

Temporal relationship between infestation with lice (*Bovicola ovis* Schrank) and the development of pruritic behaviour and fleece derangement in sheep

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Received 30 April 2007; received in revised form 25 July 2007; accepted 1 August 2007

Abstract

Pruritic behaviour and deranged fleece are often used as indicators of sheep louse infestation but the exact relationship between infestation and the observation of signs of pruritis was unclear. Two studies were conducted to examine this association. In the first, 24 castrate Merino sheep were randomly assigned to six pens in groups of four and the sheep in three pens infested with 10 lice each on the right mid-side. Louse numbers were counted, fleece derangement scored and pruritic behaviour assessed periodically on each sheep until 38 weeks after infestation. In the second study a single moderately infested sheep was paddocked for 15 weeks with 32 uninfested sheep and louse numbers and fleece derangement monitored for 41 weeks.

In the pen studies, differences between infested and non-infested sheep in fleece derangement and pruritic behaviour first became significant ($p < 0.05$) at 8 and 14 weeks, respectively and at louse densities of 0.06 and 0.27 per 10 cm wool part. Some sheep showed definite signs of deranged fleece as early as 5 weeks after initial infestation. In the paddock studies, it took 37 weeks until lice were detected on all sheep in the flock. The correlation between louse numbers and fleece derangement score first became significant ($r = 0.44$ and $p < 0.05$) at 9 weeks after introduction of the lousy sheep, reached a maximum of $r = 0.79$ ($p < 0.001$) at 22 weeks when 84% of sheep had lice detected and the mean louse density was 0.29 per part, and then declined to $r = 0.12$ (n.s.) at 41 weeks when all sheep were infested and the mean louse density was 3.04 per part.

It is concluded that fleece derangement is a powerful early indicator of the presence of lice and that sheep may exhibit signs of pruritis well before lice can be readily found by direct inspection. Fleece derangement may be useful as a basis for establishing economic thresholds for the application of long wool treatments in developing louse infestations but appears to be a poor indicator of louse numbers once the infestation is advanced.

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Keywords: Phthiraptera; Detection; Pruritis; Population dynamics; *Ovis aries*

1. Introduction

The traditional approach to sheep lice control in Australia is annual treatment of all sheep with a chemical lousicide after shearing regardless of whether or not they are known to be infested. Concerns about residues, occupation health and safety issues with some chemicals (Murray et al., 1992; Savage and Russell,

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1999) and continuing economic pressure for cost and labour efficiencies have stimulated a movement away from routine annual treatment in favour of treatment only when lice are found (James and Riley, 2003; Walkden-Brown et al., 2006). Under this approach, the prevention of new infestations and prompt and effective treatment when they occur, assumes increased importance. Both of these elements of good louse control programs require the efficient detection of lice.

Currently detection is achieved almost exclusively by direct inspection of sheep. