagnose PPR clinically on the basis of its highly characteristic disease signs including necrotic stomatitis and a frequently high mortality rate. The presence of scabs along the borders of the mouth characterize animals which have recovered from PPR and is a useful diagnostic aid.

While there was any possibility of RP being involved, it was necessary to use a diagnostic test that would differentiate the two conditions, either of which could exhibit a necrotic stomatitis. For this task the immunocapture ELISA test [11] was developed [27] and should still be used extensively when confirming the recent appearance of PPR in a country. In India, this bivalent test has been replaced by a sandwich ELISA test directed at an epitope of the N protein of PPRV [28]. Another technique now widely used for the differential diagnosis of the different morbilliviruses is reverse transcription polymerase chain reaction. Developed in the mid-1980s, it was quickly applied to virus diagnosis and has proved extremely sensitive and effective. It has the advantage that genetic information can be derived from the sequenced product which allows the distribution and movement of the various virus lineages to be followed [29].

However, where the presence of PPR is well established, and now that RP has become a diminished consideration, for routine diagnostic work simpler tests should be introduced. An agar-gel immunodiffusion test can be applied using either RPV or PPRV immune sera or the recently standardized haemagglutination test of Wosu [30]. Histopathological findings and the detection of viral antigen using immunohistochemisty may also be used [31].

Virus isolation is seldom considered as a diagnostic method but there is a growing need to build a collection of international strains for virulence studies and this technique should not be neglected. Appropriate methods are described [27].

Antibody detection ELISAs are available for use in seromonitoring or epidemiological surveys [27, 32]. Unfortunately, due to the absence of a marker vaccine, these tests are unable to differentiate between a post-vaccinal or a field reaction.

In differential diagnosis of PPR, other conditions to be considered are foot-and-mouth disease, which is common in several PPR-infected countries, undoubtedly infects small ruminants and may cause mortality in goats. Affected animals will be febrile and lame but will not show catarrhal discharges, oral necrosis or diarrhoea. Contagious caprine pleuropneumonia is widely reported among small ruminants on rangelands but pneumonic changes will predominate without either mouth lesions or diarrhoea. Enterotoxaemia is also widely reported but will give rise to diarrhoea and fever, again without mouth lesions or catarrhal discharges. Bluetongue is commonly and erroneously considered a disease exclusive to fine wool sheep

breeds of European origin whereas, in the coarse-haired sheep of south and central India it is a well-recognized cause of severe clinical disease with attendant mortality, coronitis serving as the distinguishing feature.

Epidemiology

PPR is an acute contagion. Infection is acquired by the respiratory route through the inhalation of infected aerosol particles produced by the coughs and sneezes of infected stock. Under these circumstances the disease spreads more rapidly when animals are crowded together. Livestock markets appear to be particularly dangerous and numerous outbreaks have occurred when owners have added newly purchased, incubating animals, to their flocks. Similarly, communal grazing may spread the disease between flocks belonging to the same village. The trade in infected animals and the contamination of vehicles is another way in which the virus can be spread. Over longer distances the movement of transhumant and nomadic flocks represents a means of spreading the virus and villagers near migration routes frequently associate outbreaks of PPR with the passage of such animals.

The historical accounts of the epidemiology of PPR may be somewhat misleading. For a long time PPR was considered a west African condition following its first description in the Ivory Coast in 1940 [1] and, because of a demonstrable relationship with RP, it was assumed that PPRV was a variant of RPV. However, between 1968 and 1976, workers in the USA, Britain and Nigeria reassessed the epidemiology and virology of PPR concluding that PPRV was not an RPV variant but a fourth *Morbillivirus* within the family Paramyxoviridae [33]. Molecular techniques later confirmed this classification [34]. Finally, PPRV has a far wider geographical distribution than west Africa – one that takes in that continent's entire sub-Saharan belt, continues northwards through Sudan and Egypt into the Middle East, the Arabian Peninsula and then eastwards through Afghanistan, Iran, Pakistan, India as far as Bangladesh. This understanding grew in part from the improved reporting of PPR following its classification as an Office International des Epizooties (OIE) List A disease but also from the reduced incidence of bovine RP in Asia. Undoubtedly, the demise of RP threw into relief the continuing presence of a second, RP-related virus, causing death and destruction in sheep and goats.

Of the four PPRV lineages, 1 and 2 are confined to Africa and lineage 3 partly so. The first indications that PPRV occurred outside Africa was the discovery of lineage 3 in Oman and the United Arab Emirates in 1983 [35]. Of even greater significance, however, was the unveiling in 1988 of a fourth lineage, this time from the state of Tamil Nadu in India [36]. Since then, its evolving epidemiology, allied to a retrospective understanding of its distribution and an appreciation of a potential for further spread, has placed lineage 4 at the forefront of PPR concern.

Setting aside the fact that, as discussed above, PPR was a well-established disease in India from an earlier period, for years it was apparently unknown across northern India and northern Pakistan. Certainly in India, where a reliable disease-reporting system was in operation, no contemporary RP-like condition of small ruminants had been seen. Then, in the early 1990s and for no apparent reason, this situation changed. The first indications of the dramatic upsurge that was to follow came in 1991, in the Punjab Province of Pakistan [37] where the emergence of a PPR epidemic represented a novel situation. Although the dates do not fall in a perfect chronology there seems little doubt but that a highly virulent form of the virus subsequently spread to neighbouring countries at all points of the compass. In late 1992 and early 1993 a series of PPR outbreaks occurred in goats in the west of Maharashtra State, India. Then, in early 1994 it was recorded across the plains of northern India, in migratory and non-migratory flocks several thousand head strong. Where observed, morbidity rates in sheep were between 20 and 40 per cent with mortality rates of 40–60 per cent in lambs and 10–20 per cent in adults. Subsequently, the disease was found in the goat markets of Calcutta and finally, late in that year, in migratory sheep and goats from Himachal Pradesh, returning from summer grazing at high altitude [38]. However, its possible introduction into Nepal and its certain introduction into Bangladesh, in 1993, suggest that the virus had actually spread across northern India a year before it was first recorded.

Starting in the west, PPR spread across Bangladesh in an epidemic involving all districts of that country [39]. Traditionally, goat keeping had been popular among poor Bangladeshi farmers because of the goat's high reproduction rate and, compared with

cattle and poultry, its relatively high disease resistance. Now, in combination with goat pox, millions of goats died and the whole industry became endangered. Moving south in India, from 1994 to 1998 a similar epidemic severely affected the sheep industry of the southern state of Andhra Pradesh [14].

Moving westwards from Pakistan, epidemic PPR arrived in northern Afghanistan in 1995 with cases being recognized in 1996. It is possible that the virus was introduced through nomadic movements among sheep and goats moving from summer grazing in the mountains of Pakistan. In Iran, PPR was confirmed in 1996, while in Iraq outbreaks were seen in 1998 and possibly earlier (Obi, personal communication). The onset of epidemic PPR in Turkey now appears to date from 1996 [40] after which the incidence has continued to escalate, with recent reports from Turkish Thrace. In Israel, PPR was first recognized in December 1993 having possibly entered from Lebanon [41] and is now considered as endemic (A. Shimshony, personal communication).

Within the domain of lineage 4 (Turkey to Bangladesh) several pointers suggest that PPR was present prior to the events of the mid-1990s. In Saudi Arabia, the virus was killing local and imported sheep as early as 1977 and 1979 [42] but owing to the annual importation of animals from both Africa and Asia this cannot be taken as evidence of an endemic presence. However, in Lebanon, PPR was reported in 1987, and in Jordan serological screening on samples collected during 1987–8 confirmed the presence of the virus in that country although there had been no reports of the disease [43]. Evidence compatible with the presence of PPR in India in the 1950s and 1970s (and even earlier) has been noted already and PPR has probably existed in other parts of Asia for a long time but has been confused with other diseases such as pasteurellosis or RP [44]. Perhaps then, the virus was already widely present across its domain as a low-virulence, endemic infection, but one with a potential for periodic increases in virulence and associated wide-ranging epidemics.

Unfortunately, the end of the present cycle is not yet in sight and currently the disease is epidemic in the Pakistan province of Sindh. In addition, there is growing evidence that PPRV is spreading northwards from Afghanistan. Tests on sera collected in 1997–8, identified a tentative presence of the virus well north of Afghanistan in central Kazakhstan [45]. Further, the presence of clinical disease has been confirmed from the Badakshan region of Tajikistan, where it might have been present for a number of years (P. Roeder, personal communication). Apparently, Kazakhstan has not yet experienced the disease but recognizes the likelihood of this happening due to the importation of large numbers of small ruminants from Afghanistan and Tajikistan. It remains to be seen if the virus is present in the other central Asian countries of Kyrgyzstan, Uzbekistan and Turkmenistan.

Treatment and control

PPR is the major viral killer of small ruminants in west Africa with mortality rates of up to 100 per cent. Affected goats with stomatitis, enteritis and pneumonia were treated with penicillin and streptomycin reinforced with broad-spectrum chloramphenicol [46]. Therapy for diarrhoea and ionic imbalance was also instituted. Considerable time and energy was devoted to easing the discomfort caused by the presence of labial scabs which cleared in a few days when rubbed with lemon juice. A 58.8 per cent recovery rate was attributed to clearing the scabs and allowing a return to natural feeding. In Pakistan, substantial recovery rates were linked to the use of broad-spectrum antibiotics and fluid replacement therapy [37].

Vaccination is the most effective way to gain control over epidemic PPR. Taking advantage of the cross-relationship between the two viruses, and its ready availability, RP tissue culture vaccine was initially used to protect small ruminants. Latterly, the need to undertake serological surveillance for the presence of RPV under GREP has led to international insistence on the use of a homologous, live attenuated PPRV vaccine conferring a long-lived immunity [27, 47].

Anticipating the need to be able to detect the continued circulation of endemic strains within a vaccinated population, genetically modified viruses are proposed. In many PPR-infected countries, sheep and goat pox are additional burdens to the farmer and vaccines delivering a multivalent protection against all three conditions without prejudicing serosurveillance will probably characterize the next vaccine generation.

Currently, two approaches are being pursued to produce marker vaccines for PPRV. The first is to produce recombinant poxvirus vaccines which express one or more immunogenic genes from PPRV. An example is

the recently described capripox recombinant carrying the fusion (F) protein gene of PPRV responsible for the fusion of the viral and cellular membranes which plays a key role in infection. Interfering with this process greatly reduces the viruses' ability to replicate and cause disease. This vaccine has the advantage of being a dual vaccine which can protect small ruminants against both PPR and capripox infections [48]. The other approach is to modify the genome of the current live attenuated PPR vaccine using reverse genetics, the process whereby viruses with RNA genomes can be manipulated through DNA copies and the altered virus genome copy 'rescued' back into an RNA form in the virus. This technology has been used to make marker vaccines against RPV infections, for example by modifying the genome so that it expresses a foreign protein that acts as a serological marker to identify vaccinated animals, and is now being applied to develop marker PPRV vaccines.

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Rift Valley fever

G.F. Bath

Synonyms: slenkdalkoors, zinga, lunyo, enzootic hepatitis.

The name of this disease is associated with the Great Rift Valley in East Africa, where it was first recorded and described. Its cause was established in 1930 [1] as a mosquito-transmitted viral disease of domestic ruminants. It can assume epidemic proportions under conditions that favour multiplication of the vector, specifically, prolonged and abnormally high rainfall. The disease is characterized by high mortalities in newborn lambs, massive abortion storms in pregnant ewes, necrotic hepatitis and widespread haemorrhages. It has been identified in many sub-Saharan countries [2] and extends as far as Senegal [3, 4], Egypt [2], Madagascar [2] and has more recently been confirmed in Asia for the first time, in the Arabian peninsula [5, 6].

CAUSE

The causative virus is a member of the family Bunyaviridae, in the genus *Phlebovirus*, and is related to the causes of Nairobi sheep disease, Akabane disease, sandfly fever, simba and Congo-Crimean haemorrhagic fever. It is a single-stranded three-segmented RNA virus that shows little significant antigenic variation, although different isolates may vary considerably in their pathogenicity. The virus has two envelope glycoproteins, which are responsible for viral recognition on receptor cells and induction of the immune response, as well as for haemagglutination [2]. Rift Valley fever (RVF) virus is pantropic, and it can infect a wide variety of tissues, including liver, lymphoid and nervous tissues.

CLINICAL SIGNS

The incubation period is short, as little as 12 hours in young lambs or up to 72 hours in adults. Lambs under 2 weeks old are highly susceptible and mortality may exceed 90 per cent. A short, severe fever of >41°C may be biphasic with a terminal decline in body temperature. The disease progresses rapidly, with most young lambs dying within 2 days of the onset. Therefore, clinical signs may not be observed in the field. Affected lambs may be anorexic, listless, recumbent and breathe rapidly [2, 7].

Adult animals may develop an inapparent infection or show such mild or non-specific signs that the disease can go unrecognized. Older lambs and adults may, however, develop the peracute form of the disease, characterized by unheralded deaths. More often, the disease follows an acute course of 1–4 days, with a fever of 41°C as well as non-specific signs such as listlessness, anorexia, tachypnoea and weakness [2]. More specific (although not more frequent) signs include a blood-tinged or fetid diarrhoea, even melaena, a bloody mucopurulent nasal discharge and occasional icterus [2, 7]. Vomiting has also been recorded [2]. Haemorrhages and congestion in the mucous membranes of the eye, mouth, rectum or vulva are suggestive of RVF, and sometimes blood can be seen dripping from these orifices [7]. In pregnant ewes, massive abortions approaching 100 per cent of the flock are very characteristic of an outbreak. Aborted fetuses are often autolysed, and retained afterbirth may be a sequel [2, 7]. Mortality in adults is quite variable from flock to flock and ranges from 5 to 35 per cent in susceptible sheep during field outbreaks. Clinical signs of encephalitis which are a feature of the disease in humans have not been described in ruminants [2].

Sometimes, the clinical picture is accompanied by a mild hyperaemia and crusting of exposed skin areas such as the perineum and face, but this may be due to a concurrent infection by bluetongue [2, 7]. Anaemia is occasionally a clinical feature but may be unrelated [7].

PATHOLOGY

Lambs under 2 weeks old may become viraemic within 16 hours of infection. The virus undergoes primary multiplication, most probably in the regional lymph nodes, from where it spreads by primary viraemia to the target organs, especially the spleen and liver. A secondary and much more severe viraemia follows [2]. In young lambs, the liver undergoes massive necrosis that causes peracute death, and thus precludes the development of other lesions which are seen in less acute cases. The liver appears pale grey and may be mottled with areas of congestion and haemorrhage. Lymphoid necrosis, nephrosis and widespread haemorrhages also are characteristic of peracute cases [2, 8]. Older lambs and adults become viraemic in 1 or 2 days, a state that can persist for 7 days. As in young lambs, the acute form of RVF is characterized by hepatic necrosis, but this tends to be more focal, giving the liver a mottled appearance with small to larger foci of grey necrosis interspersed with normal tissue and haemorrhages. In less acute cases, the parenchyma appears more yellow. Widespread haemorrhages are common, but have special diagnostic significance in the gastrointestinal tract and the lungs [2, 7]. Streaky subserosal haemorrhages in the rumen, giving it the appearance of being smeared by a paintbrush, are typical. Small to large haemorrhages may be seen in the folds of the abomasum, often with blood which is usually dark brown and clotted in the lumen [2, 7, 8]. Haemorrhages are common anywhere in the intestine, on the omentum and mesentery, and are often large and irregular. Frequently, the lungs are haemorrhagic, a feature of diagnostic significance. Haemorrhages, often accompanied by oedema, in the wall of the gall bladder also are of special value, if present [2, 7, 8]. Pulmonary oedema may be present and ascites, hydropericardium and hydrothorax, frequently blood-tinged, are common. Cases that survive a few days quite often exhibit light to moderate icterus. The spleen is invariably enlarged, sometimes with infarcts, while lymph nodes are swollen, oedematous and often haemorrhagic.

Microscopically, the liver picture is dominated by necrosis and haemorrhage [2, 8]. In the severely affected neonate, almost the entire liver is necrotic and superficially resembles lung tissue due to pyknosis and destruction of hepatocytes [2, 8]. In less acute cases, there are areas of primary necrosis, usually paracentral, with hyalin degeneration and pyknosis predominant. Acidophilic cytoplasmic inclusion-like bodies and intranuclear inclusions have diagnostic value [8]. Haemorrhages, vasculitis, thrombi and fibrin are indicative of disseminated intravascular coagulopathy, both in the liver and in other organs. Lymphoid necrosis (including splenic infarcts) and nephrosis are further typical features.

DIAGNOSIS

In addition to the presence of favourable climatic conditions and the characteristic clinical and post-mortem features outlined, initial leucopenia, thrombocytopenia and subsequent leucocytosis, as well as elevated liver enzymes, have some diagnostic value. The virus is quite stable in serum at 4°C and, provided

blood is taken from a viraemic animal and kept chilled, the chances of isolation are good. Various tissues, but especially the liver and spleen, also are suitable specimens for virology if dispatched on ice from fresh carcasses. RVF virus grows easily in a variety of cell cultures, embryonated eggs and laboratory animals [2]. Specific identification depends on neutralization tests. Although there are some antigenic similarities with related viruses, serological tests (e.g. complement fixation, enzyme-linked immunosorbent assay, immunodiffusion and immunofluorescence) are reasonably reliable and specific [2]. Differential diagnosis may include Wesselsbron disease (lambs only) and seneciosis, both of which can closely resemble RVF. Other diseases that may cause initial confusion include poisoning with *Cestrum*, *Microcystis*, *Lasiospermum* and other hepatotoxins, systemic pasteurellosis, enterotoxaemia, bluetongue, pregnancy toxaemia and chronic copper poisoning [2, 7]. With abortion storms, other causes of abortion have to be considered [2, 7].

EPIDEMIOLOGY

Outbreaks occur at irregular intervals. The single most important factor responsible is above-average rainfall, which favours multiplication of the vectors. Investigations [9–11] have shown that non-ruminant vertebrate reservoirs are not significant for maintaining the virus, but rather that certain mosquito vectors maintain the virus in endemic areas by transovarial transmission, with occasional infections of ruminants, which may often go unrecognized. Mosquitoes of the genus *Aedes* are the most important maintenance vectors; they are biological transmitters and can pass infection transovarially. Aedine mosquitoes must perch on vegetation to lay eggs, and therefore flooding and marshy areas are needed for big outbreaks. They overwinter as eggs, even in mud, and require periods of drying between floods to hatch. Very low levels of virus activity are found between outbreaks, and amplification of virus takes some time before outbreaks of disease are recorded, usually in late summer at the end of the seasonal rains [2, 3, 6, 9–12]. Subsequently, major epidemics are worsened by secondary or mechanical transmitters, mainly *Culex* species, but also other biting insects [9–11]. Increased viral dose and higher ambient temperatures augment the rate of infection of mosquitoes, which, in turn, results in less efficient feeding and therefore the chances of multiple infection of a series of hosts. Furthermore, infected hosts appear more attractive to mosquitoes, which become more active in feeding on these animals and are thus able to spread infection more. The movement of infected livestock, or high-altitude winds which can convey infected mosquitoes over long distances, may be responsible for spread to non-endemic areas [9, 11]. The onset of cold weather (especially frost) will slow or suspend an epidemic, but a combination of drier weather, predation of mosquitoes and development of an immune host population (whether by infection or by vaccination) is necessary to end an epidemic [2]. The pattern of outbreaks can be quite different on individual farms since sometimes the sheep are more infected, and at other times the cattle. The explanations for such variations are unknown.

TREATMENT, PREVENTION AND CONTROL

The course of the disease is usually too acute and the lesions too severe for treatment to be of much value. Treatment for liver failure and impaired blood clotting may be considered in very valuable animals. Risk of infection can be lowered by removing livestock from marshy areas, and applying insecticidal sprays or ear tags. Stabling can be indicated for valuable sheep but cannot be used for flocks for long periods [2, 7]. Although genetic susceptibility and resistance to infection has been established [2] it is unlikely that breeding resistant sheep will ever be a viable option, given the number of other genetic characteristics which have to be considered as well as the unpredictable and sporadic nature of epidemics.

Practical control measures rest mainly on the timely use of effective vaccines. The Smithburn vaccine [13] has been attenuated and neuro-adapted by serial passage in mice and embryonated eggs. These strains grow readily in cell cultures and yield live attenuated vaccines, which cause no symptoms in non-pregnant sheep and a durable, usually lifelong immunity within a week of inoculation. There is no record of reversion to virulence. Formalin-inactivated vaccines made from wild

strains are useful for pregnant sheep and for cattle, but the duration of protection is short [2]. Non-pregnant commercial breeding stock should be vaccinated once with the live vaccine before joining the breeding flock, while valuable stud animals can be given annual booster doses. Vaccination of rams should take place at least 6 weeks before mating. During outbreaks, strict precautions must be taken to prevent needle transmission of wild virus, which could be present in individual sheep. Lambs of vaccinated ewes should preferably be older than 6 months when inoculated, but in the face of an epidemic that age can be lowered. Pregnant ewes should be vaccinated only with the formalin-inactivated vaccine, especially when 5–10 weeks pregnant. If pregnant ewes are mistakenly vaccinated with the live vaccine, economic losses can occur. Early pregnancies may be resorbed and late pregnancies terminated by abortion. Between 5–10 weeks gestation, vaccination with the neuro-adapted live virus can cause developmental abnormalities of the brain (usually hydranencephaly), which results in secondary arthrogryposis and hydrops amnii. Ewes carry such living fetuses to term and beyond have massively distended abdomens and are unable to deliver the fetuses naturally [2, 7]. Unless a Caesarian section is performed, the ewe eventually dies. Lambs are not viable and die soon after removal. Although these sequelae are not as common as with the Wesselsbron disease vaccine, they are nevertheless economically significant [14]. Since epidemics are difficult to predict, and vaccine is not always readily available during the panic that ensues in an outbreak, it is wiser to vaccinate all animals intended for breeding just once in regions of potential epidemics, even when weather conditions are unfavourable for RVF. An immune population makes an epidemic unlikely.

RVF has been reported in a wide variety of habitats in a very extensive geographical area, and has potential for further spread globally due to the suitability of a range of mosquitoes as vectors, either as transovarially infected reservoirs or as transmitters during epidemics. In addition, RVF is a dangerous human pathogen. Its potential for causing serious epidemics outside Africa should therefore not be underestimated.

ZOONOTIC IMPLICATIONS

RVF poses a definite danger to humans, and strict precautions must be taken in outbreaks [2]. The usual manifestations in humans are the sudden onset of flu-like symptoms after a short incubation of 2–6 days. Fever, often biphasic, may last several days. Headaches, photophobia, loss of visual acuity, nausea, vomiting, dizziness and muscle or joint pains are often experienced, followed by recovery, which may take a few weeks. Occasionally, the infection follows a more serious course, particularly in patients with pre-existing liver disease. Sequelae may include widespread haemorrhages, with subcutaneous and mucous petechiae and ecchymoses, bleeding from the gums, haematemesis and melaena. Severe liver damage can result in jaundice, shock, liver and kidney failure, and death. Ocular lesions can occur in 20 per cent of patients up to 4 weeks after infection and are initiated by focal thrombosis, resulting in retinal ischaemia and opacity, occasionally culminating in haemorrhage and retinal detachment. Encephalitis may follow up to 4 weeks after onset in about 1 per cent of cases. Confusion, hallucinations, salivation and teeth grinding may be seen, and can be followed by coma and death. Focal necrotic lesions may be found in the brain with leucocytic infiltrations indicative of a delayed hypersensitivity. Human infection is contracted either by contact with infected sick or dead animals, less commonly from meat or milk, or by insect bites, mainly mosquitoes. Person-to-person transmission has not been recorded. Particularly at risk are veterinarians, farmers, shepherds and abattoir workers, and around 15 per cent of farm residents can be infected. Fatalities are usually less than 1 per cent, but deaths can run into several hundred in outbreaks as recorded in Egypt, Senegal and Arabia. It is therefore clear that strict measures are indicated to minimize the chances of infection. Should the disease be suspected, wearing eye protection, protective clothing, gloves and masks should be mandatory, and cleaning or sterilization of equipment done with due care. If suspicious disease occurs within 2 weeks of contact, a medical practitioner should be consulted and informed of the risk so that the possibility of RVF infection can be investigated and treatment instituted at an early stage.

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Akabane disease

P.D. Kirkland

'Akabane' is the name of the Japanese town in which the virus was first isolated from a mosquito in the late 1950s [1, 2]. The virus was subsequently isolated from biting midges (*Culicoides* species) in Australia in the 1960s, and it was found that antibodies to this virus were common in ruminants and horses held in areas where *Culicoides* were abundant [3, 4]. It was not until 1973 that the virus was linked to any animal disease. It was shown to be pathogenic to cattle in Japan, causing abortions, stillbirths and the birth of offspring with congenital defects, especially arthrogryposis (AG) and hydranencephaly (HE) [5, 6]. In the following year, Akabane virus was also linked to a major epidemic of AG/HE in Australia [7, 8]. The term 'Akabane disease' was suggested for the syndrome of AG/HE after the connection was established between the virus and the disease [9]. However, this name can be misleading as these defects can also

be induced by several other viruses. Significant outbreaks of disease have been confined to Australia and Japan [7, 8, 10] but disease has also been reported sporadically in the Middle East [11–14].

CAUSE

Akabane is an arthropod-borne virus (arbovirus), whose insect vectors serve as obligatory biological hosts. Although the virus has been isolated from a number of species of mosquitoes (*Culex* spp., *Aedes* spp., *Anopheles* spp.), the principal vectors are small biting midges from the genus *Culicoides*. It is believed that many of the isolations of the virus from mosquitoes were probably the result of the insects bearing a blood meal from an infective mammalian host. At best, mosquitoes appear to play a minor role as vectors.

Akabane is an enveloped RNA virus with a diameter of approximately 130 nm and is a member of the *Orthobunyavirus* genus of the family Bunyaviridae. The virus is very labile and is readily inactivated by heat, lipid solvents and low pH. The virus can be easily propagated in a range of cell cultures [15].

Infection of livestock in nature is always the result of bites from insects carrying the virus. Antibodies to Akabane virus can be found in many mammalian species, predominantly ruminants and equids, both domestic and wild, in many parts of the world. The virus is widespread in Asia, Africa and Oceania, although it has not been reported in Europe or in the Americas.

CLINICAL SIGNS

In countries where Akabane virus is common, disease is infrequently observed. The virus is almost exclusively a pathogen of the fetus and, in endemic regions, breeding animals are usually infected before reaching reproductive age. Outbreaks of disease may occur at the margins of the distribution of *Culicoides* following transient expansions of the range of the vector, usually as a result of favourable weather conditions or when susceptible stock are introduced to an endemic area [16]. Disease is most frequently observed in cattle as vectors are uncommon in regions that are conducive to the raising of sheep. Disease is occasionally observed in goats as they may be raised in vector areas. Fetal infection is also less common in sheep and goats than in cattle because of their seasonal breeding and shorter period of gestation. Nevertheless, sporadic outbreaks of disease have been observed in small ruminants in Israel [12, 13] and Australia [17, 18]. A combination of the optimal breeding time for small ruminants and the shorter period of gestation means that, even when held in vector areas, these animals are less likely to be pregnant when vectors are active.

Infection of animals after birth is invariably asymptomatic and, following a brief period of viraemia lasting about 5–7 days, animals develop a strong antibody response. Rarely, some strains of Akabane virus in Japan have been associated with encephalitis in postnatal cattle [19, 20]. Disease outbreaks are almost exclusively the outcome of infection of susceptible pregnant animals, where the virus rapidly crosses the placenta and replicates in the tissues of the developing fetus. A distinct succession of clinical signs (encephalitis, AG then HE) are observed as the outcome of infection of the bovine fetus at different stages of gestation [10]. However, in sheep and goats, through a combination of a shorter period of gestation and narrower periods of fetal susceptibility, a range of congenital defects can be seen in the one fetus or neonate. The ovine (and probably the caprine) fetus is most susceptible between 28 and 56 days of gestation, when infection can result concurrently in severe HE (in some cases almost anencephaly), AG and hypoplasia of the spinal cord and lungs [21–23]. The virus also crosses the placenta up to 50 days of gestation, but the defects are usually less severe and brain lesions are restricted to porencephaly [24, 25]. Infection at later stages of gestation has failed to result in any disease. The incidence of fetal defects also varies markedly with different strains of virus, ranging from 16 to 80 per cent of fetuses affected, depending on the virulence of the virus [23, 26, 27].

Clinical signs in sheep include abortion, dystocia, stillbirths and the birth of weak lambs that usually die soon after birth. The congenital defects that are grossly apparent at birth include AG, doming of the cranium, brachygnathia and spinal-cord deformities (scoliosis, kyphosis, torticollis) [28]. The AG may be seen affecting multiple joints on one or more limbs, but the forelimbs are more frequently affected than

the hind ones. Dystocia often occurs due to the limb defects. The skeletal deformities are the result of both a direct effect of the virus on skeletal muscle and a neurogenic muscular atrophy following the damage to the fetal brain and spinal cord. Newborn animals that are born alive may show a variety of neurological signs, from paralysis, paresis to ataxia, incoordination, blindness, inability to suckle and other disorders consistent with severe central nervous system (CNS) damage. These signs can be observed without any apparent external gross anatomical changes to the skull. In some animals displaying definite neurological signs, lesions in the brain and spinal cord may only be apparent histologically but are often still severe. Neonates that survive at birth usually die within a few days. Some may, however, survive longer if hand-raised and taught to suck but may die from misadventure.

PATHOLOGY

The typical lesions caused by Akabane virus (but by other pathogens too, hence the need for confirmatory tests) are usually apparent grossly, consisting of musculoskeletal defects (arthrogryposis, kyphosis scoliosis) and varying degrees of damage to the brain, ranging from porencephaly to severe hydranencephaly. In some cases there may be hypoplasia of the spinal cord and lungs. Histological examination of the brains of full-term lambs is usually unrewarding, with few histological indications of a viral infection, rather, absence of large areas of brain surrounded by tissue with relatively normal architecture. In cases where infection has occurred later in gestation, there are few if any gross lesions in the brain, and the presenting signs are arthrogryposis and skeletal defects with degenerative histological lesions apparent in the ventral horn neurons. If an aborted fetus is presented for examination, there may be acute CNS lesions suggestive of a viral infection, including a mild-to-moderate non-suppurative encephalomyelitis. The lesions can be detected in all parts of the CNS, with perivascular cuffing, neuronal degeneration and cavitation of the brain, and neuronal degeneration in the motor neurons of the spinal cord. Muscular dystrophy may also be observed. The acute encephalitis that has been described in calves infected at or near birth has not been observed in sheep.

DIAGNOSIS

Serology is the most reliable method for the diagnosis of Akabane virus. Definitive confirmation of Akabane virus infection can be achieved serologically only by obtaining a positive result in blood or body fluids (pericardial, pleural or peritoneal fluid) from a fetus or neonate that has not suckled. The colostrum-free status of a neonate should be confirmed by checking that the stomach contains no milk. In areas where Akabane virus is not usually encountered, although not diagnostic, positive serology in the dam can be highly suggestive. Conversely, a negative result in the dam conclusively excludes Akabane virus infection.

Virus isolation from newborn fetuses is usually unsuccessful, as virus replication ceases long before delivery. Experimentally, Akabane virus has been isolated from fetuses removed from dams at mid-gestation so that, at least in theory, virus may be isolated from aborted fetuses but has been rarely achieved in practice.

The virus neutralization (VN) test is recognized worldwide as a suitable test to detect antibodies to Akabane virus but does rely on the availability of the virus and cell culture facilities. Although there is some antigenic diversity among different strains of Akabane virus, there appears to be minimal variability in the production of neutralizing antibodies [28, 29]. The enzyme-linked immunosorbent assay (ELISA) gives results that are comparable to the VN test and has the advantages of being very quick, less expensive, and can be conducted without the need for cell culture facilities and live Akabane virus (provided key reagents have been sourced from a laboratory that regularly tests for Akabane virus). Other serological methods that can be applied include the agar gel immunodiffusion test (AGID) but it is less sensitive than the ELISA or VN test and suffers from cross-reactivity with related viruses. The haemagglutination-inhibition test (HI) has also been used in some countries but is not in regular usage.

Histopathological examination of brain, spinal cord and other tissues can only be complementary to serology and virology as the lesions inflicted on the fetal CNS are not pathognomonic.

DIFFERENTIAL DIAGNOSIS

The clinical and pathological features of the disease in sheep and goats can be caused by several other viruses including Wesselsbron, Cache Valley, border disease and perhaps bluetongue virus, especially vaccine strains. Non-infectious causes include genetic disorders and some plant poisonings. The need for serological confirmation of infection by a specific agent is, therefore, essential. It should be noted that high levels of immunoglobulins that are detected in the precolostral serum and fluids of newborns or fetuses indicate exposure to an infectious agent *in utero* and can be used to distinguish infectious causes from genetic diseases or a toxic insult.

EPIDEMIOLOGY AND TRANSMISSION

As an obligate vector-borne virus, the epidemiology of Akabane virus infection is inevitably closely linked to the distribution and abundance of the local vector species. When investigating the possibility of Akabane virus infection in a disease outbreak, as this disease is almost entirely acquired *in utero*, the location of the dams of affected animals and the local environmental conditions that were prevailing several months ago must be taken into careful consideration. Environmental factors that influence the life cycle of the insects are important both to initiate transmission and for the cessation of spread. Some *Culicoides* species utilize decaying moist vegetable matter or sludge in the environment while others depend on cow dung to oviposit and for larvae to develop [30–32]. These ecosystems provide an intrinsic degree of moisture and temperature control but are nevertheless affected by environmental extremes. Generally, warm moist conditions favour midge development, breeding and survival. On the other hand, dry conditions retard breeding and spread, and very cold conditions, especially the occurrence of weather that is associated with frosts, prevent breeding and is often lethal for adult midges. In general, Akabane virus infection depends on the development of a sufficiently large vector population to sustain transmission from animal to animal and occurs in the mid to late summer and autumn months in most temperate regions and ceases with the onset of winter. While a sustained period of warm moist weather conditions are usually required for local spread, in regions that are in proximity to competent vector populations, the possibility of transient spread through the introduction of wind-borne midges must be considered. There are well-documented instances of the long distance dispersal of midges by air currents, with the introduction of Akabane and other insect-borne viruses into new habitats, far from the original endemic focus [33, 34]. While mosquitoes are not considered to be the principal vectors of Akabane virus in most countries, they may play a role in local transmission and, like midges, are also dependent on the occurrence of warm moist conditions for their development and spread.

In general, midges have a preference for cattle as a source of a blood-meal so the occurrence of disease in cattle may give a strong indication that Akabane virus has been spread in a district. In areas where Akabane virus is endemic regular, usually annual, virus transmission provides a high level of population immunity and breeding stock are usually exposed and immune before reaching reproductive maturity. In those areas the virus is usually transmitted very efficiently and extremely high levels of infection (often close to 100 per cent of animals) are observed. However, in regions where virus transmission is intermittent, some animals may not be exposed before becoming pregnant and sporadic cases of disease can occur. As indicated previously, the timing of infection in relation to the stage of gestation is also important and while there are some stages of gestation (e.g. 28–56 days in sheep) where a high incidence of fetal disease can occur, at other stages (e.g. after 80 days of gestation), infection may not occur or be inapparent.

Significant outbreaks of disease are usually observed when vectors move into otherwise Akabane virus-free regions following favourable climatic conditions [35]. Usually, these regions will have a history of the previous occurrence of Akabane virus, although incursions may occur at irregular and sometimes quite long intervals, perhaps of 10–20 years. Although the introduction of virus is likely to be transient, high levels of seroconversion can occur. The incidence of disease will be the product of the seroconversion rate, the stage of gestation at which infection occurred and the teratogenicity of the strain of virus involved.

The movement of livestock from vector-free areas into zones endemic with Akabane virus can also

result in substantial losses, especially when pregnant animals are moved during the vector season [16]. If non-pregnant animals are introduced, the immediate likelihood of disease will be eliminated, but the possibility of disease at the next pregnancy will depend on the time of breeding in relation to virus transmission and the efficiency of transmission in the area.

PREVENTION

As Akabane virus transmission is integrally linked to the insect vector, there is little likelihood of preventing spread of the virus. Although modification of the immediate environment by improving drainage and the extensive use of insecticides has been considered, these are generally considered to be impractical and ineffective on a broad scale as it is impossible to eliminate the insects completely and re-introduction from nearby areas is inevitable. Further, as Akabane virus is transmitted with very high efficiency compared to some arboviruses, the presence of even low numbers of insects has the potential to cause disease.

The most effective methods to prevent infection must be directed at the level of the mammalian host. Vaccines have been successfully developed against Akabane virus in several countries [36–40], but their use has not been adopted widely or they have limited availability. Therefore, careful management of breeding animals in most cases is the only practical solution available. The introduction of pregnant animals from virus-free regions into endemic areas should be carefully controlled. The introduction of non-pregnant animals abolishes the immediate risk but prevention of a later problem will depend on delaying breeding until the animals have been exposed to the virus. However, none of these measures provides a solution to the problems that occur following the unexpected introduction of the virus into a region that is usually virus-free.

ZOONOTIC IMPLICATIONS

The vector species that spread Akabane virus appear to be dependent on equids and ruminants as a source of the blood-meal that is essential for their reproduction. These insects are not known to feed on humans and there is no evidence of human infection with Akabane virus [41].

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Part XIV
Regional problems

64

Middle East and North Africa

M.M. Rweyemamu and J. Berrada

The Middle East is the name used to designate the countries on the eastern coast of the Mediterranean Sea and eastward, towards the centre of Asia. This geographical entity extends from Turkey in the north, to Afghanistan in the east and to the Arabian Peninsula and Egypt in the south.

North Africa is divided from west to east into five countries (Morocco, Algeria, Tunisia, Libya and Egypt) and extends over a huge area. Much of the geology and population across this region is remarkably similar [1].

This chapter will deal only with certain countries of the 'Near East' and North Africa, i.e. the countries in the close vicinity of the Mediterranean, namely Syria, Lebanon, Israel, the Palestinian Autonomy, Jordan, Saudi Arabia, Egypt, Libya, Tunisia, Algeria and Morocco. This region shares many similarities with regard to sheep management practices and social organization, and has been the seat of sheep and goat breeding since the dawn of history and, to the present day, its rural economy is based, to a very large extent, on the breeding, management and trade of those species.

The Near East is a very important historical and geographical junction: the meeting point of three continents, namely: Europe, Asia and Africa. Over the centuries, this location has allowed not only the exchange of populations and cultures into and from the vast areas adjacent to the junction, but also the transfer of animal (and human) pathogens from one continent to the other, with consequent outbreaks of diseases. Those pathogens might have been conveyed from one zone to another as a result of geographical, climatic conditions and human activity. Today, the risk of pathogen transmission into the Near East from Europe is very low, but the region is often invaded by epizootics that originate either from the south–central Asian axis or from the Horn of Africa. This is a direct consequence of trade, as there is a thriving import of livestock from these regions into the Middle East. Disease spread within the region is further complicated by centuries-old animal management systems still practised by the vast majority of the region's sheep and goat breeders. By contrast, North Africa, which has far less livestock trade connection with south Asia and the Horn of Africa, does not experience epizootics from these regions with such frequency as the Middle East [2].

Small ruminant feeding in the Middle East and North Africa is based almost entirely on grazing. As both rainfall and surface water in the region are limited, transhumance and nomadism are practised. The long-range transfer of large flocks in the search of good grazing areas makes their contact with other populations inevitable, with a probable exchange of pathogens that may, in that way, bridge long distances.

In periods of drought or pasture shortage without feed supplementation, severe loss of body condition takes place at the end of summer (August to September) almost each year. During those periods, flocks become particularly sensitive to diseases, either to pathogens already carried by them or to new infections acquired from the environment or from neighbouring animal populations.

Livestock are used as currency in traditional Near Eastern and North African societies. Farming income is, therefore, invested in animals that, in case of need, can be sold for cash. The result is that Middle Eastern and North African flocks and herds are never closed; there is always movement of animals into and from them. Moreover, for traditional rural society sheep and goats are not only an economic asset but a symbol of wealth. Therefore, farmers tend to enlarge their flocks (even beyond their true economic ability and/or beyond the real carrying capacity of the pastures at

their disposal) without questioning the origin of the bought-in animals.

In the Middle East, traditional households, even in non-rural communities, own a small number of sheep and goats, which are unprofessionally managed on their property. They are used for domestic consumption and, as an asset for small family savings, are constantly subject to uncontrolled trade.

Meat consumers in the Near East prefer to witness the slaughter of the animal they are going to eat, rather than buying pre-slaughtered meat. That tradition, together with the fact that power and road infrastructure in many of the regional zones do not allow the maintenance of an efficient refrigerated food chain, further enhances live animal movement throughout the region and thereby exacerbates disease spread in the region.

Animal trade in the Middle East and North Africa is practised mainly by livestock dealers who purchase animals over large areas and offer them to potential buyers in open public markets, where no information as to the origin of the displayed animals can be given. Thus, markets become an excellent point for interchange of pathogens.

The traditional Middle Eastern and North African society is tribal, with family links maintained between remote households, who permanently or occasionally exchange animals between their flocks and herds. Livestock are traditionally used as presents in religious and family celebrations as on the occasion of a wedding or childbirth.

In addition, large numbers of sheep are slaughtered and a tremendous amount of sheep meat is consumed during the special religious Muslim holiday called 'Aid Al Adha'. Millions of sheep are slaughtered domestically for this single-day ceremony both in urban and rural communities of Middle East and North Africa. For example, in Morocco alone some 4.5 million sheep were slaughtered in December 2004. Accordingly, prior to this event, there is extensive sheep movement both within and between countries as well as imports.

The degree of cooperation of the farming community with the local veterinary authorities is generally poor, especially in the Middle East: traditionally, the nomadic society is unwilling to cooperate with any of the authorities. The result is a lack of important epidemiological data and difficulties in imposing animal health control regulations. Epidemiological enquiries and surveys as well as eradication and vaccination campaigns are almost impossible to apply. However, a fair degree of cooperation between farmers and local veterinary authority exists in some countries of North Africa (i.e. Morocco and Tunisia), who regularly conduct, with the assistance of mandated private veterinary practitioners, epidemiological surveys and large-scale vaccination campaigns against major notifiable diseases.

The veterinary infrastructure in most of the Near Eastern countries is not adequate to cover modern needs for animal disease diagnosis and control and for setting up a good epidemiological database. Fieldwork generally is not carried out promptly and intensively in response to livestock epidemics. State control of animal movements, both within and between the national boundaries, is lacking in most Middle Eastern and North African countries. Large flocks and herds from countries outside the region, traditionally from the Horn of Africa or from central Asia, may freely cross into the Near Eastern countries, either on vehicles or, very often, by foot. The same phenomenon is also witnessed among Maghreb countries, especially between Morocco and Algeria.

BRUCELLOSIS

While *Brucella ovis* is reported only from Lebanon and from Saudi Arabia and does not seem to be a region-wide problem, *B. melitensis* is reported from most of the Near East and North African countries [3]. *B. melitensis* is transmitted exclusively by the sheep and goat population but constitutes the most serious zoonotic hazard in all the Near Eastern countries, with hundreds or thousands of human cases each year. Sheep and goat dairy products, mainly yoghurts and fresh cheeses, are important traditional dishes in Arab cuisine. As public awareness of the risk of disease transmission from animals to man is lacking and public health education is not carried out throughout rural communities, farmers keep on breeding brucellosis-affected animals, the milk of which is transformed, generally on the farm, into products without any heat treatment to prevent the transmission of brucellae and other pathogens to the consumer [4]. National health control of domestic dairy manufacturing is practically impossible. As *B. melitensis* has become endemic to the Near East, much of its small ruminant population has become partially immune. Its presence within the

flock does not cause substantial abortion waves and therefore has almost no economic implications for the farmers themselves, who are not aware of the link between the health status of their own animals and disease in their families and community. Consequently, it is difficult to gain the farming community's cooperation in any vaccination and/or eradication campaigns against *B. melitensis* on behalf of the regions' governments. Human brucellosis (Malta fever) is prevalent in most Near Eastern countries and will remain so unless a well-coordinated scheme of eradication, vaccination and health education takes place, either at single government level or, preferably, as intergovernmental cooperation throughout the region.

B. melitensis infection is also reported in North Africa. However, accurate and updated information on the prevalence of this infection in sheep and goats and its public health significance is scarce [5, 6]. The disease seems to be more prevalent in Algeria and Libya than in Tunisia or Morocco. In 1990, the national incidence of human *Brucella melitensis* infection in Algeria was estimated at 0.36 per 100 000 inhabitants (1.68 per 100 000 inhabitants for the population at risk) [7]. In Morocco, *B. melitensis* infection in sheep seems to be confined to the eastern part of the country along the Algerian border, with an estimated prevalence of 2 per cent among herds. The disease is subjected to a control programme based on vaccination with Rev1 vaccine (see Chapter 20).

GASTROINTESTINAL PARASITES

Many of the grazing areas are located at the 'desert threshold', i.e. in areas where the stocking rate may vary considerably between one year and another, according to the amount of rainfall, which determines growth of vegetation. Drought is a common phenomenon and, in years of drought, the nutritive value of many pastures decreases considerably towards the end of the summer. As it is impossible to respond to the drought by reducing stocking rates, sheep and goats become undernourished from July–August to December–January. The population of worm larvae, which had been maintained latent in the mucosal wall of the digestive system since the previous winter, becomes active and produces severe clinical disease. Instead of the 'spring rise' of faecal egg shedding known in other countries, the Near East experiences an 'autumn rise' which, unlike the European situation, has nothing to do with the gestational and lactational status of the animals.

ECTOPARASITES

The permanent movement of animals into and from flocks and herds without any health control makes transmission of lice and mites very easy. Many breeders accept that their livestock will be permanently infested and do nothing to stop the fleece loss, skin lesions and intensive pruritus, as they know that a new introduction of ectoparasites may take place immediately after the end of the treatment. Sarcoptic or psoroptic mange is not always a reason for shepherds to avoid the purchase of animals that display the characteristic skin changes, not to mention the purchase of animals during the first stages of the infestation, before macroscopic changes are visible. While spraying or dipping whole flocks in insecticides seems difficult for poor rural communities in arid or semi-arid areas, the introduction of the avermectins provides breeders with an effective injectable preparation for the treatment of their livestock against ectoparasites.

The cat flea, *Ctenocephalides felis*, adapted itself during the early 1980s to sheep, goats and cattle in Israel. Since this adaptation, flea infestation of sheep and goats has become the most devastating of all ectoparasitisms in the country [8]. The damage inflicted by fleas to the adult animal population is generally limited. They suffer moderate to severe anaemia due to the bloodsucking activity of the parasites, intensive pruritus and serious hair loss as the result of the scratching, which is due to the mechanical stimulation of the animals' skin and the eventual allergy that may develop to flea excreta on the skin. The end result is decreased milk and meat production.

The fleas' activity, however, may be devastating to young kids, which may die as a direct consequence of anaemia or may develop allergic dermatitis. Young lambs are less likely to die, but they become severely anaemic and therefore much more sensitive to any secondary infection, mainly to *Mannheimia haemolytica* and/or to coccidiosis, with resulting high morbidity and mortality rates.

The northern parts of the Near East are the southernmost breeding area for the *Ixodes ricinus* tick. Outbreaks of tick-paralysis, due to a toxin secreted by the ixodid tick into the blood circulation of parasitized goats, are reported from time to time during the relatively cold and humid winter months.

Ticks generally occur only in flocks/herds that graze on the northern slopes of the hilly areas, as *I. ricinus* is unable to survive on the southern, sunny slopes. *Rhipicephalus bursa* is the tick species that transmits *Babesia ovis* to sheep and goats in the near east. As the tick becomes active at the beginning of spring, outbreaks of tick-borne fever in the region occur between March and July each year.

MYCOPLASMOSIS

As mentioned, traditional animal management and trade in the Near East favour the transmission of pathogens from neighbouring areas. Outbreaks of contagious caprine pleuropneumonia (CCPP), caused by *Mycoplasma capricolum* var. *capripneumoniae* [9] are reported often from some countries, generally as the result of the introduction of animals from East Africa or central Asia, although it is possible that limited CCPP-endemic zones exist in the Near East. CCPP has been reported from Saudi Arabia, from Lebanon and from Tunisia. Contagious agalactia, caused by *M. agalactiae*, prevails in the countries of the Near East as it does in other Mediterranean countries. The introduction of mycoplasmas into the sheep and goat populations is favoured by the fact that mycoplasma-affected animals may remain pathogen carriers long after they have stopped displaying any clinical sign of the disease.

OTHER DISEASES

Rabies in farm animals, and in humans, prevails in many parts of the Near East and North Africa as the result of the large stray-dog population in close cohabitation with the human, mainly rural, population. Efficient control measures to stop the spread of rabies are not used. Reports of rabid sheep and goats are published from time to time from all the Near Eastern and North African countries. It is safe to say that brucellosis, rabies and echinococcosis are present throughout the Near East and North Africa, and are the main zoonotic diseases. All three can be transmitted quite easily to humans as the result of the poor sanitary conditions and lack of knowledge of hygiene among both the rural and urban populations. Information is lacking as to some transmissible diseases that may be of concern to other countries producing small ruminants.

There are no data on the existence and/or incidence of scrapie, maedi-visna, caprine arthritis encephalitis (CAE) and pulmonary adenocarcinoma in most of the Near Eastern and North African countries. Only limited information is available concerning infectious abortion agents such as border disease virus, *Chlamydophila abortus*, *Coxiella burnetii*, *Salmonella abortus ovis* (which has been reported only from Lebanon and Morocco [10]), *Toxoplasma gondii* and others.

THE MAJOR PLAGUES AND THEIR DIAGNOSIS

This name is given to those diseases of small ruminants characterized by a sudden onset with high morbidity and mortality rates. Because of their great economic importance both directly to the rural communities and indirectly to the national economies of the Near Eastern and North African countries, they were included in former List 'A' of the Office International des Epizooties (OIE). These diseases are generally referred to as transboundary animal diseases (TADs), which are defined as those animal diseases that are of significant economic, trade and/or food security importance for a considerable number of countries; which can easily spread to other countries and reach epidemic proportions; and where control/management, including exclusion, requires cooperation between several countries.

The important TADs of sheep for the Middle East and North Africa are: Rift Valley fever (RVF), foot-and-mouth disease (FMD), sheep pox and goat pox, bluetongue (BT) and peste des petits ruminants (PPR). All share some common clinical signs, namely sudden onset in a large part of the population with high fever, anorexia and recumbence, and later, visible lesions on the face and high rate of lameness amongst the

affected animals. To the inexperienced eye, especially under the pressure of the emergency field situation, they may look alike, but it is important to know the clinical features that differentiate one disease from another.

Although most of these diseases are not endemic in the near east, their causative viruses are present in the surrounding areas of Africa and central Asia.

Rift Valley fever (RVF)

Rift Valley fever (see Chapter 62) is a mosquito-transmitted zoonotic disease, which affects humans, ruminants and camels. In sheep, it manifests itself with a sudden onset of abortion in a large proportion of the flock and is associated with a high mortality rate in lambs. RVF has been reported from Egypt, first in 1974 with further reports in 1993 and 1997, and in Yemen and Saudi Arabia in 2000 [11, 12]. The outbreaks are of concern to the neighbouring Near Eastern countries, as the pathogen may be carried to them, either by non-vertebrates or by vertebrate vectors.

Foot-and-mouth disease (FMD)

FMD (see Chapter 40) is generally mild in adult sheep. However, it can be severe and result in high mortality rates in lambs. Both in the Middle East and North Africa there have been epidemics which have resulted in heavy losses of the lamb crop, which die from myocardial necrosis.

Outbreaks occur in most of the Near East and North African countries periodically. Nevertheless, the epidemiology of FMD in the Middle East differs from that in North Africa mainly as a consequence of livestock trade patterns [13]. Although there are areas where FMD, especially serotypes O and A, are locally endemic, in general, epidemics of FMD in the Middle East can be linked to virus strains which are genetically similar to those known to circulate either in the south–central Asian axis or the Horn of Africa. Notable examples include incursion into the Middle East of exotic serotypes SAT-1, SAT-2 (both from Africa), serotype Asia 1, the Pan-Asian topotype of serotype O and various topotypes of serotype A (from south-central Asia).

There is no endemic FMD in North Africa, except possibly Egypt where Type O virus was considered to be endemic until recently. All outbreaks of FMD in Morocco, Algeria, Tunisia and Libya since the late 1970s could be generally linked to introductions through imported livestock commodities. In the past these could be associated with viruses from either South America or the Middle East or even south-eastern Europe. Recently, in 1999 and 2003 outbreaks of serotype O and serotype SAT-2, respectively, in Algeria and Libya could be linked to virus strains from south of the Sahara. This is probably a result of newly established trade links.

Sheep pox

This disease (see Chapter 43) is reported from all the Near Eastern and North African countries, except Egypt, and constitutes the major epidemic threat to the health of their sheep and goat populations. It is one of the most important and enzootic diseases of sheep in North Africa. Despite annual reports of a few outbreaks, the disease has been brought under control in Morocco and Tunisia by large-scale vaccination campaigns. Eradication of sheep pox requires a coordinated programme throughout North Africa and the Middle East.

Both the morbidity and mortality rate of pox disease are very high and an outbreak can ruin the economy of single breeders or even whole areas based on sheep and goat breeding. The disease is, generally, easy to recognize on the grounds of its clinical features, and does not have a very long incubation period, but permanent, uncontrolled movement of livestock amongst the flocks and herds perpetuates the endemic situation of poxvirus in the region.

Bluetongue

Cattle are the vertebrate hosts of bluetongue virus in nature (see Chapter 60). In the Middle East and North Africa, cattle seldom suffer clinical disease but are the reservoir from which *Culicoides imicola* midges acquire the virus, which is highly pathogenic to sheep. As the midges are not active during the winter months and their infective populations need some weeks to build up to a substantial threshold number, outbreaks of clinical BT always take place between July and

December. Clinical BT in the Near East almost always occurs only in imported, European breeds of sheep, as the indigenous Near Eastern 'Awassi' and other sheep breeds are naturally resistant to BT virus. There are also a few reports of clinical BT in imported goat breeds in the region.

The disease in imported sheep is characterized by inflammation of the mucous membranes of the mouth, nose and the coronary band of the foot, widespread haemorrhages and oedema.

Since 1997, BT has emerged in European Mediterranean countries and in North Africa. The disease caused by serotype 2 appeared in Algeria in 1999 and in Tunisia in 2000. Large outbreaks occurred in Morocco in the autumn of 2004, especially in the north-western part of the country. Serotype 4, genetically typed as identical to the one prevailing in Corsica, has been isolated from sheep in Morocco. A few weeks later the Iberian peninsula (Spain and Portugal) also became infected with the same serotype. Presently, there are four serotypes, i.e. serotypes 2, 4, 9 and 16, circulating in the countries of the Mediterranean basin [14].

Peste des petits ruminants (PPR)

This sheep and goat disease (see Chapter 61), which was first described in Côte d'Ivoire in west Africa in 1942, is endemic in several countries of tropical Africa and in south Asia. It has been introduced to the Middle East relatively recently, i.e. during the past 20 years (Table 64.1), and has never been described in North Africa, except Egypt.

Most cases of PPR are acute, with a sudden onset of fever that may last for 5–8 days before the animal either dies or begins to recover. The characteristic signs begin with a serous nasal discharge that becomes mucopurulent. The nasal discharge may remain mild or may progress to a severe catarrhal exudate that crusts over, blocking the nostrils and causing respiratory distress. The nasal mucous membranes may develop small areas of necrosis. The conjunctiva may be congested, with crusting at the medial ocular canthus and profuse catarrhal conjunctivitis with matted eyelids is often seen. Necrotic stomatitis is also common and can be severe. As the disease progresses, a characteristic foul smell exudes from the mouth. In severe cases diarrhoea commonly appears about 2–3 days after the onset of fever. Severely affected cases show difficult and noisy breathing marked by extension of the head and neck, dilation of the nostrils, protrusion of the tongue and soft painful coughs – the obvious signs of pneumonia [15].

The Food and Agriculture Organization's RADISCON programme has summarized the spread of PPR in the Middle East as follows [16]:

- In Jordan, a new disease was seen in 1989 in flocks of sheep and at the same time in flocks on both the Syrian and Saudi Arabian borders in the east of the country. In December 1990 some 700 serum samples were evaluated and 23 per cent were found to be PPR-positive. Since 1989–90 there does not seem to have been any PPR epidemic at field level, although problems have occurred in imported Australian sheep kept in feed lots. In 1993, the reporting system recorded a high incidence of nasal discharge, coughing and diarrhoea in both sheep and goats, but it cannot be assumed that this was due to PPR.
- In Syria, a retrospective serological study undertaken in 1996, using sera collected in the winter of 1991–2, demonstrated antibodies in 96 per cent of the flocks sampled and a prevalence rate of 50 per cent in the ewes from which blood was taken. On the basis of these reports there can be

Table 64.1: Peste des petits ruminants in the Middle East [3]

Country	Year reported
Egypt	1989
Iran	1994
Iraq	1998
Israel	1993
Jordan	1994
Kuwait	1991
Lebanon	1995
Palestinian Autonomous Territories	1997
Saudi Arabia	1996
Syria	Serological evidence 1991–2; but no clinical report
Turkey	1999
United Arab Emirates	1996
Yemen	Suspected in 1997 and confirmed in 2000

little doubt that PPR has occurred in Syria in the recent past, although there is no reported incidence of PPR by the Directorate of Animal Health and no clinical samples have been referred for diagnosis. Sheep and goats are not vaccinated with rinderpest vaccine.

- In Turkey, PPR virus type N4, dated 1996, has been sequenced. The disease-reporting system has no information relating to any outbreak of PPR in Turkey. However, the same virus type was found in Bangladesh in 1993, in India in 1994–6, in Nepal in 1995, in Pakistan in 1994, in Iran in 1994, in Saudi Arabia in 1994, and in Israel in 1993, 1994 and 1995. From September 1997 a national research programme has been operating to determine the distribution of PPR in Turkey based on serosurveillance. Furthermore, as of October 1997, PPR has been made a notifiable disease.

In conclusion, PPR may be endemic in most of the above-mentioned countries but is not being reported or investigated. To undertake the necessary investigations on PPR, particular attention will be needed to ensure clear differentiation of PPR especially from CCPP and pasteurellosis. None of the affected countries has developed sufficient understanding of the epidemiology to be able to advance sound control policies.

There has undoubtedly been spread of PPR from the Horn of Africa to the southern Arabian peninsula, as the recorded lineage (PPR Lineage 3) appears to be common to the two regions. Trade in livestock has undoubtedly fostered this spread. A second lineage (PPR Lineage 4) is common between Bangladesh, India, Pakistan, Iran, Israel and Turkey. This virus appears to have spread within the Middle East in the past decade, probably through a combination of nomadic movements by small ruminants and also by trade.

DIFFERENTIAL DIAGNOSIS

Because of their different ecological and epidemiological features, each of the small ruminant major plagues requires a distinct prophylactic approach and therefore accurate and prompt diagnosis is crucial whenever an outbreak occurs. However, their shared clinical signs may make differential diagnosis quite difficult, especially during the early phase of an outbreak when clinical signs are not yet very clear. The following guide, based on clinical and pathological features, may assist in reaching a provisional differential diagnosis before specimens are sent for laboratory analysis.

The guide excludes Rift Valley fever, which has distinct clinical features, but includes orf (Chapter 42), a disease of low mortality but with a high morbidity rate and clinical signs similar to those of the more fatal plagues. More detailed accounts of each of these diseases are given elsewhere in this book. Fever may occur in any of the plagues but is not normal in orf. Lameness is not a typical feature of pox or PPR but can be predominant in FMD, BT and in orf when the feet are involved. However, the distribution of lesions together with their character is a key in differential diagnosis (Table 64.2).

Table 64.2: Lesion type and distribution in the major plagues of sheep and goats

		Head lesions						
Disease	Lesion type	Lips	Tongue	Gums	Nose	Skin	Foot lesions	Chapter
FMD	Vesicular	–	++	+	–	–	+++	40
BT	Haemorrhagic	+++*	+	++	++	+	+++†	60
Pox	Pustular	–	–	+	–	+++‡	–	43
PPR	Erosive, necrotic	+	+++	+++§	++	+	–	61
Orf	Proliferative	+++	+++	+++	++	–	+	42

* See Figure 64.1. † See Figure 64.2. ‡ See Figure 64.3. § See Figure 64.4.

Figure 64.1: Bluetongue. Swollen, oedematous, hyperaemic lips.

Figure 64.2: Bluetongue. Haemorrhagic line on the coronary band.

FMD lesions are vesicular, stand above normal tissue and are filled with limpid fluid (Figure 64.5).

BT lesions are typically small haemorrhages beneath the mucosae or in the depth of the skin. Pox lesions are reddish to dark pustules about 1 cm in diameter, which protrude from the skin. They are easy to see on the udder, tail, perineum and abdomen. Lesions of PPR occur mainly on the digestive tract mucosae, the superficial necrotic layers sloughing to leave a raw surface

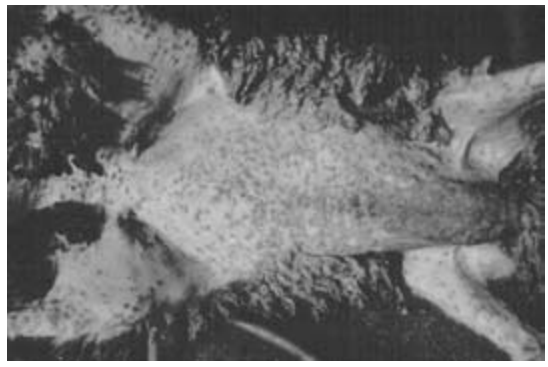

Figure 64.3: Pox. Extensive lesions on the skin surface.

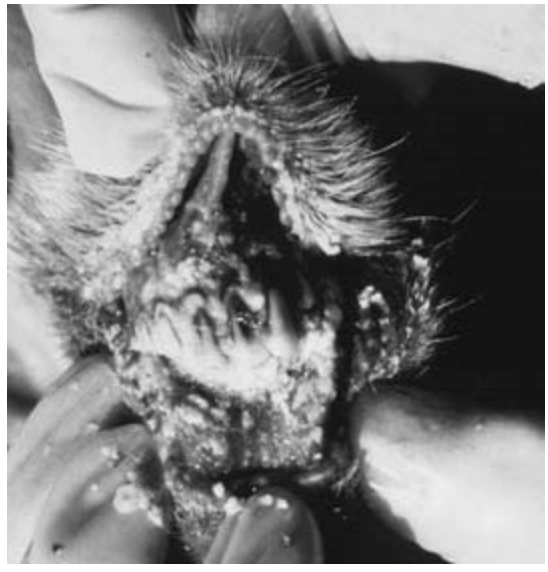

Figure 64.4: Peste des petits ruminants. Diffuse necrotic areas on the gums.

prone to secondary bacterial infection, which smells. The proliferative lesions of orf on the skin or buccal mucosa can also be invaded by bacteria and acquire an unpleasant smell.

Necropsy findings also may aid differential diagnosis. The main post-mortem findings in FMD are white foci of myodegeneration seen from the outside and in the depth of the heart of young animals (Figure 64.6). Quite rarely, vesicular changes may be seen on the rumen mucosa, mainly on the rumen pillars. In BT, a pathognomonic change almost always present is red-

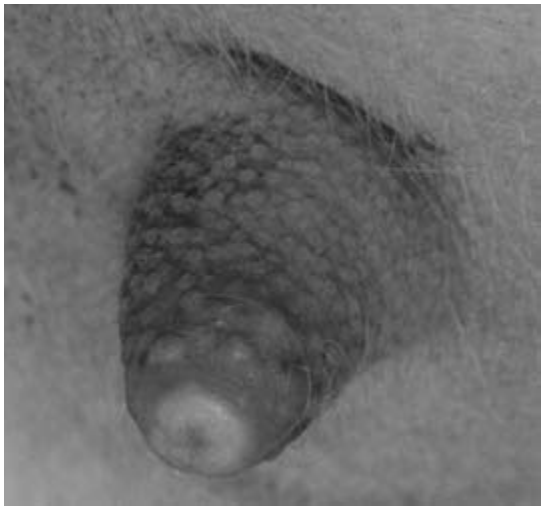

Figure 64.5: Foot-and-mouth disease. Vesicles around teat opening of a sheep.

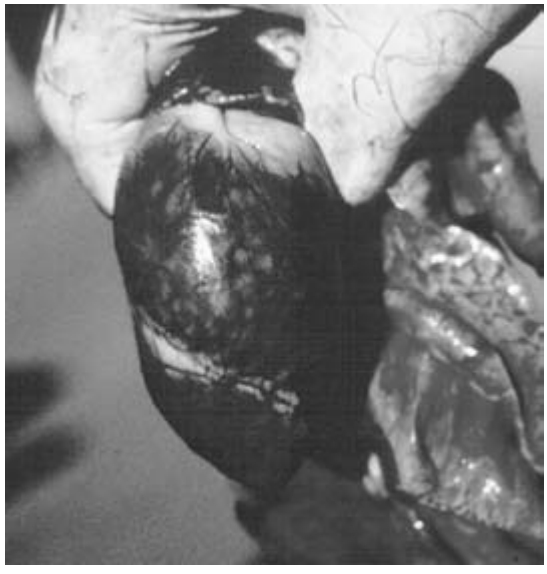

Figure 64.6: Foot-and-mouth disease. Typical foci of degeneration on the heart of a lamb.

dish brown petechiation in the depth of the whitish intima of the pulmonary artery (Figure 64.7), and can be seen when the large vessel is incised upon its exit from the right ventricle. In pox, typical pustules often can be seen on and in the depth of the lung parenchyma. Less often, such changes may be observed in the kidneys, liver, rumen mucosa (Figure 64.8) and

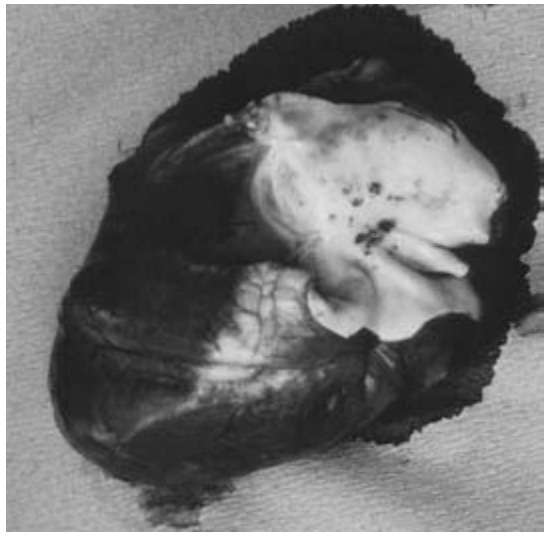

Figure 64.7: Bluetongue. Typical haemorrhages under the intima of the pulmonary artery.

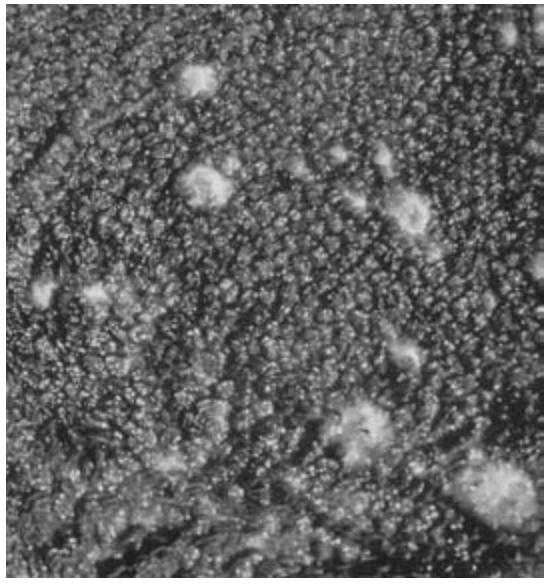

Figure 64.8: Pox. Typical pustules on the internal surface of the rumen.

on placental cotyledons. PPR is characterized by a very acute, necrotic gastroenteritis along the digestive tract (Figure 64.9). Large surfaces of mucosal tissue slough and fill the gut lumen with necrotic–haemorrhagic material. There are no internal changes in orf.

Figure 64.9: Peste des petits ruminants. Acute necrotic enteritis.

Because of their crucial epidemiological and economic importance, it is imperative to confirm the field diagnosis of the major plagues by laboratory tests, preferably by virus isolation and typing (either in cell cultures or by using more sophisticated, molecular techniques such as PCR). For FMD, good specimens for virus isolation are the liquid contents of the vesicles, aspirated by a sterile syringe before they rupture, or the wall of the same vesicles once ruptured. The specimen of choice, however, is throat scrapings of diseased animals, obtained by 'probang cup', a cup-like metallic device with cutting edges, that may accumulate infected material once inserted into the throat and pulled back and forth several times.

In BT, sterile heparinized or citrated blood samples can be obtained from diseased animals, from which the virus can be isolated, while from dead animals, spleen is the specimen of choice. In pox, pustule biopsies can be cut (under local anaesthesia) from the skin of diseased animals. Such specimens are suitable not only for virus isolation but also for electron-microscope observation of the virus particles within the cells. Virus isolation in PPR is very difficult, as the virus is very labile in environmental conditions. Only very fresh digestive tract tissue must be sent cooled and as fast as possible.

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65

Southern Africa

G.F. Bath

The region can be divided into the northern countries of Angola, Zambia, Malawi, Mozambique and Zimbabwe, with a more tropical climate and thus less suited to sheep farming, and the southern countries of Lesotho, Namibia, Botswana, Swaziland and South Africa, which are more temperate and better suited to sheep farming. Livestock numbers are therefore greater, farming is more developed, and more is known about sheep problems in the latter group of countries.

Standards of sheep welfare (Chapters 2–6) vary considerably in the region. Some countries have reasonably good legislation in place, while others do not, and surveillance and enforcement are often not satisfactory.

This overview adopts the sequence of groups of diseases followed in the book, but it should be noted that southern Africa has a multitude of parasites and plant poisons to which sheep are vulnerable.

REPRODUCTIVE DISEASES

Apart from most of the reproductive problems outlined elsewhere in this book, predation of young lambs (and even adults) is a special problem [1]. Near towns, domestic dogs are usually responsible, while elsewhere the jackal and caracal are most commonly involved. Several other wildlife species, including baboons and raptors, have been incriminated but are far less important. A number of control measures have been developed, which vary in effectiveness. Exposure can be a major lamb mortality factor in winter when cold fronts sweep across the southern parts. Ram infertility due to *Brucella ovis* is still a major problem [1–3] in spite of the availability of effective vaccines, while infertility caused by *Actinobacillus seminis* has become quite rare. Mastitis is often encountered, caused chiefly by *Mannheimia haemolytica* but also by *Pasteurella multocida* and *Staphylococcus aureus* [1, 2]. While several vaccines for mastitis are available, they give only partial protection, and farmers are advised to concentrate more on sound management practices to control the syndrome.

Reproductive losses caused by toxoplasmosis are generally unimportant while chlamydial abortion can be significant and many farmers vaccinate for the latter disease. Border disease (BD) is occasionally diagnosed, while the status of *Salmonella* abortion is uncertain. *Campylobacter* and *Coxiella* organisms are identified from time to time as abortion agents, while

B. melitensis is present only in the northern countries. Ulcerative balanoposthitis and vulvitis constitutes a major problem in Dorper sheep. The underlying cause is thought to be a mycoplasma [2] but genetic predisposition probably accounts for its clinical significance chiefly in Dorpers. Posthitis occurs frequently as a result of high protein diets and subsequent bacterial degradation of urinary urea in the sheath of male animals [1, 2, 4].

DISEASES OF THE ALIMENTARY SYSTEM

Virtually all the diseases of the alimentary system mentioned elsewhere in this book occur, but braxy, black disease, bacillary haemoglobinuria and watery mouth have not been diagnosed. Infection of the lips and cheeks by *Actinobacillus lignieresii* as a result of eating thorny material, particularly 'spineless' prickly pear leaves, can be a particular regional problem [1, 2]. Clostridial infections (Chapter 23) are of major importance; overwhelmingly, pulpy kidney disease (*Cl. perfringens* type D) is implicated but occasionally other types may be involved [2]. Abomasal bloat is seen in young lambs raised on surrogate milk [1].

Johne's disease (Chapter 24) is an emerging problem which appears to be spreading inexorably. Redgut, as described in New Zealand, has been important on lush leguminous pastures, but adoption of the appropriate control measures has minimized its impact [3]. Intussusception of the small intestine associated with *Oesphagostomum* infection was once a problem, but modern anthelmintics have reduced this parasite to minor importance.

By far the most important internal parasite in the region is *Haemonchus contortus*, and rampant resistance to all the anthelmintic groups as well as intensification of farming on pastures has exacerbated the problem [5]. Development of the FAMACHA© control system in South Africa has dramatically changed the situation for the better [5]. Ostertagiosis (*Teladorsagia and Ostertagia* spp.) is the major worm problem of the winter rainfall region (Western Cape), while *Trichostrongylus* infection is quite common over a wider area. In the drier Karoo areas, *Nematodirus* worms are significant, while in the Sandveld, *Gaigeria* and *Strongyloides* can cause problems. *Trichuris* and also *Chabertia* are rarely responsible for deaths. *Moniezia* spp. are very common and are regarded by some as economically important.

Liver fluke is a threat where marshes occur. To the south, *Fasciola hepatica* is the main parasite, but northwards the giant liver fluke (*F. gigantica*) is the predominant species. Under similar epidemiological conditions paramphistomiasis (*Calicophoron* spp.) can be an important problem on individual farms. Clinically, the disease features extremely watery diarrhoea, emaciation and deaths. The pathology is characterized by a swollen, oedematous and hyperaemic small intestine and an oedematous mesentery. Migrating immature flukes can be found in large numbers in the small intestine.

Another disease frequently seen in the region is rumen acidosis, which usually arises as a result of feedlot systems, bad ration formulation or grazing sheep on grain crop residues, particularly maize and wheat. It is characterized by depression, dehydration, apparent bloat due to the osmotic effects of rumen lactic acidosis, sour smelling greyish-yellow diarrhoea and laminitis [1].

DISEASES OF THE RESPIRATORY SYSTEM

Acute viral infections may frequently be the forerunners of pulmonary pasteurellosis, although their role is often overlooked and seldom confirmed. Pulmonary adenocarcinoma (Jaagsiekte) is an important regional problem that is difficult to control and, short of destocking, almost impossible to eradicate [1, 2]. Among Karakul sheep, it has been a particular problem owing to the practice of bringing ewes into close confinement over the lambing period, which facilitates transmission. Maedi-visna was recognized under the local name of Graaff–Reinet disease but is seldom diagnosed clinically. It appears to be a milder strain of the virus than described elsewhere, and sheep may suffer only subclinical mastitis or focal pneumonia with minimal or no production effects [2]. Pneumonic pasteurellosis is of major importance and is commonly seen in feedlots, intensive housing and under conditions of stress. Dust often appears to be an important predisposing factor. Although most isolations reveal various *Mannheimia haemolytica* types, *P. multocida* is sometimes involved [2]. Parasitic bronchitis is rare while nasal bots (*Oestrus ovis*) are a

common problem. Pulmonary abscesses, caused mainly by *Corynebacterium pseudotuberculosis*, are quite frequent and, if severe enough, cause a clinical disease known locally as 'harsslagsiekte ('pluck disease'), characterized by emaciation, anaemia, tachypnoea and occasionally mild icterus [1, 2].

DISEASES OF THE NERVOUS SYSTEM

Scrapie is not found in the region, although it was introduced and then promptly eradicated in South Africa in the 1960s. Nervous manifestations of maedi-visna have not been seen in sheep. Louping-ill is absent, while listeriosis is rare. Apart from daft lamb disease, most of the other diseases described in Chapter 38 are present. Swayback and polioencephalomalacia may become particularly important in certain areas. Heartwater is probably the single most important disease to the north [1, 2], and prevents sheep farming on any scale in many areas.

Tetanus is a threat especially if sheep farmers use the rubber ring method of castration or docking, and other techniques are thus preferred. Vaccination against tetanus is effective if administered to the ewes. Rabies is very rare in sheep, probably owing to their tendency to flee from, rather than confront, a rabid animal. Botulism can reach serious proportions, either where broiler litter is used as a feed component, or in animals suffering from pica, or if water is contaminated by animals (e.g. baboons) that fall into water reservoirs and drown. A number of plant and other toxins cause neurological signs, and are listed in the poisons section of this chapter. The Karoo paralysis tick (*Ixodes rubicundus*), of major importance in the south, is a three-host tick which causes paralysis when adults attach to sheep, goats and other ungulates in autumn and winter. *Rhipicephalus evertsi evertsi* and *R. simus* can also cause paralysis in lambs, usually in spring.

DISEASES OF THE FEET AND LEGS

By far the most important syndrome in the region is foot abscess. Apart from the predisposing factors mentioned in Chapter 39, an important avenue of infection in Africa is infestation by ticks with long mouth parts, especially *Amblyomma* and *Hyalomma* species but also *Rhipicephalus* species [1–3]. These ticks can also cause lameness without bacterial infection, presumably due to constituents in the saliva. Foot-rot is only important locally, while the other foot diseases occur to a varying degree. Shelly toe is common and can sometimes lead to toe abscess, while strawberry foot is much less of a problem. Post-dipping lameness is sometimes encountered, if dipping follows shearing too quickly and bacteriostatic agents are not included in the dip. Other forms of joint infection are also seen occasionally. Foot-and-mouth disease has been largely or completely eradicated in the southern countries, but continues to be a problem in the north [2].

DISEASES OF THE SKIN, WOOL AND LIPS

Orf is a problem throughout the region, whereas sheep pox is absent. Caseous lymphadenitis assumes major proportions, especially in woolled sheep. Culling sheep with excessive skin folds has considerably reduced skin nicking during shearing, and thus infection. Emphasis is placed more on management than vaccination to achieve control [1, 2]. Bolo disease [1, 2] is a local problem of wool sheep characterized by circumscribed fleece patches that are darker and shorter. On opening the fleece, these patches appear greyish-white to yellow, and sticky. Fleece-rot caused principally by *Pseudomonas* spp. is seen mainly in Merinos during seasons of above-average rainfall. Lumpy wool is one of the commonest skin diseases, more often seen in wool sheep. Another important skin disease, seen mainly in non-wool sheep, is ringworm [1, 2], which is characterized by itchy bare patches, found especially on the head and legs. Necrotic staphylococcal dermatitis has been diagnosed recently for the first time.

Sheep scab is a major threat throughout the region, in spite of attempts at control or eradication [1]. Itch mite (*Psorergates ovis*) and *Chorioptes ovis* were prevalent in the past. Both conditions may be confused with sheep scab. The major ectoparasite in the region is without doubt the blowfly *Lucilia cuprina*; other species are less important. Blackflies

(*Simulium* spp.) can be a problem along major rivers where irrigation projects result in regular and reliable water flow, whereas sandflies (*Leptoconops* spp.) are encountered in areas with dry, sandy, saline soils [1]. Blue and red lice (Chapter 48) are a factor, particularly in winter [1], while *Rhipicephalus glabroscutatem* [1, 2], and *Hyalomma* species (bont-legged ticks) can cause lameness and lead to foot abscess, while *Amblyomma* species (bont ticks) cause abscesses and transmit heartwater. Sand tampans (*Ornithodoros moubata*) are troublesome in the arid western regions, where they attack livestock resting under trees. The larvae of *Gedoelstia hassleri*, normally parasitic in the sinuses of blue wildebeest, may be found in sheep and cause 'uitpeuloogsiekte' (bulging eye disease) due to aberrant migration. The old world screw worm, *Chrysomia bezziana*, may cause strike in wounds.

Photosensitization is of major importance in the south. Wilted *Tribulus terrestris* is the major cause, while *Panicum maximum* will cause indistinguishable lesions. Other causes include the fungi *Pithomyces chartarum* on grasses, *Phomopsis leptostromiforme* on lupins, and the plants *Lasiospermum bipinnatum*, *Asaemia axillaris* and *Athanasia trifurcata*. Histopathology, area and season are important in distinguishing the causes [3, 4, 6].

Infectious keratoconjunctivitis is widespread and troublesome, and seems to be associated with several factors including dust, eye-frequenting moths and flies. Organisms that have been incriminated include *Moraxella, Mycoplasma* and *Chlamydophila* species [2]. Entropion is a common congenital condition seen especially in Merinos [1], while 'vrotvel' (dermal asthenia) is seen as a recessive gene problem in breeds originating from Dorsets, e.g. the Dorper [1]. Trypanosomiosis transmitted by tsetse flies (*Glossina* spp.) can be important in the north [2]. *Anaplasma ovis*, transmitted by ticks, is seldom diagnosed, while leishmaniosis (transmitted by *Phlebotomus* spp.) has been confirmed on only a few occasions [2].

METABOLIC DISORDERS

Pregnancy toxaemia is currently less frequently seen, as farmers have come to appreciate the value of adequate feeding in the last trimester. However, milk fever (hypocalcaemia) occurs often but can easily be misdiagnosed [1, 3]. 'Bowie' is seen mainly in fast-growing ram weaners on high phosphorus pastures or diets [1, 3]. Trace element deficiencies are most troublesome in the leached, sandy soils of the extreme south-west of the region [1]; cobalt deficiency has been diagnosed only in this area, where it is an important problem. Selenium deficiencies are more widespread and associated with lucerne grown on long-term irrigated and fertilized soils. Copper deficiencies are recorded mainly from coastal areas, while copper toxicity on natural pasture, known as enzootic icterus, is a problem in the arid Karoo [1, 4, 6]. Iodine deficiencies are seen with the feeding of *Brassica* spp. or *Cynodon* spp. (stargrass) that has been heavily fertilized [1, 4]. Zinc-responsive conditions have also been described. Urolithiasis is encountered mainly as a result of excessive phosphorus in the feed, but may be due to other factors [1, 3, 4]. Manganese deficiency may cause infertility.

POISONS

Plant poisoning is one of the special risks in the region, and many of the problems are not found elsewhere [4, 6]. Apart from the plants causing secondary photosensitization (see above), seneciosis and *Hertia pallens* ('Springbokbos') can cause icterus. The latter causes cirrhotic lesions and thickened bile ducts. A peculiar problem of the southern regions is 'waterpens', caused by *Galenia africana*, characterized by severe ascites and cirrhosis. Another plant of the arid regions, *Salsola tuberculatiformis* may cause prolonged gestation if consumed in excess, while *S. barbata* is suspected of causing a nephrosis/oedema syndrome [4, 6].

Gaigeria spp. cause 'vermeersiekte' (vomiting disease) due to damage to the oesophageal muscles and can be financially devastating. Krimpsiekte, caused by ingestion of *Tylecodon* and *Cotyledon* plants, is one of the oldest known intoxications in the region and can cause major financial losses. It is characterized by acute or chronic paralysis. 'Bitterbos' (*Chrysocoma tenuifolia*) causes alopecia (kaalsiekte), diarrhoea (lakseersiekte), ataxia (valsiekte) and phytobezoariasis. The latter problem has also been associated with Bushman grass (*Stipagrostis* spp.), *Eriocephalus* spp., *Gnidia polycephala* and seradella [1, 4]. Grass and other awns can cause damage to the skin and natural openings [4].

Thesium spp., *Dichapetalum cymosum* and various 'gousiekte' plants [4, 6] as well as *Dipcadi glaucum* and several members of the Iridaceae (tulips) are important causes of sudden death resulting from heart failure. *Helichrysum argyrosphaerum* causes amaurosis and *Trachyandra* spp. cause progressive paralysis, while occasional outbreaks of *Phalaris* staggers may be encountered. Annual ryegrass toxicity has been largely overcome by grazing management, but diplodiosis (caused by the ingestion of *Diplodia maydis* fungus-infected maize), characterized by reversible paralysis and stillbirths or non-viable lambs, can still be significant as maize stover remains a very important element of sheep feeding in the region. The important oxalate-containing plants include *Opuntia* spp. ('prickly pears') and *Agave americana* (century plant), both introduced as drought-resistant fodder banks. Prussic acid and nitrate are both important poisonings and are found in a range of indigenous plants as well as heavily fertilized pastures [4]. *Cynodon*, *Sorghum*, *Acacia* spp. as well as several indigenous daisy species are the most important sources of prussic acid, while fertilizer, water, pigweed, brassicas, sorghums, oats, rye, wheat and maize are the more usual sources of nitrate poisoning [4].

Urea poisoning is common as a result of its misuse as a source of non-protein nitrogen. Organophosphate poisoning is another result of the misuse of agricultural products. Ionophores, used to control coccidiosis and improve feed efficiency, can cause loss in production, disease and death if fed in excess. They may be included in the ration intentionally or by inclusion of chicken litter. The signs are anorexia, ataxia and diarrhoea, and the lesions comprise oedema, haemorrhage and muscle necrosis [1]. Lead and arsenic poisoning have become very rare since the withdrawal of potentially dangerous products.

TUMOURS

The only tumour of economic significance in the region is squamous cell carcinoma, seen almost exclusively on exposed unpigmented skin on the face, ears, perineum and udder. Selection for animals with pigmented eyelids, perineums and elsewhere has significantly reduced the incidence, together with correct tail-docking practices [1]. Cornu cutaneum, a horny outgrowth of the skin presumed to be due to prolonged irritation, is a metaplastic condition that is also frequently seen [1, 2].

OTHER DISEASES

Bluetongue is the second most important infectious disease of sheep after enterotoxaemia, and is especially dangerous to imported sheep [1, 2]. Rift Valley fever can cause severe losses when it breaks out in epizootics following abnormally high rainfall [1, 2]. Anthrax poses an important threat if vaccination is not carried out in affected areas, due to the very long persistence of viable spores [2]. Schistosomiosis may be found in the warmer, north-eastern regions suitable for the intermediate host. It causes severe diarrhoea, debility and emaciation in sheep, and the post-mortem picture is dominated by greyish discoloration of the lungs and liver. Vitamin A deficiency is encountered only after prolonged droughts, as sheep can store retinol for up to 6 months in the liver. Signs of deficiency include reproductive failure, digestive and respiratory disorders, and increased susceptibility to infections. For this reason, it may be overlooked and the secondary infection diagnosed. While lightning strike and snakebite are quite rare, the veterinarian has to be aware of these possibilities, and how to diagnose them, when investigating cases of sudden death. Although not a veterinary matter, theft of livestock is a major problem in most countries in the region and puts severe pressure on the viability of sheep farming in many areas, particularly those close to towns.

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66

Australia

J. Plant

In 2004–5, there were 107 million sheep shorn in Australia, producing 475 million kg of greasy wool, 340 000 tonnes of lamb and 220 000 tonnes of mutton. Wool exports were valued at $A2.5 billion approximately (£1 billion), with China now being the major market for Australian wool. In 2003–4, domestic consumer expenditure on lamb was $A1.7 billion with another $A575 million in export lamb. Figures for mutton were $A311 million in the domestic market and $A367 million in export markets. In the same period, 3.8 million live sheep, worth $A268 million were exported, mainly to the Middle East. The number of farms where sheep are a significant source of income has been declining in recent years, largely because of an extended drought and the reduced income from wool. In June 2003, there were 47 200 farms where sheep made a significant contribution to farm income. In Australia, there are no subsidies for sheep or wool producers.

Traditionally, the sheep industry in Australia has been based on the income from wool, but in recent years there has been an increasing emphasis on lamb, mutton and live sheep exports. A number of new breeds have been introduced including the Dorper, Dohne Merino and the South African mutton Merino. These have become popular because of their fecundity and the increased value of the lambs with a higher body weight at a younger age. There have been several attempts to establish sheep dairying for cheese production. The Awassi breed was imported from Israel, but the numbers of sheep being milked is small.

The environment ranges from highly productive improved clover and lucerne pastures in the higher rainfall areas, running five or more breeding ewes per hectare, to natural pastures in the semi-arid areas running one sheep to 10 hectares. Depending on the location and the pastures, ewes are normally joined for 6–8 weeks any time from October until April, and lamb under extensive conditions. There is no intensive supervision of lambing except in some stud flocks.

MAJOR DISEASES

Because of its island situation and its strict attention to quarantine, Australia has kept out many diseases seen in other countries. It is free of sheep pox, jaagsiekte, maedi-visna, *Brucella melitensis*, ovine enzootic abortion and foot-and-mouth disease. Sheep scab was eradicated in the late 1890s. Scrapie was introduced in imported sheep in the 1950s, but all introductions and progeny were slaughtered and the disease has not been seen since. The Australian sheep flock has a high proportion of sheep with the susceptible scrapie genotypes. In terms of economic importance, the major sheep diseases include footrot, internal parasites (including liver fluke), external parasites (including lice and blowfly), ovine Johne's disease (OJD), plant poisoning and perinatal mortality.

In 1994, it was estimated that internal parasites cost the sheep industry $A222 million per annum, lice $A169 million and blowfly $A161 million [1]. A significant part of this cost was in control and prevention programmes ($A111 million for sheep lice and $A130 million for sheep blowfly).

Other diseases of concern, controlled by vaccination programmes, include clostridial diseases, caseous lymphadenitis and contagious pustular dermatitis (orf).

DISEASE-CONTROL PROGRAMMES

The first major sheep disease-control programme was developed for sheep scab, culminating in the eradication of this disease from Australia in the 1890s. However, other programmes that have been supported by government and based on regulations have not been successful. They were perceived to be a government rather than an industry problem. Examples include attempts to control sheep lice and foot-rot prior to the 1980s. More recently, attempts to control the spread of OJD by regulation have been unsuccessful.

Since the 1980s, there has been a major change in direction of industry-based control programmes for the significant diseases. These began in 1961 with the Ovine Brucellosis Accredited-free Flock Scheme, followed by the Wormkill Programme in 1981, a Footrot Advisory Programme in 1984, the Foot-rot Strategic Plan and the Licekill Programme in 1988, Flywise in 1990 and the Ovine Johne's Disease Strategic Plan in 1996. All of these programmes have recognized the need for technology transfer of existing and new information. In the past, many research findings were not being applied in the field. The emphasis is now on industry owned and managed disease-control programmes [2], to improve the transfer of information from the laboratory to the field.

The basis of these programmes has been the coordination of the activities of all the key players, including government, farming organizations, chemical manufacturers and retailers, veterinarians, and other advisory groups and research workers. Packages have been developed that include existing technologies that can be applied in the field, research deficiencies have been identified and the programmes are continually monitored and adapted as new information becomes available. Where disease-control and eradication programmes are put in place by industry, they are supported by regulations that allow action to be taken against producers who, by their actions or negligence, put others at risk. These programmes are being aided by an increasing awareness of the importance of quality assurance on farms and the demands of the market place for products that meet specifications and are free of chemical residues.

The major disease-control programmes in Australia are described briefly below.

FOOT-ROT CONTROL

Virulent foot-rot is a severe debilitating disease, which causes losses in wool production, affects ewe fertility and growth rates, increases susceptibility to fly strike and reduces the value of sale sheep. Programmes for its control and eradication are in place in Western Australia and New South Wales (NSW).

In NSW, a Foot-rot Strategic Plan was developed in 1988 with the aim of having all of NSW in Control or Protected Areas for virulent strains of *Dichelobacter nodosus* by 2000 [3].

Benign foot-rot is not considered a major cause of economic loss, but it is of significance on those farms where the environment is not suitable for the full expression of the disease, and where the differential diagnosis of benign and virulent foot-rot on clinical examination may be difficult. The success of the programme in NSW is summarized in Table 66.1.

The increase in the number of infected flocks between 1988 and 1991 was a result of the advisory programme, encouraging sheep owners to seek advice on foot-rot control and eradication.

The development of the disease is influenced by moisture, temperature, and by pasture type and density. When requirements for moisture are not met, virulent foot-rot may not fully express itself but may progress only to separation of the skin horn junction in the interdigital space with little under-running of the soft horn on the inside of the claw. Many of these cases will resolve and may be confused with benign foot-rot. As a guide, any under-running of the hard or soft horn associated with *D. nodosus* infection is viewed with suspicion, and further investigations are undertaken to determine whether it is due to benign or virulent foot-rot.

Table 66.1: Foot-rot statistics in New South Wales, 1988–2005

	1988	1991	1999	2001	2003	2005
Total flocks in NSW*	45 399	41 244	32 378	29 631	25 158	23 651
Total infected flocks	3 820	6 179	588	446	236	129
Quarantine releases†					166	109
New quarantines†‡					64	57

* Figures from 1999 relate to flocks with more than 50 sheep.
† Relate to the previous 12 months.
‡ All infected properties quarantined at this stage.

Control and eradication in individual flocks require careful planning, taking into consideration the need to fit the programme into sheep management and other farm enterprises. Such programmes have been more successful when they have been organized on a district basis using voluntary farmer control groups [3].

Eradication programmes were more successful where control schemes reduced the number of infected sheep in the flock to a low level before the start of eradication. This was achieved either by vaccination prior to the start of the transmission or spread period or by foot-bathing in zinc sulfate or formalin every 5–7 days during the spread period. A newer copper preparation has also been successful [4].

The multivalent vaccines have given protection for 6–10 weeks, depending on the environmental conditions. In some areas, more than one booster vaccination has been required. Field experience has shown that the greater the number of strains of *D. nodosus* incorporated in the vaccine, the shorter the protection period.

Foot-bathing has provided effective control in flocks where labour and facilities were available, but this was not always possible in larger flocks or where lambing or other sheep operations coincided with the spread period. Eradication is attempted only in non-spread periods. Where control programmes have been effective, eradication involves culling all infected sheep from the flock, followed by repeat inspections of all feet of all sheep in the flock until two successive clean flock inspections have been achieved.

In other flocks, salvage of affected sheep is attempted. The preferred treatment is the use of antibiotics such as long-acting oxytetracycline or lincospectin [5]. Streptomycin is no longer available for use in sheep. However, there are now limitations to their use because of concern with meat residues, and the need to observe withholding periods after treatment before sending sheep that do not respond to treatment to slaughter. Antibiotics are successful only where the feet of the sheep are kept completely dry for at least 24 hours after treatment. Experience has shown that sheep that do not respond to the initial treatment are not likely to respond to further treatments.

Foot-bathing is not recommended in the eradication phase. It will suppress the expression of the disease in infected sheep, making it difficult to detect all infected feet on clinical inspection.

Using a well-planned programme under professional supervision, virulent foot-rot has been successfully eradicated from a large number of flocks in NSW over a wide range of environmental conditions.

EXTERNAL PARASITE-CONTROL PROGRAMMES

The main economic loss from external parasites is in the cost of preventive programmes on the farm. For example, blowflies were estimated to cost the industry $A161 million in 1994, but only $A35 million was due to lost production [1]. The major costs were labour for flock supervision and crutching (clipping wool in the perineal area), mulesing (surgical removal of folds of skin in the perineal region) and jetting (spraying).

Sheep lice

Lice infestations due to *Bovicola ovis* significantly reduce wool value. Sheep showing visible signs of lice

infestation, i.e. rubbing and cotted fleeces, are estimated to lose 30–40 per cent in fleece value at the point of sale, depending on the type of wool. Historically, lice-control programmes in most states have been based on regulation, but that had no effect on the prevalence of infested flocks and resulted in a reluctance by owners to seek advice because of the fear of regulatory action. Because of this concern, many flocks were treated annually, whether they were infested or not.

Until the introduction of the organochlorines in the 1950s, control relied to a large extent on the use of arsenic in plunge dips, although shower dips were developed at this time. Organophosphate (OP) chemicals were first used in the late 1950s and the synthetic pyrethroid (SP) backline (pour-on) applications in 1981. Insect growth regulators (IGRs) were first used for lice control in Australia in the 1990s. In the past 10 years, the advisory approach has put more emphasis on the correct application of the correct chemical at the correct time. This has resulted in a recognition of the reasons for eradication failures and the targeting of these reasons in research programmes.

Surveys showed that very few owners were successful in eradicating lice from their flocks, even with plunge dips and often despite more than one treatment. This was due to the failure to wet thoroughly all of the skin or the failure to maintain a lethal concentration of chemical in the dip wash. Automatic jetting races were not effective because they did not wet the sheep completely. Recent research has shown that increasing the length of the swim in plunge dips and increasing the time in a shower dip improved the effectiveness of these techniques.

With the low volumes applied using some SP backline formulations, it was shown that lethal chemical concentrations for lice were not achieved over all of the body. The new backline chemicals, triflumuron and diazinon, are being applied with better applicators, using higher volumes to ensure a more effective chemical concentration over all areas of the body. SP-resistant lice are widespread in Australia and, on many farms, SPs are ineffective, even when used in plunge or shower dips. Field evidence indicates that the development of resistance was hastened by poor application techniques and by low dose rates. Cross-resistance between all the SP chemicals, including cyhalothrin, cypermethrin and deltamethrin, has been demonstrated. There is now field evidence of resistance to the IGRs, diflubenzuron and triflumuron. There is no evidence of resistance to diazinon, but its continued use for lice control is under threat because of concerns with occupational health and safety when applying the chemical to sheep.

There is increasing concern over chemical residues in greasy wool, from treatment for ectoparasite control or prevention. These chemicals are present in the effluent discharge from wool-scouring plants. A number of chemical groups are presently under review because of these environmental concerns.

The advisory approach in NSW has resulted in a significant increase in the number of farmers who no longer treat their flocks because they have eradicated lice; from less than 10 per cent in 1988 to nearly 40 per cent in 1997.

Future control will be in improved application of chemicals to eradicate lice from sheep flocks, thus avoiding the need for additional flock treatments. Current research work aimed at improved diagnostic tests on a flock basis will assist in achieving this.

Blowfly

The main cause of fly strike is *Lucilia cuprina*. Other flies, including *L. sericata* and the *Calliphora* spp., are of lesser importance as a cause of primary strike. *Chrysomia rufifacies* will attack already struck wounds.

Fly strike is associated with warm, humid weather, often following rain, and the presence of susceptible sheep. The most common form is body or breech strike in weaner or young sheep, associated with fleece-rot, dermatophilosis or urine stain in unmulesed sheep.

Fleece-rot, due mainly to *Pseudomonas aeruginosa* infection, sets up conditions attractive to the fly and provides a suitable environment for the development of the larvae. Control, based on the selection of sheep less susceptible to fleece-rot, has reduced the incidence of fly strike in many flocks. Control by vaccination has been unsuccessful, partly because of other bacterial infections of the skin capable of setting up conditions attractive to the fly.

In some areas, infection with *Dermatophilus congolensis* is considered more important than fleece-rot. It is usually spread when wet sheep are held in yards and can be controlled in many flocks by management.

Breech strike, associated with the urine-staining of the wool in the perineal region, is common in weaner

ewes. Research over many years has shown that mulesing as lambs or weaners is the most effective means of control of breech strike in young sheep and that it will improve survival rates in the first year of life [6]. The sheep industry has set a target date of 2010 for the elimination of surgical mulesing and is devoting considerable effort to develop alternatives [7].

Chemicals are an essential part of any fly-control programme, although there is widespread resistance against the use of OP chemicals, as they only give 4–6 weeks' protection. Cyromazine, which has been used for many years, is still giving 14 weeks' protection in the field, if applied correctly and, at present, there is no evidence of resistance against this chemical. Dicyclanil is giving longer protection applied either as a backline or by jetting. Another IGR, diflubenzuron, became available in the late 1990s but resistance has already developed against this chemical.

Internal parasites

Internal parasites, estimated to cost the Australian sheep industry over $A200 million per annum [1], cause significant mortalities, especially in younger sheep, and are responsible for significant losses due to reduced meat and wool production. The major worm species involved are *Haemonchus contortus, Trichostrongylus* and *Teladorsagia* spp. *Fasciola hepatica* also causes problems in areas suitable for the host snails.

Anthelmintic resistance is now emerging as one of the most important problems facing the sheep industry [8]. Since benzimidazole-resistance in *H. contortus* was first reported in Australia in 1968, the prevalence has increased rapidly and resistance occurs in all areas where regular drenching for the parasite is carried out. Closantel-resistance in *H. contortus* is also widespread in the summer rainfall areas of NSW.

Surveys have shown that, in the major sheep-raising areas of Australia, approximately 80 per cent of farms now have worms that are resistant to both the benzimidazole and levamisole groups of anthelmintics, involving *Haemonchus, Teladorsagia* and *Trichostrongylus*. More recently, macrocyclic lactone-resistant *H. contortus* and *Teladorsagia* spp. have been detected on commercial farms.

One of the major contributing factors has been the frequent drenching of all classes of sheep, often with lower than recommended dose rates. Resistance in *Teladorsagia* is becoming common in winter rainfall areas, where the hot dry conditions in the summer months are not conducive to the survival of refugia on pasture. In this environment, the larval pasture contamination occurring after anthelmintic treatment comes mainly from the worms that have survived treatment. With the development of closantel, which provided effective control against *H. contortus* for up to 60 days, a regional worm-control programme based on strategic drenching was developed [9]. The response to this Wormkill Programme encouraged the development of similar programmes for other regions. These rely on strategic treatments to minimize the contamination of pastures with worm eggs before environmental conditions become favourable for the development of eggs into infective larvae. They also rely on correct dose rates and grazing management to provide 'low-worm' pastures, especially for younger sheep. Rotation of the different groups of broad-spectrum anthelmintics has been recommended, but is still under review. More recently, resistance in *Fasciola hepatica* to triclabendazole has been detected, which will require the development of control programmes to reduce the reliance on flukicides.

Research into vaccine development has been ongoing over many years, but there are no effective sheep vaccines available at present. More recently, programmes aimed at the genetic selection for natural immunity have been developed [10] and the biological control of the free-living stages on pasture by nematophagous fungi [11] is being examined.

With the development of resistance against the newer anthelmintics, in particular the macrocyclic lactones, there is an urgent need to develop alternative strategies that do not rely on drenching to provide effective worm control. Several strategies are being investigated to take advantage of the natural immunity of sheep, thus reducing the need for frequent treatment. All programmes are emphasizing the importance of regular monitoring of parasite burdens and regular testing for anthelmintic resistance [12]. Veterinarians and sheep advisers must become more involved in the development of individual property programmes to control internal parasites.

Ovine Johne's disease

OJD, caused by the S(sheep) strain of *Mycobacterium paratuberculosis*, was first diagnosed in Australia on a

farm in the Central Tablelands of NSW in 1980. The disease has now been diagnosed in most sheep-raising areas in Australia. It is estimated that over 40 per cent of flocks in some districts are now infected [13].

The disease has spread largely through the movement of sheep from infected properties. The spread to neighbouring properties also suggests that transmission of infection has occurred by the movement of faecal material between properties. In infected flocks, mortalities range from 1 to 15 per cent per annum, depending on stocking rate and management practices with replacement sheep. The early programmes aimed to control the spread of disease by regulatory restrictions on the movement of sheep off infected properties, other than direct for slaughter. This had a major financial impact where the major source of income is from the sale of surplus or stud sheep for breeding purposes. This programme failed because of the difficulty in diagnosing infection in flocks with a low prevalence, especially those in which the diseases had been introduced recently. The disease was more infectious than previously believed with flocks becoming infected from neighbouring properties or from the introduction of a small number of infected sheep. Sheep, as distinct from cattle, can become infected as adults, making any attempt at control by segregation of ewes and lambs difficult. Also, the organism can survive for many months in soil and water, making land decontamination difficult on infected properties. The OJD policy now relies largely on the use of a killed vaccine to control the infection in infected flocks [13].

In the past, the diagnosis of OJD relied on clinical examination and histopathology to confirm the presence of typical lesions and the demonstration of the presence of acid-fast organisms. Attempts to culture the organism using traditional techniques were largely unsuccessful, even in those sheep with large numbers of organisms present in intestinal smears and tissues. Serological tests, including the agar gel immunodiffusion test and an enzyme-linked immunosorbent assay, have a low sensitivity, requiring large numbers of animals to be tested and examination of any reactors to confirm the presence of the disease in a flock. Culture of the organism from tissues and from faeces using a radiometric culture medium, in both multibacillary and paucibacillary forms of the disease, has been shown to have a high sensitivity and specificity and is now being used routinely on pooled faecal samples for flock diagnosis [14]. There is no reliable method for the diagnosis iof infection in an individual animal [15].

The killed Gudair vaccine being used in Australia has shown evidence of a 90 per cent reduction in mortality and a delay in the onset, and 99 per cent reduction in shedding of *M. paratuberculosis*, in vaccinated animals [14].

THE FUTURE

With the success of the industry-based control programmes, rather than control by regulation alone, it is anticipated that further progress will be made against diseases that have a significant economic effect on the sheep industry. Collaboration between research and advisory staff will be essential to ensure that new developments are packaged and promoted in a user-friendly manner.

OJD is a major challenge for the industry to control or eradicate from the national flock. Sheep lice and ovine brucellosis are being addressed by regional groups, while anthelmintic resistance and internal parasite control are high priorities in the research and advisory areas.

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New Zealand

A.L. Ridler and N.D. Sargison

New Zealand's temperate climate is ideally suited to pastoral farming and, unlike many other sheep-producing countries around the world, a system of sheep production has evolved which is wholly dependent on pasture nutrition. Most sheep are grazed on hill country, which includes steep mountainous native pastures, easy rolling hills, and level plains of improved grass species and clover. Phosphatic fertilizer application is used to promote clover growth for nitrogen fixation. Stocking rates are generally high and range from seven to 20 ewes per hectare, depending on the class of country. In the early settlement of New Zealand, the Merino was used extensively but, with the advent of refrigeration, the dual-purpose Romney became the basis of the national sheep flock. While finished lamb production has been a feature of the New Zealand sheep industry since the 1930s, in recent years there has been a further shift away from the production of cross-bred wool. This has been accompanied by an increase in the use of

terminal sires, and of cross-bred ewes incorporating Finnish Landrace and East Friesian genetics, with the aim of improving prolificacy, carcass conformation, and lamb growth rates. A further change has been the increasing number of farmers choosing to mate ewe hoggets at 6–9 months of age. Fine-wool Merino sheep and their crosses are still farmed in summer-dry high country areas not suited to other types of animal production.

While natural grazing of pasture is generally considered to be healthy for animals, dependence on a solely pasture diet can predispose to certain animal health problems such as trace element deficiencies and gastrointestinal parasitism. Many of New Zealand's soils are derived from volcanic eruptions and are severely deficient in trace minerals such as selenium and cobalt. New Zealand's temperate climate and the agronomic selection of pasture cultivars for resistance to disease, predispose to mycotoxicoses such as facial eczema, ryegrass staggers and zearalenone-induced infertility, which are uncommon elsewhere [1]. Iodine deficiency appears to be an emerging problem in sheep, associated with the selection of pastures that contain high levels of goitrogen precursors.

New Zealand agriculture has to compete on export markets largely without the aid of subsidies. As a result, most sheep flocks are large and are dependent on the efficient utilization of pasture. Veterinary input is based on flock and pasture management and the prevention of disease, rather than on the treatment of disease in individual animals. Another consequence of the large flock sizes is that a system of production has evolved to ensure minimum handling of the animals. The development of flocks that lamb naturally with a minimum of human intervention has been a significant advance, without which current large-scale sheep farming would be impossible. However, as sheep have become less accustomed to handling, they may have also become more susceptible to stress-induced diseases such as pneumonia and salmonellosis, and to the effects of disturbance of newly lambed ewes.

New Zealand's sheep production is dependent on the export of sheep meat and wool, and the sheep industry is responsible for 30–40 per cent of New Zealand's agricultural export receipts. Thus, in addition to the management of economically limiting animal health problems, veterinary attention is focused on wider issues such as ectoparasiticide residues in wool, animal welfare and meat quality.

DISEASE PREVENTION AND CONTROL

New Zealand's present sheep flock of approximately 30 million ewes is derived mostly from the importation of a limited number of animals from the UK and Australia during the nineteenth century. Fortuitously, few infectious diseases were introduced with these animals, probably because any infected sheep died during the 6-month sea journeys from the UK. New Zealand is geographically isolated, has a thorough disease surveillance policy, enforces strict control over the import of live animals and has successfully operated a number of disease eradication programmes. Consequently, many diseases that are economically or politically important elsewhere, such as *Chlamydophila* abortion, maedi-visna, sheep pulmonary adenocarcinoma, sheep scab and scrapie do not occur in New Zealand. New Zealand has a large rural veterinary presence, which is involved in the passive and active surveillance for exotic diseases. Strict import control policies and surveillance programmes based on current epidemiological understanding also apply to exotic diseases that potentially could be economically important to New Zealand sheep farmers, or compromise the country's export trade.

In contrast to the UK situation, New Zealand sheep production is not stratified so there is less movement of animals between flocks and therefore less opportunity for the spread of infectious disease. Furthermore, the universal use of effective fencing, an essential tool for the efficient management of pasture, contributes to the low spread of disease.

MYCOTOXICOSES

Facial eczema

Facial eczema is the name given to the disease that occurs following ingestion of toxic levels of sporedesmin, the spores of the fungus *Pithomyces chartarum.* The fungus is present in leaf litter beneath shaded pasture, and spore numbers increase rapidly during warm, moist conditions such as those in late summer and early autumn. The liver damage induced by sporedesmin results in acute disease related to

hepatogenous photosensitization (see Chapters 49 and 56). In many affected animals, long-term liver damage results in associated production effects. Prevention is by recognizing high-risk periods when climatic conditions are conducive to spore multiplication, and instituting control measures which include moving stock to safer pasture, protective treatment with zinc salts, application of fungicides to the pasture or a combination of these practices. Selection for genetic tolerance is practised by some ram breeders.

Ryegrass staggers

Ryegrass staggers is a temporary neurological disease caused by the mycotoxin lolitrem B, which is produced by the perennial ryegrass endophyte *Neotyphodium lolii* [2]. Ryegrass staggers occurs under close grazing conditions during summer and autumn, and affects all grazing livestock species including horses. The disease is characterized by tremors and incoordination which may be inapparent at rest but become more severe following disturbance of the animal. Losses are rare and are due to misadventure, but the disease can lead to decreased animal performance and management difficulties.

Fusarium infertility

Poor reproductive performance in ewes has been associated with high pasture concentrations of zearalenone, an oestrogenic toxin produced by *Fusarium* spp. fungi present in some pastures. Warm humid conditions, such as those in late summer and autumn, favour the growth of *Fusarium* spp. High daily intakes by cycling ewes lead to changes in oestrous behaviour and a reduction in ovulation and fertilization rates. The severity of effect is dependant on the period of exposure and the daily dose [3].

TRACE ELEMENT DEFICIENCIES

Selenium deficiency

About 30 per cent of farmed land in New Zealand is considered to be selenium (Se) deficient [4]. Deaths of newborn to 6-week-old lambs due to nutritional muscular dystrophy were commonly reported before the recognition in the early 1960s of the role of Se. This disease is now rare, owing to awareness of Se deficiency and widespread supplementation (Chapter 54).

The important Se-responsive conditions in New Zealand sheep are ill-thrift in lambs up to 15 months and infertility in ewes. Improved growth responses in lambs of 5–10 per cent can be achieved by supplementation on most typical deficient pastures. Infertility in ewes is a result of embryonic mortality 3–4 weeks after conception [5]. Se-responsive ill-thrift and poor wool production also occur occasionally in adult sheep.

Iodine deficiency

Iodine (I) deficiency occurs in many areas of New Zealand, often associated with the prolonged feeding of brassica crops during late pregnancy (Chapter 54). However, reports of I deficiency in New Zealand sheep flocks are becoming more common. Modern varieties of white clover present in New Zealand pastures contain extremely high concentrations of thiocyanate goitrogen precursors [6] and it has been suggested that selection of clover cultivars with high hydrogen cyanide levels has improved their resistance to slug and insect predation, but inadvertently may have resulted in an emerging problem of I deficiency.

The prevalence of severe I deficiency, where the thyroid glands of newborn lambs are obviously goitrous, appears especially high in Merino flocks. Marginal I deficiency in flocks with no history of clinical goitre may result in poor perinatal lamb survival, the effect being greatest during periods of inclement weather, owing to the roles of thyroid hormones in fetal maturation and thermoregulation. There is some evidence that I deficiency also affects ewe fertility, probably associated with reduced embryonic survival [7].

SPECIFIC DISEASES

Salmonellosis

Sporadic outbreaks of salmonellosis caused by *S. typhimurium*, *S. bovis-morbificans* and *S. hindmarsh*

occur throughout New Zealand, generally during summer, autumn and early winter. The disease is usually precipitated by stress such as high stocking density, nutritional stress or transport. Salmonellosis usually occurs in adult sheep and is characterized by diarrhoea, severe systemic illness and rapid death. The average mortality rate in affected flocks is around 1 per cent. Unlike the situation in Europe, these serovars rarely cause abortion (Chapter 19).

Since 1996, many flocks in parts of the South Island of New Zealand have been affected by *Salmonella brandenburg*. Infection with this serovar is characterized by abortion, predominantly in ewes carrying multiple fetuses, and approximately half of the affected ewes subsequently die [8]. It would appear that grazing ewes at very high stocking densities (strip-grazing) during mid- to late-pregnancy is an important risk factor for this disease.

Enzootic pneumonia

Sudden deaths in lambs due to acute pneumonia are a common and important source of loss to individual sheep farmers. Furthermore, chronic forms of pneumonia are also common, and downgrading of carcasses at slaughter due to chronic pneumonia and pleurisy is an important problem to the New Zealand sheep industry [9].

The pathogens associated with enzootic pneumonia in New Zealand are similar to those reported in other countries and the appearance of disease is essentially determined by predisposing management factors. These include hurried mustering, droving and yarding in dry and dusty conditions, presumably because panting and mouth breathing associated with these procedures aids the establishment and proliferation of pathogens. Thus, most cases of pneumonia occur in lambs during the summer.

Leptospirosis

Leptospirosis is New Zealand's most important direct zoonosis, with the major serovars present being *Leptospira hardjo* and *L. pomona*. Most human cases are associated with direct or indirect contact with dairy cattle or pigs, and occur in meat workers or livestock farmers. However, *L. pomona* infection sporadically causes clinical disease in sheep, primarily lambs [10]. Most of these cases have been associated with high rainfall events and surface flooding. The disease causes intravascular haemolysis with resulting haemoglobinuria, jaundice and death. Sheep can also be subclinical carriers of *L. hardjo*.

Necrotic dermatitis

Outbreaks of severe dermatitis associated with *Pseudomonas aeruginosa* occur periodically, particularly in high-rainfall areas of New Zealand. Most outbreaks occur within 6 weeks of shearing and are associated with persistent wet weather or dipping 3–4 weeks beforehand. The disease results in the formation of thick, crusty, closely attached scabs underneath which caseous purulent material extends to the full depth of the dermis. Mild cases may resolve but severe cases usually waste and die and, at necropsy, often have abscesses in multiple body organs, particularly the lungs. Flock incidences are usually around 2–10 per cent, although they can be higher [11].

DISEASE CONTROL: RAM SOUNDNESS

Veterinary examination of rams prior to breeding has become routine for many farms in New Zealand. This intervention was initially driven largely by the need to control *Brucella ovis* in flocks, but it has also allowed farmers to mate large numbers of ewes to rams with confidence. Ram-to-ewe ratios of 1:100 or even 1:150 are common in group mating situations. In addition, most young rams that are sold for breeding are subjected to veterinary examination prior to sale. Inspection of rams, and palpation of their scrotal contents, allows assessment of their preparation for mating, detection of infectious diseases such as *B. ovis* epididymitis, Gram-negative pleomorphic epididymitis and scrotal mange, as well as detection of congenital abnormalities in young rams.

Brucella ovis epididymitis

In common with many sheep-producing countries, *B. ovis* is present in New Zealand and is associated with

epididymitis of rams [12]. A voluntary national control scheme for *B. ovis* was launched in 1986 with the primary aim of controlling the disease in ram-breeding flocks, thus preventing the spread of infection by purchased rams. This scheme is based on annual palpation of all rams used for breeding and for sale, and blood testing a proportion of the flock including any with epididymitis. Most ram-breeding flocks belong to the scheme, as do a smaller number of commercial flocks. This control scheme has been effective in reducing the incidence of infected ram-breeding flocks and has been accompanied by a substantial reduction in infected commercial flocks.

Epididymitis caused by Gram-negative pleomorph infection

The term 'Gram-negative pleomorphs' is used to describe a group of bacterial organisms with similar morphological and biochemical properties. *Actinobacillus seminis* and *Histophilus ovis* have been isolated from cases of epididymitis in New Zealand. Gram-negative pleomorphic infection is an important cause of epididymitis in New Zealand, resulting in an annual wastage of around 2–3 per cent of 6–15-month-old rams in many stud flocks. In a minority of flocks the incidence may be even higher, affecting 10 per cent or more of the flock [13]. This high incidence of the disease in New Zealand only became apparent with the advent of routine ram soundness examinations.

Scrotal mange

Scrotal mange, caused by *Chorioptes bovis*, is a common cause of reduced ram fertility in New Zealand. Outbreaks of severe scrotal mange are unpredictable and can appear suddenly in a large proportion of the ram flock. Severe scrotal mange can cause a serious reduction in the fertility of individual rams due to overheating of the scrotum.

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North America

C. Wolf

The North American continent spans the USA, Canada and Mexico. Within this area, sheep are a minor livestock species and much of the land, although well suited, is under-utilized for sheep grazing. On 1 January 2006, the National Agricultural Statistics Service reported that there were 68 280 sheep operations in the USA with 6.23 million sheep [1]. The majority of sheep in the USA are maintained in flocks of fewer than 500 ewes [2]. The breakdown in the size of breeding flocks in the USA is shown in Table 68.1.

In the western part of the USA and Canada, range flocks of 1000 ewes or more are common. The major threats to the existence of range flocks are predation, increasing environmental regulations, reduced availability of public lands for grazing and market volatility. The latter is also the major obstacle to profitable farm flock operations. The states with the most sheep are Texas, California, Wyoming, Colorado, South Dakota, Montana, Utah, Idaho, Iowa and Oregon. Unlike other parts of the world, many of the lambs are moved from ranches and farms into feedlots and fed nearly all grain-based rations until they reach slaughter weight. This practice results from heavier target slaughter weights, which demand a longer feeding period then elsewhere in the world. Lambs are at risk of developing metabolic diseases unless management strategies are implemented to minimize their occurrence. Unique to the USA is the fact that fewer tools are available for preventing and controlling some of these diseases, due to a lack of availability of vaccines and pharmaceutical products approved for use in sheep.

The estimated Canadian sheep inventory was 975 600 sheep and lambs on 1 January 2003. Mexico's sheep population is increasing due to imports and as of 2005 is estimated at 6.8 million head. The majority of sheep in Mexico belong to small producers who also have other species such as goats, dairy cows and an equine animal for transport. These flocks are often communally grazed. Larger flocks are found in the northern states of Chihuahua and Colima.

Diseases of major importance are similar to those found in other parts of the world. In all of the above countries, many diseases, commonly classified as foreign animal diseases, do not exist. Predation is a very important cause of both lamb and sheep losses. A common predator across the entire North American continent is the coyote, whose population has steadily increased; others include domestic dogs, red fox and bobcats. Protected predators include the timber wolf (not protected in Canada), grizzly bear, black bear, mountain lion and eagles.

REPRODUCTIVE DISEASES

Brucella ovis is the prevalent aetiological agent of epididymitis in mature rams living west of the Missouri

Table 68.1: Breeding sheep: survey percentage by group size, USA, 2005*

	1–99 Head	100–499 Head	500–4999 Head	>5000 Head
Per cent of operations	90.8	7.6	1.5	0.1
Per cent of national flock	28.7	24.0	33.8	13.5

* Percentages reflect distributions from annual survey.

River. The incidence of this condition has decreased significantly, as many rams are now routinely examined prior to the breeding season. Rams that have suspicious findings on scrotal palpation are usually enzyme-linked immunosorbant assay (ELISA)-tested or cultured for *B. ovis* infection. Ranchers that have recently identified a *B. ovis* problem usually ELISA test all of their rams annually and cull those that test positive. Public ram sales in much of the USA and Canada require proof of a recent negative *B. ovis* ELISA test within 30 days prior to sale.

The three most common causes of infectious abortion are *Chlamydophila abortus*, *Campylobacter jejuni* and *Campylobacter fetus* subspecies *fetus*, and *Toxoplasma gondii*. Chlamydophilia abortion is diagnosed more commonly in the western USA and Canada, whereas campylobacteriosis and toxoplasmosis occur more frequently in midwestern and eastern farm flocks. Outbreaks of chlamydophilia abortion have occurred when some ewes from western Canada or the USA have been moved eastward to other parts of the continent.

Bluetongue occurs in immunologically naïve sheep, primarily in western parts of the USA. Owing to climatic factors, only specific areas of the USA are able to support the vector *Culicoides variipennis*. Bluetongue virus serotypes 2, 10, 11, 13 and 17 have been identified, and some states have available a modified live, multivalent vaccine that is reported to be effective.

Cache Valley virus occurs sporadically throughout North America. If naïve ewes are infected while gestating, the results can be abortion, stillbirth, birth of weak lambs, dystocia, arthrogryposis and hydranencephaly. In endemic areas, the best means of control is to delay the start of the breeding season until the mosquito vector has been killed by cold autumn temperatures.

Bacterial mastitis commonly occurs in all flocks in the lactation and early dry period, and can be either clinical or subclinical. It is an economically significant cause of culling of ewes.

DISEASES OF THE ALIMENTARY SYSTEM

Enterotoxaemia is a sporadic problem in flocks. Most breeding sheep and lambs are vaccinated annually. Most flock owners believe that the protective immunity conferred from regular vaccination is the reason that losses do not frequently occur from this disease.

An ill-defined syndrome of sudden abomasal bloat, associated with high mortality, is recognized in young lambs fed free-choice milk replacer. While the aetiology is still in question, some believe that *Clostridium perfringens* type A or *Sarcina* spp. [3] causes this syndrome. Some milk replacers are formulated to have an acidic pH, which appears to have reduced the occurrence of the syndrome. The addition of small volumes of formalin to milk replacer also seems to help.

Johne's disease exists throughout the continent. In the US National Animal Health Monitoring System (NAHMS) 2001 Sheep Study, it was found that producers frequently house thin ewes with young lambs in an attempt to improve the body condition score of thin mature sheep. This common practice may positively affect the transmission of Johne's disease. The significance of Johne's disease will become clear as better tools are developed to diagnose the infection. It has been observed that accelerated lambing flocks are recognizing the problem with an increasing frequency [4]. Formal control programmes, including vaccination, are not available.

In a national survey of sheep producers in the USA, nematode infections were the greatest health concern [5]. Anthelmintic resistance to all classes of chemicals has been documented in multiple regions of the USA [6]. *Nematodirus battus* is important in lambs raised in the north-western USA and western Canada. *Thysanoma actinoides*, the fringed tapeworm, is a common cause of liver condemnations in lambs and is of regional importance as well. In the same national survey, producers revealed that they do not have a good understanding of parasite-control methods and producer practices such as inappropriate rotation of classes of dewormers may be contributing to the development of anthelmintic resistance [5]. Parasite infections are the leading health problem in sheep in Mexico, especially when the sheep are on a poor diet. Liver flukes, *Fasciola hepatica* and *Fascioloides magna* exist in regions of the continent that support their life cycle, i.e. marshy ground with lymnaeid snails. As no approved flukicides are available in the USA, producers have learned to try to avoid grazing sheep in these areas whenever possible and to use albendazole at regular intervals.

Coccidiosis is common in both confinement-reared and grazing sheep. Only arid parts of the continent

do not have a problem with this protozoal infection in young growing lambs. Most producers include anticoccidial agents such as lasalocid or decoquinate in either the sheep mineral or feed. These prophylactic agents are often fed intermittently or continuously throughout the lambs' first 6 months of life. Potentiated sulfonamides are frequently used for treatment. Improving sanitation in the animals' environment is also critical in the control of this condition.

Rumen acidosis is frequently seen in both feedlot lambs and in pregnant or lactating ewes that have experienced rapid changes in their ration.

DISEASES OF THE RESPIRATORY SYSTEM

Mannheimia spp. pneumonia occurs sporadically in individual sheep and as flock outbreaks. Many viruses such as ovine adenovirus, parainfluenza virus type 3, respiratory syncytial virus and reovirus have also been associated with the development of pneumonia. The highest incidence of pneumonia occurs in lambs raised in facilities with inadequate ventilation. Deficiencies of vitamin E or selenium in young lambs have also been identified as predisposing causes of *Mannheimia* spp. pneumonia [7].

Mycoplasma ovipneumoniae and *M. haemolytica* have been isolated from pneumonic lesions found in lambs at slaughter. *M. ovipneumoniae* and *M. arginini* have been isolated from the upper respiratory tract of lambs. These agents play a role in the chronic paroxysmal coughing syndrome observed in midwestern feedlots. It is associated with reduced weight gain and rectal prolapse.

Ovine progressive pneumonia (OPP) virus in the USA and maedi-visna virus in Canada are antigenically similar ovine lentiviruses that are endemic in both sheep populations [8]. The seroprevalence rates range widely in both countries. An average OPP seroprevalence of 24.2 per cent was found in the 2001 NAHMS Sheep Study based on 21 000 sera from 22 states [9]. Other USA studies [10–13], with the exception of a study of native Texan sheep [14], report similar seroprevalence rates. Two Canadian studies report seroprevalence rates of 19 and 20.9 per cent, respectively [15, 16]. These viruses cause persistent infection that results in chronic lymphoproliferative changes in mammary gland, lung, lymph nodes, central nervous system and joints (see Chapter 31). Subclinical infection occurs much more commonly than clinical disease. Infection results in any of the following signs: reduced milk production and a hard udder, a progressive arthritis, dyspnoea, progressive weight loss and paresis that develops to paralysis. The economic significance of infection across flocks is still debated. Sheep in some flocks experience more clinical problems than others. Many veterinarians and producers have observed that the economic impact of OPP is related to age and breed or breed cross of sheep, management system, level of production and climate. Control and eventual eradication has proved to be a costly process that requires a serious commitment. Control is achieved through culling positive animals that are identified through whole flock serological testing every 6 months [8]. Research is underway to determine what gene loci control disease progression and viral lode [17].

Caseous lymphadenitis

In a national review of 6 years of data available from slaughter ewes in the USA, caseous lymphadenitis was identified as the most common cause of condemnation. Lesions of caseous lymphadenitis have been reported in the brain and spinal cord, lung, lymph nodes in the thoracic and peritoneal cavity, liver, kidney, testes, epididymes, mammary gland and external lymph nodes. Vaccines are in widespread use in the USA and Canada. Anecdotal reports suggest that implementing a proper vaccination programme and selective culling can reduce the incidence of infection.

DISEASES OF THE NERVOUS SYSTEM

Polioencephalomalacia occurs in sheep of all ages. It is diagnosed most frequently in those sheep being fed cereal grains as part of their ration, but also occurs in grazing sheep not receiving concentrate. Producers successfully treat many cases with injections of thiamine early in the disease process.

Listeriosis occurs both on sheep farms that feed silage and those that do not. The organism can contaminate other feedstuffs via flooding, carrier animals or soil contamination, e.g. from gopher mounds in

hay fields. Many larger confinement-type flocks in the midwestern USA feed silage because of its low cost and availability. There are more cases diagnosed during the first year that silage feeding is introduced, than occur in subsequent years. Most confirmed cases in ewes are the meningoencephalitic form rather than the abortion form.

Scrapie is most commonly reported in a few breeds in the USA [18]. Mexico reports that its national sheep flock is scrapie-free. In March 2003, the US Department of Agriculture completed a national slaughter surveillance study to determine the prevalence of scrapie in US sheep. This study sampled 54 per cent of the cull sheep population and found the prevalence to be 0.20 per cent. Prevalence in black-faced sheep was 0.84 per cent while only 0.01 per cent in white-faced sheep. All 33 scrapie-positive sheep were QQ (homozygous glutamine) at codon 171 [19]. Ongoing regulatory slaughter surveillance of cull mature sheep targets black-faced and mottled-face sheep. In 2001, the National Scrapie Eradication Program changed significantly. The programme relies heavily on mandatory ear-tagging of mature sheep as they engage in interstate commerce and exhibition. Also, breeding stock of any age must be ear-tagged prior to leaving the farm of birth. The ear tag enables traceback capability to the flock of birth. Producer compliance is reasonably good in large part due to a provision of farm-friendly tags at a very low cost. Scrapie-positive sheep found at slaughter are traced back to the farm of origin and to farms where they have lambed. All sheep are genotyped and the high-risk genotype sheep are depopulated. Additional testing is conducted at farms that have acquired sheep from infected flocks. Previously infected flocks are surveilled for 5 years after the removal of the last infected and high-risk sheep. As part of the control programme a flock clean-up plan is cooperatively developed and carried out by the producer, state and federal veterinary health officials once the disease is diagnosed. Research data [20, 21] strongly suggest that US producers may be able to control the disease effectively through the simultaneous use of genotype selection towards more resistant sheep and elimination of those animals that test positive with third eyelid or rectal lymphoid monoclonal antibody staining. Producers can also enrol voluntarily in the US Scrapie Flock Certification Program. This voluntary monitoring scheme is geared primarily for seedstock producers as a means of demonstrating freedom from scrapie through continuous surveillance.

Tetanus is seen in all ages of sheep but most cases are associated with the use of elastrator bands to dock and castrate unprotected lambs.

Parelaphostrongylus tenuis is a nematode that normally infects white tail deer. Its intermediate host is a terrestrial snail or slug. When an aberrant host such as the sheep ingests this nematode, it begins to go through its life cycle by migrating through the abomasal wall and finding a spinal nerve root, where it travels to the spinal cord and begins migrating in the cord. The most common initial sign is posterior paresis that within a few days develops into paralysis. Most cases are seen in young sheep under 1 year old, suggesting a partial age-acquired immunity. Most cases occur in the fall and winter, which may be due to increased foraging close to woods during those seasons when grass becomes sparse.

DISEASES OF THE FEET AND LEGS

Virulent foot-rot occurs throughout the continent. Most producers attempt to avoid purchasing the disease. Control strategies include a combination of the following: partial or total depopulation, selective culling, foot-bathing, tactical antibiotic treatments and vaccination. Even though the condition is reportable in some parts of the USA and Alberta, no regulatory programme exists to control and eradicate the disease.

Foot scald can be a common problem during the wet periods of the year. Additional risk factors have been identified in flock outbreaks. Foot abscess is also seen sporadically in individual sheep.

DISEASES OF THE SKIN, WOOL AND LIPS

In the USA, orf is commonly called soremouth. The disease is the same as is seen in the rest of the world. Many producers vaccinate their flocks once the disease has been introduced. They vaccinate all breeding age animals initially, well ahead of moving to the lambing area. In housed winter lambing flocks, it is common practice to vaccinate young lambs in the medial aspect of the thigh. Lambs born outside on grass are usually not vaccinated until the following

fall if at all. Most show lambs are also vaccinated to minimize the risk of developing disease and to prevent their exclusion from show grounds.

Over the past 15 years, an unusual fungal infection has been seen in lambs that are shown frequently. This condition has been named 'Club lamb fungus', even though positive fungal cultures are not found in all affected sheep. In many cases, either *Trichophyton verrucosum* or *T. mentagrophytes* is cultured. Risk factors include close shearing, as is commonly done for shows, frequent bathing resulting in the removal of the protective layer of lanolin, contact with other lambs at public exhibitions and the use of common non-disinfected shearing equipment to shear lambs from multiple farms. This condition may be strictly ovine ringworm, where the risk factors make the clinical presentation more severe than would be expected if the wool and skin of these animals were left in their natural state. Many cases respond to topical antifungal treatments, while some lambs develop generalized infection. This condition is zoonotic.

Chewing or biting lice (*Bovicola ovis*) are a problem in some flocks. Chemical resistance is not as large a problem as it is in other parts of the world. Clinical signs are more noticeable during the winter when the staple length is longest. 'Wool-slip' is observed in shorn late-pregnant ewes.

Myiasis occurs throughout the USA during the wet, warm summer months. The common sites are the perineal area when faecal soiling is present, docking and castration sites especially following secondary infection, in feet affected with foot-rot and in polls of fine wool sheep.

Infectious keratoconjunctivitis or pink eye occurs in sheep of any age. Common predisposing eye irritants include confined ewes in late pregnancy that are fed large round bales, and lambs on rank pasture in humid areas. Otherwise, the condition is the same as that found elsewhere in the world.

METABOLIC DISORDERS

Vaginal prolapse occurs most commonly in prolific ewes that are housed with limited feeder space or are being fed poor-quality roughage. Pregnancy toxaemia occurs in both under- and over-conditioned ewes. Hypocalcaemia also occurs, but is most probably under-recognized. It may occur as a flock outbreak when ewes experience forced exercise, long distance transport and sudden deprivation of feed or grazing oxalate-containing plants or green cereal crops.

White muscle disease occurs in regions where soils are deficient in selenium, or in sheep consuming feeds deficient in vitamin E or selenium. Two syndromes are recognized: weak neonatal or stillborn lambs due to *in utero* acquired selenium deficiency, and rapidly growing lambs (frequently 2–4 months old) develop an acute stiffness affecting the rear limbs. These older lambs become recumbent within a few days. When this latter form is recognized and treated early, most lambs return to normal. Progressive producers supplement their flocks' ration based on either historical levels or actual test results.

Copper toxicity occurs in farm flocks usually as a result of a feeding error. Sheep in affected flocks are supplemented with treatment levels of sodium sulfate and ammonium molybdate. Occasionally, the problem occurs because the flock consumes forages from molybdenum-deficient soils thus resulting in relative copper excess. Toxicity also occurs in sheep pastured on fields where poultry or pig manure is spread frequently.

Urolithiasis occurs in feedlot lambs when the high phosphorus concentrate ration is not properly balanced for calcium and phosphorus levels. Most of these uroliths are composed of ammonium magnesium phosphate.

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69

South America: pampas areas

L.A.O. Ribeiro

The pampas, an extensive area of flat grassland, starts in southern Brazil, extends to most of Uruguay and at least half of the Argentinean pastoral country. The growing area between latitudes 27° and 55°S has a mean annual rainfall of 1300 mm in Brazil and Uruguay, and very dry conditions in Argentinean Patagonia, with 150–300 mm of rainfall in autumn/winter. This area has a sheep population estimated at 52.4 million. Four main breeds – Corriedale, Polwarth, Merino and Romney Marsh – are grazed throughout the year in natural 'pampa' country.

The sheep industry is based on wool. The annual wool production of the area is 179 000 tonnes most exported to European and Asian countries. The mean fleece weight is estimated at 3.0–3.5 (Brazil), 3.7–4.1 (Uruguay) and 4.5–5.0 (Argentina) kg/ewe per year. The lamb industry is secondary, with only male lambs going for slaughter after weaning (90–110 days old) with a mean live weight of 20 kg. This chapter covers data on management-production and pressing problems of sheep flocks from southern Brazil and Uruguay.

PRODUCTION SYSTEMS

Sheep and cattle are grazed together (2:1) extensively on natural pastures at 0.8 animals per hectare. Sheep are shorn after lambing during the spring. The reproductive performance of breeding ewes depends very much on their nutritional status and weather conditions around lambing time. The mating season is during the autumn (March to April) with artificial insemination being used on many farms. Usually, lambs are born at the beginning of spring, but some farms are changing to a later mating season and lambing, when much better weather conditions reduce mortality. Reproductive failure is a factor that most limits the expansion of the sheep industry, since a low lambing percentage not only affects lamb production, but severely restricts the selection of the best ewe lamb replacements. Data on lamb marking (weaning) of Uruguayan sheep flocks show that it rarely rises over 70 per cent (Figure 69.1). Improvement is difficult under pampas conditions, as farmers do not keep flock records of ewe body weight, condition score or lambing and weaning percentages.

DISEASES AND OTHER CAUSES OF LOSS

Various diseases cause losses to the industry. Most are common to other countries where sheep are grazed commercially. The diseases most frequently diagnosed are shown in Table 69.1.

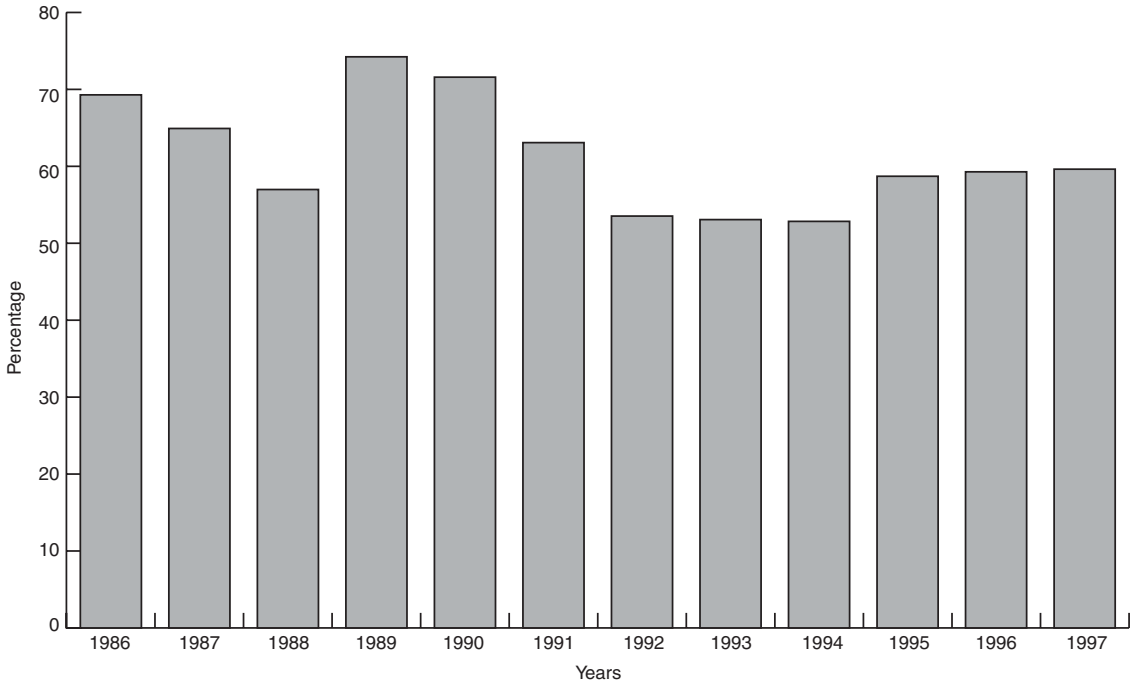

Figure 69.1: Lamb marking rates at weaning in Uruguayan sheep flocks, 1986–97.

Table 69.1: Frequency of diagnosis of diseases in flocks from southern Brazil

Disease	Frequency (%)
Nematode gastroenteritis	16.5
Chronic copper poisoning	15.5
Foot-rot	13.0
Skin diseases*	12.5
Malignant oedema	8.0
Keratoconjunctivitis	4.5
Enterotoxaemia	4.0
Pasteurellosis	4.0
Pregnancy toxaemia	2.0
Others	20.0

* Dermatosis, dermatophilosis and contagious ecthyma (orf).

Conception rates and lamb mortality

Data from a 5-year ewe scanning of Brazilian commercial flocks showed a mean pregnancy rate of 84 per cent, suggesting that annually 16 per cent of the ewes are barren [1]. The main cause of the low conception rate seems to be the low nutritional provision during the tupping time. In the study, body condition score of ewes that failed to conceive was 1.5–2.0. The main corrective measure is to score the ewes 6–8 weeks before exposing then to rams and improve the nutrition of those with a body score below 3.0, but the recommendation is difficult to adopt, as body scoring is not commonly practised. Perinatal lamb mortality associated with ewe undernutrition is a pressing problem, as usually no extra food or improved pastures are provided between tupping and parturition. In bad winters, the weaning percentage can be below 50 per cent. The causes of lamb mortality found in three different surveys pointed to starvation/exposure syndrome as the most important cause of the casualties. Reduction of this mortality probably could be achieved by better shepherding and shelter together with higher energy supplements (such as molasses) provided during late pregnancy. Furthermore, recent work on commercial flocks [2] showed that shearing pregnant ewes around day 70 of pregnancy improved the lamb birth weight by 700 g. The mean birth weight of lambs in such flocks is 3.3 kg, which is within the range of high mortality risk. Thus, birth weight enhancement, produced by shearing, will reduce the mortality risk.

Gastrointestinal helminthosis

The climatic conditions of the pampas suit many of the gastrointestinal nematodes of sheep, although *Haemonchus contortus* is the most important, causing outbreaks of disease, usually in autumn. Lambs can become infected while with their dams in spring, but after weaning (December to January) they are exposed to a greater challenge, and losses occur during their first autumn. Adult sheep do not develop a good immunity to *Haemonchus* and also can develop acute clinical disease in autumn. Other important nematodes are *Teladorsagia* spp. *Trichostrongylus axei* in the abomasum and *T. colubriformis* and *Nematodirus spathiger* in the small intestine. Other gastrointestinal nematodes such as *Strongyloides papillosus*, *Cooperia* spp., *Moniezia expansa*, *Oesophagostomum columbianum*, *O. venulosum* and *Trichuris ovis* occur in low numbers, as do the lungworms *Muellerius capillaris* and *Dictyocaulus filaria.*

As the highest numbers of *H. contortus* are present on pasture during autumn and as weaning takes place in early to mid-summer, the control strategy has been to recommend the use of drugs with residual protection (e.g. disophenol or closantel) against this parasite at weaning and again 8 weeks later, with the aim of reducing the summer contamination of pastures: this effectively eliminates haemonchosis during the autumn.

The high level of pasture contamination with trichostrongyle larvae during the autumn has encouraged farmers to administer an excessive number of treatments to lambs during their first year of life. Some may drench at 20-day intervals during autumn and once monthly thereafter. Although this policy has been successful in controlling parasitism and providing production benefits, it has precipitated anthelmintic resistance [3]. A survey conducted in South America (Table 69.2) found anthelmintic resistance in most flocks. In some cases not even the ivermectins can be used, as large-scale resistance to them has evolved, leaving the sheep industry in a very difficult situation unless alternative parasite control methods are developed.

Table 69.2: Percentage of farms found to have anthelmintic resistance in four surveys in Latin America

Country	BZ	LEV	COMB	IVM	CLOS
Argentina	37.0	8.0	5.0	2.0	–
Brazil	89.6	83.5	72.5	12.6	19.5
Paraguay	73.0	68.0	–	73.0	–
Uruguay	80.0	71.0	–	1.2	–

BZ, benzimidazole; LEV, levamisole; COMB, combination BZ+LEV; IVM, ivermectin; CLOS, closantel.

Foot-rot

Foot-rot is a major problem, probably ranking slightly below internal parasitism. Virulent foot-rot causes locomotor and reproductive difficulties as well as wool losses, but there is little information on its economic impact. In Brazil, the annual wool loss caused by foot-rot is about 713 tonnes, corresponding approximately to about £600 000. A field trial [4] showed that the percentage of barren ewes in the group affected by foot-rot during tupping time was almost three times greater (26 per cent) than in the contemporary unaffected group (9 per cent).

Outbreaks of foot-rot occur most frequently during spring and early summer, when prevalence of infection may vary from 20 to 70 per cent. In southern Brazil, with an annual monthly rainfall of 90–170 mm and a mean temperature of 16.5–19.6°C, foot-rot may occur throughout the year, with perhaps some interruption during the winter months when the temperature drops below 10°C. Autumn outbreaks are also common and are associated with wet weather and intensive management such as artificial insemination. Observation of the prevalence of foot-rot in Merino, Polwarth and Corriedale sheep in Uruguay [5] gave figures of 16, 17 and 16 per cent, respectively, suggesting little difference between breeds, which does not support the common believe that sheep with coloured hooves are more resistant to foot-rot. Up to seven different serotypes of *Dichelobacter nodosus,* the causative organism, have been recovered from cases of virulent foot-rot in Brazil and Uruguay. Practically all serotypes described in Australia are present with only a small divergence of serotype frequency between Brazil and Uruguay.

The most common treatment is formol (10 per cent) in footbaths, usually with no foot care or segregation of infected animals. The results are poor and an alternative method of control is needed. A polyvalent vaccine prepared with local serotypes A, B, D, E and F strains is available commercially. Field trials revealed that double vaccination produced antibody titres up to 1/5000 by 8 weeks. In some trials, the protection and cure rates were 87 and 77 per cent, respectively. Intensive vaccine use is limited by the relatively high cost of the dose (£0.20) and the short period of immunity, not greater than 16 weeks.

Chronic copper poisoning

Cases of copper poisoning are quite common in valuable pedigree sheep prepared for shows and fed concentrates with excessive amounts of copper (30–40 mg/kg). The condition normally does not occur in sheep at grass, but losses of sheep grazed in an apple orchard have been recorded [6]. The orchard was sprayed with copper sulfate 4 weeks after the ewes were introduced, and a month later some showed depression, jaundice and haemoglobinuria. Twenty-one ewes died, representing 17.5 per cent of the flock. High liver copper levels of 1313 mg/kg were found and a sample from the pasture revealed 60 mg/kg of copper. More recently, cases of copper poisoning occurred in a flock of breeding ewes fed poultry litter and citrus pulp [7]. Forty-five of 98 ewes died within 4 weeks showing icteric livers and dark kidneys and urine (see Figure 69.2 in the colour plate section). The copper level in the poultry litter and citrus pulp fed to the sheep were 171 and 40 mg/kg, respectively. Diagnosis is based on signs of jaundice and haemoglobinuria associated with high concentrations of the element in the pasture or supplementary feed. Control is based on estimation of copper in the diet and monitoring sheep at risk by assaying serum aspartate aminotransferase (AST).

Emerging diseases

Scrapie

Scrapie is an exotic disease in most pampas flocks. Although not recorded in Uruguay and Argentina, scrapie was introduced accidentally on many

occasions to southern Brazil. The first case was a ewe imported from the UK that died without progeny [8]. The second outbreak also involved sheep imported from UK in 1985; an emergency plan carried out by the Brazilian animal health authorities led to the slaughter of 143 animals and a ban on sheep importation from that country. New cases of scrapie have been confirmed recently in pedigree sheep imported from Canada and USA. So far, no cases have been recorded in commercial indigenous sheep of any breed. Current control measures include a total ban on importation of live sheep from countries where scrapie is endemic, obligatory notification of new cases, and destruction of clinical cases and descendants. Preliminary results of scrapie genotyping in Suffolk flocks, showed that more than 50 per cent of the sheep were QQ^{171} homozygous, followed by 48 per cent. RQ^{171} and just one animal (1.3 per cent) was RR^{171} homozygous. Presently, the Brazilian animal health authorities are implementing a certification scheme for scrapie and other diseases in free flocks.

Maedi-visna

Maedi-visna (MV), in its pneumonic form, has been observed mainly in pedigree Texel flocks in Brazil with a history of importation of live sheep from European countries. The prevalence of serologically positive animals in a flock can be as high as 31 per cent and demonstration of the MV virus by polymerase chain reaction has been described [9]. The disease seems to be restricted to Texel pedigree flocks, no cases having been found in commercial sheep. Argentina and Uruguay apparently are free of this condition. The clinical signs are mainly respiratory, but joints and udder can be affected also. Diagnosis depends on serology, clinical signs and post-morten findings. Control is based on serology and elimination of positive cases.

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South America: Andean highlands

R. Rosadio

Sheep farming is of primary importance in the rural economy of the Andean highlands. In Peru, more than 15 million sheep are raised, of which 60 per cent are of the Criollo breed kept by traditional rural communities. These sheep, degenerate descendants of Spanish Merinos and Churros, are well adapted to the harsh environmental conditions of the high Andes. The remainder of the population is made up of improved breeds including Corriedale (19 per cent) and Junin (10 per cent) as well as some Suffolk, Merino, Blackbelly and Pelibuey. The Junin is a Peruvian breed developed in the central sierra by crossing Corriedale and Romney sheep with the American Warhill, Columbia and Panama breeds [1]. Prior to the agrarian reform initiated in 1969, improved sheep were raised in large enterprises (haciendas) each averaging 100 000–150 000 head. Under the reform, these haciendas were transformed into Agriculture Social Interest Enterprises (SAIS) and progressively split into smaller rural cooperatives holding at most 20 000–30 000 animals. Today, only a small percentage of the sheep population is raised using appropriate technical knowledge. In well-managed enterprises, mortality rates are similar to those in any sheep-producing country, but losses are quite high among poorly managed communal herds.

This chapter covers the most important sheep diseases in Peru. It is divided into three parts; diseases of neonates, young sheep and adult sheep.

DISEASES OF NEONATES

Neonatal diarrhoea, enterotoxaemia, acute pneumonia and navel infections are the main causes of mortality.

Colibacillosis or white and yellow scours occurs during the first 10 days of life, and clostridial infections associated with *C. perfringens* types A and B (lamb dysentery), are frequently responsible for diarrhoea in neonates between 2 and 3 weeks of age. Colibacillosis caused by pathogenic strains of *Escherichia coli* is common among animals born in unhygienic conditions. The animals scour profusely and become weak and dehydrated. Death is usually due to dehydration or, more rarely, to terminal coliseptisaemia.

C. perfringens, type A, infection has been associated with sudden death, enteritis, hepatitis and/or hydropericardium in lambs in the central sierra. This infection, observed frequently within the first 2 weeks of life, produces sudden death or depression, with some lambs showing tremors or abdominal pain. At necropsy, there is an accumulation of fluid and gas, especially in the small intestine, hepatomegaly and, in some cases, lung congestion. Haemorrhagic enteritis is also observed occasionally, although, sometimes, the only pathological finding is fluid in the pericardic sac, so that this disease is frequently referred to as hydropericardium [2].

C. perfringens, type B, infection occurs primarily in the south sierra and clinically is characterized by profuse blood-stained diarrhoea (lamb dysentery, bloody scours) and occasional intestinal ulcers during the first 10 days of life. Even in well-managed enterprises, mortality rates may be as high as 20–39 per cent [2].

C. perfringens, type D, the cause of enterotoxaemia in suckling lambs, produces sudden death among lambs in good body condition, and neurological signs such as rapid convulsions and opisthotonos. Heavy rain, poor sanitation and flock concentration encourage the disease, which is endemic throughout Peru, and mortality rates can be as high as 20–30 per cent of the lamb crop. At necropsy, an enlarged abomasum

containing undigested milk, petechial haemorrhages in the intestinal serosa, lungs, diaphragm and even in the subepicardium are all highly suggestive of enterotoxaemia [2, 3]. Vaccination with multiple clostridial antigens has reduced losses.

The role of rotavirus and protozoal infections as a cause of lamb diarrhoea has yet to be determined, although rotavirus has been observed by electron microscopy (EM) in faeces of diarrhoeic lambs, and *Crytosporidium* has been found in diarrhoeic neonatal alpacas throughout the Peruvian Andes.

Acute pneumonias are commonly observed during the first weeks of life, affecting primarily immunologically weak lambs and animals raised in poor conditions. Peruvian sheep have been found to have antibodies to common respiratory viruses, especially to parainfluenza 3 (PI-3) virus and respiratory syncytial virus (RSV) [2, 4]. Most fatal field cases, however, are caused by the interaction of PI-3 virus and *Pasteurella multocida* or *Mannheimia* (*Pasteurella*) *haemolytica*. Neither organism has been serotyped, but field cases of animals dying from septicaemia and exhibiting lesions highly compatible with systemic pasteurellosis have been reported [4].

Navel infections are very common in 3–7-day-old lambs in poorly managed flocks with unsanitary lambing paddocks. Most of the affected animals develop septicaemia, with infection in the joints producing suppurative arthritis [2].

DISEASES OF YOUNG ANIMALS

Young sheep is used here to describe lambs from 4 weeks of age to weaning (6 months). In this population, enteric and respiratory infections are very predominant, but contagious ecthyma, necrotic stomatitis, keratoconjunctivitis, verminous bronchitis, taeniosis and enzootic ataxia are common.

The diarrhoea complex in young animals involves salmonellosis, coccidiosis and parasitic gastroenteritis. Clinical cases of coccidiosis in Peruvian sheep are rarely reported, but coccidiosis appears to be a common cause of haemorrhagic diarrhoea and even sudden death. Normally, oocysts first appear in the faeces when lambs are about 2 weeks old, but incidence peaks at 4–8 months and then progressively declines. Parasitic gastroenteritis caused by helminth parasites in lambs is a major economic drain on the sheep industry. Losses are due not only to lamb mortalities and weight loss during clinical outbreaks of the disease, but also to the more insidious but equally important effects of unthriftiness, poor growth rates and inefficient feed conversion which accompany subclinical infection. In Peru, the major helminths involved are *Teladorsagia* and *Nematodirus* spp., less frequently *Cooperia* and *Trichostrongylus* spp. The disease normally results from a build-up of infestation on pastures repeatedly grazed by ewes and lambs over several years [3].

Atypical pneumonia, in contrast to fatal acute pneumonia, is often subclinical but frequently fatal. It affects animals aged 2 weeks to 12 months and appears to be stress-related. The disease is produced by various micro-organisms including viruses, mycoplasma and bacteria. These agents act alone, or in cooperation, to damage the lungs of animals from poorly managed flocks and/or those exposed to the extreme daily temperature variations characteristic of the Andean dry season. The age of these animals helps to differentiate atypical pneumonia from the chronic virus-induced pneumonia found in adult livestock.

Verminous bronchitis is still a problem among young animals from poorly managed flocks. Affected stock cough, have respiratory difficulties, lose weight, and, in some cases, develop nasal and ocular discharges. The disease is usually confirmed at necropsy by identifying adult parasites in the main airways and even blocking terminal air passages.

Taeniosis causes problems in sheep from 2 to 4 months of age. The most common taenia is *Moniezia expansa* but, in communal animals, *Thysanosoma* and *Thysaniezia* are also important tapeworms [3].

Enzootic ataxia (swayback) is a crippling disease that affects both newborn and young animals. It is usually observed endemically in Criollo lambs raised near smelting plants on the central sierra. The mineral-heavy fumes released by the plants are thought to interfere with the lambs' uptake of copper from native pastures. In southern Peru, however, the disease is associated with low copper content in the soil. Clinical diagnosis is based on the characteristic ataxia of affected lambs, which lose limb coordination, particularly in the hind legs, before progressing to ataxic gait and posterior paralysis [2].

Contagious ecthyma is common when vaccination programmes are not properly conducted. The disease is diagnosed by scabs on the lips, nose, eyelids,

or other parts of the face, as well as on the udder, feet, and occasionally the mouth and gums.

Necrotic stomatitis and keratoconjunctivitis can be problems associated with poor management systems or overgrazing the typical poor-quality ligneous pastures of the Andes, predisposing the epithelial surface to further infection. Necrotic stomatitis is characterized by a painful and swollen throat, breathing difficulties and necrotic, malodorous lesions on the soft and hard palate, tongue and glottis. The lesions sometimes descend, producing aspiration pneumonia and necrotic gastritis (abomasitis).

DISEASES OF ADULT ANIMALS

Chronic respiratory disorders are the most prevalent cause of losses in adult sheep. Virus-induced chronic pneumonia and parasitic pneumonia are responsible for both high mortality and morbidity in Peruvian mature sheep [4]. Chronic respiratory diseases are a major limiting factor to development of the sheep industry in Peru. Since 1945, ovine pulmonary adenocarcinoma (OPA) regularly has been reported as 'poliadenomatosis' [5], but there is little evidence of its existence prior to this date [1]. The disease apparently was introduced with importation of American breeds used to develop what is now the Peruvian Junin sheep [1]. The first outbreaks were restricted to the central sierra, but, since the 1970s, the disease has spread throughout Peru with the movement of Junin stock to other areas [5].

In Peru, both OPA retrovirus and ovine lentivirus (maedi-visna virus) produce chronic disease [4]. The coexistence of OPA and maedi was documented in 1983 [6], but early descriptions of OPA in Peru suggest that both agents were introduced in the 1940s. The economic impact of chronic respiratory disease in the Peruvian sheep industry is unknown. However, mortality records collected over a 13-year period (1971–83) at one enterprise in the central sierra document that 12.5 per cent of 222 516 sheep losses were due to chronic respiratory diseases [1, 6]. Current estimates indicate that retrovirus-induced pneumonopathies cause annual losses of 1.6 per cent in the central region, but morbidity is much higher. The prevalence of lentivirus antibodies averages 25 per cent throughout the country [7, 8].

Both OPA and maedi affect mature animals, but the maedi-affected population is much older [6]. Both viruses produce respiratory dysfunction, and affected animals progressively lose weight. The production of watery, clear nasal exudate can suggest OPA, but definitive diagnosis can only be made by histopathology. In Peru, OPA is more prevalent than maedi but both viruses are commonly found in affected flocks and occasionally in the same animal [8, 9]. Epidemiological data collected over 10 years in the central sierra shows an increase in the incidence of OPA in animals reared above 4000 m as well as in those from areas adjacent to the large ore-smelting and refining plant of this region. However, OPA has also been reported from lower altitudes and even at sea level [5]. Periodic culling of all affected animals helps to reduce the prevalence of the causative virus in the flock.

Caseous lymphadenitis caused by *Corynebacterium pseudotuberculosis* is highly prevalent. The infection is not restricted to peripheral lymph nodes, it is also responsible for generalized or visceral abscessation. When lung and regional lymph nodes are affected, progressive weight loss is followed by respiratory difficulties.

Mastitis is common during the lambing season and almost invariably associated with bacterial infection [3]. Ovine lentivirus also has been found to induce chronic indurative mastitis in both young and mature ewes in the central sierra.

Foot-rot is by far the most common disease of adult sheep. It is highly contagious, particularly during the rainy season, and both *Dichelobacter nodosus* and *Fusobacterium necrophorum* have been isolated from clinical cases. Treatment includes foot trimming, bathing and soaking, supported by topical medication and/or parenteral therapy, but vaccination is not yet used [3].

Clostridial infections of mature animals are highly prevalent and cause economic losses in some areas. Malignant oedema and blackleg are frequently reported as causes of death. Multicomponent vaccines to protect against clostridial infections are administered at the beginning of the rainy season in the larger enterprises [3].

Rabies has been reported sporadically in sheep, usually associated with outbreaks in dogs [10]. The tendency of sheep to huddle together when scared may result in many animals being bitten by a rabid dog in a short period of time. Rabid sheep exhibit marked sexual arousal and try to mate indiscriminately. They

are generally very aggressive, attacking people who enter the corral, fighting among themselves, and biting the wires and posts of their pens, while some stare at the horizon, and others grind their teeth and yawn. As the disease advances, the rabid sheep become more and more nervous and uncoordinated, and exhibit involuntary movements of face muscles (nervous tics), which end in paralysis before death.

Epidermoid carcinoma of the ear (squamous cell carcinoma) has been observed mostly in sheep in the central sierra . Although the cause is unknown, a virus is suspected and it is believed that ear wounds (e.g. from eartags) and exposure to intense sunlight predispose animals to epidermoid carcinoma. Genetic predisposition is also possible. The tumours, which are located mainly in the cephalic region, ears, eye (cornea and eyelids) face and lips, are cauliflower-like growths with a wide base and an ulcerated surface. They become necrotic and infected by saprophytic microorganisms, thus producing an offensive odour. Spread of the tumour to regional lymph nodes has been observed [11, 12].

Liver fluke is the most important parasitic trematode of domestic ruminants and a serious zoonotic disease [13]. High prevalence has been reported in rural and urban people, particularly in the central and northern sierra where the disease is endemic. Infections in sheep may be acute or chronic, depending on the degree and duration of infection, the time of year and the number of immature and adult larvae. The acute form results in sudden death with no prior clinical sign. The chronic form is more common and is characterized by progressive weight loss leading to severe emaciation with typical submandibular oedema ('bottle jaw').

Hydatid disease is a very important zoonotic disease that affects both animals and people [14]. It is caused by a parasitic larva whose adult form (*Echinococcus granulosus*) lives in the intestines of dogs. Infected dogs eliminate parasite eggs in their faeces. Sheep and other ruminants are infected by ingesting eggs on contaminated pastures. The eggs pass through the gut into the blood to settle in the lungs and liver, where they grow into larval cysts (water bags). Cyst growth destroys normal tissues and may eventually kill the host. People are also very susceptible to this disease and can be infected by ingesting eggs from, for example, petting carrier dogs. The dogs are infected in turn by eating the cysts in the lungs, liver or kidneys of infected sheep.

Coenurosis is a parasitic disease that causes much ovine mortality in cooperative enterprises. It is caused by the larval stage (*Coenurus cerebralis*) of an intestinal tapeworm (*Taenia multiceps*) of dogs. Coenurosis is recognized by the affected animal's habit of walking in circles, a consequence of the pressure exerted by cysts in the cerebral cavity.

Abdominal cysticercosis is the larval stage of *Taenia hydatigena*, a common intestinal tapeworm of dogs. Sheep and other ruminants are infected by grazing forages contaminated with dog faeces that contain *T. hydatigena* eggs. Larval cysts form on the wall of abdominal organs but produce no visible signs. Most diagnoses are established at necropsy, when larval cysts are observed on the omentum, mesentery and intestinal organs.

Photosensitization (jacapo) is a severe acute dermatitis characterized by irritation, oedema and necrosis of non-pigmented skin (ears, eyes, eyelid and lips). The causes in Peru are not known, but the disease occurs mainly during the dry season when grazing is very poor and sheep consume aquatic plants in wet, boggy areas [3]. Although one possible cause of jacapo is ingestion of a photodynamic substance from plants (primary sensitization), liver damage is characteristically associated with clinical cases from the central sierra, suggesting that the secondary hepatogenous form of this disease is the most common. Abortion is frequent in pregnant ewes [3].

Astragalus poisoning is characterized by progressive muscular and nervous incoordination in animals grazing where *Astragalus* spp. (locoweed) are present and/or where the soil contains an abnormally high level of selenium. There are at least six suspected species of *Astragalus* (*garbanzo* or *garbancillo*) in the Peruvian sierra, but data on selenium content are lacking. The disease is usually observed during the dry season after sheep have fed on these drought-resistant plants for several weeks. Initially, affected animals put on weight, but, thereafter, become progressively emaciated and show nervous signs such as staggering, impaired sight and hearing, and ultimately severe muscular incoordination. During the course of the disease, abortion may occur at any stage of pregnancy. Stillbirths and weak and/or deformed lambs are also observed [3].

Ram epididymitis is usually associated with infertility and sometimes with ewe abortion. The disease is highly prevalent throughout Peru, particularly in poorly managed flocks [3]. Epididymitis is caused

mainly by *Brucella ovis*, which has a predilection for the testicles and for the placenta and fetus. *B. ovis* is transmitted heterosexually or homosexually from ram to ram [15]. A rapid evolution of the bacterial infection over the 2-month breeding season, from seropositive to overt clinical lesions, has been observed in mature rams [16]. The disease must be suspected in flocks with low fertility rates. Ram epididymitis may be diagnosed clinically by manual palpation to detect testicular lesions and/or orchitis. If epididymitis is found in 6 per cent or more of the rams in a flock, it is probable that *B. ovis* is the cause. The disease is more prevalent where mature and immature animals are raised together. A combined programme of REV 1 vaccination and thorough culling of infected animals has yielded good results at one large central sierra enterprise [17, 18]. The success of this programme is based on complete separation of adult and young rams and vaccination of all of the latter.

Neospora caninum, an abortigenic cyst-forming coccidian, has been found in alpacas and llamas from southern Peru, but its role in Andean sheep abortions has not been clarified [19].

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South America: Patagonia

C.A. Robles

Patagonia's vast region of 782 112 square kilometres lying between 36° and 55°S, is bounded by the Andes to the west and the Atlantic Ocean to the east. Comprising the five southernmost provinces of Argentina and 28 per cent of that country's land mass, the region has three agro-ecological areas (Figure 71.1) but most is a large steppe covered by shrubs and perennial grasses. The climate is dry with temperatures ranging from 30°C in summer to −30°C in winter. Annual rainfall is 3000 mm in the narrow fringe of the Andes and 200 mm in the steppe, and occurs mainly in autumn and winter.

Patagonia has 28 000 farms and a large number of smallholders, mainly devoted to livestock production. Sheep (8.2 million) are the most important species, followed by Angora and Criolla goats and Hereford cattle (850 000). About 95 per cent of sheep enterprise is focused on production of fine Merino and Corriedale wool, with meat as a secondary product. Only in some Andean valleys and artificially irrigated areas have Hampshire Down, Ile de France, Suffolk, Texel, Scottish Blackface, Southdown and Border Leicester breeds been introduced specifically for meat production [1].

Over the past decade sheep numbers have fallen from 23 million to 8.2 million mainly due to the world wool crisis but numbers are beginning to rise again, evident in an 8.9 per cent increase in wool production between 2003 and 2005 [2].

Flock management is predominantly extensive through year-round use of natural pastures without any supplementary feeding. Depending on the area, about 4–8 hectares are required per adult sheep. Agro-ecological characteristics preclude cultivation of fodder crops or grassland improvement. Mating takes place in autumn with a ram-to-ewe ratio of around 1:25. Females are first bred at 18–20 months, depending on body weight and condition.

Three different production systems prevail. Large companies with high management standards each own around 30 000–120 000 sheep in several 'estancias', individual family units run flocks of 500–10 000, while subsistence-level systems have fewer than 500 sheep. In each case, income is dependent on the year's climatic conditions and the current international price for wool, as most wool is exported. Patagonia has 64 per cent of Argentina's sheep and most are Merinos. Provincial numbers and wool production are given in Table 71.1.

Sheep production parameters are not ideal due to desertification which leads to poor nutrition, adverse climatic conditions, predation by foxes and puma, and several health problems. Although female fertility rate is usually high (>90 per cent) the average weaning percentage varies from 30 to 60 per cent according to the year and farmers' management practices.

Perinatal mortality, several infectious, toxic and metabolic diseases as well as endo- and ecto-parasitism contribute to these low levels of performance. However, from the international standpoint, the region has a favourable sheep health status, being free of all the Office International des Epizooties (OIE) List A diseases and most List B diseases defined by the OIE. Bluetongue, scrapie, border disease, rabies, Q fever, foot-and-mouth disease and sheep pox, among others, are not found in Patagonia [3]. Moreover, as the region is free of ticks, none of the tick-borne diseases is present.

This advantageous status is partly due to the region's geographic isolation. The Andes, along the western border with Chile, and the Barrancas and Colorado rivers, to the north, form natural sanitary barriers thereby preventing live animals as well as beef, sheep or pork meat from entering Patagonia from northern Argentina.

Given the extensive system of production with, for example, a single flock of 2500 sheep on 10 000 hectares of open grazing, it is difficult to exercise close management or to develop surveillance activity.

Figure 71.1: The five provinces and three agro-ecological areas of Patagonia.

Flocks are gathered routinely for mating in April, for pre-parturition shearing in September and for weaning in December/January. On some of these occasions animals may be treated for endo- and ectoparasites and vaccinated against clostridial diseases.

DISEASES CAUSED BY BACTERIA AND VIRUSES

Brucella ovis infection in rams

Brucella ovis, the agent responsible for contagious epididymitis of rams, is widespread, with an approximately 8 per cent regional prevalence. Farms with up to 52 per cent of infected rams have been reported [4]. Epididymo-orchitis due to *Histophilus ovis* and *Actinobacillus seminis* are diagnosed sporadically [5], so a differential diagnosis between these agents and *B. ovis* is required when infertility problems arise. Contagious epididymitis is transmitted at tupping when a healthy ram mates a ewe that was previously covered by an infected ram. The homosexual behaviour that rams usually show before the start of the reproductive season also contributes to transmission of the disease.

Lesions vary from slight enlargement to large indurations of the epididymis with testicular atrophy and fibrous adhesions between parietal and vaginal layers of the tunica vaginalis (see Figure 71.2 in the colour plate section). In Patagonia *B. ovis* infection produces clinically detectable lesions (i.e. epididymitis) in as little as 30 per cent of cases (Table 71.2). This makes clinical examination alone an unreliable technique for diagnosing and controlling the disease [6].

When epididymal lesions are present, in approximately 70 per cent of cases they are located in the tail of the epididymis (Table 71.3). Affected rams have normal libido. However, semen quality is variable, with reduced concentration and motility of spermatozoa together with morphological defects on the tails and detached heads. Neutrophils are also commonly present.

The complement fixation test (CFT) and agar gel immunodiffusion test (AGID), each employing a hot saline extraction (HSE) of *B. ovis* REO 198 as antigen, have been used for serological diagnosis. However, in the last decade these tests have been replaced by the indirect enzyme-linked immunosorbent assay ELISA using HSE or R-LPS from *B. ovis* as antigen, with improved sensitivity and specificity [7]. *B. melitensis* has never been detected in sheep and goats in Patagonia. Although this is an advantage, the use of the only vaccine (REV I) that has proved to be effective in controlling *B. ovis* infection is precluded. In this regard the development of new vaccines for use in areas free from *B. melitensis,* like Patagonia, would be desirable.

Table 71.1: Numbers of sheep and wool production of each province of Patagonia relative to national ouptut. Data relate to 2004 [2]

Province	Number of sheep	Wool production (greasy tonnes)	Wool production breakdown expressed as per cent of national wool production
Chubut	3 868 997	24 140	31.6
Santa Cruz	2 161 536	11 405	14.9
Río Negro	1 412 662	10 510	13.7
Tierra del Fuego	522 288	2 380	3.1
Neuquén	167 556	625	0.8
Total Patagonia	8 133 039	49 060	64.1

Table 71.2: Occurrence of epididymal lesions in *Brucella ovis*-affected rams [6]

	Rams with lesions	Rams without lesions	Total
Brucella ovis (+)	22 (28.2 per cent)	56 (71.8 per cent)	78

Table 71.3: Distribution of clinical lesions in 75 *Brucella ovis*-affected rams [6]

Parts of the epididymis	Number of cases	Per cent
Head	1	1.3
Body	3	4.0
Tail	52	69.3
Head and body	3	4.0
Tail and body	5	6.6
Complete epididymis	11	14.7
Total	75	

As there is no official control programme for ovine brucellosis in Argentina, control of *B. ovis* infection in Patagonia is carried out only by those farmers interested in improving overall flock health. The National Institute for Agricultural Technology (INTA) has developed a voluntary control scheme based on periodical serological testing, culling of positive animals and introduction of changes in management geared to avoiding the transmission and spread of the disease.

Clostridial diseases

Clostridial diseases are the most important causes of death of sheep in Patagonia. They usually occur as severe outbreaks which are difficult to control and, as a consequence, high mortality rates are recorded.

Gas gangrene

This is an exogenous toxi-infectious disease caused by *Clostridium septicum*, *Cl. novyi*, *Cl. perfringens*, *Cl. chauvoei*, *Cl. histolyticum* and *Cl. sordelli* alone or in combination. Shearing wounds, drug injections and vaccine applications associated with poor hygiene are the most common routes of infection. A rapid diagnosis can be achieved from typical post-mortem lesions (see Figure 71.3 in the colour plate section) and immunofluorescence performed on smears from affected areas. Cases of bighead due to *Cl. novyi* have been detected in rams when fighting and ramming their heads into each other [8].

Enterotoxaemia

Caused by *Cl. perfringens* type D, known also as pulpy kidney disease, this is probably the most common clostridial disease of sheep in Patagonia. This peracute and almost always lethal disease frequently

follows changes in grazing management when animals are switched from a poor to a rich pasture or when a dry period is followed by rainy days with temperate to warm weather. This causes annual grasses to growth rapidly, with low fibre and high ammonia contents. As the course of disease is very short affected animals may or may not show clinical signs and lesions at post-mortem. Diagnosis is usually based on history and detection of epsilon toxin in the intestinal contents of dead animals [8, 9].

Infectious necrotic hepatitis

Also called black disease, this is caused by *Cl. novyi* type B and is a serious and concomitant problem where *Fasciola hepatica* is prevalent. However, many cases without the presence of liver flukes have been reported and *Thysanosoma actinioides* has been incriminated as the trigger factor (Figure 71.4). Sudden death with subcutaneous oedema and congestion, abundant fluid in thoracic and abdominal cavities, and generalized jaundice are the most common findings observed [8–11].

Tetanus

Tetanus caused by *Cl. tetani* has been reported on some farms, affecting lambs shortly after castration and docking. Contaminated paddocks and poor hygiene during the procedures are thought to trigger the outbreaks [8].

Control

Although there is no official programme to control clostridial diseases, farmers are advised to protect their flocks with polyvalent vaccines formulated against most common risks. The scheme recommends two doses 30 days apart to all lambs at around 3 months of age and then an annual booster dose. Ewes are vaccinated 1 month before parturition to afford passive protection to their lambs during the first months of life.

Caseous lymphadenitis (pseudotuberculosis)

Caseous lymphadenitis due to *Corynebacterium pseudotuberculosis* is a chronic disease, endemic in most Patagonian flocks. It produces caseous abscesses in the superficial lymph nodes that can be seen with the naked eye. In more severe cases the bacteria spread to the lungs and mediastinal lymph nodes. Within-flock prevalence as high, as 70 per cent has been observed in Merino and Corriedale flocks. Disinfection of castration, docking and shearing wounds helps to control the disease. Vaccines, although used in other countries, have not yet been introduced in Argentina.

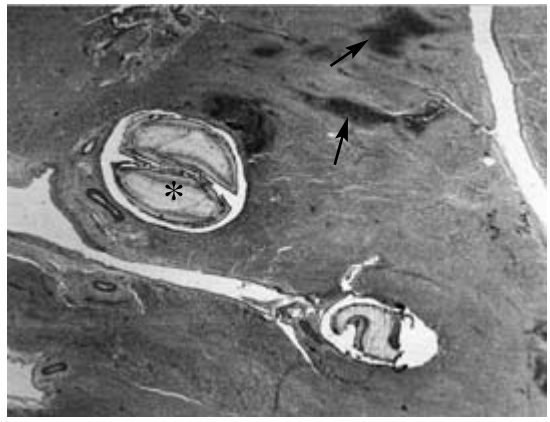

Figure 71.4: Black disease caused by *Clostridium novyi* type B. *Thysanosoma actinioides* inside a biliary duct (asterisk) and necrotic foci (arrows) distributed in the liver parenchyma. Haematoxylin and eosin.

Contagious keratoconjunctivitis

Seasonal outbreaks of contagious keratoconjunctivitis occur under dry warm weather conditions. Lacrimation, hyperaemia of ocular mucosa and increasing opacity of the cornea are the most common clinical signs. Its aetiology has not been fully elucidated but, in Patagonia, *Branhamella ovis* has been isolated consistently from affected Merino sheep. Other agents are thought to be involved in the development of clinical disease. Affected animals are treated with antibiotics, usually long-acting oxytetracyclines, administered as sprays in both eyes or by subcutaneous or intramuscular injection.

Dermatophilosis

Dermatophilosis due to *Dermatophilus congolensis* is a sporadic disease on most farms. It affects animals

under 1 year of age, usually becoming apparent at first shearing, when horn-like formations are discovered in the fleece. Bathing to control mange has been incriminated in the past as the main source of contamination and transmission of the disease. Outbreaks affecting up to 30 per cent of adult animals have occasionally been recorded. Cases of strawberry foot-rot (see Figure 71.5 in the colour plate section), related to the same agent, have also been reported.

Waxy wool/sisal wool

This is a relatively new disease in Merino flocks and probably is related to the Bolo disease described in South Africa. Its aetiology is not yet clear but *Propionibacterium acnes* has been isolated consistently from affected animals. A genetic risk factor is believed to be involved in the development of the disease. Clinically, waxy wool can be recognized by well-defined dark patches on the fleece which, when opened, reveal greasy clumped wool fibres (Figure 71.6). The underlying skin is congested and cracks easily when handled. Chemical and physical analysis of the affected wool demonstrated that the fibre itself is not affected, but the wax content of the fleece is noticeably increased. Culling of affected animals decreases on-farm prevalence but more research is needed to characterize the disease fully and to develop a reliable control method [12].

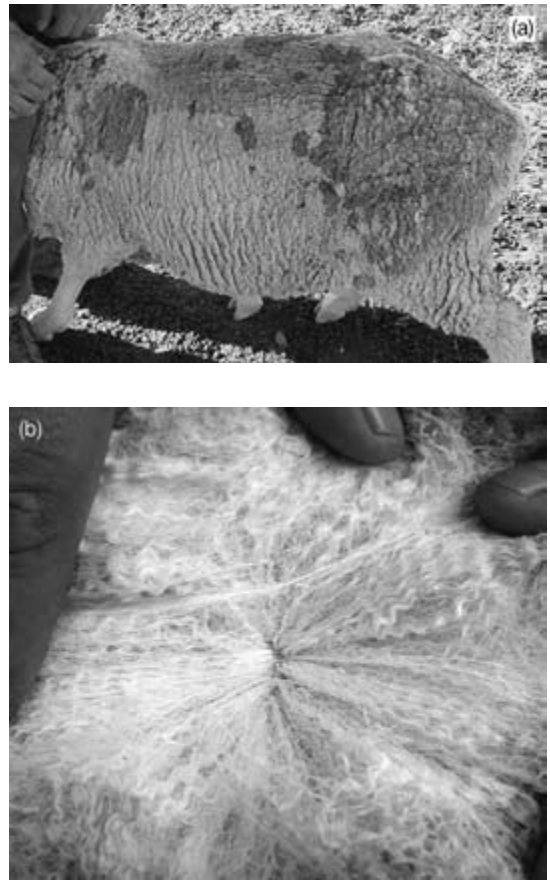

Figure 71.6: Sisal/waxy wool. (a) Characteristic dark patches on the fleece. (b) Greased and clustered wool fibres.

Pneumonia

Pneumonia is not an important issue in wool sheep in Patagonia. However, *Mannheimia* (*Pasteurella*) spp. and *Erysipelotrix rusiopathiae* have been isolated occasionally from diseased adult animals. Pneumonia associated with *E. rusiopathiae* is characterized by respiratory distress including coughing and abdominal breathing. Deformity of pulmonary lobes and several abscesses (see Figure 71.7 in the colour plate section) can be seen at necropsy. Microscopically, vast areas of liquefactive necrosis, containing small calcified foci and surrounded by a fringe of necrotic inflammatory cells, bacterial colonies and large number of macrophages and lymphocytes, can be observed [13].

Recently, cases of ovine pulmonary adenocarcinoma (OPA) and ovine progressive pneumonia/maedi were diagnosed on a dairy farm in Río Negro province, where Milchschaf sheep had been imported from Europe. No other cases of OPA have been reported. However, some animals serologically positive for OPP/maedi have been detected in other dairy sheep units that had purchased animals from the above-mentioned farm in the past. Testing (by agar gel immunodiffusion) of 6380 sera from wool sheep across the five Patagonian provinces revealed only 0.19 per cent of the samples to be positive. In neither dairy nor wool farms have any clinical cases been reported to date [14].

Contagious ecthyma

Contagious ecthyma, orf or soremouth is very common. Most outbreaks occur in spring and summer in young animals, causing severe lesions and pain in the mouth, leading to poor body condition due to difficulties in feeding. In more severe cases the feet, vulva and teats of animals can be affected. After approximately 1 month of clinical illness, and if proper nutrition and management are provided, animals recover fully and become immune, usually for life. As the only vaccine available is an attenuated live strain, its use is recommended only in flocks where the disease is highly prevalent.

Listeriosis

Listeria monocytogenes has been isolated from outbreaks of encephalitis in sheep which have been consuming contaminated silage. The resulting nervous disease ends with the death of the affected animals. Nervous signs in animals that were fed with silage are enough to suspect listeriosis. Confirmation can be obtained by isolation of the causative agent and by histopathological examination of the brain in which characteristic inflammatory foci can be found. Listeriosis is a rare disease in Patagonia as feeding sheep with silage is restricted to some Andean or coastal valleys.

Infectious abortion

Under the extensive management systems ewes are grazed in open fields throughout pregnancy and parturition, which makes it difficult to detect and examine aborted fetuses and placentas. *Brucella ovis*, *Campylobacter fetus* and *Listeria monocytogenes* have been incriminated as causative agents of abortion but, since this problem is systematically underestimated and misdiagnosed, other causes should be considered.

Mastitis

Mastitis is detected sporadically in ewes when they are examined clinically before the start of the reproductive season. However, it is not considered a significant problem in Merino and Corriedale sheep under Patagonian conditions.

DISEASES CAUSED BY HELMINTHS AND ARTHROPODS

Fascioliasis or distomatosis

Parasitism due to *Fasciola hepatica* is a serious endemic problem. *Lymnaea viatrix* is the intermediate snail host involved in the life cycle of the parasite. The disease is widespread in most of the humid areas of north and central Patagonia, where both adequate ambient conditions (meadow areas with acid or neutral pH) and the intermediate snail are found, thus providing the conditions required for the complete life cycle of the trematode [15, 16]. The disease in sheep is more severe than in cattle as sheep, unlike cattle, do not produce a strong immune response against the parasite. Consequently, outbreaks with moderate to high mortality occur in sheep. In those cases the liver is enlarged and sometimes deformed due to hypertrophy and fibrosis of hepatic lobes with high numbers of adult and juveniles parasites inside the hepatic ducts. In acute cases, scars are visible on the surface of the liver due to the migration of larval stages through the hepatic capsule and parenchyma. The lesions produced by the parasite can act as the trigger factor for the onset of infectious necrotic hepatitis (black disease).

Control of the disease can be achieved by periodic drenching of the flock within a rational grazing strategy. Despite full knowledge of its epidemiology in the region, fascioliasis continues to be a highly prevalent problem perhaps because farmers find it difficult to sustain the activities (drenching and pasture management) demanded by the control scheme [15, 16].

Gastrointestinal and pulmonary parasitism

Teladorsagia spp. and *Nematodirus* spp. are the most important nematodes of sheep in Patagonia. Several other genera, namely *Trichostrongylus*, *Cooperia*, *Trichuris*, *Chabertia*, *Oesophagostomum* and *Dictyocaulus*, are also present [15–17]. The nematode

considered most dangerous for sheep, *Haemonchus contortus*, has never been detected in Patagonia [15–17]. Health problems caused by nematodes are virtually restricted to sheep grazing in humid areas such as the cordillera and pre-cordillera of the Andes and the meadow areas spread across the steppe. Poor body condition and low production levels are the most commonly noticed effects. In spring, when they are still with their mothers and have started to consume grass, lambs are exposed to a high challenge of nematode larvae from the pasture. Because they have not yet developed immunity, clinical cases with high faecal eggs counts may appear during the autumn and winter, when treatment with appropriate drugs and changes in grazing routine become necessary [3].

Sheep scab

With the exception of the southern parts of Santa Cruz province and Tierra del Fuego island, classical mange (scab) due to *Psoroptes ovis* is highly prevalent. Affected animals are restlessness and small areas of deranged fleece can be detected at the start of the infection. In more advanced cases wool loss from different parts of the body and a hyperkeratinized skin with dry and yellowish scars are usually seen. If not treated, severely affected animals can die during the winter. At post-mortem examination, the carcass is characterized by a total lack of body fat reserves and enlarged, oedematous lymph nodes [18]. Scab is a notifiable disease in Argentina and, when the disease is detected, the affected farm is put under quarantine until all the sheep are treated and the outbreak is controlled. Historically, control was carried out by dipping, although the lack of facilities on most premises made eradication of the disease difficult. With the systemic treatments now available and regionalization of the National Control Programme, the situation has changed, and in recent years, scab has progressively been brought under control in many areas of Patagonia.

Sheep ked and lice

The sheep ked *Melophagus ovinus,* a wingless fly which has the sheep as its unique host [17], has always been present in humid areas of Patagonia but over the last decade keds have been expanding to the drier parts of Patagonia.

Chewing (*Bovicola ovis*) and sucking (*Linognathus pedalis*) lice [17] are detected sporadically in some flocks at very low prevalence and never to the point of attracting the farmer's attention. However, as with the sheep ked, these parasites are experiencing territorial expansion and increased prevalence.

It is thought that the changes in scab control, switching from dipping to pour-on or injectable treatments, have caused the increase in the populations of these two ectoparasites. Shearing combined with periodic animal treatments has been highly effective in controlling these parasitic diseases. This approach is recommended to farmers who want to deal with the problem.

Hydatid disease

Hydatid disease due to the larval stage of the taenia *Echinococcus granulosus* [15–17] is widespread. Several cysts full of fluid and oncopheres, within the lungs and liver, are the most common post-mortem findings in affected sheep (see Figure 71.8 in the colour plate section). Although not having much impact on sheep health, the disease is an important issue in public health, constituting one of the most important zoonoses in rural Patagonia. The disease remains prevalent in sheep due to several factors, the most important being farmers: (a) feeding their dogs raw offal from sheep slaughtered on-farm for home consumption; and (b) not submitting their dogs to a routine worming.

TOXIC AND METABOLIC DISORDERS

Astragalus poisoning

Locoism or locoweed intoxication due to the ingestion of *Astragalus* plants occurs in correlation with the distribution of *Astragalus pehuenches* (see Figure 71.9 in the colour plate section). It affects sheep of all ages and usually occurs when *Astragalus* plants are the only green forage available in late autumn or winter. Outbreaks, lasting for more than 2 months and in

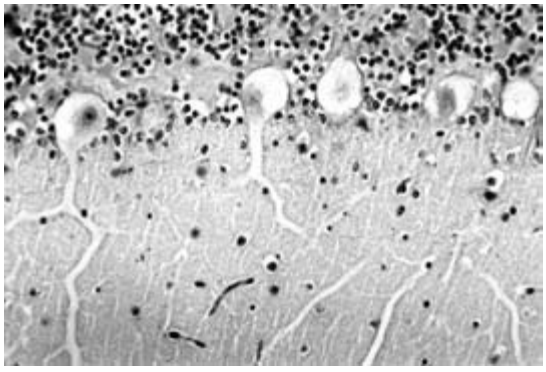

Figure 71.10: *Astragalus* intoxication. Severe vacuolation of Purkinje cells of cerebellum. Haematoxylin and eosin.

which up to 73 per cent of the animals died, have been recorded. The clinical course of the disease ranges from 8 to 12 days and is characterized by abnormal behaviour including blindness, weakness, loss of appetite, ataxia, recumbency and death. No significant gross features are found at necropsy but marked vacuolation of neurons and astrocytosis throughout the central nervous system is visible histologically. Eosinophilic spheroids are present in both grey and white matter. In the absence of scrapie the prominent vacuolation usually seen in Purkinje cells of cerebellum (Figure 71.10) can be a pathognomonic finding [19]. To date, no practical treatment has been found to cure affected animals under field conditions. The control of *Astragalus* plants by use of selective herbicides is recommended where possible.

Huecú and tembleque

These two diseases are due to the ingestion of either *Poa huecú* or *Festuca argentina* (see Figure 71.11 in the colour plate section), two native perennial grasses that grow in some areas of Patagonia. Affected animals develop nervous signs, consisting of tremors, ataxia of fore limbs, loss of appetite, recumbency and death [20]. Histologically, focal lesions of the status spongiosus in the brain and cerebellum are characteristic. Degenerative lesions can be observed in the myelin and axons and in the ventral and lateral funiculi of the spinal cord. Focalized myodegenerative changes in the skeletal muscles have been observed also [21]. While the plants have been considered to be toxic by themselves, studies in which endophyte fungi were detected in the plants suggest that mycotoxins could be the real cause of the diseases [22]. Preventing sheep from grazing fields where the plants are present is recommended, as no treatment has been found to improve the health of affected animals.

Mycotoxins

Poisoning by mycotoxins has been recorded in sheep grazing Patagonia's natural pastures. Most commonly, the disease occurs as large outbreaks that, depending on weather conditions, can recur year after year. When made to move, affected sheep begin walking quite normally but soon develop progressive limb incoordination. If forced to continue walking they fall and remain recumbent, displaying fine body tremors. After a few minutes, and if not disturbed, they recover fully. In more advanced stages of the disease, the animals develop severe incoordination, marked ataxia, recumbency and after a few days die of starvation. Secondary pneumonia can be detected at post-mortem examination. Histologically, a moderate number of myelin sheets in the dorsal and ventral roots and in the dorsal, ventral and lateral bundles of the spinal cord show signs of degeneration that can be detected by the Schwan–Davenport technique. Several species of *Penicillium* spp. which produce tremorgenic toxins such as penitrem A, penitrem B and roquefortine have been isolated from pastures on affected farms and suspected as putative causes of the disease. No treatment has been found to alleviate the conditions of affected animals. Prevention is by avoiding overgrazing and improving grazing management [23].

Drug and product overdose

Cases of overdosing with mineral supplements and anthelmintics have been reported. Usually, they occur because of errors in estimating body weights or in calculating correct doses when veterinary supervision is not available. The most common intoxications are acute and chronic copper poisoning because of incorrect use of mineral supplements. Problems arise in the use of both oral and injected products. In some

cases mineral supplements indicated for cattle are injected as a single dose to sheep and, as sheep are more susceptible to copper toxicity, they usually became acutely intoxicated. In other instances, several doses are given over a short period of time and the animals develop a chronic intoxication [24]. A clinical diagnosis of copper poisoning can be confirmed at necropsy by the generalized jaundice and liver haemorrhage of acute cases, and by jaundice, orange-hued liver and congested and dark kidneys of chronic cases.

Pregnancy toxaemia

This condition usually follows a sudden drop in feed availability and/or adverse climatic conditions that lead to starvation. Clinically, it is characterized by dullness, lagging behind the flock, walking in circles, blindness, recumbency and death. Since treatment is rarely possible in Patagonia's extensive systems, prevention of the disease by improved management and nutrition is the sole option. A clinical condition similar to pregnancy toxaemia, with ketone bodies in urine, has been seen on occasions during winter in castrated male sheep and in non-pregnant ewes, when the animals were deprived of grazing for several days because of snow storms.

Hypomagnesaemic tetany

Tetany due to hypomagnesaemia has been described in Merino sheep under extensive grazing systems where overgrown pastures and poor nutrition were the norm. Most commonly, it occurs under situations of intense stress, such as shearing. At that time, animals are confined in small paddocks without water or forage, to allow for shearing, dipping, sometimes deworming and, finally, vaccination against clostridial diseases. As a consequence, on the way back to their normal pasture, some animals develop clinical signs consisting of incoordination, severe tetany, opistotonous, recumbency with limb extension and death in 48–72 hours (Figure 71.12). Treatment is usually impossible, so prevention through better nutrition and improved management at shearing time is the best way to overcome with this problem [25].

Urolithiasis

Urolithiasis occurs most frequently in pedigree rams on stud farms. It is associated with concentrated diets, with high levels of protein and a low water intake. Affected rams can be treated to prevent urinary infections and to encourage the release of urethral calculi. In cases where calculi are not expelled, surgery is

Figure 71.12: Hypomagnesaemia. Severely affected sheep with all four limbs extended.

necessary to save the animal's life. Control and prevention of the disease can be achieved by making changes in the diet and increasing water consumption.

DISEASES CAUSED BY PROTOZOA

Coccidiosis

Coccidiosis is mainly a disease of lambs and rarely of adult animals. It is found mostly within intensive production systems. Clinical coccidiosis is most prevalent under conditions of poor nutrition, poor sanitation, overcrowding, worm infestation and stressful situations such as weaning or bouts of severe weather. Catarrhal diarrhoea, dullness, anorexia leading to dehydration and marked loss in weight and body condition, weakness and death are the main clinical characteristics of affected animals. The disease is not common in Patagonia's extensive, open-field management system but sporadic outbreaks in lambs have been recorded on stud farms and in feedlot-like production units [3].

Sarcocystiosis

Both forms of sarcocystiosis have been detected in sheep in Patagonia. The macrocystic disease produced by *Sarcocystis ovifelis* (*S. gigantea*) is characterized by the presence of elliptical cysts, having the appearance of rice grains, found most commonly in the connective tissue of the oesophagus (see Figure 71.13 in the colour plate section) at slaughter of Corriedale sheep in Tierra del Fuego Island. They are not considered to pose any health threat to sheep. The microcystic form, due to *S. ovicanis* (*S. tenella*), is an incidental histopathological finding in heart muscle samples processed for any diagnostic purpose. Small cysts containing the bradyzoites are usually seen in cardiac muscle. No disease or negative health effects have also been associated with this form (unpublished observations).

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Part XV
Technical section

72

Flock health programmes

R.N. Spedding, J.C. Hindson and J.A. Earl

Some 25–30 years ago annual losses to the UK sheep industry from death were reported as being between 17 and 25 per cent [1, 2], considerably higher than those for cattle and pigs (8 and 5 per cent, respectively). There is little reason to suppose that there has been a significant reduction in these rates of loss since those figures were compiled. While a significant part of this loss is a function of exposure to an environment with temperature variations (snow-storms, floods, drought and predation, particularly in the USA and Australia) much will be due to management failure and overt disease. The latter often is a function of intensification.

Such losses are clearly unacceptable, and open to reduction by coordinated flock health control measures. Some years ago Europe's largest sheep keeper with some 20 000 breeding ewes, stated that 'there must be a continuous downward pressure on costs for profitable sheep keeping, but this must never extend to veterinary costs!'.

While this chapter is concerned mainly with coordinated flock health schemes involving client and clinician, these schemes, of which there are five general categories, proceed within a context of multilayered sheep health control:

- statutory schemes
- certified national/regional schemes (non-statutory)
- coordinated flock health programmes
- planned, unsupervised schemes
- basic response to specific episodes.

The objectives of flock health control are to reduce disease and other loss, to improve welfare, and to increase production and/or profitability. In addition, state-imposed schemes aid national and international trade in sheep. Although the aims of individual flocks are different, the basic philosophy in flock health control can be applied equally to hill or lowland breeding sheep, sheep dairying and specialized wool production.

STATUTORY SCHEMES

Several nations reserve the right to impose by statute such control measures as are deemed necessary to maintain an internationally accepted statement of absence of specific sheep diseases. In the UK, this includes foot-and-mouth disease, while in Denmark, Australia and New Zealand the priority is scrapie. Individual national lists will be freely available to those involved in the sheep industry.

A further example of statutory schemes is the National Scrapie Plan for Britain, implemented by the Department for Environment, Food and Rural Affairs (Defra), the Scottish Executive and National Assembly for Wales. Its purpose is to eradicate sheep scrapie by selecting for resistant genotypes used for breeding. Initially a voluntary scheme, certain components have become statutory in line with European Union (EU) legislation. Details of the plan can be found on the Defra website [3]. The basis for genetic selection is described in Chapter 35.

CERTIFIED NATIONAL/REGIONAL SCHEMES (NON-STATUTORY)

These may be national, regional or even based on breed. Although driven primarily by a search for added value, considerations for animal welfare and a general reduction in disease incidence are major factors.

Such schemes, although led by industry, have been operated initially by government bodies and then reverted to industry control in both the UK and Denmark. Other examples can be found in Australia with reference to foot-rot and internal and external parasites [4, 5]. These schemes are based on a very short list of diseases for which group certification offers a guarantee of freedom to purchasers at the point of sale. More recent initiatives are the Ontario (Canada) and Brazilian Sheep Health Programmes [6, 7].

In the UK, a scheme started after the arrival of maedi-visna (MV) was initially breed-based. Ovine pulmonary adenocarcinoma (OPA), enzootic abortion (EAE) and scrapie were added later, and the whole scheme adopted by Defra's predecessor (Ministry of Agriculture, Fisheries and Food) as the Sheep and Goat Health Scheme, but now MV and EAE modules are run by the Scottish Agricultural College [8], together with a recently introduced ram monitoring scheme for caseous lymphadenitis.

Whatever conditions are selected, all such schemes must operate within accepted guidelines, which are:

- the existence of infectious disease that is identifiable and eradicable
- a diagnostic test of high sensitivity and specificity
- eradication to be cost-effective
- a certifying body of recognized integrity
- permanent identification of individual sheep
- adequate record keeping.

COORDINATED FLOCK HEALTH PROGRAMMES

It has been recognized that the rates of loss given in the introduction were open to cost-effective reduction if stock keepers and their advisers were able to cooperate within an agreed framework and objectives to produce a flock health programme. A definition of such a programme would be one drawn up by a veterinary practitioner in consultation with his or her client, which would enhance the health, welfare and profitability of the client's sheep enterprise.

The first schemes were initiated in Australasia in the late 1970s and were operated both by private groups and by university 'Outreach' teams. The report on a New Zealand scheme included a cost–benefit analysis of between −3 and +24 per cent [9]. By 1982, the first UK scheme was detailed [10], followed by one in The Netherlands in 1984 [11]. Schemes to control disease and promote sheep health now operate in Norway [12], Sweden [13] and Denmark [14].

In the UK, heightened interest has been prompted by the industry's need for improving productivity given the greater financial pressures on it and by the accompanying requirement to meet higher enforceable welfare standards. Consequently, many clinicians have adopted the original formats to suit their clients' requirements and the Sheep Veterinary Society, led by Earl [15], has developed a 'gold standard' computerized flock health programme (see later).

All health schemes will vary with the challenge presented and with consideration of local disease patterns and environmental limitations. However, there is an overall structure within which all must operate if they are to succeed. These are:

- an identified area of suboptimal production or direct loss, which is accepted and agreed by all parties
- identifiable, key points in the production cycle
- available external expertise on ovine systems of management
- willingness by the veterinary surgeon to acquire and maintain such expertise and to develop the ability to communicate this to the client
- willingness of the client to recognize and accept the advantage of the advice offered
- target-setting
- cost-effective veterinary fees, which are discussed and agreed at the start.

The whole package relies on the three 'Rs' for successful operation: regular visits, records and reports.

Flock health programmes do not differ fundamentally from those that have operated successfully for a number of years in both cattle and pig industries, to which the aim of such schemes defined above apply equally.

There are, however, two serious limitations that have made widespread adoption of non-statutory sheep health schemes difficult. The first is that of continuity. The problem stems from the pattern of production; since this is episodic, veterinary involvement also tends to be episodic. Extra motivation from both veterinarian (especially) and client is therefore essential. The second problem, also a function of sheep production, is that of cost–benefit analysis. Since the output and profitability of the sheep industry fluctuate due to seasonal

variation, there can be great difficulty in identifying the benefit from the effect of the scheme within the pattern of other variables. In common with all animal health schemes, flock health programmes will succeed only if clients are fully motivated and convinced that the balance of advantage is in their favour and not that of their advisers. Schemes tend, therefore, to be problem-initiated and success-terminated. The veterinary surgeon who keeps asking the following questions will stay out of trouble and find that, by constant re-evaluation, new dimensions of involvement will continue to present themselves:

'Is what I am doing continuing to fulfil the client's objectives?'
'Is what I am doing worth anything to the farmer?'

For any innovation to be meaningful, it must convey greater benefit to the clients than to the adviser. Clients should recognize their own wisdom in recruiting veterinary involvement, but the consultant should not fear being supplanted.

Initial review

The first step in any programme must be an initial review at a planned meeting, when the whole scheme to be followed should be established. This will give clients the opportunity to indicate their needs and degree of commitment, both in enthusiasm and finance.

The following categories should form the basis for this programme:

1. Analysis of past performance and future objectives:
   - pedigree breeding
   - maximum output
   - maximum profit
   - results of any previous tests.
2. Identification of problems:
   - overt disease
   - macro-/micronutrient deficiencies
   - reproductive performance.
3. Stock assessment:
   - body score/body weight at the appropriate stage of the productive cycle
   - disease
   - ram health
   - breed suitability for area/objectives.
4. Plan of disease control:
   - essential – clostridial/worms/fluke/pasteurella
   - optional – orf/EAE and toxoplasma/foot-rot.
5. Plan the recording system:
   - manual/computerized
   - details; whose responsibility, client and/or veterinary surgeon?
6. Target-setting:
   - high but achievable.

Although it would appear to be a truism that the maximum return on working capital will be the sole aim of all sheep keepers, this is not exclusively so. The sheep enterprise may not be the principal activity on the farm and the production of prime pedigree stock may be for social prestige! However, on most units wishing to be involved in a health programme, maximum profitability will be the key motivation. When areas for improvement cannot be identified, the clinician should have the courage to say so.

Where a practical 'hands-on' approach to the programme is necessary, an assessment should be made of the stock and its problems. The practitioner should have local knowledge of problems of macro- or micronutrient deficiencies (Chapters 53 and 54) but should not hesitate to call in other sources of expertise if required. Stock assessment should include breed suitability for the agreed objectives. In addition, a method for either body scoring (Chapter 8) or weighing at key points in the production cycle should be agreed with the client. As to ram health, suitability for the aims established should be agreed and fertility assessed (Chapter 9). Levels of fertility vary significantly within a group of rams, but it is possible, by examination, to assess how each ram conforms to the accepted 'normal' with regard to its reproductive function [16].

The disease control plan is central to any scheme. This can be set down in detail for reference, but is best displayed as a chart in a prominent place at the central point of sheep handling. If the chart is displayed and procedures noted as applied, there can be no cause for disagreement as to the advice, its application and how to improve if the result is less than ideal. Comprehensive charts of disease occurrence and control methods, planned animal health and production performance, annual health measures chart, performance targets and flock health scheme records are available [17]. However, these will be far too demanding for anything other than very intensive or pedigree breeding units. Such records are of use only if fully maintained and if the information can be utilized. An example of a basic health action chart is given in Table 72.1, but the clinician and client together

should establish such records as fit the particular demands of the enterprise.

Since the control programme must be as cost-effective as possible, it must be limited to those treatments that are essential plus those that are optional, but indicated in any one unit. It is absolutely vital that the client be acquainted with the whole spectrum of possible diseases and the reasons for omitting some agreed with the client, otherwise there is scope for litigation.

Table 72.1: Health action chart

Disease	Nutrition
Weaning to mating	
Ewe flock	
Culling for udder/fee/teeth/production	Body score and group for varying points to achieve uniform body score at mating
Metabolic profile for micronutrient levels; correct where indicated	
Foot care	
Establish abortion status – appropriate vaccinations	
Lamb flock	
Worm dose at weaning; 'hold' overnight	Transfer to clean/safe grazing
Routine drenching if no 'Spring suppression' used	
Booster vaccinations where indicated	
Rams	
Check fertility/feet/body score; drench and vaccinate	Adjust intake for body score
Pregnancy	
Mid pregnancy **full** metabolic profile	Maintain high inputs to time of implantation
Correct where indicated	
Scan-divide groups as indicated when housing	Adjust intake for fetal load and body score
Foot care	
Booster vaccines plus orf if indicated	
Lambing and lactation	
Detailed plan of lambing logistics	
Equipment check	
Staff expertise check and update	Maintain nutritional intake
At turn-out check for bonding	Decrease concentrate feeding slowly to match grass growth
Ewes; feet	
Lambs; tailing and castration	
Ewes; monitor for:	
Mastitis	
Metritis	
Ca and Mg status	
Lambs; monitor for:	
Hypothermia	
Hypoglycaemia	
Post-natal septicaemia	
Enteritis	
Watery mouth	
Post-turn-out monitor for:	
Ewe spring suppression sequence	
Coccidia suppression	
Lamb growth rates and enteritis	
Monitor for worm egg counts and coccidial oocysts	

Table 72.2: Performance targets. This example is based on sheep meat production under intensive conditions, utilizing prolific 'hybrid' ewes of 70–75 kg body weight

Lambing percentage	
Hoggs; 70 per cent lambing with 100 per cent born	
Two tooths; 95 per cent lambing with 180 per cent born	
Mature ewes; 98 per cent lambing with 190 per cent born	
Avoidable losses	
Lambs born dead	5 per cent
Lambs born alive	0 per cent
Deaths 0–48 hours	0 per cent
Deaths 48 hours to weaning	0 per cent
Ewe deaths	Less than 4 per cent
Culling rate less than	15 per cent
Target weights	
Total lamb birth weight per ewe	10 kg
50-day weights	20 kg per lamb
Weaning weight	28–30 kg
20-week sale weight	36 kg
Hogg tupping weight	45 kg
Shearing tupping weight	65 kg

Targets can be expressed only in terms of physical output, not economic returns.

Targets are essential if the success of any scheme is to be assessed objectively. The targets should be high but achievable. If met, they can be raised; if not attained within an agreed timescale, the reasons can be identified. The clinician should not hesitate to accept his or her part in any shortfall. An example of performance targets for an intensive lowland unit in the UK is shown in Table 72.2.

In common with all other management programmes, the keeping of records is essential for this monitoring process. However, there will be a wide variation in the form and sophistication that records take. In the case of a pedigree unit, these cannot be too wide, but commercial units should not be overloaded with information that cannot be utilized. If too complex, they will not be maintained. As the records will frequently have to be maintained during periods of severe stress, such as lambing time, they should be stress-proof, idiot-proof and waterproof!

Schedule of visits

The timing and frequency of visits will vary with both the enterprise and the clinician, but must be synchronized with key points in the production cycle. The scheme outlined below is based on a four-visit programme suitable for UK conditions. There should be sufficient interval between each visit and the key points of production so that correction of problems can be put into place that are likely to be effective, such as correcting the micronutrient intake and body score.

Post-weaning; pre-tupping visit

Since ovulation rates are largely a function of body condition and nutrient intake, and thus can be influenced and freed from other constraints, stock assessment is vital at this stage in the production cycle. Blood assay for nutrient status should be included.

Rams should be assessed for general health, body score/weight, condition of feet and teeth, and for fertility. The clinician must note that certain breeds will not emerge from the refractory period due to the photoperiodic effect until early September in the UK and that spermatogenesis can take up to 8 weeks to achieve full fertility.

Mid-winter; pre-lambing visit

Again, the nutritional status of the flock is central to both fetal growth and milk production. If the ewe does not lamb with her lactation potential fully realized, this cannot be corrected on a short-term basis with the lamb at foot. A full blood profile of a sample of ewes should be routine, and action taken at least 8 weeks before anticipated lambing date. Tupping results from scanning should be assessed and detailed planning of lambing facilities completed. Disease control strategies such as vaccinations should be re-emphasized. Perhaps most important, the expertise of the staff in correcting dystocias should be checked. If any shortcoming is identified, training should be instigated.

Post-lambing visit

Although important, if fewer visits are required this one can be omitted. The results of the lambing should be analysed in detail, with losses of those born alive being central. Ideally, this should be zero but will rarely be achieved. Anything less than 5 per cent for the total of lambs born dead, or dying within 48 hours, is praiseworthy, and this figure should be used when setting targets. At this time, lactation levels, periparturient problems and any lamb scouring problems should be considered.

If infectious abortion has been diagnosed during the season, control measures should be agreed for following years. The correction of any shortcomings in the facilities can be written into the programme for future seasons. Finally, early grazing management and anti-parasitic programmes should be confirmed.

Details of all disease control methods will be found in other chapters.

Mid-summer visit

At this visit, lamb growth will be the central factor to consider as, if suboptimal, this can erode much of the effort during the other production cycles. Parasitism (coccidia and helminths) will be the obvious areas to examine but also macro- and micronutrient intake will be important. Beware of low dry matter intake, as high spoilage levels of food during wet seasons may complicate what appears to be adequate availability.

Reports

Each visit should be closely followed by a report outlining details of the relevant decisions and date of next visit. Reports have two main purposes: (1) for reference to prevent possible disagreement on strategies; and (2) to familiarize other colleagues in the practice with the programme should the veterinarian responsible be absent.

COMPUTERIZED FLOCK HEALTH PROGRAMS

As the aforementioned reports and written protocols can be time-consuming for the veterinary surgeon to produce, computer programs have been purposely designed to ameliorate this restraint.

Program format

The Sheep Veterinary Society (UK) has produced a computerized plan that is intended to be the gold standard. The plan can be purchased to stand alone or as a bolt on to the British Cattle Veterinary Association Herd Health Plan. Its list of contents is shown in Table 72.3. As can be seen, this plan starts with flock details and production records before going on to deal with more specific issues. It covers all the management, husbandry, nutritional and disease aspects of a flock, and, as such, it allows either a single problem or every possible aspect of monitoring a healthy flock to be discussed with the farmer. Thereafter, appropriate preventive measures can be instigated.

Table 72.3: Sheep Veterinary Society flock health plan menu

Farm and veterinary details
Flock production records
Management and husbandry
Veterinary care
Medicines
Biosecurity and isolation
Infectious disease
Parasitic disease
Lameness
Mastitis
Nutrition
Fertility and reproduction
Lamb health
Reasons for culling
Calendar

System requirements. The minimum specifications required are:

- Pentium 233 processor or equivalent
- 32MB RAM
- CD-Rom drive
- Windows 95/98/2000/XP/NT. (The software will not run on Windows 3.1.)

The CD ROM is available from the:
Sheep Veterinary Society, Moredun Research Institute, Pentlands Science Park, Bush Loan, Penicuik, Midlothian EH26 0PZ.

Summary

By having a pre-written plan that includes all the requirements for a complete health plan and that allows a plan to be specifically tailored to an individual farm – and farmer – the time required to produce a gold standard flock health plan can be dramatically reduced. If the time is reduced, so is the cost, and consequently the idea of a flock health plan has one

less hurdle, or at least a lower one, to overcome when being promoted to farmers.

PLANNED UNSUPERVISED SCHEMES

Many and perhaps most stock keepers will operate some form of flock health control to avoid high economic losses. They will have a programme, which may or may not be written down, and usually will not be properly evaluated. These programmes may well have been formulated either as a result of experience, formal education, attendance at practice meetings or organized courses.

Even at this level there are certain prerequisites:

- areas of loss must be identified and accepted
- cost-effective control measures such as vaccines and anthelmintics must be available at cost-effective prices
- control of disease, which will have to be integrated into other management constraints (dairying or arable) and operated within the limitations of the environment, such as a high hill area.

If requested, the veterinarian should not hesitate to give full support in establishing such a level of health control, possibly limited to the provision of progress charts. By doing so, he or she retains goodwill, a confirmed commitment to health control and may even be invited to more detailed involvement later, in the establishment of a full scheme.

BASIC RESPONSE TO SPECIFIC EPISODES

At the lowest level of stock management, where cost pressures are most severe, a large percentage of sheep keepers will simply respond to an individual episode by applying methods based on tradition, suggested by neighbours or simply on commercial pressure from pharmaceutical or feed companies. There will be little or no evaluation, coupled with a philosophical acceptance of the death-wish of sheep. Even at this level, the veterinarian should be able to offer some valuable inputs.

CONCLUSION

While undoubtedly presenting a challenge to the motivation, commitment and competence of the practitioner, the operation of coordinated flock health programmes can achieve the stated objectives and add to the job satisfaction and remuneration of both client and clinician. Let there be no doubt that the halcyon days of almost 'cost-plus' farming economics are gone forever, even in the UK. Both clients and veterinary practitioners are therefore under increasing pressure from their customers to produce sheep products in a welfare-friendly manner, to standards of farm assurance and traceability that can be strictly maintained. With the distant possibility that stock keepers may have to be subject to certified competence, veterinary surgeons have a vital role to play in helping to achieve those standards. One aspect of that role is the setting up and sustaining of coordinated flock health programmes.

If, after a period of 2–3 years, the client is sufficiently confident to continue with less support, then this is success, not failure. If the clinician originally is presented with a standard of health control that is less than ideal and raises this level, the outcome can be regarded as satisfactory.

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Pharmacology and therapeutics

S. Page and D. Hennessy

Sheep production is a significant undertaking in many countries including the UK, Australia, New Zealand and South Africa but, on a global basis, sheep are considered a minor species by the pharmaceutical industry. Total sales of veterinary medicines (parasiticides, biologicals, anti-infectives, feed additives and other pharmaceuticals) in 2005 was almost £8 billion with sales of £400 million (or 5 per cent) in sheep. This can be compared with sales of 40 per cent in companion animals, 27 per cent in cattle, 16 per cent in pigs and 11 per cent in poultry. While the animal health market is forecast to grow by 7 per cent over the next 4 years, sales in sheep products are expected to decline. Because sheep are the smallest market segment and in decline, and because the development of a new chemical entity can cost approximately £27 million, it is unlikely that new pharmaceutical products (drugs) will be developed specifically to address the animal health and welfare needs of sheep. Vaccines, which are significantly less costly to develop, will continue to become available. Products currently available will need to meet the ongoing demands of veterinary practice well into the future. This reinforces the need to use available therapeutic resources judiciously.

Vaccines, while widely used, are not the principal focus of this chapter. The pharmaceutical products most commonly used in sheep include the endoparasiticides, ectoparasiticides, antibacterials and drugs for reproductive manipulation. A summary of products approved for use in the UK, Australia, South Africa and the USA is set out in Table 73.1. Sources of regulatory and pharmacological information about these products can be obtained from the websites listed in Table 73.2.

Two areas currently not well served, but likely to require increased attention in the near future, are antibacterial treatments for use in lactating dairy sheep and analgesic drugs to provide pain relief during the conduct of routine husbandry practices. A third area that is rapidly being challenged by the selection and dissemination of drug resistance is that of the endoparasiticides. If new anthelmintic drugs are not developed the success of sheep production may be threatened in an increasing number of countries within the next decade.

Table 73.1: Active constituents approved for use in sheep in Australia, South Africa, the UK and the USA

Class	Active constituent
Anaesthetic, central	Halothane,[1,2,4] Pentobarbital sodium,[1–3] Thiopentone[1,2]
Anaesthetic, local	Lignocaine[1,2]
Antibacterial	Amoxicillin,[1–3] Ampicillin,[3] Bacitracin zinc,[*1] Ceftiofur,[4] Chlortetracycline,[1,3,4] Cloxacillin,[1,3] Doxycycline,[2] Erythromycin,[1,4] Kanamycin,[2] Lincomycin,[*2] Neomycin,[1–4] Oxytetracycline,[1–4] Penethamate hydriodide,[1] Penicillin,[1–4] Sodium iodide,[2] Spectinomycin,[2,3] Streptomycin (dihydro),[2,3] Sulfadoxine,[*1,2] Sulfatroxazole,[*1] Sulfadiazine,[*1,2] Sulfadimethoxine,[2] Sulfadimidine,[1,2] Sulfamethoxazole,[*2] Sulfapyridine,[2] Tilmicosin,[3,4] Trimethoprim,[*1,2] Tylosin,[2] Virginiamycin[1]
Antibloat	Dioctyl sodium sulfosuccinate[1,2,4]
Antihistamine	Chlorpheniramine[1]
Anti-inflammatory, non-steroidal	Aspirin[4]
Anti-inflammatory, steroidal	Dexamethasone[1,2]
Antiprotozoal	Decoquinate,[3,4] Diclazuril,[2,3] Isometamidium,[2] Lasalocid,[1,2,4] Monensin,[2,4] Salinomycin[2]
Autonomic	Adrenaline,[1,4] Atropine[3,4]
CNS	Acepromazine,[1,2] Doxapram,[3] Ketamine,[1] Xylazine[1]
Growth promoter	Zeranol[2,4]
Parasiticide, anthelmintic	Albendazole,[1–4] Albendazole oxide,[1–3] Closantel,[1–3] Fenbendazole,[1–3] Levamisole,[1–4] Mebendazole,[1–3] Morantel,[1–3] Naphthalophos,[1] Netobimin,[3] Niclosamide,[1,2] Nitroxynil,[1–3] Oxfendazole,[1–3] Oxyclozanide,[2,3] Piperazine,[2] Praziquantel,[1–3] Pyraclofos,[*1] Rafoxanide,[2] Resorantel,[2] Triclabendazole[1–3]
Parasiticide, endectocide	Abamectin,[1,2] Doramectin,[1–3] Ivermectin.[1–4] Moxidectin[1–3]
Parasiticide, external	Alpha-cypermethrin,[1–3] Amitraz,[1–3] Chlorfenvinphos,[*1,2] Cyfluthrin,[2] Cypermethrin (±PBO),[1–3] Cyromazine,[1–3] Deltamethrin (±PBO),[1–3] Diazinon,[1–3] Dicyclanil,[1,3] Diflubenzuron,[1,2] Esfenvalerate,[2] Fenthion,[2] Flumethrin,[2] Magnesium fluorosilicate,[*1] Permethrin (±PBO),[4] Propetamphos,[1,2] Pyrethrins* (±PBO),[1,4] Rotenone,[*1] Spinosad,[1] Temephos,[1] Triazophos,[2] Triflumuron[1,2]
Reproductive	Clenbuterol,[1,2] Flugestone,[1–3] FSH-ovine,[1] Gonadotrophin-serum,[1,2] Luprostiol,[2] Medroxyprogesterone acetate,[2,3] Melatonin,[1,3] Oestradiol benzoate,[1] Oxytocin[1–4] PMSG,[1–3] Polyandroalbumin,[1] Progesterone,[1] Testosterone (enanthate, propionate)[1,2]
Respiratory	Etamiphylline[2,3]

* Only available in a combination product. Approvals (November 2005): [1]Australia (APVMA online); [2]South Africa (IVS October to December 2005); [3]UK (NOAH Online Compendium); [4]USA (Compendium of Veterinary Products, online).
CNS, central nervous system; FSH, follicle stimulating hormone; PBO, piperonyl butoxide; PMSG, pregnant mare serum gonadotrophin.

CLINICAL PHARMACOLOGY

Pharmacology and therapeutics fall within the domain of clinical pharmacology, the discipline devoted to the rational use of drugs whereby dosage regimens are selected and designed to provide an optimum balance of efficacy and safety in the individual or flock under the care of the veterinarian. Although an often subconscious iterative process, the ideal approach to initiation and management of drug therapy can be represented by the steps presented in Figure 73.1. Of pivotal importance to optimal performance is ongoing and continuous reassessment of the response to treatment. Only

Table 73.2: National regulatory bodies for veterinary medicines: Sources of regulatory and drug information

Country/Region	Website Address	Description
Australia	http://www.apvma.gov.au/	Veterinary medicines must be approved by the Australian Pesticides and Veterinary Medicines Authority. This website contains information on all approved sheep products and their labels as well as summaries of adverse drug reactions (ADRs)
Europe	http://www.emea.eu.int/index/indexv1.htm	European Medicines Evaluation Agency (EMEA) Information on veterinary medicines, guidelines, Maximum Residue Limits (MRLs) and European Public Assessment Reports (EPARs), pharmacovigilance
Europe	http://pharmacos.eudra.org/F2/mrl/index.htm	European Union MRLs of veterinary medicinal products in foodstuffs of animal origin, including Annexes I–IV
New Zealand	http://www.nzfsa.govt.nz/acvm/	The Agricultural Compounds and Veterinary Medicines (ACVM) Group is responsible for registration of veterinary medicines. The site contains information on all approved products and copies of many labels
South Africa	http://www.nda.agric.za/act36/main.htm	Act 36 (Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act, 1947) applies to over-the-counter products. This site contains information on the reporting of ADRs
South Africa	http://www.mccza.com	Act 101 applies to veterinary prescription medicines and is administered by the Medicines Control Council
UK	http://www.vmd.gov.uk	The Veterinary Medicines Directorate is an Executive Agency of the Department for Environment, Food and Rural Affairs (Defra). This site contains information on legislation, ADRs, Veterinary Medicines Regulations 2005, and many useful links
UK	http://www.noah.co.uk/	National Office of Animal Health (NOAH). The site contains information on withholding periods and access to an online compendium of approved products
UK	http://www.ruma.org.uk	RUMA. Responsible Use of Antimicrobials in Sheep Production (September 2005)
UK	http://www.rcvs.org.uk	Royal College of Veterinary Surgeons. Guide to Professional Conduct, including 'The Use of Veterinary Medicinal Products'
USA	http://www.fda.gov/cvm	The Center for Veterinary Medicine (CVM) regulates the manufacture and distribution of food additives and drugs that will be given to animals. This site contains a database of all approved products (Green Book), information on ADRs, Freedom of Information (FOI) summaries and the Animal Medicinal Drug Use Clarification Act of 1994 (AMDUCA)
USA	http://www.aphis.usda.gov http://www.aphis.usda.gov/vs/cvg	The US Department of Agriculture regulates animal vaccines and bacterins within its Center for Veterinary Biologics (CVB)
USA	http://www.epa.gov/pesticides http://www.epa.gov/pesticides/fifra6a2	The US Environmental Protection Agency (EPA) regulates topically applied external parasiticides. The Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) requires pesticide product registrants to submit adverse effects information about their products to the EPA and voluntary reports are also welcome
USA	http://www.farad.org	The Food Animal Residue Avoidance Databank (FARAD) is an excellent resource for guidance on withholding period (WHP) estimation after extra-label use. A compendium of drugs approved in the USA is also provided

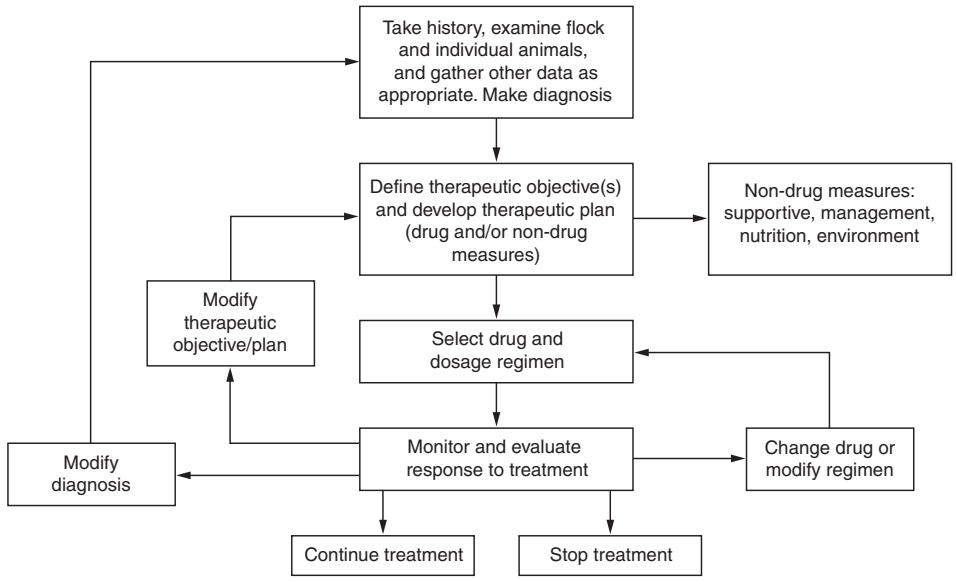

Figure 73.1: Steps in the initiation, management and reassessment of drug therapy.

by understanding the factors that can influence the response to treatment is it possible to institute appropriate modifications to diagnosis, therapeutic objectives and plans. William Osler, the renowned Canadian physician said 'One of the first duties of the physician is to educate the masses not to take medicine'. Similarly in veterinary practice, the management of a disease or disorder may not necessarily include drug treatment.

Many factors lead to variability and unpredictability in response to drug treatment. Some of the most significant sources of variation are set out in Table 73.3 and include factors that influence the pharmacokinetic and pharmacodynamic phases which logically separate the dose–response relationship into dose–concentration and concentration–effect divisions. While a number of important sources of inter- and intra-animal variation are presented in Table 73.4, other factors include:

- *Age*: hepatic drug metabolism of neonatal sheep increases significantly during the first week of life [1]. Rumen development occurs rapidly to approach adult proportions by 8 weeks, although the time is dependent on diet [2]. Body composition changes substantially as animals mature, notably total body water declines from around 74 per cent in neonates to 58 per cent in adults [3], possibly necessitating a change in dose schedule of drugs such as oxytetracycline that distribute in body water [4].
- *Disease*: a number of disease conditions can affect the distribution of drugs to their site of action [5, 6] as well as having impacts on gastrointestinal function and hepatic and extrahepatic biotransformation reactions [7]. External [8] and internal [9–12] parasitism have been associated with unfavourable changes in drug disposition.
- *Season*: while seasonality of reproductive activity is a well-known source of changes in the physiology of ewes, other effects with potential impact include seasonal changes in blood acetylcholine esterase activity of sheep and the efficacy of drugs against the itch mite *Psorergates ovis*, assumed to reflect the eubiotic or hypobiotic activity of the mite. Analogous seasonal effects are also apparent with a number of gastrointestinal nematodes.
- *Breed*: xylazine activity appeared to be breed-specific when assessed in Clun, Swaledale and Welsh Mountain breeds, although breed-specific body weight differences may have explained the observations [13]. Pentobarbital sleeping time is approximately twice as long in Romney as in Merino sheep (96 vs 46 min after intravenous dose of 20 mg/kg) [14]. The activity of plasma A esterase varies within sheep populations, with

Table 73.3: Factors influencing the relationship between dose and effect

Prescribed dose	
● Compliance ● Medication errors ● Pre-treatment interactions ● Product quality	**Pre-administration phase**
Administered dose	
● Route and site of administration ● Drug formulation characteristics ● Drug physicochemical properties ● Body size and composition ● Feeding regimen ● Bioavailability (rate and extent of absorption) ● Physiological/pathological state ● Protein and tissue binding ● Genotype and ADME ● Drug and non-drug interactions ● Clearance (metabolism and excretion)	**Pharmacokinetic phase** Dose–concentration (absorption, distribution, metabolism, excretion) (ADME)
Concentration at site of action	
● Drug–receptor interactions ● Resistance ● Tachyphylaxis ● Realistic potential to respond ● Measurement of response ● Concentration–time profile	**Pharmacodynamic phase** Concentration–effect
Effect	
● Quantitative effect (e.g. microbial eradication) ● Qualitative effect (e.g. lameness improved) ● Impact of placebo and nocebo phenomena	

ADME, absorption, distribution, metabolism, excretion.

two distinct phenotypes leading to significant differences in the toxicity of certain organophosphates such as haloxon [15], with approximately 25 per cent of sheep in Britain displaying the presence of the enzyme.

An approach to studying and predicting the concentration of drugs following administration that is increasingly being adopted is physiologically based pharmacokinetic (PBPK) modelling. Such models can be combined readily with pharmacodynamic models to allow biologically based dose–response models to be constructed. An example of the structure of a PBPK model applicable to sheep is set out in Figure 73.2. Absorbed drug enters each tissue in arterial blood and returns to the heart in the venous blood. Elimination occurs primarily from liver (with potential for enterohepatic cycling) and kidney and milk in lactating sheep. Uptake of drugs by tissue is usually blood-flow limited, but permeability limited uptake can also be accommodated by modelling. Clearance of drug by each tissue can be determined by combining the blood flow and the arteriovenous difference in concentration, which in turn is related to the drug permeability of each tissue. Tissue concentrations (which may be important from an efficacy and residue perspective) are dependent on amount of drug extracted from the arterial blood and the volume of each tissue.

It is helpful to consider this mechanistic approach as it can represent a template of normal or pathological physiology, allowing the absorption and distribution pathways from different routes of administration to be assessed, emphasizing the circulatory links between all body systems and highlighting the possible routes of elimination. Furthermore, changes due to disease, age, diet and other factors can be considered (Table 73.4).

Table 73.4: Pharmacokinetic variation due to physiology and pathology [50, 76–79]

	Physiological or pathological condition		
Pharmacokinetic stage	Neonate	Pregnancy	Pyrexia
Absorption	Immature rumen Elevated abomasal pH Decreased gastric emptying Increased gut permeability	Decreased gastric emptying Increased intestinal transit time Increased skin perfusion	Decreased feed and water intake Decreased rumen motility Fluid diarrhoea Blood flow redistribution (from skin and GIT to shivering muscle)
Distribution	Increased total body water Decreased body fat Decreased plasma albumin Decreased blood–brain barrier	Increased plasma volume Increased total body water Increased body fat Decreased plasma albumin	Increased cell permeability Increased α_1 acid glycoprotein Decreased plasma albumin Increased free fatty acids
Metabolism	Decreased gut wall metabolism Decreased hepatic function	Increased hepatic Phase I and II activity	Decreased hepatic Phase I and II activity
Excretion	Decreased GFR* Decreased tubular secretion* Lower urine pH	Increased GFR Increased renal blood flow	Decreased GFR Decreased tubular secretion Decreased urine pH

* Neonatal renal function in lambs rapidly reaches mature activity (GFR by 2 days, tubular secretion within 2 weeks). GFR, glomerular filtration rate; GIT, gastrointestinal tract.

It is clear for example, that administration of an antibacterial agent will lead to distribution throughout the body, the extent of which will be drug- and physiology-dependent. While the site of infection may be present in one tissue (e.g. the lungs) and the objective will be to achieve appropriate concentrations of unbound drug at this site, other tissues, possibly including the gut, will be exposed to the agent and may be a focus of unintended resistance selection. Whenever a drug is administered a composite of therapeutic and subtherapeutic concentrations will be present at any one time amongst the tissues of the body. In many cases this will have no impact, but in the case of toxicity, resistance or tissue residues, non-target tissue exposures may be highly significant.

Because the physicochemical properties of a drug substance are fixed, can be readily measured, and determine the likely absorption and biotransformation characteristics, this information can be combined with knowledge of key physiological processes (e.g. the distribution of cardiac output, flow rates to and volumes of different tissues) to predict drug behaviour and tissue concentration kinetics even before undertaking *in vivo* investigations [16, 17]. Indeed, this approach has been applied to predict oxytetracycline behaviour in sheep accurately [18] after intramuscular injection.

ANATOMICAL AND PHYSIOLOGICAL CONSIDERATIONS

Sheep have unique anatomical, physiological and biochemical characteristics, which confer specific pharmacokinetic and pharmacodynamic attributes on drugs administered to them [19, 20]. It is not possible to extrapolate dosages from monogastric species and it is inappropriate to consider the sheep as a small cow when determining drug-dosage regimens.

The sheep's large and complex forestomach permits the pregastric microbial fermentation of cellulose and

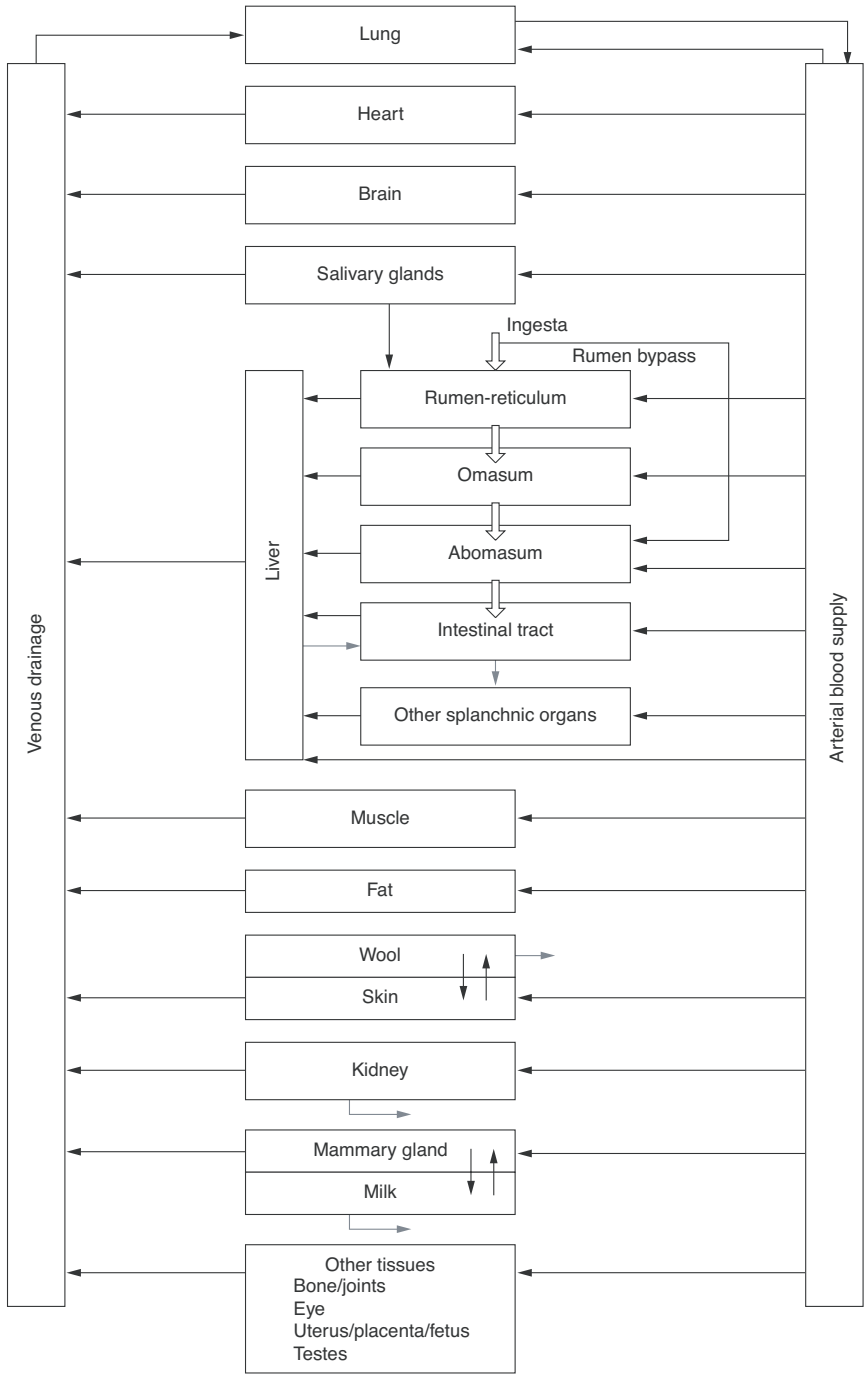

Figure 73.2: Physiologically based pharmacokinetic model of sheep.

thus the utilization of herbage fodder. The relationship between the ruminant and its microbial population is a true synergism with the ruminant providing an anaerobic environment (with homeostatic mechanisms to maintain temperature, pH and nutrient supply) while the anaerobic flora ferment cellulose and other nutrients to provide energy, vitamins and protein. Members of the rumen ecosystem are continuously removed by ruminal movements into the omasum, abomasum and small intestine where they are digested and nutrients absorbed. Ruminants thus cultivate and harvest a continuous culture of high-quality nutrients. There are around 20–25 billion bacteria, 0.2–2 million protozoa, and a variable number of fungi per ml of ruminal fluid.

During ruminal fermentation, feedstuffs are converted to short-chain acids, ammonia, methane, carbon dioxide, cell material and heat. Animal growth and maintenance is dependent on the balance of these products, and ultimately this balance is controlled by the types and activities of micro-organisms in the rumen. The acids are used as an energy source, while the microbes serve as an important source of amino acids for protein synthesis. Ammonia, methane and heat, in contrast, represent losses of either nitrogen or energy to the animal.

The efficiency of food and fibre production is determined by the balance of fermentative microbial digestion (principally but not exclusively in the rumen) and host hydrolytic digestion (mainly in the small intestine). Because of the central role of the rumen flora and fauna, drug treatments that directly (e.g. antimicrobial agents) or indirectly (e.g. those that affect rumen movements) impact on the composition or function of this complex microcosm should be used with great care.

The rumeno-reticulum exerts a major influence on drugs administered orally and also may affect those delivered parenterally, which distribute into the rumeno-reticulum by passage across its epithelium or by passage into saliva and thence to the forestomachs. The rumeno-reticulum is of variable size but can occupy 20 per cent of body volume [21], equivalent to that of the extracellular fluid, and thus can accommodate drugs as a body compartment of substantial capacity. The stratified squamous epithelium projects into the organ as highly vascularized keratinized papillae, increasing the surface area around sevenfold and constituting approximately 1.2 per cent of body weight, comparable to that of the liver. Weak bases such as levamisole should transfer and concentrate in the rumen after systemic administration while weak acids such as acetylsalicylic acid, pentobarbitone and the sulfonamides with favourable pKa and lipid solubility should be rapidly absorbed from the rumen. However, experimental evidence suggests that the rates of flux are slow [22], probably related to the poor mixing of the aqueous phase in the rumen, adsorption of drugs to particulate matter, relatively low blood supply and low surface area to volume ratio, despite the increases due to papillae. Other considerations in drug disposition include:

- *Rumen metabolism*: the rumen environment is anaerobic and metabolic activity is restricted to reductive reactions. Of toxicological significance, tryptophan, present in high levels in lush pasture, is deaminated to indoleacetic acid then decarboxylated to 3-methylindole, and nitrate is reduced to ammonia unless enzyme activity is limited in which case nitrite can accumulate and react with haemoglobin after absorption. However, on high carbohydrate diets, microbial populations and enzyme activity are increased and nitrite accumulation is less likely. Of pharmacological importance, drugs can be activated or inactivated by rumen biotransformation. Some sulfonamides and trimethoprim, digitalis, nitroxynil and chloramphenicol are inactivated. Bioactivation is exemplified by netobimin being cyclized to albendazole, and the reduction of albendazle sulfoxide and oxfendazole to their respective more active sulfides, albendazole and fenbendazole. Dietary changes can enhance these reductions [23] and antimicrobial agents (e.g. monensin) can reduce them [24].
- *Rumeno-reticulum bypass*: using video-taped fluoroscopy of barium sulfate the role of reticular (or oesophageal) groove closure has been studied in sheep from 3 to 9 months of age. Rumeno-reticulum bypass of more than 20 per cent was observed in 25–60 per cent of recently weaned lambs and 35 per cent of 8–9-month-old hoggets that had been gathered and held without food for 24 hours and administered barium sulfate (3 ml/10 kg) orally but not when a lower volume (1 ml/10 kg) was administered [25]. However, this effect was not sufficient to reduce the efficacy (assessed by faecal egg count reduction) of oxfendazole against resistant *Trichostrongylus* spp. [26]. Nonetheless, it is worthwhile considering reticular groove closure as a source of variable response in young animals as it is possible that in some circumstances, especially of high volume

administration, reduced efficacy or toxicity of drugs with low margins of safety (e.g. levamisole, closantel or organophosphates) could be associated with rapid delivery to the abomasum.

- *Gut transit*: the frequency of primary cycle rumeno-reticular movements has been shown to increase from the second month of pregnancy (1/76 s) peaking in the second month of lactation (1/58 s) coinciding with an increased rate of passage of foodstuff through the digestive tract of sheep [27]. The rate of digesta flow is also closely governed by the quality and quantity of feed intake. Reticular fluid outflows of 9, 11 and 17 l/day and duodenal fluid flows of 11, 16, and 25 l/day have been measured in 45 kg wethers with daily consumption of 700 g mixed wheat and lucerne chaff, *ad libitium* fresh clover, or 1700 g lucerne chaff, respectively [28]. Reducing feed intake from 800 to 400 g/day is associated with a change in fluid digesta rumen outflow half-life from 5 to 13 hours, and such slowing of flow has been shown to improve anthelmintic activity of benzimidazoles, macrocyclic lactones and salicylanilides [29–32]. Febrile diseases are frequently associated with inappetance which markedly increases gut transit time and can significantly affect oral bioavailability.

ABSORPTION

Cell membranes consist of a bilayer of amphipathic lipids with hydrocarbon chains oriented inwards to form a continuous inner hydrophobic phase sandwiched between outer hydrophilic layers. Membrane-embedded proteins serve as receptors, ion channels and transporters. Passage of substances across the triphasic membranes demands either special molecular characteristics or special processes. Solute or drug transport across biological membranes includes passive diffusion, facilitated diffusion and active transport. Passive diffusion consists of three processes, partition from the aqueous to lipid phase, diffusion down a concentration gradient and repartition into the aqueous phase of the receiving environment. Facilitated diffusion frequently requires a membrane transport system but does not require expenditure of energy and occurs down a concentration or electrochemical potential gradient. For both these forms of diffusion, steady-state is achieved when electrochemical potentials across the membrane have equalized.

Passive diffusion of non-polar substances involves dissolution in non-polar membrane lipids and penetration of cell membranes by diffusion. The rate of transfer across a membrane (number of molecules per unit area per unit time) is described by Fick's law of diffusion and is related to the lipid solubility of the molecule, the concentration gradient across the membrane, the length of the diffusion path and the permeability coefficient of the cell membrane (often estimated by octanol to water partition). Many drugs are weak acids or bases and exist in both ionized and unionized forms, with the latter molecular species having a vastly higher lipid solubility and greater potential for transmembrane diffusion. In physiological environments, the dissociation reaction of acids and bases can be represented as follows:

$$\text{Acid dissociation: } \underset{\text{neutral}}{AH} \rightleftharpoons \underset{\text{anion}}{A^-} + \underset{\text{proton}}{H^+}$$

$$\text{Base dissociation: } \underset{\text{cation}}{BH^+} \rightleftharpoons \underset{\text{neutral}}{B} + \underset{\text{proton}}{H^+}$$

In environments with an excess of protons (lower pH), protonated species will predominate, thus for acids the neutral AH will dominate and for bases the cation BH^+. The concentration ratio of protonated to unprotonated species is described by the Henderson–Hasselbach equation:

$$\text{pKa} - \text{pH} = \log\left[\frac{\text{protonated}}{\text{unprotonated}}\right]$$

pKa − pH	Species	Acid	Base
0	Protonated = unprotonated	Equal concentration of ionized and neutral forms	
<0	Unprotonated dominant	↑Anion (A^-) (ion trapping possible)	↑Unionized (absorption potential)
>0	Protonated dominant	↑Unionized (absorption potential)	↑Cation (BH^+) (ion trapping possible)

This relationship is very relevant pharmacologically as most drugs are either weak bases or weak acids and they can become trapped and concentrate in environments that favour ionization. For example, weak bases can be trapped in the high volume rumeno-reticulum which has a pH lower than that of plasma. Similarly, weak bases can be trapped in normal milk but to a lesser extent in mastitic milk with its higher pH. In contrast, weak acids can be readily absorbed from the abomasum but become trapped in saliva as anions and returned to the rumeno-reticulum. Table 73.5 describes the pH of pharmacologically relevant tissue environments.

Active transport, in contrast, requires energy to transport solutes and drugs against their electrochemical gradient, and frequently involves membrane channels or transport proteins. The critical role of transport proteins has recently been the subject of intensive interest and investigation [33].

The adenosine triphosphate (ATP)-binding cassette (ABC) family of transport proteins represents one of the largest families of proteins in procaryotic and eucaryotic organisms and plays a central role in cellular physiology and pharmacology. P(permeability)-glycoprotein (Pgp) (also known as multidrug resistance protein) is the prototype transport protein and is involved in unidirectional drug efflux from cells, one of the mechanisms by which cells may be resistant to the action of a drug, as for example resistance by nematodes to ivermectin, many bacteria to antibiotics, and neoplastic cells to many chemotherapeutic agents. The other superfamily of transport proteins are the solute carrier transporters (SCTs) and in contrast to the ABC transporters mediate bidirectional flux – both drug uptake and efflux.

Table 73.5: pH of ovine tissue fluids [50, 80]

Environment	pH
Saliva	8.0–8.4
Plasma	7.4
Urine, high roughage diet	7.0–8.5
Caecal fluid	7.0–7.5
Milk, mastitis	6.8–7.2
Intracellular (cytoplasm)	6.8
Milk, normal	6.5–6.8
Rumen fluid, high roughage	6.0–7.5
Urine, high concentrate diet	6.0–7.5
Rumen fluid, high concentrate	5.5–6.5
Phagolysosome	3.5–4.0
Small intestinal fluid	3.0–7.0
Abomasal fluid	1.0–3.0

↓ Increasing H^+ concentration ↓
↓ Acids: increasingly unionized ↓
↓ Bases: increasingly ionized ↓

Transporters are determinants of drug absorption, distribution, metabolism and excretion. For example, the blood–brain barrier is a physical phenomenon associated with tight junctions between endothelial cells but is supported by the presence of many transport proteins that regulate influx and efflux of drugs and endogenous compounds. Pgp is located on the luminal (blood-vessel facing) side to prevent drug uptake. Similar vectorial efflux of drugs has been described in association with the blood–placenta barrier and the blood–testis barrier. A specific transport system appears to increase the transport of benzylpenicillin into the mammary gland as concentrations observed were greater than suggested by passive diffusion considerations [34]. Ivermectin and albendazole efflux into the intestinal tract via the mucosa has also been described [35, 36].

Multiple transporters are involved in the transcellular transport of drugs in the liver, kidney and intestine. Transcellular transport of drugs necessitates transfer across two different membranes on the basal and apical sides. Distinct transport proteins are present within each membrane. In the intestine, drugs are absorbed from the luminal side (brush border membrane) and excreted into the portal blood across the basolateral membrane. In the liver, drugs are taken up into hepatocytes across the sinusoidal membrane and excreted across the apical canalicular membrane into bile. In the kidney, drugs undergo secretion crossing basolateral and apical membranes respectively into urine, a process that is reversed with reabsorption.

Transporters include a diversity of organic anion-transporting polypeptides (OATPs) and organic cation transporters (OCT).

Highly lipid-soluble unionized drugs may cross the rumen wall in either direction, thus facilitating absorption of orally administered drugs and distribution of parenterally administered drugs. Although the ruminal epithelium is papillary, its surface area is relatively small compared with that of the intestine, and the thick keratinized papillae slow the passage of drugs. The large content of the rumen (5–10 kg in a 50-kg sheep) also acts to dilute orally administered drugs, thus reducing the concentration gradient between rumen and plasma, and slowing the absorption of drugs administered by this route. Rumen microbial fermentation produces volatile fatty acids, which are buffered by the large volume (6–16 l) of bicarbonate-rich

(pH 8.2) saliva [37] such that the pH (5.5–6.5) is optimal for fermentation and in the range that encourages weak bases (high pKa) to concentrate in the rumen. The relatively low pH of the rumen content means that it can 'trap' weakly basic drugs to the extent that 80 per cent of the total body content of such a drug could occupy this compartment. Conversely, administration of an acidic drug based on body weight could result in a 20 per cent overdosage, since it may not distribute well into the rumen [22].

The small intestine is the most important site of gastrointestinal absorption of most drugs. Morphological and morphometric analysis of the absorptive surface area of the sheep small intestine has demonstrated that in the 42.5-kg sheep, when including the contribution of the microvilli, the surface area is 4.242 m^2 [38].

METABOLISM AND EXCRETION

The characteristics of drugs that enable them to cross biological membranes to their sites of action also impede their excretion. For example, lipophilic agents that are filtered by the glomerulus are candidates for reabsorption from the renal tubules into the systemic circulation. Metabolic pathways have evolved to convert endogenous compounds and xenobiotic drugs into more hydrophilic metabolites to terminate their biological and pharmacological activity and promote excretion. Drug metabolism can be divided into phase I functionalization reactions that, by oxidation, reduction and hydrolytic reactions, expose functional groups on the parent molecule (such as –OH, –COOH, –SH or NH_2) to permit further biotransformation, and phase II biosynthetic or conjugation reactions. Generally phase I reactions lead to inactivation but, in the case of prodrugs (such as netobomin and diazinon), drugs can be activated. Similarly, phase II reactions, which involve conjugation with glucuronic acid, sulfate, glutathione, amino acids or acetate, usually create inactive polar compounds, although a rare exception is morphine-6-glucuronide which is more potent than its parent compound. The enzyme systems involved in metabolism are concentrated in the liver, although all tissues manifest some metabolic capability, with key extrahepatic sites of biotransformation including the gastrointestinal tract, kidney and lung.

The liver is the major metabolic organ of herbivores and has a large capacity for conjugative metabolism, a trait that may have been selected to metabolize and excrete potentially toxic plant products. Many substrates have been used to determine the activities of hepatic enzymes in sheep and to compare them with those of other animal species. Sheep are generally but not consistently more active in glucuronosyl transferase than cattle or swine and have higher mixed-function oxidase activity than swine. The very rapid metabolism of pentobarbital by hydroxylation and oxidation in sheep accounts for its very short half-life and rapid biological activity (duration of anaesthesia) in this species [39]. Gastroenteric bacterial metabolism clearly affects the pharmacology of many drugs and the gastrointestinal mucosa contributes substantially to extrahepatic metabolism. The overall contribution (per gram of wet tissue) to glucuronidation, glutathione conjugation and acetylation is less from gastrointestinal mucosa than from the liver, but metabolism does occur and duodenal *N*-acetyltransferase activity is higher than that in the liver when expressed per ng of cytosolic protein [40]. Within the ovine gastrointestinal mucosa, the duodenum had highest (per gram wet tissue) *N*-acetyltransferase and glucuronosyltransferase activity, and the omasum and jejunum had highest glutathione-*S*-transferase activity [40].

It is also probable that metabolism contributes significantly to recovery from thiopentone anaesthesia in sheep, thus distinguishing it from other species in which redistribution is thought to account largely for its short duration of action. Clearance of thiopentone by hepatic metabolism may be facilitated in the sheep in which regional hepatic blood flow differs from monogastric species. Because of the voluminous forestomach, a relatively larger proportion of cardiac output goes to the abdominal organs (42 per cent) than to muscle (10 per cent) [41].

A study of hepatic drug-metabolizing enzyme activity in healthy sheep and sheep experiencing pneumonia, foot-rot, parasitism or systemic bacterial infections revealed a greater range of activity within the healthy sheep than that associated with disease [42]. Nonetheless, in individual animals changes specifically attributable to disease have been observed. For example, infection with *Haemonchus contortus* or *Fasciola hepatica* has been found either to induce or to inhibit the activity of specific pathways [43–48]. After comparing the activities of biotransformation enzymes in sheep and a number of other ruminant

and monogastric species it was concluded that the significant differences in level and activity rendered extrapolation between species potentially misleading [49].

Drugs are excreted from the body by several routes; inhalational anaesthetics are largely excreted by exhalation and many drugs may be excreted in saliva (acidic drugs as described above can be concentrated in saliva by ion trapping) and milk (lipid-soluble basic drugs). Most drugs, however, are excreted in urine, bile and transmucosally via Pgp into the intestinal tract. In sheep, urine tends to be less acidic than in carnivores. Basic drugs are excreted more rapidly in acidic than basic urine because of ion trapping (and vice versa). Since most drugs and their metabolites are weak organic bases, they would be expected to be excreted less rapidly in sheep than in carnivores. Some compounds, particularly glucuronide metabolites with molecular weights greater than 300 and which possess polar groups, may be excreted in bile. On the basis of the extent of biliary excretion of drugs with molecular weights between 300 and 500, sheep have been classified as moderate biliary excretors [50]. Drugs excreted in bile enter the small intestine and ultimately are eliminated in faeces. Drugs conjugated as glucuronides, however, are susceptible to deconjugation by intestinal glucuronidases and can be reabsorbed and enter an enterohepatic cycle. In this way the systemic residence of some drugs such as albendazole can be extended.

PHARMACODYNAMICS

Pharmacokinetic and metabolic characteristics account for major differences in drug disposition, and thus activity, in sheep compared with other animal species, but there may be other differences associated with the specific drug receptor interactions. The α_2-agonist, xylazine, is a particularly potent sedative analgesic in sheep. In horses, the effective intravenous dose of xylazine is 1.1 mg/kg, whereas a dose of 0.05 mg/kg is appropriate in sheep.

It has been shown also that the non-steroidal anti-inflammatory drug, carprofen, is a particularly potent inhibitor of the ovine cyclo-oxygenase enzyme, which may confer good anti-inflammatory and analgesic activity on carprofen in this species.

ANTIMICROBIALS

The selection of an appropriate antimicrobial for use in bacterial infection depends on the susceptibility of the target pathogen and the physiochemical properties of the drug, which allow therapeutic concentrations to be achieved at the site of infection. Formulation characteristics should be considered when selecting convenient dosage preparations. Toxicity to the host animal and withholding periods for meat or milk must also be considered.

Pathogen susceptibility may be determined qualitatively using antimicrobial-impregnated (Kirkby Bauer) disks applied to an agar plate seeded with the causal organism. Quantitative data [minimum inhibitory concentrations (MIC)] can be determined in the laboratory by assessing growth of a pure culture of the organism in serial dilutions of the antimicrobial being tested. Susceptibility testing is useful for organisms in which resistance is known to develop rapidly or if initial antimicrobial therapy has been unsuccessful, and its routine use within a practice helps to assess prevalence of antimicrobial resistance. In many cases, therapy may be instituted before the results of sensitivity testing are available if the infecting organism can be identified, since many bacteria have predictable sensitivity.

Antimicrobial selection should be restricted to drugs with distribution characteristics that ensure sufficient concentrations of free drug are achieved at the site of infection. Preparations with inherent physicochemical (e.g. tilmicosin) or formulation characteristics (e.g. long-acting preparations of oxytetracycline) for sustained delivery permit treatment of animals when frequent administration may not be possible. The tolerance and residue potential of the selected drug must be considered. Appropriate antimicrobial drug use has been defined as use that maximizes therapeutic impact while minimizing toxicity and the development of resistance. This has been translated as use of the right drug for the right patient in the right amount at the right time. Table 73.6 summarizes the principles of appropriate use.

In sheep, clinical signs, bacteriological identification (stain and culture) and knowledge of the husbandry and management systems should be used, together with thorough understanding of the drug characteristics, to permit the rational selection and may be supported by reference to formularies such as that outlined in Table 73.7. Many microbial diseases of sheep are best

Table 73.6: Principles of appropriate drug use

Parameter	Example: Antimicrobial therapy
Professional intervention	Establish a veterinarian–client–patient relationship
Diagnosis	Clinical diagnosis history, physical examination, other assessments Microbiological diagnosis sampling of appropriate fluids or tissues likely aetiological agent identified (i.e. not normal flora) culture and susceptibility testing
Therapeutic objective	Options include: eradication of infecting organism clinical improvement
Therapeutic plan	Individual animal or flock treatment Therapeutic choices (drug and non-drug) Supportive therapy (drainage, debridement, foot baths, wool removal, nutrition, management, etc.) Drug treatment Host factors (concurrent illness, age, immunocompetence, pregnancy, lactation)
Drug treatment	Selection of appropriate drug, considerations include: activity against infectious agent activity against non-target agents factors influencing effective concentration at site of infection availability of approved product acceptable dosage form target animal and environmental safety public health implications withholding period cost Dosage regimen pharmacokinetic–pharmacodynamic factors dose rate route of administration site of administration dosage interval duration
Monitoring	Institute plan to monitor response to treatment to enable ongoing reassessment of the objectives and plan, and identification of any significant adverse events.
Record keeping	Date of examination Diagnosis Animal identification and numbers treated Drugs used Dosage regimen (including dose rate, route and duration) Date(s) administered Withdrawal period Other treatment advice and measures implemented
Flock health and disease prevention	Prevention plan flock health programme, including consideration of vaccination, hygiene, nutrition, environment, genetics, husbandry, routine monitoring

Table 73.7: Antimicrobial formulary

Condition	Common infecting organism	Suggested antimicrobial(s)
Infectious abortion		
Campylobacteriosis	*Campylobacter fetus* ssp. *fetus, C. jejuni*	Penicillin G, oxytetracycline
Chlamydial abortion	*Chlamydophila abortus*	Oxytetracycline (cure not likely, shedding continues)
Coxiellosis (Q fever)	*C. burnetii*	Oxytetracycline
Leptospirosis	*L. pomona, L. grippotyphosa*	Streptomycin; oxytetracycline
Listeriosis	*L. monocytogenes*	Oxytetracycline
Salmonellosis	*S. typhimurium, S. abortus ovis, S. montevideo, S. dublin*	Oxytetracycline, trimethoprim-sulfonamide
Toxoplasmosis	*T. gondii*	Decoquinate, monensin (prevention only)
Other infectious reproductive disorders		
Endometritis	*Arcanobacterium pyogenes, E. coli*, mixed anaerobes including *Clostridium* spp.	Penicillin G; oxytetracycline
Enzootic posthitis	*C. renale* group	Penicillin G; oxytetracycline
Epididymitis, lamb	*Haemophilus somnus, Actinobacillus seminis Corynebacterium pseudotuberculosis*	Oxytetracycline
Epididymitis, ram	*Brucella ovis*	Oxytetracycline (successful control based on culling)
Infectious disease of lambs – Systemic		
Omphalophlebitis	*A. pyogenes, E. coli,* mixed anaerobes	Penicillin G
Watery mouth (lambs)	*E. coli* (endotoxaemia)	Oral amoxicillin; systemic trimethoprim-sulfonamide
Tick-borne fever (tick pyaemia, staphylococcal arthritis)	*Ehrlichia phagocytophilia* and/or *S. aureus*	Oxytetracycline
Erysipelothrix polyarthritis	*E. rhusiopathiae*	Penicillin G, tylosin
Infectious diseases of lambs – Digestive		
Colibacillosis	Enterotoxigenic *E. coli*	Oral amoxicillin; systemic trimethoprim-sulfonamide
Yersiniosis	*Y. enterocolitica, pseudotuberculosis*	Oxytetracycline, penicillin G
Campylobacter enteritis	*Campylobacter* spp.	Oxytetracycline
Coccidiosis	*Eimeria* spp.	Decoquinate, diclazuril, sulfonamide, monensin; lasalocid; salinomycin;
Infectious conditions of lambs – Respiratory		
Pneumonic pasteurellosis	*M. haemolytica*	Tilmicosin; oxytetracycline; ceftiofur
Septicaemic pasteurellosis	*P. trehalosi*	Tilmicosin; oxytetracycline; ceftiofur
Mycoplasma pneumonia	*M. ovipneumoniae, M. arginini*	Oxytetracycline; tylosin
Ocular Infections		
Infectious keratoconjunctivitis (pink eye)	*C. psittaci, M. conjunctivae*	Oxytetracycline (cure unlikely, carrier state persists)
Infectious conditions of the skin		
Dermatomycosis (lumpy wool)	*Dermatophilus congolensis*	Penicillin-streptomycin, oxytetracycline
Caseous lymphadenitis	*Corynebacterium pseudotuberculosis*	Antimicrobial therapy ineffective, vaccination protective
Infectious conditions of the foot and joints		
Virulent foot-rot	*Dichelobacter nodosus, Fusobacterium necrophorum*	Oxytetracycline; penicillin G. Outcome improved by keeping sheep dry for 24 hours
Ovine interdigital dermatitis	*F. necrophorum*	Penicillin G; oxytetracycline

(*Continued*)

Table 73.7: (Continued)

Condition	Common infecting organism	Suggested antimicrobial(s)
Strawberry foot-rot	*D. congolensis*	Penicillin-streptomycin, oxytetracycline
Streptococcal arthritis	*Streptococcus* spp.	Penicillin G, oxytetracycline
Erysipelothrix arthritis	*E. rhusiopathiae*	Penicillin G, oxytetracycline
Polyarthritis	*Chlamydophila pecorum*	Oxytetracycline
Infectious conditions of the mamary gland		
Contagious agalactia	*Mycoplasma agalactiae*	Oxytetracycline; tylosin (shedding and recurrence likely)
Subclinical and clinical mastitis	*S. aureus, M. haemolytica,* environmental streptococci, coagulase negative *Staphlococcus* spp.	Penicillin G, oxytetracycline
Infectious conditions of the nervous system		
Bacterial meningoencephalitis	Many species, including *E coli, Mannheimia (Pasteurella), Staphylococcus*	Trimethoprim-sulfonamide, oxytetracycline, ceftiofur
Listeriosis	*L. monocytogenes*	Penicillin G, amoxycillin, oxytetracycline

prevented as treatment can be unrewarding and ineffective. Judicious and strategic implementation of vaccination programmes can reduce the need for antimicrobial drugs. Vaccines available for use in sheep include those providing protection against infection with *Bacillus anthracis*; a variety of clostridia (*C. botulinum* type C, D; *C. chauvoei*; *C. haemolyticum*; *C. novyi*; *C. perfringens* type A, B, C, D; *C. septicum*; *C. sordellii*; *C. tetani*); *Campylobacter fetus* sub-species *fetus*; *Chlamydophila abortus*; *Corynebacterium pseudotuberculosis*; *Dichelobacter nodosus* serotypes A–I; *Erysipelothrix rhusiopathiae*; *Leptospira hardjo*; *Leptospira pomona*; *Mannheimia haemolytica*; *Pasteurella trehalosi*; and *Toxoplasma gondii*.

To minimize the likelihood of the development of antimicrobial resistance, appropriate narrow-spectrum agents should be selected in preference to broad-spectrum agents, which exert a greater selection pressure on non-target commensal bacteria. Optimal therapeutic dosage strategies must take account of pharmacokinetic and pharmacodynamic characteristics of selected antimicrobial agents. The β-lactams (penicillins and cephalosporins), tetracyclines, macrolides and sulfonamides can all be classified as time-dependent with respect to antimicrobial activity. Serum concentration of free drug (assumed to be in equilibrium with concentrations at the site of infection) should exceed the MIC of the target organism for at least 50 per cent of the dosing interval. Post-antibiotic effects and post-antibiotic leukocyte enhancement allow antimicrobial activity to continue during the sub-MIC periods. Increasing the dose to produce serum concentrations more than small multiples of the MIC is not associated with any increase in efficacy. By contrast, aminoglycosides (and the fluoroquinolones, although not currently widely used in sheep) have concentration-dependent activity, with high ratios of peak concentration to MIC best correlated with efficacy [51]. In human studies, clinical responses in greater than 90 per cent of patients required the peak to exceed the MIC by 8–10-fold. Once-daily dosing was associated with high efficacy and reduced toxicity as uptake of aminoglycosides by renal tubular cells and the endolymph of the ear was more pronounced at low sustained concentrations rather than high intermittent levels [52].

The diversity of sheep management and husbandry systems may constrain strategies for antimicrobial administration. In intensive stocking units with many animals in a single air space, spread of bacterial disease can be rapid. Antimicrobial sensitivity testing may be particularly useful so that new cases can be dealt with in the most appropriate way. The complex microbial ecosystem of the ruminant forestomach makes it necessary to consider carefully the choice of antimicrobial agent to administer by mouth, although this route may be more generally useful in pre-ruminant lambs. However, the oral absorption characteristics of the drug should be known since only bioavailable antimicrobials will be effective in treating systemic disease. Drugs such as the aminoglycosides and virginiamycin have poor systemic bioavailability after

oral administration, but may nonetheless be very effective in the management of enteric conditions (such as enteritis or acidosis, respectively) when given by this route. Rumen fermentation may be affected also by parenteral administration of drugs that diffuse across the ruminal epithelium or are secreted in saliva, although adverse effects may be limited by ruminal degradation or metabolism of the antimicrobial. Dysbiosis of the intestinal flora can result from biliary excretion of systemically administered drugs. The most notable example is probably that following use of systemic lincomycin, much of which is excreted in active form in the bile. Spectacular episodes of diarrhoea have been reported sporadically with up to 25 per cent of sheep dying in the 2–3 weeks after treatment. Death appears to be associated with salmonellosis, a disease caused by organisms not susceptible to lincomycin but whose enteric niche may be amplified by removal of competing flora.

A key principle of clinical pharmacology is assessment of response to treatment. All cases of apparent antibacterial failure should be investigated in order to elucidate the contribution of factors that can be overcome or managed. Possible causes of treatment failure are summarized in Table 73.8.

ENDOPARASITICIDES

The major endoparasitic problems in sheep are associated with gastrointestinal nematodes, most notably *Haemonchus contortus, Teladorsagia circumcincta, Trichostrongylus* spp. and *Nematodirus battus* (see Chapter 27), with relative importance dependent on many geographical and epidemiological factors. The liver fluke *Fasciola hepatica* (see Chapter 28) can be extremely important in areas in which its snail intermediate host is present, and lungworms and tapeworms may present adverse challenges to health.

Anthelmintic drugs approved for use in sheep in various countries are presented in Table 73.1 and additional details are provided in Table 73.9. There are currently five principal classes of anthelmintic, each with a different mechanism of action. The macrocyclic lactones (MLs), benzimidazoles and levamisole are broad-spectrum anthelmintics effective against most gastrointestinal nematodes and lungworms. The salicylanilides target the haematophagous *Haemonchus*, as do the organophosphates (OPs) which are also active to a variable extent against small intestinal nematodes. The benzimidazoles, levamisole, morantel and the OPs in conventional delivery systems (drench or injection) do not confer persistent or sustained activity as they are cleared from the animal within hours or days. On the other hand, the MLs are lipophilic and associate with bodyfat (especially moxidectin which, unlike the avermectins, lacks sugar substituents) while the salicylanilides bind to plasma protein (albumin); this behaviour confers variable periods of sustained activity associated with their persistence in plasma at concentrations that distribute to parasitic loci and are effective against ingested parasites. Clearly, for drugs that persist because of their physicochemical or formulation characteristics, the associated meat withdrawal periods are likely to reflect their persistence.

Extending the duration of activity can be achieved by appropriate formulation. The intraruminal controlled release capsule (CRC) has variable geometry which changes from a cylinder for oral administration to a 'winged' device that resists regurgitation and is retained in the rumen or reticulum. The active constituent (either albendazole or ivermectin in currently available CRCs) is released from an orifice at a fixed rate designed to provide a sustained, but low, enteric and plasma drug concentration over a period of 100 days. The value of this continuous presentation is that it inhibits establishment of ingested infective larvae. A less sophisticated and reliable approach to continuous anthelmintic delivery can be achieved by incorporating anthelmintics in feed supplement blocks.

Regardless of the chemical or mode of administration, exposure of parasites to anthelmintic drugs will ultimately result in the development of anthelmintic resistance which has developed more rapidly where frequent treatments have been given and where there are few or no refugia of susceptible parasites to dilute resistant forms. Anthelmintic resistance is a particular problem in the major sheep-producing regions of the southern hemisphere, but is now emerging as a significant issue in the UK [53]. Development of resistance may be delayed by using anthelmintics as infrequently as possible and by adopting strategies that use knowledge of parasite epidemiology to permit targeted therapy. A recent survey of the rapidity of onset of resistance to ivermectin in Western Australia has shown that those farms seeking professional advice on worm control from veterinarians benefited by experiencing a greater time to resistance than those

Table 73.8: Apparent treatment failure: antibacterial example

Diagnosis
- Condition not of bacterial origin (non-infectious, other infectious – viral, protozoal, etc.)

Therapeutic goals
- Unrealistic objective (bacterial eradication vs disease control)

Pathophysiology
- Progression of underlying disease
- Poor management of mixed infection (e.g. mixed aerobic and anaerobic infection)

Host factors
- Predisposing factors uncorrected
- Impaired immune function (e.g. failure of passive transfer of colostral immunoglobulins)
- Nutritional deficits

Pharmaceutical factors
- Substandard product (expired, inappropriate storage)

Treatment
- Compliance
- Misadministration (e.g. animal avoided treatment, oral dosage regurgitated, injection misdirected)

Pharmacology
- Inappropriate drug selection
- Inappropriate dosage regimen (inadequate dose rate, route, frequency, duration)
- Pharmacokinetic issues (especially changes in absorption, distribution and clearance)
- Impaired perfusion and penetration (blood–brain barrier, abscess, oedema, swollen milk ducts, etc.)
- Interaction with concurrent medication

Supportive therapy
- Omission of concurrent supportive measures (nutrition, hydration, nursing, abscess drainage, sequestrum removal)

Microbial factors
- Toxin elaboration
- Drug resistance
- Reinfection
- Bacterial dormancy (e.g. non-growth phase)
- Bacterial L-forms
- Phenotypic tolerance (e.g. small colony variants)
- Dense bacterial loads in infected tissue
- Biofilm formation
- Superinfection (bacteria or fungal)
- Poor correlation of *in vitro* susceptibility and clinical outcome (e.g. *in vitro* rapid growth vs slower growth in milk)

Epidemiology
- External bacterial challenge unabated

Toxicology
- Adverse drug reaction

Failure investigation
- Inappropriate samples collected
- Non-representative animal(s) investigated (e.g. necropsy of untreated animal)

that did not seek advice [54]. When nematode parasites are selected for resistance, it is generally common to all drugs with a similar mode of activity, although the 'degree' of resistance and potency of the drug may mask its selection in the early stages.

Confronted with developing resistance to current anthelmintic compounds, and a paucity of new antiparasitic agents with a unique mode of action, combination formulations are of increasing importance in some countries (e.g. Australia and New Zealand). In this

Table 73.9: Antiparasitic drugs for use in sheep for (A) nematodes (B) cestodes and trematodes

Compound	Administration route	Dose rate (mg/kg)	Comments
(A) Treatment of nematodes			
Macrocyclic lactones (MLs) (avermectins/mylbemyins)			
Abamectin	Oral	0.2	Broad spectrum activity against nematodes.
Doramectin	i.m.	0.2–0.3	Persistent action, particularly with the very
Ivermectin	Oral, s.c.,	0.2	lipophilic moxidectin. Ivermectin CRC
	intraruminal CRC	0.02 mpkpd	provides 100-day efficacy. Reducing activity due to
Moxidectin	Oral, s.c.,	0.2	development of resistance in trichostrongylids,
	Long acting injection	1.0	particularly southern hemisphere countries
Benzimidazoles (BZs)			
Albendazole	Oral	3.8–7.5	Netobimin is a pro-drug of albendazole. BZs
	Intraruminal CRC	0.5 mpkpd	control most gastrointestinal nematodes and
Albendazole oxide	Oral	4	lungworm, albendazole has some control of
Fenbendazole	Oral	4–5	adult liver fluke at increased dose. Albendazole CRC
	Medicated lick blocks	10–15	provides 100-day efficacy BZ resistance emerging and disseminating quickly in trichostrongylids
Mebendazole	Oral	15	
Netobimin	Oral	7.5	
Oxfendazole	Oral	4–5	
Tetrahydro-imidazoles and -pyrimidines			
Levamisole	Oral	7.5–10	Broad spectrum of activity, but becoming greatly limited due to widespread development of resistance in trichostrongylids.
Morantel	Oral	5.94 (as base)	Narrow spectrum, some in trichostrongylid nematodes
Salicylanilides and nitrophenols			
Closantel	Oral	7.5–10	Specific efficacy against *H. contortus*, although activity
Rafoxanide	Oral	7.5	reducing due to development of resistance. Also active
Nitroxynil	s.c.	10	against *F. hepatica*
Organophosphates (OPs)			
Naphthalophos	Oral	37.5	Narrow spectrum to abomasal/upper intestinal
Pyraclofos	Oral	30	nematodes, few reports of resistance
(B) Treatment of cestodes and trematodes			
Albendazole	Oral	7.5–10	Efficacy against adult *F. hepatica, Monieza* spp.
Closantel	Oral	10	Efficacy against 6-week larval/adult *F. hepatica*
Netobimin	Oral	20	No activity against immature fluke
Niclosamide	Oral	50	Broad-spectrum activity against cestodes
Nitroxynil	s.c.	10	Efficacy against immature and adult *F. hepatica*
Oxyclozanide	Oral	15	Efficacy against immature and adult *F. hepatica*
Praziquantel	Oral	3.76	Broad-spectrum activity against cestodes
Rafoxanide	Oral	7.5	Efficacy against 6-week larval/adult *F. hepatica*
Resorantel	Oral	62.5	Efficacy against paramphistomes and cestodes
Triclabendazole	Oral	10	Efficacy against all stages of larval and adult *F. hepatica* Resistance emerging

CRC, controlled release capsule; i.m., intrasmuscularly; mpkpd, mg per kg per day; s.c., subcutaneously.
Note: not all drugs are authorized in every country.

way as many as four different chemical classes have been combined in a single formulation (ML, BZ, levamisole and salicylanilide). Further details can be obtained from the APVMA website (Table 73.2). Parasites able to survive exposure to one or two components may be susceptible to the remaining compounds and thus killed. Epidemiological modelling has demonstrated that starting with a relatively low resistant gene frequency the use of combination products can significantly delay the development of resistance compared to alternate (sequential) use of single preparations. Even when higher resistance gene frequencies are present the use of combination products, with multiple modes of biochemical action, can be effective in treating worm populations that may be resistant to one or more of the anthelmintic components.

Benzimidazoles bind to nematode tubulin and disrupt cytoplasmic microtubules. Parasite cells thus affected are unable to transport intracellular secretory granules or complete mitosis. Benzimidazoles have greater affinity for parasitic than mammalian tubulin and consequently have a large margin of safety in sheep and other animals. However, the tubulin-binding activity of some but not all benzimidazoles may cause teratogenicity in some species when administered at high dosage at the sensitive stage in early pregnancy. This was first described in 1965 when high doses of parbendazole (no longer approved for use) were administered to ewes.

The MLs increase parasite neuronal membrane permeability to chloride ions through glutamate-gated cell membrane channels. They are distributed into the central nervous system of mammals to a very limited extent and are actively pumped from this site by transport proteins, thereby explaining their relatively large therapeutic index. Salicylanilides uncouple oxidative phosphorylation, thus increasing energy loss and reducing effective metabolic activity in the target pathogen. They have a similar but less intense effect in mammalian cells, which accounts for their rather narrow therapeutic index and require caution when used in flocks with a large range in the weight of the animals to be treated and where dosage is based on the heaviest animal. Levamisole and morantel have broadly similar mechanisms of action as cholinergic agonists at the parasitic ganglion, bringing about spastic paralysis of parasite musculature. Levamisole is well absorbed following oral administration and distributes to the gut following parenteral administration. This differentiates it from the commonly used salts of morantel, which are not absorbed following oral administration and are given only by this route and have activity limited to gastrointestinal parasites. Levamisole has a relatively narrow therapeutic index and care is needed when administering levamisole to lambs, particularly if administered by injection, and especially in sheep that have been held without water for a prolonged period.

It should be noted that the action of BZ, ML and salicylanilide anthelmintics is greatly influenced by the duration for which a parasite is exposed to a concentration of drug or active metabolite that exerts a 'toxic' effect. These anthelmintics are relatively slowly absorbed from the gastrointestinal tract and after systemic distribution and metabolism are secreted back into the gastrointestinal tract in bile. Thereafter, a proportion of biliary metabolites are reabsorbed from both the small and large intestine with up to 30–40 per cent of the dose following this enterohepatic cycle. Clearly, every opportunity must be taken to maximize the duration of these compounds at sites of absorption. First, and most importantly, the orally administered drug must be wholly deposited into the rumen to mix with rumen contents. This provides a 'reservoir' effect with the slow passage from the rumen prolonging presence of drug at the sites of infection and absorption. Young sheep possess a unique characteristic where the muscle of the reticulum can form a groove (the reticular groove) which functions to direct suckled milk past the developing rumen to the abomasum to maximize nutritional benefit. While the action of the reticular groove decreases after weaning it can be stimulated by deposition of drench in the mouth.

Slowing the rate of gastric transit will also increase the duration of drug availability. Because they are highly associated with rumen particulate material BZ and ML anthelmintics are absorbed over a period of time which increases when the flow of digesta from the rumen is reduced. For example, oxfendazole was more extensively absorbed in sheep given reduced feeding (400 g lucerne/wheat chaff) and had a longer rumen fluid half-life than in those on increased feeding (800 g lucerne/wheat chaff). The efficacy of oxfendazole against benzimidazole-resistant *Haemonchus contortus* and *Trichostrongylus colubriformus* increased from 19 and 60 per cent to 60 and 94 per cent, respectively, when the rations were reduced from 800 to 400 g lucerne/wheat chaff [29]. Similar results have been reported with use of albendazole [31], ivermectin [30] and closantel [32].

If there is no opportunity to reduce feed intake, repeated dosing can provide an alternative option for increased anthelmintic effect. Owing to the 'first-order' pattern of drug clearance administering a second or even third therapeutic dose at intervals of the half-life of the drug can significantly extend drug presence. For example, almost all resistant worms were eliminated when three therapeutic doses of oxfendazole were administered at 12-hour (the approximate oxfendazole half-life) intervals compared to less that 60 per cent efficacy when the three doses were administered as a single dose. While this may be a successful approach to resistant parasite control, the cost, residue and withholding period implications need to be carefully considered. In a similar approach using recommended dose rates of albendazole administered as two divided doses, a threefold increase in bioavailability has been reported [55]. Improved efficacy with divided doses of fenbendazole have also been reported [56].

The MLs are lipophilic and they associate with body fat which prolongs their presence. Of the available MLs, moxidectin is the most lipophilic, about 100 times more lipophilic than ivermectin, endowing moxidectin with a longer residence time which results in prolonged anthelmintic activity. However, the extended depletion profile has the potential to increase the selection pressure for resistance of parasites exposed to discriminating concentrations. Variations in fat composition of sheep has also been associated with changes in moxidectin efficacy. Persistent efficacy of moxidectin against *Ostertagia circumcincta* was reduced in sheep with low fat reserves [57].

Successful gastrointestinal parasite control relies on an integrated approach of strategic chemotherapy combined with nutritional and pasture management [58]. Excellent resources providing comprehensive details of parasite management have been developed in the UK [Sustainable Control of Parasites in Sheep (SCOPS) www.defra.gov.uk.animalh/diseases/control/parasite_control.htm] and Australia (Wormboss www.wormboss.com.au).

ECTOPARASITICIDES

Important ectoparasitic infestations of sheep include those caused by mites (*Psoroptes ovis* – sheep scab; *Sarcoptes scabiei* – head scabies; *Psorergates ovis* – itch mite), flies (*Lucilia cuprina* and *sericata* – cutaneous myiasis; *Melophagus ovina* – ked), ticks (*Ixodes ricinus*) and lice (*Bovicola ovis*, the biting or body louse and *Linognathus* spp. – sucking lice). Further details of ectoparasites of sheep can be found in Chapters 47 (Sheep scab), 48 (Other ectoparasitic conditions) and 51 (Tick-borne diseases). Ectoparasiticides may be applied topically or systemically [59]. Topical application can be in the form of high-concentration, low-volume backline preparations or may rely on the saturation of the fleece with an aqueous solution or emulsion of the ectoparasiticide, generally by plunge dipping, shower spraying or hand jetting.

Plunge dipping is labour-intensive and has been described as the most complex animal health-related task that a sheep farmer carries out in the course of a year. Nonetheless, when carried out diligently, dipping can deliver an appropriate concentration of drug to the target site. In general, active constituents of sheep dips that are relatively lipid-soluble will be retained in the fatty sebaceous environment of the skin and wool to a greater extent than the aqueous carrier. Dip returning to the bath from draining pens will therefore contain less active constituent than that in which the animals were originally submerged. It is often essential to top-up plunge dips (and the reservoirs for spray systems) with more concentrated solutions than used to charge the initial tank. Maintaining an effective concentration of the active constituent in the dip is a challenging task and product-specific. Therefore, it is essential that labelled directions for each product be read carefully, understood and implemented. Investigations of failed parasite control frequently identify improper dipping practice as the cause. Mixing inaccuracies, dip maintenance errors, and inadequate wetting and saturation of the head and fleece to skin level are commonly encountered.

Plunge baths are readily contaminated with dirt and faecal material, and are therefore excellent environments for the multiplication of bacteria. This has been associated with the infection of lambs with *Erysypelothrix rhusiopathiae* and development of post-dipping lameness. While bacteriostats may be added to control microbial contamination, it is unlikely that this can be achieved effectively. Therefore measures should be instituted to minimize dip contamination (e.g. by withholding feed prior to dipping, pre-treatment of sheep with dermatophilosis, diarrhoea, dags or foot infections, regular replenishment) and to ensure that susceptible animals are not dipped (e.g. by allowing shearing wounds to heal and vaccinating sheep against clostridial diseases and caseous lymphadenitis).

Disposal of excess dip wash must be undertaken to comply with prevailing regulations. Dip wash is nutrient-rich but also contains pesticides that can be very toxic to aquatic invertebrates and fish. As mentioned above, inappropriate disposal of synthetic pyrethoid (SP)- and organophosphate (OP)-containing dip wash in the UK has led to adverse effects on waterways.

An alternative to plunge or shower dipping is topical application of ectoparasiticide in a formulation that facilitates spread through the sebum to distribute over the surface of the animal. Pour-on and spray-on formulations are conventionally administered along the backline immediately after shearing – 'off-shears' – with the intention of dispersing through the fresh grease [60]. In the first few hours after shearing a 'shock' response by the sheep to wool removal increases wool grease secretion by 20–25 per cent but within 24–48 hours the fresh grease rapidly oxidizes to a more waxy consistency. The changing consistency entraps the drug which slows and finally halts lateral dispersion, retaining a large portion of the dose at the backline application site. While this dispersed drug will exert its effect it can be short-lived as wool growth takes the entrapped ectoparasiticide away from the skin surface the predilection site of the ectoparasites. Thus, it is important to administer 'off-shears' treatments as soon as possible after shearing, certainly within a day. Thereafter, their performance will quickly decline as distribution around the pelage is limited. Even when high concentrations of an ectoparasiticide are present in samples of fleece, biological activity (i.e. lousicidal efficacy) may have declined, indicating that bioavailability falls with time [61, 62]. Pour-on formulations remain of value in the management of lice and fly infestations, particularly with application of insect growth regulators (IGRs), but more effective uniform coverage of insecticide is only obtained with whole body dipping.

Products used for ectoparasiticidal activity are given in Table 73.10. Dip and spray products contain OP, SPs amitraz, IGRs or spinosad. OPs inhibit the enzyme acetyl cholinesterase, causing accumulation of acetylcholine in the insect and continuous stimulation of insect nerve function. Differential toxicity between arthropod and host is ensured by limited uptake in the host and interspecies differences in metabolic inactivation of acetyl cholinesterase, allowing development of OPs with greater affinity for arthropod than mammalian enzymes.

OP toxicity in the mammalian host or man may occur if exposure is sufficient and presents as an acute syndrome with muscarinic and ultimately nicotinic stimulation. Thus, increased gastrointestinal motility, bronchoconstriction and miosis are followed by involuntary muscle fasciculation. While well known and infamously associated with massive outbreaks of paralysis from contaminated alcohol in the USA during the prohibition era of last century and more recently from contaminated cooking oil, OP-induced delayed neuropathy (OPIDN) is not a consequence of exposure to OPs used to control sheep parasites. OPIDN results from inhibition of neuropathy target esterase (NTE), causing paralysis with swelling and degeneration of distal parts of long nerves in the legs and spinal cord. OPs for use in livestock are assessed prior to approval in sensitive avian models and do not demonstrate evidence of NTE activity. However, other more subtle neurological syndromes have been described as a long-term complication of acute OP poisoning. A recent systematic study of 471 UK individuals with OP exposure related to dipping sheep did not find unequivocal evidence of safety or toxicity [63], and concluded that further research should be supported. A recent review of diazinon in Australia concluded that occupational exposure during conventional plunge dipping was unacceptably high and such uses of diazinon would be phased out. In recognition of the hazards of occupational exposure to OPs (and other chemicals) most countries make recommendations on appropriate precautions to reduce the risk. Fundamental approaches include properly designed facilities with adequate ventilation, engineering controls to reduce splash and other sources of exposure, combined with personal protective equipment and application of good working practices. In the UK, the sale and supply of sheep dips is regulated and those responsible for dipping must hold a Certificate of Competence in the Safe Use of Sheep Dips, and in England and Wales must have a Licence to Dip Sheep.

The lethal activity of the synthetic pyrethroids is mediated by effects on presynaptic sodium channels in the insect nerve membrane by impeding conformational changes in the proteins at the lipid–protein interface, leading initially to repetitive neuronal discharges and thence to irreversible neuromuscular blockade. Pyrethroids are inherently very safe to most mammals (cats are an exception) and birds, although, at high dosages, they may cause tremor and ataxia associated with neurological intoxication. Pyrethroids are highly toxic to fish and many aquatic invertebrates

Table 73.10: Antiparasitic drugs for treatment of arthropod ectoparasitism of sheep

Compound	Administration route	Dose rate (mg/kg)	Comments
Cyfluthrin Cypermethrin Deltamethrin Esfenvalarate Flumethrin Permethrin Pyrethrins	Plunge dip, shower spray, dust, backline topical (off shears)	Saturation of fleece or high concentration backline application	**Pyrethrins and pyrethroids**. High margin of safety for sheep. Toxic to aquatic invertebrates. Broad spectrum against flies, lice, mites and ticks. Action enhanced by mixed function oxidase inhibitors (piperonyl butoxide). Activity against biting lice (*Bovicola ovis*) becoming limited due to widespread development of resistance. Environmental toxicity is major limitation to future use
Chlorfenvinphos Diazinon Fenthion Propetamphos Temephos	Shower spray, plunge dip, jet	Saturation of fleece	**Organophosphates**. Caution required in use to reduce human exposure. Broad-spectrum pesticides, well-established resistance in flies, but less so in other arthropods
Abamectin Doramectin Ivermectin	Oral i.m. injection Oral, s.c. injection	0.2 0.2–0.3 0.2	**Macrocyclic lactones.** Control of feeding insects, flies, mites, sucking lice. Control of sheep scab and periods of protection unique to each active and labelled dosage regimen should be followed carefully.
Moxidectin	Oral, s.c. injection Long-acting injection	0.2 1.0	Efficacy spectrum-dependent on route of administration
Spinosad	Plunge dip	Saturation of fleece	**Spinosyn.** Efficacy against flies, ticks and lice. Resistance not yet reported
Amitraz	Plunge dip and spray	Saturation of fleece	**Formamidine.** Active against ticks, mites, lice, keds.
Cyromazine Dicyclanil	Topical, backline application	High-concentration backline application	**Insect growth regulators (cyclopropylamino)**. Efficacy in prevention of blowfly strike for up to 19 weeks (dependent on active, formulation and administration)
Diflubenzuron Triflumuron	Plunge dip, jet, backline topical (off shears)	Saturation of fleece or high-concentration backline application	**Insect growth regulators (benzoyl ureas)**. Sustained efficacy in prevention of blowfly strike and control of lice. Resistance in lice emerging and first reports of resistance in flies recorded

(up to 200 times as toxic on a molar basis as some OPs) and must be disposed of with very great care.

Amitraz is a formamidine insecticide and, while inhibiting monoamine oxidase, an important metabolic enzyme for amine neurotransmitters in ticks and mites, the lethal biochemical lesion appears to be inhibition of mixed function oxidases. It is also an agonist of octopamine receptors responsible for modification of tonic contraction in parasite muscles. It is not extensively absorbed following topical administration, and thus is relatively safe on target mammalian hosts. The IGRs cyromazine and dicyclanil disrupt cuticle turnover during ecdysis thereby altering the elasticity of the cuticle in first instar and later stage fly larvae. Parasites cannot move, feed or grow normally and do not progress normally to the second- and third-stage instars, which inflict most damage. These IGRs have only prophylactic activity and exert a protective effect for up to 19 weeks, depending on product and method of administration. The benzoyl ureas are also IGRs but active against both flies and lice. They act by inhibition of chitin synthase, an enzyme absent from mammals

but vital for growth and development of insects. The mode of action of spinosad is characterized by activation of nicotinic acetylcholine receptors resulting in excitation of the insect nervous system, leading to involuntary muscle contractions, prostration with tremors and paralysis. Spinosad also has effects on γ-aminobutyric acid (GABA) receptor function that may contribute further to its insect activity.

The activity of avermectins and milbemycins against arthropods has been exploited to allow systemic control of *Psoroptes ovis*. The MLs have broad-spectrum activity against itch mite, nasal bots and parasitic nematodes, and their mode of action has been described previously. The avermectins (doramectin, ivermectin) and milbemycin (moxidectin) for use in scab control are given by parenteral injection. Doramectin may be given as a single intramuscular injection but ivermectin must be administered twice by subcutaneous injection with a 7-day interval. For clinical cure, moxidectin must be administered twice at the recommended dosage 10 days apart, but a single dose will protect against *P. ovis* infestation for at least 2 weeks. It is important that label directions are followed closely.

SUSPECTED ADVERSE DRUG REACTIONS

Judicious drug treatment requires balancing the benefits and risks to minimize harm (*primum non nocere*) and to optimize response. Each use of a veterinary medicine constitutes a therapeutic trial and in many ways each use is unique. It is therefore prudent to maintain the same vigilance in monitoring responses to established treatments as would be applied to new approaches. Whenever unexpected findings present themselves they should be investigated to establish their origin and to enable changes in treatment regimens to be implemented if necessary (Figure 73.1). Such an adverse drug reaction (ADR) or adverse drug experience (ADE) can be defined as 'an unintended or unexpected effect on animals, human beings or the environment, including injury, sensitivity reactions or lack of efficacy associated with the clinical use of a veterinary chemical product'. Generally, the causality of ADRs or ADEs is uncertain and so it is common practice, and in most cases more accurate, to refer to these occurrences as 'suspected ADRs' (sADRs). Not all unwanted clinical phenomena encountered in practice are related to drug use and it is therefore important to differentiate between ADRs or ADEs and adverse events (AEs) that are defined as 'untoward occurrences that may be present during treatment with a veterinary product but which do not necessarily have a causal relationship with this treatment' [64, 65]. An algorithm describing the logical process of classification of untoward observations is presented in Figure 73.3.

The study of ADRs is now termed pharmacovigilance, the science and activities relating to their detection, assessment, understanding and prevention [65]. Veterinary pharmacovigilance has additional dimensions not relevant to its medical counterpart, particularly its interest in tissue residues, environmental toxicity and resistance selection [66, 67] and has become the cornerstone of post-marketing surveillance. In most countries the veterinary medicine regulatory authorities mandate the submission of sADR reports by licence holders but veterinarians can submit reports voluntarily and it is recommended that any sADRs be reported simultaneously to both manufacturer and regulatory authority. The rate of spontaneous reporting of sADRs bears little relationship to the actual incidence of sADRs and caution needs to be taken in determining the impact or importance of a particular sADR. Furthermore, the rate of spontaneous reporting is susceptible to a number of well-known influences such as the Weber effect (increased reporting frequency of sADRs for the 2 years following introduction of a new product) and the Panorama effect (which describes increased reporting frequency immediately following widespread publicity about a particular adverse reaction).

The principal objectives of pharmacovigilance include [68]:

- identifying previously unrecognized drug safety hazards and assessment of rate
- elucidation of factors predisposing to toxicity
- obtaining evidence of safety
- refutation of 'false positive' ADR signals.

Clearly, the outcomes of pharmacovigilance can be disseminated quickly and have the potential for substantial improvement of therapeutic decisions and plans. In reviewing the sADRs reported to the Australian Pesticides and Veterinary Medicines Authority (APVMA) over the period 1995–2004,

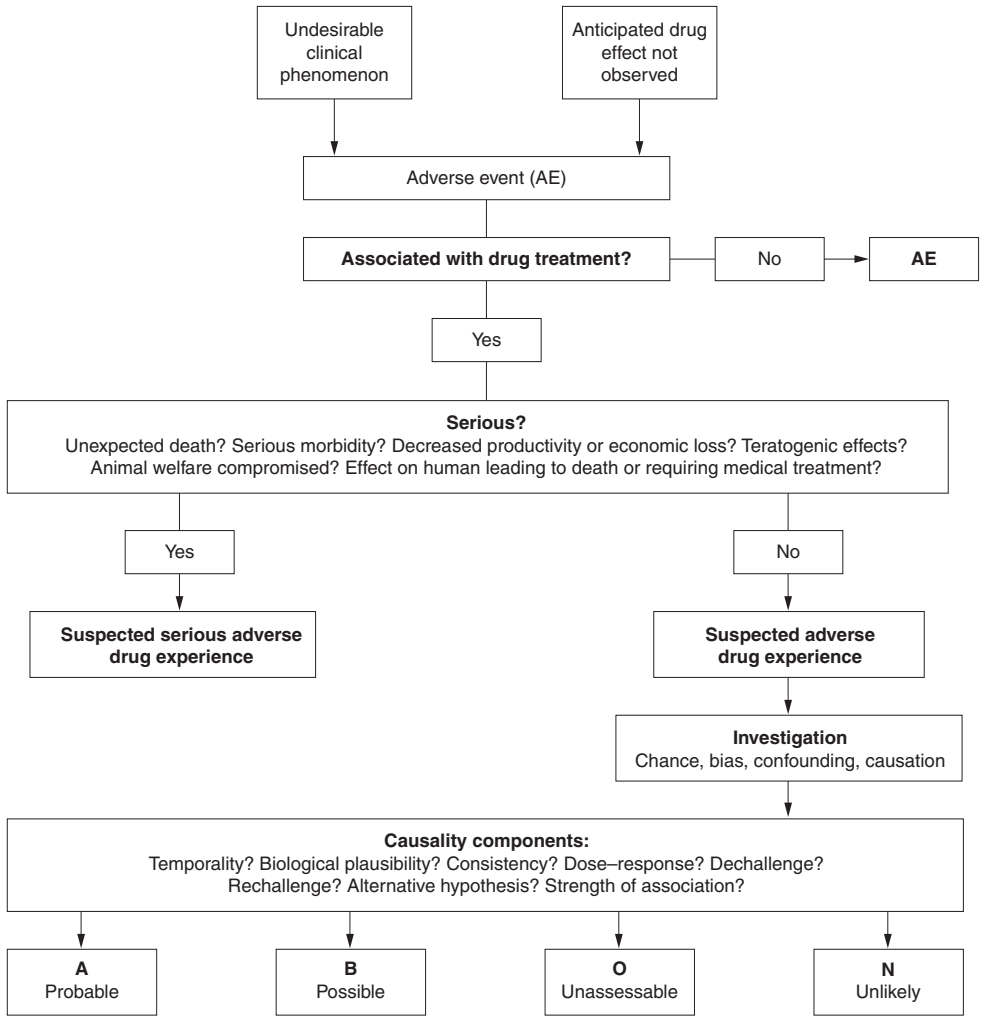

Figure 73.3: Adverse experience classification.

reports of treatment failure are dominant. Factors contributing to apparent treatment failure are set out in Table 73.8 in an example pertinent to antibacterial treatment but readily adaptable to other situations. The APVMA reports demonstrate the growing importance of anthelmintic resistance (benzimidazoles, salicylanilides and triclabendazole represented) and resistance by *Bovicola ovis* to the synthetic pyrethroids and benzoyl ureas. In addition, safety concerns with levamisole in sheep deprived of water and numerous deaths in sheep following misadministration into the parapharyngeal tissue of rumen boluses or controlled release capsules were noted. Knowledge gained from these episodes led to label changes and improved use of veterinary medicines. In the UK, the Suspected Adverse Reaction Surveillance Scheme (SARSS) identified triclabendazole resistance in *Fasciola* and environmental toxicity following inappropriate disposal of sheep dips containing cypermethrin [69, 70]. The published literature is also a source of pharmacovigilance information with the cautious use of ionophores, closantel and lincomycin–spectinomycin (administered orally or systemically) in sheep following reports of toxicity (reviewed by [71]).

Details of authorities to whom reports can be sent are set out in Table 73.2. In the UK, Australia and New Zealand a single agency is responsible for all veterinary ADRs but in the USA separate agencies deal with veterinary medicines (CVM), pesticides (EPA) and biologicals (USDA).

EXTRA LABEL USE

Because of the lack of new drugs being developed for use in sheep and the absence of any authorized products for a number of indications (e.g. analgesia and mastitis control in lactating dairy sheep) it is often necessary to consider the use of products that are not approved for the species. In these cases it is of paramount importance that prevailing laws and regulations be clearly understood and observed as they apply to such uses. Details of the requirements of the US Animal Medicinal Drug Use Clarification Act (AMDUCA) of 1994 and the UK Veterinary Medicines Regulations 2005 can be found at websites listed in Table 73.2.

Off-label or extra label use means use of a drug in an animal in a manner that is not in accordance with the approved labelling. Extra label uses include use in species not listed in the labelling, use for indications (disease or other conditions) not listed in the labelling, use at dosage levels, frequencies, or routes of administration other than those stated in the labelling, and deviation from the labelled withdrawal time based on these different uses.

In many jurisdictions it is considered acceptable under certain conditions for veterinarians to prescribe an off-label drug in order to avoid unacceptable suffering or death of animals under their care.

First, a valid veterinarian–client–patient relationship (VCPR) should be present, i.e. one in which:

1. A veterinarian has assumed the responsibility for making medical judgements regarding the health of (an) animal(s) and the need for medical treatment, and the client (the owner of the animal or animals or other caretaker) has agreed to follow the instructions of the veterinarian.
2. There is sufficient knowledge of the animal(s) by the veterinarian to initiate at least a general or preliminary diagnosis of the medical condition of the animal(s).
3. The practising veterinarian is readily available for follow-up in case of adverse reactions or failure of the regimen of therapy.

Such a relationship can exist only when the veterinarian has recently seen and is personally acquainted with the keeping and care of the animal(s) by virtue of examination of the animal(s), and/or by medically appropriate and timely visits to the premises where the animal(s) are kept.

Extra label drug use is generally permitted when there is no approved product labelled for such use, provided that a careful diagnosis has been made, an extended withdrawal period can be determined, animals to be treated can be identified and measures to assure withdrawal periods are met can be instituted.

Under the UK Veterinary Medicines Regulations 2005, the following cascade of drug selection is mandated, provided that the active substances selected are listed in Annex I, II or III of the EU maximum residue limits (MRL) index (see Table 73.2):

(a) a veterinary medicinal product authorized in the UK for use with another food animal species or for another condition in the same species; or if unsuitable
(b) a medicine authorized in the UK for human use; or
(c) a veterinary medicinal product authorized for use in a food animal species in another Member State; or if unsuitable
(d) a veterinary medicinal product prepared extemporaneously

Similar priority lists are observed in other countries. Under AMDUCA, active constituents approved in countries outside US jurisdiction may also be considered for use provided all requirements are met.

Record keeping is a very important component of extra label drug use. Records that need to be kept for a jurisdiction-dependent prescribed period (at least 5 years in some countries) include: date of examination; owner's name and address; number of animals treated; identification of animals treated; diagnosis; name of product prescribed, including batch numbers; dose rate and route; duration of treatment; withdrawal period for each commodity that can enter the food chain.

The withdrawal period recommendation varies widely from country to country and highlights the need to be familiar with prevailing regulations. For

example, in the UK, the withdrawal period should not be less than 7 days for milk or 28 days for meat. However, in New Zealand, default withholding period for veterinary medicines is 35 days for milk and 91 days for meat from ruminants. In the USA, a suitably conservative withholding period derived from all available evidence can be calculated as described by the Food Animal Residue Avoidance Databank (FARAD) (Table 73.2).

DRUG RESIDUES AND WITHHOLDING PERIODS

Withholding periods for products approved for use in food animals are based on the time it takes for tissue concentrations of drug residues (parent compound or metabolites depending on the residue definition) to deplete to the tolerance (in the USA) or maximum residue limit or MRL (most other countries). The MRL itself is calculated on the basis of the highest 'no observable effect level' (NOEL) demonstrated in a complex battery of toxicology studies, the use of a safety factor that is usually in the range from 10 to 2000, depending on the quality of available toxicology data, and finally consideration of quantitative food consumption patterns of exposed human populations.

A typical depletion graph is set out in Figure 73.4. The thicker depletion line reaches MRL_1 at time WHP_1. This represents the situation when a drug product is used according to label directions. In exporting countries such as Australia, it is possible that national MRLs are set at levels higher than those of an importing country. To ensure that the requirements of importing countries are met, an export slaughter interval (ESI), or the time which should elapse between administration of a veterinary medicine to animals and their slaughter for export, can be derived, representing the time of depletion to the lower level, MRL_2. When drugs are used in an extra label way, it is necessary to determine a withholding period that ensures that the MRL is not exceeded. An example of such a graphical calculation is presented by the dotted line. For every multiple of labelled drug dose rate, it can be assumed for most drugs undergoing first-order absorption that the blood and tissue concentration will increase by at least this multiple. The rate of elimination, again assuming that elimination processes are not saturated and first-order principles apply, will be unchanged as indicated by the parallel depletion line which attains MRL_1 at WHP_{XL}. When the dose rate is doubled, one additional half-life is necessary for depletion to the same MRL. Detailed descriptions of this approach have been published [72].

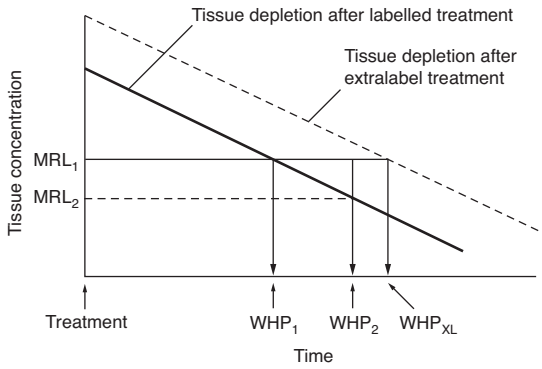

Figure 73.4: Idealized tissue residues depletion profile.

Most countries have programmes of residue surveillance to monitor compliance with withholding periods and assure food safety. It is very unusual for tissue samples to be found containing drug residues exceeding the MRL. However, when investigating cases of residue violation across all species the factors most frequently associated with violations include failure to observe recommended withholding periods, poor record keeping, and inadvertent administration of the wrong drug or dose [73]. Higher risk situations for violative residues include use of long-acting injections, tissue irritation and site reactions [74], and treatment of sick and debilitated animals [75].

While not of food safety significance, residues of ectoparasiticides in wool are subject in some countries (such as Australia) to shearing rehandling periods and wool-harvesting intervals in order to reduce occupational and environmental toxicity, respectively. Residue depletion studies and investigation of factors that influence depletion behaviour (such as formulation, ultraviolent light exposure and rainfall) are necessary to support these intervals.

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Anaesthesia and common surgical procedures

E.W. Scott

Currently, limited numbers of anaesthetics and analgesics are authorized specifically for sheep or even for food-producing animals generally in the UK, although there is a much wider variety of anaesthetic and sedative agents that are safe to use in this species. The UK's cascade system for prescribing should be used but where there are no licensed agents advice should be obtained from the Veterinary Medicines Directorate and the relevant guidance notes consulted (www.vmd.gov.uk). The small size and the manageable nature of sheep make them easy to handle and carry out procedures. Sedation with restraint is possible for minor procedures, but it is important that provision of analgesia is adequate.

ANAESTHESIA

Preparations for anaesthesia

Complications associated with sedation or anaesthesia in sheep include:

- regurgitation of ruminal contents with possible inhalation resulting in aspiration pneumonia
- accumulation of gases in the rumen with consequent distension and impairment of diaphragmatic movement
- production of a large volume of bicarbonate-rich saliva, which is lost during prolonged anaesthesia, resulting in the development of metabolic acidosis
- pressure of abdominal contents on the diaphragm reducing respiratory function.

These hazards can be minimized by starving the animal for 18–24 hours and withholding water for 12 hours prior to anaesthesia (longer periods of starvation may precipitate keto-acidosis). Use of a cuffed entotracheal tube will reduce the risk of inhaling regurgitated material, as will correct positioning of the patient in recumbency (larynx high, nose down in lateral recumbency and head-down position in dorsal recumbency). The administration of anticholinergic drugs to sheep is contraindicated, because the production of saliva is not reduced but the viscosity is increased, resulting in a greater risk of airway obstruction. The development of acidosis due to loss of bicarbonate in saliva can be counteracted by collecting the salivary material and administering it by stomach tube at the end of the procedure. Correct positioning of the animal with the pelvis lower than the thorax will reduce pressure on the diaphragm. In young lambs, hypothermia may occur during anaesthesia due to the large ratio of the surface area to body weight, and care should be taken to avoid mis-mothering during the recovery period.

All anaesthetic drugs produce a degree of respiratory depression and, where possible, provision of

oxygen is advantageous to counteract hypoxaemia (inspired air should have an oxygen concentration greater than 30 per cent).

Gaseous anaesthetics

Halothane is the agent used most commonly in veterinary practice but is not authorized for use in food-producing animals. It produces dose-related respiratory and cardiovascular depression, but no analgesic properties except at anaesthetic dose rates. The agent requires delivery using an anaesthetic machine with vaporizer and provision for delivery of oxygen. For induction, a concentration of 4 per cent halothane in oxygen delivered at a rate of 4 l/min is sufficient and, for maintenance, 1.5–2 per cent. Addition of nitrous oxide (at a ratio of 2:1 oxygen to nitrous oxide) may increase the analgesia but care should be taken that ruminal tympany is not exacerbated. When using gaseous anaesthetics, endotracheal intubation is preferred, to reduce the risk of blockage of the airways by saliva or regurgitated material. The use of isoflurane is increasing in veterinary medicine, and it has many advantages over halothane, including; more rapid onset and recovery, lack of myocardial sensitization to circulating catecholamines, more stable cardiovascular parameters, better tissue oxygenation and greater environmental/human safety. For induction, isoflurane should be administered at a concentration of 4–5 per cent followed by 1–2 per cent for maintenance. Currently, the cost of this anaesthetic, which is not licensed for use in food-producing animals, would limit its routine use in sheep. Sevoflurane is another recent addition to the group of gaseous anaesthetics providing smooth induction and recovery and, as it is non-irritant, it can be used for mask induction. It is not licensed for use in food-producing animals.

Injectable anaesthetics

There are no injectable anaesthetic agents that are authorized for use in food-producing animals. However, the use of several injectable agents has been documented [1, 2] and many are safe in sheep (Table 74.1). The animals should be weighed, and the dose rate of the anaesthetic agent should be calculated accurately, because injectable anaesthetics can have a narrow therapeutic index and overdosage may lead to respiratory depression and other signs of toxicity. Many of the agents give a short duration of action and can be used as induction agents, followed by maintenance using gaseous anaesthetics, or incremental injections can be given to maintain anaesthesia over a prolonged period. In sheep, pentobarbitone is metabolized rapidly and incremental doses can be used safely. Ketamine can be used alone to provide good analgesia but reflexes (eructation, coughing) remain, and this drug is generally used in combination with other sedative agents.

Sedation

Other approaches that are useful in sheep include use of sedatives combined with local anaesthetics. In ruminants, the use of the α_2-adrenoceptor agonist xylazine as a sedative, analgesic or anaesthetic adjunct is common. The effects of xylazine are dose related, but less predictable in sheep than in cattle. After intravenous administration, hypoxaemia may develop as a consequence of changes in pulmonary mechanics and may outlast the duration of sedation. There is initial hypertension followed by normal blood pressure. Xylazine has good analgesic properties. Normal dose rates in sheep range from 0.05 to 0.2 mg/kg. The use of medetomidine, another α_2-adrenoceptor agonist, has been documented, but this drug is not licensed for use in food-producing animals. Medetomidine at a dose rate of approximately 40 μg/kg intramuscularly (i.m.) produces sedation with good analgaesia and muscle relaxation for approximately 1 hour with full recovery after 1–2 hours. The actions of the α_2-adrenceptor agonsists can be reversed using atipamezole, although high costs limit this treatment and, in rare cases, resedation may occur with the sedatives of longer duration of action (medetomidine). Other sedatives that have been used in sheep (but are not licensed for use in food-producing animals) include the benzodiazepines, e.g. diazepam (0.5–2 mg/kg) or midazolam (4 mg/kg), both of which produce good sedation, or the phenothiazine, acepromazine (0.05–0.1 mg/kg), but there is an increased risk of regurgitation with this compound.

Analgesia

Ruminant animals are stoical, and it is very important to ensure that adequate analgesia is provided. None of the opioids is authorized for use in food-producing

Table 74.1: Injectable anaesthetic agents (none is authorized for use in sheep in the UK; see references [1, 2])

Compound	Approximate dose rate (mg/kg)	Route of administration	Appropriate duration of anaesthesia (min)	Maintenance of anaesthesia	Notes
Barbiturates					
Pentobarbitone sodium	24–33	i.v.	15	Dose to effect	Solutions containing propylene glycol may cause haemolysis and haematuria
Thiopentone sodium	10	i.v. with care	10	N/A	Irritant if injected perivenously, transient apnoea
Methohexitone sodium	4	i.v.	5–7	approximately 1 mg/(kg min)	Excitation during recovery
Others					
Alphadolone (3 mg/ml) and alphaxalone (9 mg/ml)	2.2–4.4	i.v.	10–15	0.2–0.3 mg/(kg min)	Absence of respiratory depression – useful for Caesarean delivery of lambs
Propofol	3–4	i.v.	5–10		
Ketamine	2–10	i.v. (or i.m.)	30		Unpredictable excitement when used alone. Best combined with xylazine, diazepam or other agents

i.m., intramuscular; i.v., intravenous; NA, not applicable.

animals. The opioids are powerful analgesics, but have other side effects, e.g. respiratory depression, excitement, prolonged recovery from anaesthesia. Pethidine given at a dose rate of 1 mg/kg by i.m. injection has a short duration of action in sheep with pain relief for 1.5–2 hours. Butorphanol can provide pain relief for approximately 4 hours when administered subcutaneously (s.c.) or intravenously (i.v.) at a dose rate of 0.3–0.5 mg/kg. Buprenorphine has a longer action (4–8 hours) but a slow onset (30 minutes after i.m. or s.c. administration at a dose rate of 6–10 μg/kg).

For post-operative pain, the use of non-steroidal anti-inflammatory drugs (NSAIDs) is increasing. None of these compounds is licensed for use in sheep but several have marketing authorization for use in cattle and although toxicity of these drugs show marked species variation many have been used safely in sheep. Flunixin meglumine is effective in sheep and, administered at 1.1–2 mg/kg by the i.v. or i.m. route, has proved to be effective and safe. Of the other modern NSAIDs, carprofen has been used in sheep and found to give prolonged post-operative analgesia at a dose rate of 2–4 mg/kg, administered by the s.c. route. The other possible options include use of meloxicam, ketoprofen or tolfenamic acid.

Another alternative for provision of analgesia is the use of local anaesthetics, which can be administered locally, to block specific nerves, into the epidural or subarachnoid space or i.v. Lignocaine, the most commonly used local anaesthetic, is not authorized for use in food-producing animals. Its actions persist for approximately 45 min in preparations without adrenaline and up to 90 min with adrenaline. Bupivacaine has a longer duration of action but is not licensed for use in sheep and is more expensive.

The analgesic properties of the α_2-adrenoceptor agonists can be utilized. Xylazine is authorized for use in food-producing animals but its effects are of short duration and can be associated with profound sedation and respiratory disturbances. Administration by the s.c.

or i.m. routes produces a longer duration of analgesia than after i.v. administration [3]. Administration into the caudal epidural space alone or in combination with lignocaine has been shown to produce long-lasting caudal analgesia [4].

COMMON SURGICAL PROCEDURES

Anaesthesia should be used when appropriate, and post-operative analgesia should be considered in all cases for the welfare of the patient. Where antimicrobial therapy is indicated, it should be administered before the initial surgical incision to ensure high circulating concentrations during surgery and to prevent ingress of infection.

Atresia ani

This congenital abnormality is recognized within the first few days of life, as the lamb becomes dull, anorectic and shows abdominal distension, straining and discomfort. On examination, there is absence of a patent anus. The success of surgical correction depends on the extent of the developmental abnormality.

Surgery can be conducted under local anaesthesia or using a short-acting general anaesthetic. A circular or cruciate incision is made over the anal area and the end of the rectum is located by palpation. The blind-ending rectum is opened and the walls sutured to the anal skin. If the end of the rectum is deep within the pelvic cavity and cannot be easily palpated or located then the prognosis is poor.

Caesarean section

Delivery of lambs by Caesarean section is indicated in cases of fetal or maternal dystocia or as a therapeutic measure in ewes with pregnancy toxaemia.

The ideal anaesthetic for this operation should have little or no effect on fetal survival, particularly fetal respiration. The steroid anaesthetic, alfadolone and alfaxalone combination, is a useful general anaesthetic, as is halothane. Alternatively, local anaesthetics can be used as a paravertebral block, lumbosacral epidural or by local infiltration at the wound site. Another option is extradural infiltration of xylazine [5].

The common approach is via the left flank, although a ventral abdominal approach can be used, but care should be taken to avoid the mammary blood vessels. After incision into the abdomen, the pregnant uterus is exposed and an incision made along the greater curvature and the lambs removed. The entire uterus should be checked to remove all lambs (a second incision into the other uterine horn may be necessary in some cases). Care should be taken to avoid spillage of fluid into the abdominal cavity and, unless detached, the fetal membranes should be left *in utero*. The uterine incisions are repaired using absorbable material and inverting sutures. The abdomen is then closed by stitching the individual muscle layers followed by everting sutures in the skin. Antibiotics should be administered to the ewe, together with provision of analgesia. Once revived, the lambs should receive adequate colostrum and be kept warm until the ewe recovers fully and can care for her offspring.

Coenurosis

Prior to surgery the animal can be treated with corticosteroid to reduce brain oedema, which may occur after surgery. The animal should receive a general anaesthetic. The surgical approach depends on the area of brain affected, which can be determined from the clinical signs. Prepare the skin surface, then, for a cerebral cyst, the trephine site is 1–2 cm lateral to the midline and immediately rostral to the coronal (parietofrontal) suture line. For a cerebellar cyst, the trephine site is midline between the nuchal line and the suture line between the occipital and parietal bones. After removing the 1-cm bone core, the dura mater is incised and, owing to increased brain pressure, tissue is forced into the trephine hole. The cyst is drained through an 18-gauge i.v. catheter connected to a syringe, followed by careful removal of the cyst wall and protoscolices with forceps. Postoperative analgesia should be provided along with antimicrobials.

Dehorning

Removal of a horn may be required following traumatic damage or if the horns are misaligned and growing into the tissues of the face. As removal involves

penetrating the sensitive horn, general anaesthesia or sedation plus local anaesthesia/analgesia should be used in all cases. The horn can be cut using bone cutters, but in many cases an embryotomy wire is useful because of the difficulty of access, and the sawing action can improve haemostasis. Bleeding vessels on the cut surface can be sealed by heat or bone wax, and antimicrobials should be administered systemically. In summer, the area should be protected to prevent fly strike.

Digital amputation

Amputation is indicated in cases of septic pedal arthritis with severe lameness. Removal of the digit will relieve pain more rapidly than prolonged antimicrobial therapy with sinus irrigation [6]. Local analgesia is achieved using i.v. anaesthetics ventral to a tourniquet. The distal limb is clipped and cleaned and the skin incised to a depth of 1–2 mm in the interdigital area as close as possible to the affected digit, while still removing the infected tissue. The incision is extended posteriorly, becoming deeper (6–8 mm), then laterally to encircle the hoof above the coronary band. Embryotomy wire is inserted into the interdigital incision and then the digit is removed at an angle of 15° to the horizontal, removing as much infected tissue as possible. The wound is left unsutured but is dressed with cotton-wool padding and bandaged, which should be changed after 3–4 days, when granulation tissue should cover the end of the bone. The wound should then be cleaned daily until healed adequately. Post-operative analgesia and antimicrobial treatment are required.

Embryo transfer

Manipulation of the reproductive cycle and embryo transfer are carried out to increase the rate of genetic improvement for particularly important stock production or for other valuable characteristics. The techniques require anaesthesia and surgery, and great care should be taken with asepsis and analgesia. If procedures are to be carried out repeatedly, the long-term welfare of the sheep must be considered. For both collection of embryos from the donor and insertion of the embryo into the recipient, general anaesthesia is recommended although, for speed of recovery, sedation with or without local anaesthesia is used commonly. If local anaesthesia alone is used, there will be no analgesia of the internal organs. The techniques have developed from the initial methods, where exteriorization of the organs was necessary, to the use of fibre-optic laparoscopes, which allow visualization of the organs *in situ* and manipulations to be carried out, in experienced hands, with less surgical trauma.

The reproductive cycles of donor and recipient animals are synchronized. Following anaesthesia, the donor or recipient is placed on its back with its lower abdomen raised and head down. The abdomen is cleaned and the laparoscope introduced through a small stab incision approximately 2 cm from midline and 10 cm anterior to the udder. Other instruments required for the manipulations are also introduced into the abdomen via small stab incisions. The ovaries are examined to check for the presence of corpora lutea and the number counted in the donor animal. The necessary parts of the reproductive tract of the donor are exteriorized through a small incision in the abdomen; the oviduct is cannulated via the infundibulum, and sterile fluid (phosphate-buffered saline) is introduced into the uterine horn. This fluid is massaged gently towards the oviduct, avoiding damaging the embryos, and collected through the cannula.

Care should be taken when manipulating the reproductive tract to prevent any damage or haemorrhage, as this will result in the formation of adhesions. The tract is returned to the abdomen and the incisions sutured.

In the recipient animal, the laparoscope is positioned and the uterus identified. The embryo is then placed within the uterus via a pipette introduced through a small stab incision. The stab incisions are sutured or closed with Michel clips.

Entropion

In mild cases, treatment by physical turning of the eyelid, injection of inert material or placement of a small Michel clip may be sufficient to correct the abnormality. In more severe cases, removal of a small strip of skin from the lower eyelid is required. The surgery can be carried out under local anaesthesia, but a short-acting general anaesthetic is preferable. The lower eyelid is cleaned and an incision made close to and parallel with the lower eyelid margin. Then an elliptical or V-shaped incision is made, with the widest part where the eyelid inversion is greatest. Remove the small strip of skin, taking care not to

damage the conjunctiva, and close the wound using small interrupted sutures. This condition can be inherited in some cases, and, consequently, it may be undesirable to breed from affected animals.

Enucleation of the eye

Removal of an eye after ocular trauma provides rapid pain relief and is of little disadvantage as the animal can adjust to monocular vision. General anaesthesia with appropriate post-operative analgesia is used. The periorbital area is clipped and cleaned, and the eyelids are sutured together. A linear skin incision is made parallel to and 0.5 cm away from both eyelid margins and extended around the entire circumference of the eye. The conjunctiva is separated from the lids attached to the fornix by blunt dissection and traction on the eyelid margins. The angularis oculi vein, located at the dorsomedial border of the orbit, should be identified and ligated. The orbital ligament is particularly short on the medial side and sectioning of this improves access to the deeper parts of the orbit. Section all the muscular attachments to the eye as close to their scleral attachments as possible by careful dissection. The optic nerve and associated vessels are then clamped and ligated before severing. The orbit is then packed with sterile absorbable foam or equivalent material, and the skin wound is closed. The wound should be dressed using a padded dressing, which can be removed after 2–3 days.

Fractures

The cost of general anaesthesia and internal fixation of fractures limit this technique to valuable pedigree animals. In general, fracture repair is restricted to external fixation of fractures in the distal limb area. In young animals, any splints or plasters should be checked regularly for rubbing or pressure on growing limbs. Analgesia should be given in all cases. Compound fractures or those involving proximal limb bones have a poor prognosis without internal fixation, and any affected animal should be culled.

Toe granuloma

The formation of toe granuloma is often a sequel to overzealous paring of the foot and leads to chronic lameness, deformed hoof growth and a greater risk of development of foot-rot. Removal of the granuloma must be complete to prevent regrowth [7]. Local analgesia is used either by a ring block around the cannon bone or by intravenous injection of local anaesthetic distal to a tourniquet. The area is cleaned and disinfected and a tourniquet placed on the leg. The granuloma is removed at its base with a scalpel or sharp shears, then the site is cauterized with a hot iron. Check when removing the tourniquet that all bleeding points have been cauterized. Antimicrobials should be administered to prevent infection.

Urolithiasis

Successful surgical treatment of obstructive urolithiasis provides return of urine flow and correction of uraemia. However, because of the likely accumulation of uroliths in the bladder, the long-term prognosis is poor. The area of obstruction dictates the type of surgical intervention required. In some cases, the obstruction is palpable at the vermiform appendage. Removal of this will result in resumption of urine flow. Obstruction higher in the tract (glans penis or sigmoid flexure) requires surgical intervention under general anaesthesia.

For a rapid surgical correction, a urethrotomy/urethrostomy should be performed under general or local anaesthesia or caudal epidural analgesia. The urethra is located on the outer surface of the penis. After disinfection of the area, an incision is made over the site of obstruction and the calculi removed. The urethra can be left unsutured, and the wound will close and heal rapidly, although the healed site is another potential area for blockage. Closure of the wound by suturing the edges of the urethral epithelium to the skin delays the healing process. Alternatively, penile amputation and creation of an orifice in the perineal area is an option. Under general or local anaesthesia/analgesia (caudal epidural), the perineal area from below the anus to the scrotum is clipped and disinfected. A 4–5-cm vertical incision is made in midline from the tuber ischii ventrally. Using blunt dissection the penis is identified (a firm, smooth, yellow organ) and by further dissection and gentle traction is pulled through the skin incision. Identify the area of obstruction, and sever the exposed penis above this being careful to leave sufficient tissue at the wound for suturing. The stump is then sutured to the wound, taking care not

to occlude the urethra. Post-operative wound care (daily cleaning of the area plus use of antimicrobials) is important to lessen the problems with infection and urine scalding.

Vasectomy

Vasectomized rams are commonly used to synchronize oestrus in flocks, and it is important that the operation is conducted properly and sheep are identified adequately [8]. This operation can be conducted under local anaesthesia or lumbosacral spinal analgesia [9], but may be easier under general anaesthesia. When using local anaesthetics delivered to the site, care should be taken to prevent any haemorrhage in the area of the spermatic cord, as this can make the surgery more difficult.

The neck of the scrotum is clipped and cleaned. A 2–3 cm incision is made vertically through the skin and tunica dartos on the anterior aspect of the scrotal neck towards the medial side of one spermatic cord. Using blunt dissection, the spermatic cord is identified (it contains the cremaster muscle and on the medial side can be identified by the testicular vessels and vas deferens in the tunica vaginalis). The cord is hooked from below using artery forceps and exteriorized. It can then be held at the wound by laying the artery forceps at right angles below the cord. The tunica vaginalis is opened by a 1–2 cm incision, and the vas deferens exteriorized (it is identified as a white, firm structure approximately 2–4 mm in diameter). Using artery forceps placed above and below, clamp off at least a 3–4 cm section of the vas deferens, which is removed. The stumps are tied off using catgut. One end is anchored in the fatty tissue outside the vaginal tunic, which is not repaired. The spermatic cord is then returned, the operation is repeated on the other spermatic cord, then the tunica dartus sutured followed by the skin.

Removal of a sufficiently long piece of vas deferens will reduce the risk of recanalization and return to fertility. In some cases, histopathological examination of the removable tissue can be used to confirm the success of the operation. As an initial check at the time of surgery, squeezing material from the cut duct on to a slide and examination for sperm under a microscope is reassuring. Some sperm are ejaculated for a short period after vasectomy, but motility declines rapidly by 48 hours after surgery. The rams can be put with ewes from 5 days after surgery. It is advisable that samples of ejaculate are taken from vasectomized rams before use in future seasons.

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Necropsy and sampling techniques

F. Howie

Whether a necropsy is undertaken by a livestock owner, shepherd or veterinary practitioner, they should feel capable of using the procedure to answer a question. Simple questions are easily answered, e.g. was the animal pregnant? Less obvious are questions that seek to understand the cause of a clinical problem, e.g. nervous signs or sudden death. In such instances, higher degrees of competence are called for and require consultation with a veterinary surgeon or, where possible, with a veterinary diagnostic laboratory. Such laboratories are staffed by highly trained, experienced veterinary pathologists with specialist knowledge, equipment and support facilities at their disposal. In addition, in the UK and many other countries very strict legislation covers disposal of ruminant necropsy material making a field necropsy difficult and limiting its extent [1]. If transportation of the subject is not feasible, before starting, advice should be sought from the laboratory to which samples will be sent. Otherwise,samples can be lost if not collected early in the examination, e.g. blood. Based on the history, the laboratory will recommend which samples to take and how to handle them. It may be necessary to contact the laboratory again for further advice before any material is discarded.

GENERAL NECROPSY TECHNIQUE

The procedure described here is thorough yet simple, practical and easy to adapt. Whatever method is chosen, it is crucial that it is practised consistently so that the normal appearance and location of all organs become so familiar that any abnormality is readily apparent and, on completion, the operator can be certain that all organs have been examined.

Objectives

The usual objective in performing any necropsy is to establish the cause of illness or death. However, additional information can be gained that may be of use to other animals in the group, e.g. screening intestinal content for evidence of parasitism.

Principles

The necropsy should be conducted to glean the maximum information, but examination of one organ should not be at the expense of another. All material should be retained until the gross examination is completed and all samples for analysis have been collected. If the animal is presented live, blood samples should be collected into a range of tubes, with and without anticoagulants, prior to euthanasia. Necropsies should be performed immediately after death or as soon thereafter as possible, in a well-lit area supplied with clean running water and capable of being cleaned thoroughly on completion. Chilling is acceptable when the necropsy has to be delayed, but may reduce the information available; freezing should be avoided.

Although all necropsy reports should be of a sufficient standard to withstand even the most intense scrutiny, special care should be taken if the report may be used as part of legal proceedings. In that case, photographs should be taken throughout, all samples should be individually labelled to include a unique identification, date and time of collection, and a second person should witness the findings and confirm that they have been recorded accurately. All material should be retained until the legal proceedings are completed.

Figure 75.1: A selection of safety equipment recommended for use when performing necropsies.

Safety

Many dangers must be considered when performing a necropsy but they fall into two basic categories: trauma from sharp instruments or bone fragments; and zoonotic conditions such as transmissible spongiform encephalopathies (TSEs), louping-ill and chlamydial infection. Pregnant women must avoid any contact with ovine necropsy material. Waterproof protective clothing, boots and gloves should be worn at all times. The use of cut-proof gloves, dust masks and goggles or a full-face visor is recommended, especially when handling nervous tissue in countries known to harbour transmissible neuropathogens (Figure 75.1). In the UK, both employer and employee have duties of care placed on them by legislation. Basically, employees must work in such a way as not to endanger themselves or a third party and should use all safety equipment supplied. The employer must supply adequate protective clothing and safety equipment and a safe working environment, including the provision of first aid. The law places fewer duties on self-employed people, the status of many general practitioners, but they must work with due regard for personal and third-party safety [1].

Equipment

A selection of useful instruments and equipment is shown in Figure 75.2. Maintenance of tools in a clean and, where appropriate, sharp condition is essential

Figure 75.2: A selection of equipment useful in performing necropsies.

Figure 75.3: A selection of suitable sample containers and means of labelling containers and recording necropsy findings.

and should be carried out during the necropsy as well as between necropsies. All equipment and protective clothing should be thoroughly cleaned and disinfected before leaving the site of the necropsy [1]. A vice is useful to stabilize an isolated portion of the carcass, e.g. the head or long bone, to make sawing easier. A selection of suitable sample containers, which should be watertight, and suggested means of recording the necropsy findings are illustrated in Figure 75.3. A camera is useful for recording visual abnormalities, but photography is an essential part of any necropsy involved in legal proceedings.

Procedure

Before commencing, review the history and plan ahead for any special techniques required during the necropsy. If possible, weigh the carcass. Wet the

necropsy table or area to be used and personal protective clothing to prevent adhesion of blood and other material and to make cleaning much easier.

External examination

Examine the skin, fleece, hooves, external organs, ears, eyes and orifices for abnormalities, including secretions and evidence of trauma.

Internal examination

As hair dulls the knife, incise the skin with a stab wound, insert the knife with the back of the blade towards the carcass and cut up through the skin. This procedure avoids cutting the hair and prevents damage to the tissues below.

1. Lay the animal in dorsal recumbency. Cut the skin of the axilla and muscular attachments of the scapula to abduct the forelimbs. Cut down through skin and muscle at the lateral margins of the pubis into the hip joints, severing the femoral ligaments to allow full visualization of the femoral heads. Cut beneath the prepuce or mammary gland and reflect it caudally. Make a midline ventral skin incision between pubis and mandible, and reflect the skin laterally giving access to the ventral aspect of the head, neck and body including superficial carcass lymph nodes and subcutis (Figure 75.4). With paired organs, such as lymph nodes, always examine the right before the left to help with recollection of which one was abnormal, remembering that the carcass is in dorsal recumbency.
2. Expose the contents of the abdomen by making a ventral midline incision in the body wall from pubis to sternum and, immediately caudal to the last ribs, reflect the abdominal wall latterly. If uncollapsed lung is desired, clamp the cervical trachea before puncturing the thorax. Enter the thorax by severing the costochondral junctions (saw or bone forceps: knife if the animal is very young) and removing the sternum. (Figure 75.5). Collect samples of any fluid present in the body cavities into sterile receptacles at the earliest opportunity to prevent contamination or loss. Examine the viscera *in situ* prior to removal for further study.
3. Free the tongue, by cutting through the muscle along the medial aspect of the mandible, and reflect it caudally. Whilst applying traction to the tongue, cut through the joints of the hyoid apparatus and dorsal pharynx to free the larynx. Further traction with only minimal cutting will free the tongue, larynx, trachea, oesophagus, lungs and heart to the level of the diaphragm. Cut the diaphragm close to the body wall. Cut the root of the mesentery ventral to the kidneys, taking care to leave kidneys and adrenal glands attached to the carcass. Further caudal traction and cutting of the rectum will free the alimentary tract, with the thoracic contents still attached. Remove the adrenal glands. The reproductive and urinary tracts can be dissected out as one unit, facilitated by removal of the floor of the pelvis by cutting through pubis and ischium on both sides (Figure 75.6).
4. Open the oesophagus along its length, then detach it from the trachea. Examine and section the

Figure 75.4: A sheep carcass with the skin reflected from a midline incision.

bronchial lymph nodes and tongue. Cut through the dorsum of the larynx and extend the cut down the trachea to the terminal airways in the diaphragmatic lobe of one lung. Make multiple transverse cuts into the other lung. Turn the lungs over and cut down and examine pulmonary arteries on the ventral aspect.

5. Open the pericardial sac and examine the epicardium. Cut across the right atrium. With the right side of the heart facing you, extend the cut down through the atrioventricular valve and along the left margin of the right ventricular wall close to the interventricular septum. At the apex, continue the cut up the right margin,

Figure 75.5: Removal of the sternum and ventral abdominal wall reveals the contents of the chest and abdomen without disturbance.

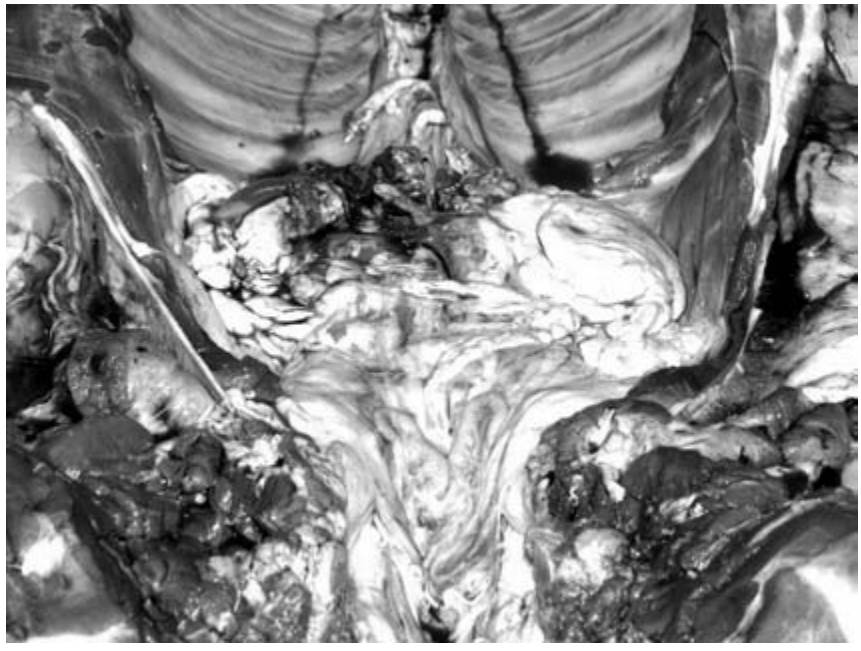

Figure 75.6: Removal of the floor of the pelvis reveals the urogenital tract without disturbance.

through the pulmonary valve and into the pulmonary artery. Turn the heart round and cut across the left atrium. Make a vertical cut down through the atrioventricular valve and left ventricle wall to the apex. To open the aorta, insert the knife under the septal cusp of the left atrioventricular valve. Cut through the cusp and wall of the atrium and up into the aorta.

6. Detach the spleen from the rumen and make several cuts into its substance. Cut a small opening into the duodenum at the level of the bile duct. Squeeze the gall bladder and check for free flow of bile. Dissect the liver free and make multiple cuts into the organ to include transection of the major bile ducts. Free the intestines from the mesentery (Figure 75.7), open the forestomachs and then the abomasum and intestines along their length noting and, if necessary, collecting the content as you go. Blunt-nosed scissors are recommended for the intestines. Do not open

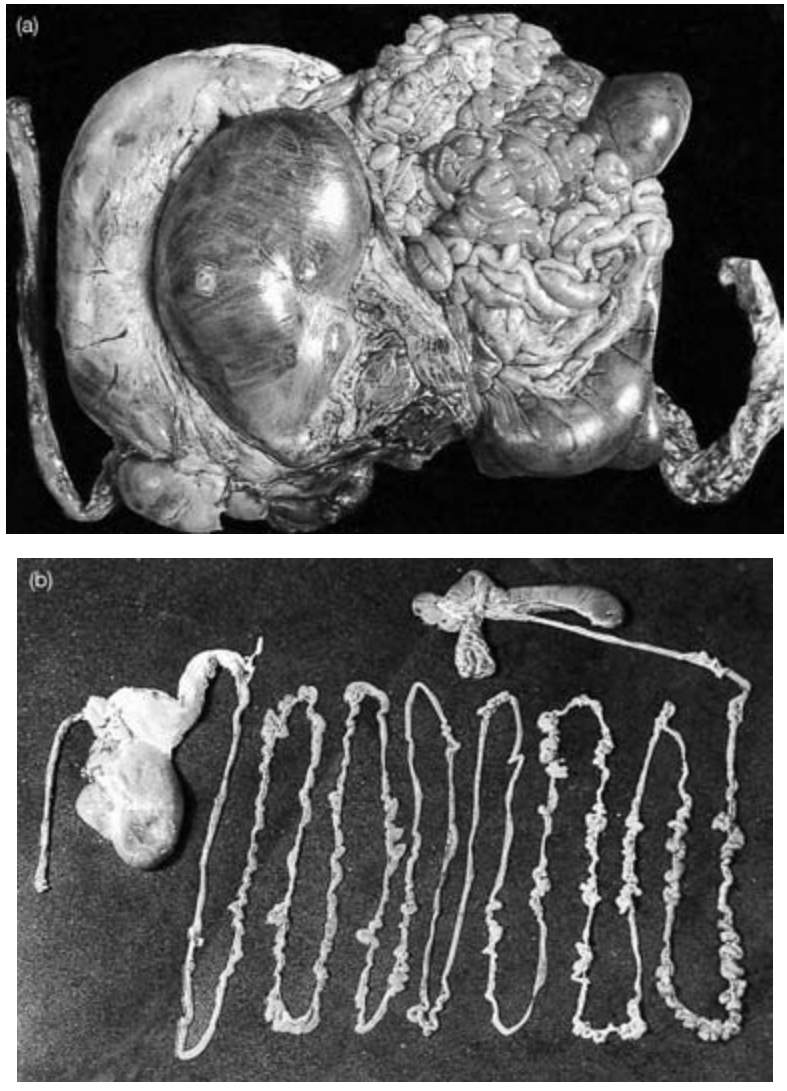

Figure 75.7 The alimentary tract (a) before and (b) after releasing the viscera from the mesenteric and other attachments.

the abomasum or intestines at this stage if total worm counts are required, see the section 'Sampling for helminthology/protozoology' later. Cut each kidney longitudinally to the pelvis and peel off the capsule. Make multiple transverse cuts through cortex and medulla. Cut down the ureters into the bladder and out into the urethra. Cut across each adrenal gland and record the cortex to medulla ratio.

7. Cut the ovaries longitudinally then transversely. Open both horns of the uterus, cervix and vagina. Make multiple cuts into the substance of the mammary tissue.
8. Open the prepuce and examine the penis. The urethra can be opened along its length. Free the testes and make a single longitudinal cut and then multiple transverse cuts into their substance.
9. Detach the head by severing the atlanto-occipital joint. Remove the horns, ears and the skin from the caudal half of the skull. Remove the roof of the cranial vault using saw cuts as illustrated (Figures 75.8). Cut and reflect the meninges and remove the brain by turning the skull upside down and severing the cranial nerves by blunt dissection. The pituitary gland should be dissected from the connective tissue of the floor of the cranium. To remove the eyes incise the skin 0.5–1 cm around the margins of the eyelids. Grasp this remaining skin and apply gentle traction. Cut the soft tissues around the eye close to the bone. Finally, transect the optic nerve and remove the eye. Dissect away the muscle and fat but leave as much optic nerve as possible.
10. To remove the spinal cord, turn the carcass over into ventral recumbency. Cut and reflect the skin along the dorsal midline. Dissect the muscles away from the spinal column. Cut through the dorsal vertebral arches using bone cutters or an oscillating saw and remove to expose the spinal cord. The cord is removed by lifting gently while severing the spinal nerve roots lateral to the dorsal root ganglia (Figure 75.9).
11. The hip and atlanto-occipital joints have been examined and the shoulder joints accessed during the initial stages of the necropsy. Examine the shoulder joints and any other joints indicated by the history. Remove a central rib to assess bone strength (try to break it). Collect bone marrow from a long bone, close to the ends in a mature animal, by cracking the bone open using rib cutters or saw through at an oblique angle and gently scoop out the bone marrow.

Disposal

Whether incineration, burial or rendering is chosen for disposal of the remains of the necropsy, as required by local legislation, it is the pathologist's duty to ensure a proper procedure, which takes account of public sensitivity as well as the possible disease hazards to livestock or human handlers. Current legislation pertaining to the UK is comprehensively covered in a recommended article [1].

SAMPLING FOR HISTOPATHOLOGY

Always sample liver, kidney, lung and all lesions. However, sampling a full range of tissues is recommended as they can be discarded, unexamined, once the case is completed. The brain should be sampled when the history indicates need and in all grossly normal necropsies.

Principles

Sterile sampling should have priority.

- Use at least ten times the volume of fixative to the volume of tissue.
- Collect and fix samples as soon after death as possible.
- Take representative samples, which may have to be multiple.
- Use a sharp blade not scissors. Use the carcass as a cutting board.
- Samples should be no more than 1 cm thick, but avoid thin slices, which will curl.
- Sample lesions at their margin to include normal tissue.
- Where appropriate sample cortex and medulla, e.g. kidney.
- Make each sample easy to identify. Place in individual, labelled containers or use sample size (left organ sample larger than right).
- Label all containers with reference number or name, date of collection and contents (see 'Transportation of samples' later).

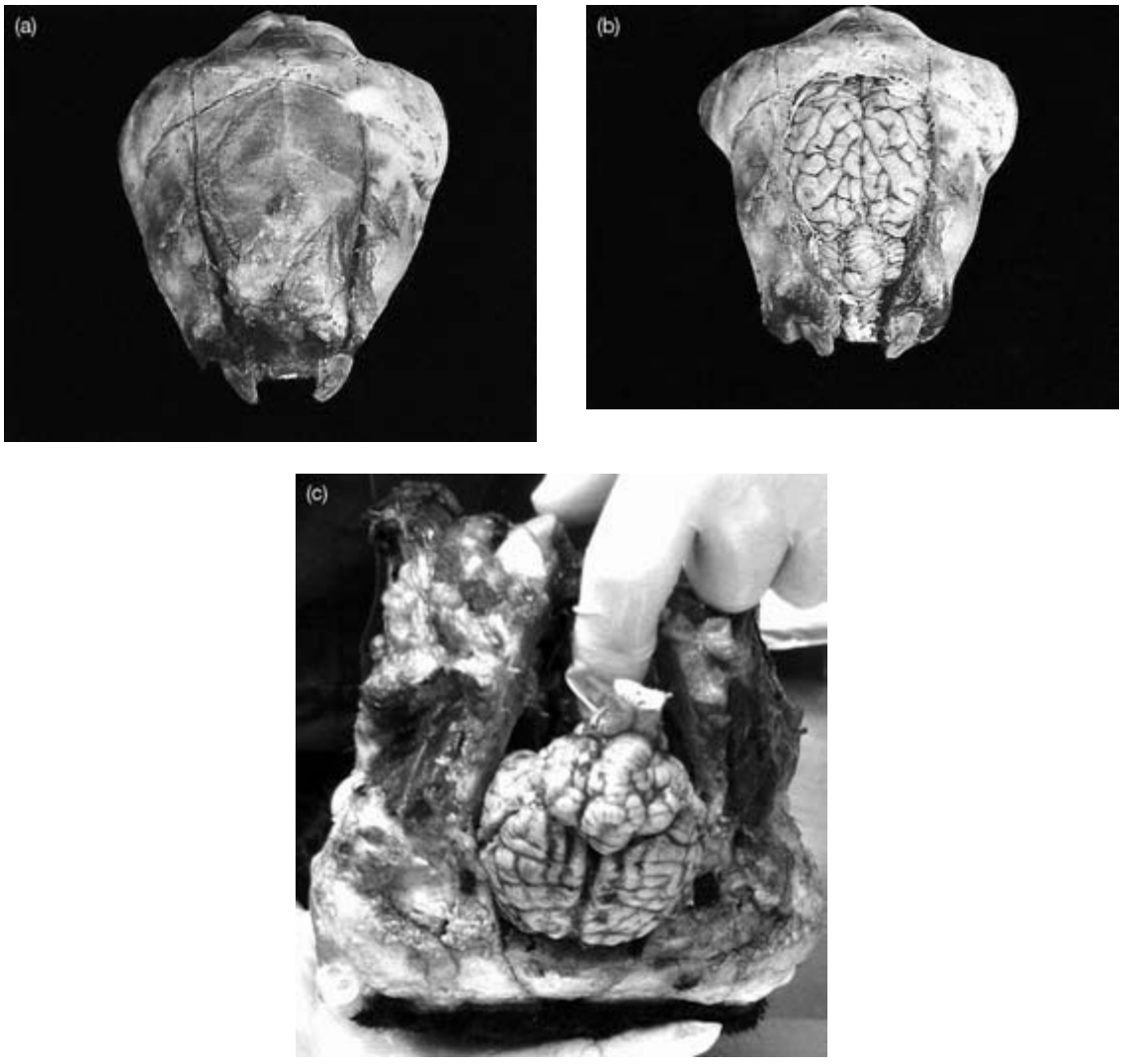

Figure 75.8: (a) Dorsal view of the skull with skin removed and saw cuts outlined. (b) Brain revealed by removal of bones forming roof of vault. (c) Removal of the brain by turning the skull upside down and freeing by blunt (finger) dissection.

Fixatives

The most commonly used fixative is neutral buffered formaldehyde (40 per cent formaldehyde 100 ml, water 900 ml, sodium dihydrogen phosphate monohydrate 4 g, disodium hydrogen phosphate anhydrous 6.5 g), which will produce adequate results for routine processing and paraffin-wax-embedding of all tissues. Many other fixatives give improved results for individual tissues, e.g. Baker's solution for the central nervous system (CNS). The histopathology laboratory to which the samples will be sent will advise on fixative selection and will provide the fixative or its recipe.

Glutaraldehyde (3 per cent in phosphate buffer pH 7.2) is used for tissues intended for electron microscopy (EM) examination. It penetrates more slowly than formalin and has an effective fixation depth of only 2–3 mm, hence the small sample size required.

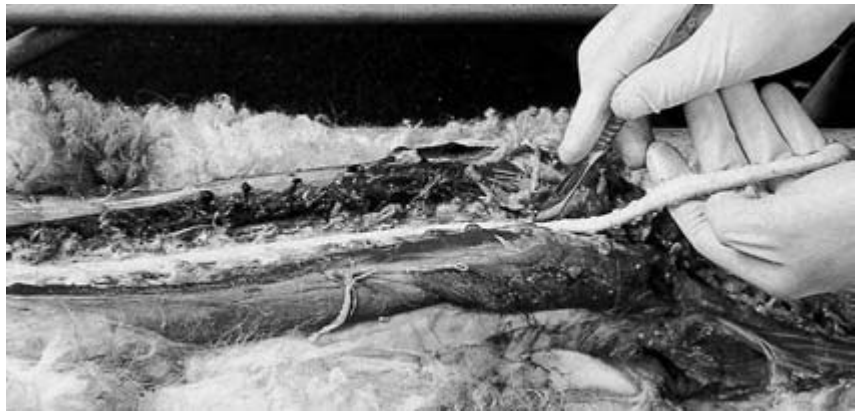

Figure 75.9: Removal of the spinal cord by lifting gently while severing spinal nerves following removal of the dorsal vertebral arches.

Fixation of tissue samples

A minimum fixative to tissue ratio of 10:1 is required. The samples should remain in the fixative until fixed throughout, which takes from a day for small samples to a month for whole organs. Replacement of the fixative during fixation reduces the time required, especially if the tissues are bloody. Bone samples should be fixed prior to decalcification or a combined solution (e.g. Gooding and Stewart) can be used. Floating tissues should be kept submerged by laying absorbent material, e.g. paper towel or cotton-wool, on top of the fixative to cover the sample. The above principles are very general and may require modification for specific tissues or purposes.

Gastrointestinal tract

Open and dislodge contents under gently running water. Do not handle mucosa. Place 1–2 cm portions, serosal surfaces down, on to card and dry for 3 min before immersing card and sample in fixative (Figure 75.10).

Lung

Before sampling, infusion of fixative into the airways, via a filter funnel inserted into the trachea, enhances maintenance of airway patency but is not necessary for routine sampling. Consolidated lesions should be sliced.

Skin

Place a 1–2 cm sample, epidermis up, on to card and dry for 3 min before placing card and sample into fixative (Figure 75.10).

Skeletal muscle

The use of muscle splints to prevent contraction artefact is ideal. However, for routine sampling take a rectangular sample, $1 \times 1 \times 2$ cm, parallel to the muscle fibres and place on card. Dry for 3 min before immersing card and sample in formalin (Figure 75.10).

Eye

This can be fixed whole, but a scalpel slit in the sclera will improve retinal fixation. Special fixatives, e.g. Zenker's, give improved results.

Nervous system

Fix the brain whole with a short length of cord attached. To fix the whole spinal cord, slit the dura along its length and divide the cord into short (6–8 cm) sections still in the dura. The cord can now be folded up to fit in a smaller pot, but will still fix in straight lengths. Portions of nerve can be allowed to dry down on to card for 3 min before placing the card and nerve into fixative.

Figure 75.10: Muscle, skin and intestine samples drying on card prior to fixation.

Biopsy samples

Tissue samples for histopathological examination should be selected and handled as above. When sampling skin, a 2-cm long ellipse taken parallel to the line of hair growth and fixed on card is preferable to a punch sample. Always include the youngest lesions.

Samples intended for EM

Autolytic specimens are unsuitable. Prepare 1–3 mm^3 blocks using a very sharp blade such as a razor and fix in glutaraldehyde for 1–3 hours. Transfer to phosphate buffer before dispatch.

Smear preparation

Express fine needle aspirate samples on to clean slides, smear if thick and air-dry. Touch preparations can be used for fluorescent antibody techniques and cytology. Blot the freshly cut surface, touch gently on to a clean slide and air-dry. Always make spare smears.

Fluid samples

Collect plain and ethylendiamine tetra-acetic acid (EDTA) samples to allow bacterial culture, biochemical analysis, nucleated cell counts and cytology. If possible, smears should be made immediately from the whole sample and from the centrifuge deposit and air-dried. Retain an unspun aliquot of the EDTA sample for a cell count.

Transportation of samples

Samples to be posted or otherwise transported must be packaged correctly [2, 3]. Current UK specifications (602 Packaging Specification) require that the sample be placed in a watertight, leak-proof receptacle, which is then wrapped in sufficient absorbent material to absorb all fluid in case of breakage. One or more of these containers is in turn placed in a second durable, leak-proof receptacle. Finally, the secondary container and completed laboratory request form, in a plastic bag, are placed in an outer shipping package. This must protect the contents from physical and water damage, and indicate the potentially toxic nature of the contents.

SAMPLING FOR BIOCHEMISTRY AND HAEMATOLOGY

Analysis of blood and tissue samples can provide valuable, often conclusive information in conditions as diverse as chronic wasting and sudden, unexpected death. However, the importance of a thorough clinical examination and review of the history cannot be overemphasized, and no tests should be carried out before both are completed. This allows selection of the most valuable tests and prevents unnecessary expense and wastage of limited sample material. All

this and more is covered in some detail in a recommended series of articles [4–6].

Object

The aim is to get the maximum information from the material available and to use it to provide an accurate diagnosis. The range of assays available is wide and recent technical advances allow most blood tests to be performed on minute volumes of sample, especially if multiple tests are carried out simultaneously by a single automatic analyser. However, care and forethought when collecting samples and selecting tests will avoid errors that cannot be rectified at a later date.

Principles

Ill-chosen tests only provide information that is irrelevant or already known, waste valuable sample material and cause unnecessary expense. Individual tests rarely provide a conclusive diagnosis and must be supported by other results and information and interpreted accordingly. Therefore, a successful outcome depends on a number of factors, principally the following:

- *Choice of tests*. Selection should be based on all available information including farm and case history, clinical and/or necropsy findings, previous results and local knowledge. Many laboratories offer packages of tests designed to assess an organ or system fully or to investigate the more common deficiencies, thereby obviating the need for specialist biochemical knowledge on the part of the clinician.
- *Choice of samples*. If in doubt, consult the laboratory prior to sampling. It is advisable to collect a full range of samples, e.g. heparinized as well as clotted blood, even if the tests initially chosen require only one type of sample. Make best use of the material available, e.g. if the animal is to be euthanased collect blood samples prior to euthanasia and tissue samples at necropsy or, if the animal died after blood sampling, collect tissue samples as well. Any unused samples can be discarded once a diagnosis is reached.
- *Handling of samples*. Some tests require samples to be chilled immediately after collection and analysed within a limited time. If clotted blood samples are to be stored or posted, the serum should be separated and frozen or chilled, respectively. Alternatively, some commercial veterinary diagnostic laboratories supply gel tubes, which, following centrifugation, stabilize sera for transit. Always clearly identify and date all samples. Samples to be posted must conform to local regulations (see 'Transportation of samples' earlier) and, to minimize transit time, should not be sent over weekends or holidays.
- *Analysis and interpretation*. Choose a dedicated veterinary diagnostic laboratory and submit all available information with the samples. Include full details of the animal sampled (species, age, breed, sex, etc.) and all individual animal and farm history.

Procedures

Live-animal sampling

Blood is the most useful and readily available sample in the live animal. The tests to be performed, the sample type and handling requirements should be considered prior to collection. The use of commercially prepared, evacuated, sterile collection tubes of the Vacutainer-type is strongly recommended [4]. Other suitable containers include pretreated self-seal syringes, which, like vacuum collection tubes, do not require immediate transfer of the sample to another container. If an ordinary syringe is chosen, it should be unused and sterile to avoid the risk of chemical contamination by cleaning agents. Remove the needle from the syringe before transferring the blood to the tube to prevent cell damage.

Some tests require only serum. Others require addition of one of a number of chemical anticoagulants to prevent clotting. Commercially prepared tubes have colour-coded stoppers for easy identification. Recommendations for anticoagulant usage [3] are summarized in Table 75.1. The tubes should be filled to the level marked on the label. Too little sample results in anticoagulant excess and damage to the cells; too much will result in clotting. The vacuum pressure in the tube automatically draws the correct volume of sample from the vein when used correctly. Samples with added anticoagulant should be gently inverted or rolled three or four times immediately after

Table 75.1: Use of anticoagulants in sampling blood for clinical biochemistry; see Appendix B for reference values

Anticoagulant	Stopper colour*	Indications	Contraindications
None	Red	Most biochemical analyses	Haematology, erythrocyte enzymes, Hb coagulation studies, glucose, endocrinology
	(Royal blue)†	Trace element assay	As above
Heparin	Green	Erythrocyte enzymes (GSH-Px, SOD, transketolase), Most biochemical analyses (including endocrinology), haematocrit, Hb	Glucose, coagulation studies, haematology. Ammonium heparin for urea
	(Royal blue)†	Trace element assay	As above
EDTA	Purple	Haematology	Glucose, coagulation studies, enzyme analyses, trace element/mineral/ electrolyte assays
Oxalate/fluoride	Grey	Glucose	Enzyme analyses, urea (urease method), protein assay, electrolytes, haematology, coagulation studies, haematocrit
Citrate	Blue (silicon coated)	Coagulation studies (fibrinogen, prothrombin time)	Enzyme analyses, ESR trace element/ mineral/electrolyte assays
	Black (uncoated)	ESR	Coagulation studies and as above excluding ESR

* Based on the Vacutainer series designation.
† Uncoaguiated and heparinized tubes specially prepared for trace element analyses. Note that zinc analysis is particularly sensitive to contamination from rubber bungs.
ESR, erythrocyte sedimentation rate; (GSH-Px), glutathione perioxidase; Hb, haemoglobin; SOD superoxide dismutase.

collection to ensure adequate mixing. Do not shake. Clotted samples should be moved as little as possible.

Whenever possible, blood samples for biochemical testing should be divided to prevent haemolysis. In the case of unclotted whole blood samples, an aliquot must first be removed and transferred to a small, clearly labelled, leak-proof, plastic container. In the case of plain samples allow clot formation and retraction to occur, which can take up to 45 min at room temperature. Centrifuge the sample (1000 rpm: 500*g*) for 10 minutes and transfer the serum or plasma into a small, leak-proof, labelled, plastic container. Only these containers need be submitted, thus reducing postal charges and chance of breakage. When plain tubes with inert separator gel are used, there is no need to remove the serum after centrifugation. EDTA samples for haematology should be submitted whole. It is advisable to prepare and submit a blood smear if any delay in analysis is anticipated [5].

Because individual tests may have very specific requirements in terms of sample type, sample handling and period between collection and analysis, these should be established before samples are taken. Always contact the laboratory prior to sample collection if the time to testing is critical so that the laboratory can undertake analysis immediately on receipt of the samples. Enzymes are particularly unstable and the problems associated with their analysis have been reviewed [7].

Biochemical analysis of body fluids other than blood can be of use in specific situations. Urine, milk and cerebrospinal, synovial, thoracic or abdominal fluid can be collected from the live animal. The plain sterile containers used should be leak-proof and clearly labelled. Collection of urine into tubes with EDTA or boric acid helps to prevent bacterial growth. Of the remaining samples useful for analysis, only wool and faeces are readily available from the live animal.

Necropsy sampling

Tissue samples should always be collected if the carcass is available. Liver and kidney tissue can be used to assess a number of deficiencies and toxicoses. In general, 15–50 g of tissue per test is required. Thyroid gland can be analysed to assess iodine levels. The tissue samples collected should be sealed in clearly labelled and dated leak-proof containers (see 'Transportation of samples'). In some cases, it is possible to obtain reasonably accurate results by analysis of formalin-fixed tissue, but this should be avoided by collection of a full range of samples at necropsy. Tissue samples can be held frozen without affecting the results, so should be collected in all cases in which a gross diagnosis is not achieved in case they are required for analysis. Other samples available include aqueous humour and cerebrospinal fluid [8], urine, milk, gastric or intestinal content, and heart blood. The latter should be centrifuged and the serum retained.

Environmental sampling

Samples derived from the animal's environment can be of relevance especially where a toxicosis is suspected. Representative samples of feed, drinking water, plants, soil, paint and unidentified substances can all be analysed to look for the presence of specific toxic agents, but this must be matched by the findings in the animal. The simple presence of the toxic agent in the animal's environment does not prove its involvement in the animal's illness or death. Samples should be submitted in labelled leak-proof plastic containers with a clear warning as to their potentially toxic nature (see 'Transportation of samples').

Results and interpretation

Most routine blood biochemical and haematological tests can be performed very rapidly and are carried out on the day of receipt, and the results despatched immediately. Results should always be accompanied by reference values and where appropriate, an interpretation. The table of reference values for commonly analysed parameters given in Appendices A and B is for general information only. Attempts to standardize reporting units and reference values, e.g. through method-specific international quality assurance schemes, so far have achieved only partial success, and interpretation still often depends on using reference ranges specifically defined by individual laboratories. Advances in information technology mean that suitably equipped submitters can receive printed results via e-mail or fax on the day of analysis, greatly reducing turnaround time.

It should be emphasized that no amount of accurate, skilled analysis and modern technology can make up for incorrect sample selection and handling. In this case, preparation makes perfect!

SAMPLING FOR MICROBIOLOGY

Some sheep diseases can be diagnosed readily by experienced clinicians, but there are circumstances when the clinician needs the back-up of a specialized microbiology laboratory to reach an unequivocal diagnosis. This section gives brief guidelines on how to obtain maximum benefit from laboratory tests. It should assist the clinician faced with establishing the cause of an unfamiliar clinical syndrome or identifying the causative agents.

Selection of samples

If feasible, live animals, freshly dead carcasses or aborted fetuses plus placentae should be submitted, but, as this is often impossible, lists of samples from diseases affecting different body systems are shown in Table 75.2. The following general principles are also important:

- Liaise with the receiving laboratory; know what tests are available and follow instructions regarding samples. When investigating serious flock problems, the early involvement of laboratory staff on the farm can save time and expense.
- Laboratory tests are of value only if the best specimens are being examined. If in any doubt, select a wide range from several sheep to allow laboratory staff to choose the most suitable.
- Numbers of infectious agents are usually highest at affected sites and during the early stage of disease, especially in virus infections, which can soon be obscured by secondary bacterial contamination. Samples taken during the later stages of disease or at post-mortem from animals that have died are less likely to yield identifiable virus.

Table 75.2: Specimens to be collected for microbiological examination

Disease	Live animal	Dead animal
Abortion and/or birth of weak lambs	Blood from dam. Vaginal swabs in TM. Precolostral blood from lambs	Placenta plus spleen, kidney, thyroid, liver and brain from fetus/lamb; dry and in TM. Fetal stomach contents. Heart blood, pleural and peritoneal fluid
Acute respiratory/ocular disease	Nasal and ocular swabs in TM. Blood	Tissues from affected areas plus draining lymph nodes; dry and in TM
Chronic/slow respiratory disease	Submit live animal showing signs. Blood from asymptomatic animals	Affected lungs, dry for bacteriology and in formol saline for histology
Gastroenteritis	10–20 ml faeces. Blood	Tissues from affected areas plus mesenteric lymph nodes; dry and in TM. Heart blood
Skin diseases	Fresh moist scabs in a dry bottle. Swabs and any fluid from lesion in TM	Tissues from affected areas plus draining lymph nodes; dry and in TM
Lesions of superficial mucous membranes	Scrapings or swabs from lesions in TM. Blood	Tissues from affected areas plus draining lymph nodes; dry and in TM
Acute nervous disease	Blood. CSF	Brain in TM and in formol saline for histology
Chronic nervous disease	Submit live animal	Whole brain and spinal cord in formol saline

CSF, Cerebrospinal fluid; TM, transport medium.

Table 75.3: Equipment required for collection of specimens

Sterile forceps, scissors and scalpels
Sterile cotton wool swabs (some available complete with transport medium)
Bijou bottles containing different transport media
Leak-proof plastic bags or pots for individual tissues
Dry sterile bottle for collection of faeces and skin scrapings
Bottles containing 50 per cent glycerol saline for large portions of brain
Large bottles containing formal saline for tissues for histology
Tubes for collecting blood; heparinized and without additive
Pencil or indelible marker for labeling specimens
Request forms to record details of samples and disease
Insulated container to keep specimens cool

Selecting the right animals to test in a flock experiencing disease is time consuming. Examine animals other than those showing severe signs, since those with early signs are more likely to yield the causative agent. A representative animal could be sacrificed and samples collected immediately at post-mortem.

Collection of specimens

The equipment required is listed in Table 75.3. Micro-organisms are killed by heat, drying, light and extremes of pH, and it is essential to protect specimens between collection and testing. Various transport media (TM) have been designed for the optimum preservation of viruses, mycoplasma, chlamydia and bacteria. Select the correct one, especially for swabs collected from live animals and do not add too large a specimen to TM, e.g. no more than two swabs per 4 ml medium. It is advisable to consult the receiving laboratory regarding their requirements.

When sampling live, clinically affected animals, always collect a clotted blood sample for serology and a heparinized sample for detection of pathogens.

Acute and convalescent stage sera from several individually identified sheep are frequently of diagnostic value and are always worth collecting. At post-mortem examination, samples for microbiological testing should be collected first. Samples for virus isolation should be collected aseptically from affected tissue, particularly the edge of any lesions, keeping the ratio of specimen to TM about 1:10. For bacteriological examination and for the direct detection of other micro-organisms in cryostat sections, tissues are best submitted dry without transport medium. The tissues should be packed separately in leak-proof containers and kept cold but not frozen during transit.

Submission of samples

Label all samples clearly. They can be transported by hand, preferably on wet ice in an insulated container to minimize delay, but, if that is not practicable, samples should be sent by courier or post. Packages must comply with local postal regulations (see 'Transportation of samples' earlier) but the following is a guideline.

Pack specimens carefully in a strong insulated container (polystyrene boxes are ideal) using sufficient absorbent material to secure the containers and soak up liquid in the event of breakage. Add 'freezer gel' bags to maintain low temperature; never use loose ice.

All specimens must be accompanied by a form supplied by the receiving laboratory, completed as fully as possible and placed in a plastic bag taped to the outside of the container before it is wrapped and addressed. Parcels must be labelled '*Pathological Specimens: Fragile, With Care*' and carry the name and address of the sender and the date of despatch.

SAMPLING FOR HELMINTHOLOGY/ PROTOZOOLOGY

The provision of a specific diagnosis in ovine endoparasitic disease may require access to centres specializing in the identification of nematode, trematode, cestode and protozoal parasites. With the increasing prevalence of anthelmintic resistance in the UK, there may also be a need to consider testing for the presence of anthelmintic-resistant parasite populations. Collection of samples for the detection of helminth and protozoal infections can, for convenience, be divided into those taken from the live or the dead animal. The aim is to identify the parasites responsible for the disease and, where necessary, to obtain information on the intensity of infection and/or the susceptibility of that population to the drugs used to control them. Since helminthoses are often chronic rather than acute, the provision of an accurate grazing and treatment history is particularly helpful in the diagnosis of disease, especially where drug resistance is suspected.

Live animal

Coprological procedures

Endoparasites infecting the liver, digestive and respiratory tracts produce eggs, larvae or oocysts that are eventually voided in the faeces, and an estimate of their number and species can be a useful aid to diagnosis. The values obtained are not absolute and need to be interpreted with caution owing to the numerous host and parasite factors that can influence the faecal egg/oocyst output. These include faecal consistency, diet, immune status of the host, species and stages of the parasite present, and age of the infection. Where specialist diagnostic services are required, it is important to contact the service provider prior to sampling to ensure that the correct sampling and delivery procedures are followed.

Helminthoses

A faecal sample (5–10 g) should be taken directly from the rectum using a plastic glove or polythene bag and then transferred to a screw top pot that is clearly labelled with the animal's identity and date of collection. The container should be filled to the top to exclude most of the air. Freshly passed faeces can be used, but after contact with the pasture or bedding there is some risk of environmental contamination. Most nematode eggs embryonate quickly and hatch within 48 hours at room temperature, but this may be delayed by storing the sample in a refrigerator until examined. However, this process can affect the survival of some species [9] and produce inaccurate larval identifications following coproculture. If enumeration is to be delayed beyond 48 hours, a few drops of concentrated formalin can be added to prevent development, but will obviously negate further coproculture.

Suspected anthelmintic resistance

The faecal egg count reduction test (FECRT) can be used to detect resistance against any of the drugs that are used to control nematodes, and is the most commonly used assay *in vivo*. Full details of the recommended procedures have been published [10]. The FECRT examines the efficacy of treatment at the recommended dose rate, efficacy being calculated by comparing arithmetic mean faecal egg counts of treated and control groups, each of which should contain 15 animals. The timing of collection of the post-treatment samples varies in accordance with the anthelmintic under test. For drugs within the imidazothiazole family, such as levamisole, samples should be collected about 7 days post-treatment [11]. For the benzimidazoles and macrocyclic lactones, samples may be collected 7–14 days post-treatment. Specialist centres may also offer *in vitro* diagnostic tests such as the egg hatch assay (EHA) and micro agar larval development test (MALDT), which require the provision of fresh, viable eggs. Advice should always be sought from these centres prior to sample collection to identify specific recommended sampling and delivery procedures. Pooled faecal samples stored and transported under anaerobic conditions [12] can also be used in some of the assays *in vitro*. Suitable containers for anaerobic transportation can be obtained from the specialist diagnostic laboratory.

Protozoal detection

Samples should be taken much as for helminthoses, a 5–10 g faecal sample collected into a polythene bag or small plastic container. There is no need to exclude air from the samples, as protozoan parasites will not hatch out in the faeces, and identification is dependent on the development of the oocyst, which is often shed unsporulated (undeveloped). Should enumeration be delayed, samples may be preserved by suspending them in 2 per cent aqueous potassium dichromate, which will prevent sample decomposition, but allow the protozoa to develop.

Examination

Helminths

For a preliminary study, a small amount of faeces can be diluted with water and examined directly under a dissecting microscope for the presence of whole nematodes and segments of tapeworms.

For estimating the number of helminth eggs in faeces, several techniques are available [13]. These are based on the removal of faecal debris, concentration of eggs and differential flotation in saturated sodium chloride or sugar (SG 1.20) for nematode and cestode eggs, and zinc sulfate (SG 1.50) or sedimentation for fluke eggs. Eggs are counted in a McMaster slide.

The higher tonicities can distort some eggs and, to obtain comparable data, the same method and conditions should be adopted. Eggs are recognized by their size, shape and morphology, but those of some species cannot be easily differentiated. For a specific diagnosis in these species, faeces need to be cultured to yield third-stage larvae, which are harvested using a Baermann apparatus and identified to generic level according to their morphology. It should be noted that free-living rhabditoid larvae may sometimes be observed, but usually in small numbers. Lungworm larvae can also be detected by flotation techniques using saturated sodium chloride, or they can be extracted from fresh faeces by the Baermann procedure. When counting worm eggs or larvae, one should be aware of artefacts such as hairs, plant cells, fungal spores, pollen grains, air bubbles, mite eggs and protozoal oocysts.

Protozoa

Estimation of oocyst numbers in faeces is usually performed in the same way as helminth egg counts, by removal of faecal debris and concentration by differential floatation, using saturated sodium chloride, sugar (SG 1.20) or zinc sulfate (SG 1.50), followed by enumeration in a McMaster chamber. Speciation of coccidia requires both observation of oocyst size and examination of sporocyst shape and number. Smaller protozoa, such as *Cryptosporidium* and *Giardia*, are better enumerated in a haemocytometer – an additional washing step is also required to remove the floatation medium – or by examination of stained faecal smears. These are air-dried prior to fixation and staining, which may be a non-specific stain or, increasingly, fluorescent-tagged monoclonal antibodies, available commercially in kit form.

Haematology/biochemistry

Migration of helminths through tissues and their feeding activity can cause damage to the structure of

the parasitized organ and increase the leakage of substances into the circulatory system. Increased enzyme concentrations in the blood or the occurrence of anaemia may assist the interpretation of the coprological findings.

Anaemia

This may be caused either by the direct blood-feeding activities of the parasite (*Haemonchus contortus, Fasciola hepatica*) and/or indirectly by haemorrhage from the damaged intestines (*Bunostomum trigonocephalum*, *Gaigeria pachyscelis*, *Chabertia ovina*, *Oesophagostomum columbianum*).

Liver enzymes

Changes in the plasma concentrations of glutamate dehydrogenase (GD), sorbitol dehydrogenase (SD), aspartate aminotransferase (AST) and γ-glutamyl transpeptidase (GGT) can provide an index of liver damage, and are thus useful aids to the diagnosis of *Fasciola hepatica* infection. However, the enzymes are not specific to the liver and are present in other tissues. Plasma GD and GGT activities are more sensitive indicators of chronic fascioliasis than SD or AST. The early increase in GD activity in fascioliasis appears to be related to the migration of flukes through the liver parenchyma, and the resulting damage to hepatocytes followed by elevation of GGT activity as flukes penetrate and enter the bile ducts [14]. Stability of enzymes at room temperature varies (AST > GGT > GD) and, for this reason, GGT may be more suitable as a diagnostic aid for field infections (see also 'Sampling for biochemistry and haematology').

Abomasal enzymes/hormones

Telodosargia circumcincta and *Haemonchus contortus* damage the integrity of the abomasal mucosa. As a result the enzyme precursor pepsinogen leaks into the circulation, and elevated plasma activity can be of use as a diagnostic aid for abomasal parasitism. Additionally, increases in the gut hormone gastrin have been associated with *Telodosargia* and *Haemonchus* infections, and raised concentrations of blood gastrin have been shown to be useful in the diagnosis of ovine haemonchosis [15].

Necropsy sampling

Tissues should be examined systematically for the presence of helminths and protozoa.

Digestive tract

Helminths. The abomasum, small and large intestines are ligated, removed from the animal, opened and washed separately in physiological saline. The washings and gut contents are combined, clarified by repeated washing and sedimentation or by sieving using a 53 μm aperture sieve. Aliquots of the contents are then examined for the presence of adult and developing worms by low power microscopy. Nematodes can be preserved by placing into hot 70 per cent alcohol or 5 per cent formol saline.

To release the larval stages of nematodes from the mucosa, the alimentary tract is either digested in 1 per cent pepsin in 3 per cent HCl at 37°C for 7–8 hours or left in physiological saline for 8–12 hours at 37°C and then processed as for the gut contents. To ensure that the larval stages are retained, sieves of 38 μm aperture should be used. Where possible, cestodes should be removed with their scolices intact, as they are invaluable for identification. This may be achieved by placing the tapeworms plus the attached piece of gut into physiological saline at 37°C and carefully dissecting out the head. Preserve in 5 per cent formal saline under slight tension.

Coccidia. Examine the duodenum, mid-jejunum, ileum, caecum and colon for lesions. Make smears from the superficial and deeper mucosal layers of suspected lesions on to glass slides, examine under a coverslip using the × 40 and × 100 objectives on a compound microscope. *Eimeria ovina* may cause raised white lesions 2–5 mm in diameter in the small intestine, visible from the serosal surface. *E. ahsata*, *E. crandallis* and *E. ovinoidalis* may also be associated with enteritis. Histopathology may also be of value in establishing schizonts and gametocytes as the stages causing tissue damage.

Cryptosporidia. Prepare smears from small and large intestine and fix in methanol. Stain using a modified Ziehl–Neelsen method by staining in cold carbol fuchsin for 5 min, differentiating in 3 per cent HCl in 64 per cent ethanol until colour ceases to flood out, and counterstaining with 0.25 per cent malachite green for 30 seconds. After rinsing in tap water, examine smears using the × 100 (oil immersion)

objective on a binocular microscope. *Cryptosporidium* oocysts appear as bright pink spheres on a pale green background. The non-specific fluorescent stain phenol auramine, when used with potassium permanganate counterstaining, will produce intense yellow/green spherules on a dark ground, and is very suitable for rapid screening of large numbers of samples.

Liver

Before removing the liver, ligate the bile duct to prevent loss of flukes. Large flukes can be removed after incision of the bile ducts and young immature flukes by dissection of the liver parenchyma into small blocks. These are gently squeezed in warm physiological saline to release the flukes, which are then fixed in 10 per cent formol saline. Young *Cysticercus tenuicollis* may also be recovered from the parenchyma in cases of hepatitis cysticercosis.

Respiratory tract

Palpation of the lung and dissection and teasing of the parenchyma will reveal metastrongyloid nodules associated with the presence of *Muellerius* spp. and *Cystocaulus* spp. in the terminal bronchioles and alveoli. To detect *Dictyocaulus filaria*, open up the bronchioles to their extremities and wash the opened bronchial tree in physiological saline; place *D. filaria* worms in a large volume of formol saline to avoid tangling. *Protostrongylus* spp. are located in the smaller bronchioles and usually fragment on dissection.

Muscle, peritoneum and CNS

Sheep act as an intermediate host for the larval stages of several tapeworms (*Taeniidae*). The cysticerci of *Cysticercus tenuicollis* may be present either singly or grouped on the omentum and mesentery or any abdominal serous surface. The intermediate cystercerci of *T. ovis* are found in the muscles of sheep, particularly those of the heart and diaphragm, but also affect the skeletal muscles. The larva of *T. multiceps* is *Coenurus cerebralis* and is located in the CNS, usually the cranial cavity, and its growth causes pressure leading to locomotor disturbance.

Sarcocysts of *Sarcocystis* spp. are found in striated muscles, including the heart and tongue, and in the CNS. They may be up to 700 μm long, with walls up to 3 μm thick. They may be viewed *in situ* microscopically, or concentrated from tissues by acidified pepsin digestion of ground material, followed by centrifugation and examination of the resultant pellet.

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Part XVI
Appendices

Appendix A

Table A: Haematological reference values for healthy sheep. *Note*: The values shown are from a range of breeds and ages of animals at pasture throughout the year

Parameter	Units	Reference range	Mean	Notes*
Erythroyctes (RBC)	$\times 10^{12}$/l	6.2–15.5	11.5	†
Haemoglobin	g/dl	8.6–15.8	12.4	
MCHC	g/dl	32–51	41	‡
MCV	μm^3	19–35	27	‡
Haematocrit (PCV)	% total blood volume	22–39	31	
Leucocytes (WBC)				
Total	$\times 10^9$/l	1.1–17.5	9.2	
Lymphocytes	% total WBC	41–83	67	
Neutrophils (PMN)	% total WBC	11–47	24	
Eosinophils	% total WBC	0–15	4	§
Basophils	% total WBC	0–3	1	
Monocytes	% total WBC	0–13	2	
Platelets	$\times 10^9$/l	0.18–0.75	–	¶

MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; PCV, packed cell volume; PMN, polymorphonuclear cells; RBC, red blood cells; WBC, white blood cells.

* This table is adapted from original data of Holman [1], except for platelet counts.

† Ranges may vary between males and females according to reference [2].

‡ *The Merck Veterinary Manual*, 7th edition [3], quotes slightly different ranges for MCHC (30–38 g/dl), MCV (25–50 μm^3) and PCV (25–30 per cent total blood volume).

§ Values above 5 per cent are unusual unless substantial parasite burdens are present.

¶ Extrapolated from references [4] and [2].

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Appendix B

CLINICAL CHEMISTRY REFERENCE VALUES

Clinical chemistry undoubtedly plays a pivotal role in supporting and confirming clinical diagnosis. However, the interpretation of abnormal values depends on numerous factors, not least the integrity of the reference values established for the parameter(s) under test. Despite numerous attempts to harmonize interpretation worldwide, most notably through the introduction of the Systeme International (SI) unit method of reporting, most veterinary laboratories have established individual reference values based on their particular practice and procedures. There can be substantial differences in reported values due to the age and reproductive status (see reference [1]), sex and breed of animals, as well as sampling techniques and/or assay methodology. Table B attempts to collate the mass of currently available data, and 25 years experience in the laboratory, into an informative and practical overview of clinical chemistry reference values for healthy adult sheep. The figures given in the table are not exhaustive, and should be used for guidance purposes only. References [2] and [3] provide more detailed discussion on diagnostic interpretation.

Table B: Clinical chemistry reference values for healthy adult sheep

Enzymes	Activity (30°C)	Reference range[2]	Conversion factors	
			30–25°C	30–37°C
AP	IU/l[1]	>250	×0.82	×1.33
ALT (GPT)	IU/l	0–38	×0.76	×1.39
AST (GOT)	IU/l	32–97	×0.73	×1.54
CK (CPK)	IU/l	0–200	×0.64	×1.59
GGT	IU/l	0–32	×0.75	×1.37
GLDH	IU/l	2–10	×0.77	×1.30
GSH-Px	IU/ml packed cells[3]	>20[4]	×0.82	×1.39
LDH	IU/l	0–450	×0.75	×1.43
Pepsinogen	IU/l	0.2–0.4	–	–

Non-enzymes	SI units[5]	Reference range	Old units	Conversion factor: old to SI units
Bilirubin	μmol/l	0–2	mg/dl	×17.1
Calcium	mmol/l	2.1–2.8	mg/dl	×0.25
Chloride	mmol/l	98–109	mEq/l	×1.0
Cholesterol	mmol/l	1.0–2.6	mg/dl	×0.026
Copper	μmol/l	9.4–23.6	μg/dl	×0.157

(*Continued*)

Table B: (Continued)

Non-enzymes	SI units[5]	Reference range	Old units	Conversion factor: old to SI units
Creatinine	μmol/l	44–150	mg/dl	×88.5
Glucose	mmol/l	2.0–3.0	mg/dl	×0.056
β-Hydroxybutyrate	mmol/l	<1.20	mg/dl	×0.096
Iron	μmol/l	18–48	μg/dl	×0.179
Lactate	mmol/l	1.0–3.0	mg/dl	×0.111
Magnesium	mmol/l	0.7–1.2	mg/dl	×0.411
Phosphorus (Inorganic)	mmol/l	0.9–2.6	mg/dl	×0.323
Potassium	mmol/l	3.9–5.4	mEq/l	×1.0
Proteins				
Total	g/l	60–79	g/dl	×10.0
Albumin	g/l	28–34	g/dl	×10.0
Globulin	g/l	32–43	g/dl	×10.0
Selenium	μmol/l	1.0–6.3	μg/dl	×0.127
Sodium	mmol/l	142–160	mEq/l	×1.0
Triglycerides	mmol/l	0.2–1.0	mg/dl	×0.113
Urea	mmol/l	2.9–7.1	mg/dl	×0.166
Vitamin A	μmol/l	0.7–1.7	μg/ml	×3.49
Vitamin B_{12}	pmol/l	>370	pg/ml	×0.738
Vitamin E	μmol/l	1–6	μg/ml	×2.31
Zinc	μmol/l	12–19	μg/dl	×0.153

Key to table
ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate transaminase; CK, creatine kinase; (CPK, creatine phosphokinase); (GPT, glutamyl pyruvic acid transaminase); (GOT, glutamyl oxaloacetic acid transaminase); GGT, gamma-glutamyl transferase; GLDH, glutamate dehydrogenase; GSH-Px, glutathione peroxidase; LDH, lactate dehydrogenase.

[1] IU (International Unit) is the globally agreed standard measure of enzyme activity. One IU is defined as the amount of enzyme required to convert 1 μmol of substrate per minute under defined reaction conditions. Note: Even using standard reagents (e.g. commercial kits), enzyme activities generally vary markedly depending on assay temperature. Temperature conversion factors are therefore included in the table.
[2] Except where stated, values refer to fresh, non-haemolysed, non-lipolytic serum or heparinized plasma.
[3] GSH-Px is reported by some laboratories as IU/g haemoglobin.
[4] Value refers to heparinised whole blood.
[5] SI (Système International) units are internationally agreed metric reporting units for clinical chemistry: mmol = millimoles (10^{-3} moles); μmol = micromoles (10^{-6} moles); pmol = picomoles (10^{-12} moles), where 1 mole = molecular atomic weight of the analyte in grams (g). Most non-enymes were formerly reported in other units (Note: dl = 100 ml; mEq = milliequivalent) and conversion factors from these to SI units are included in the table. Note also that laboratories in the USA use a different version (mass per unit volume, rather than moles per unit volume) of the metric system, and different conversion factors may then apply for some analytes.

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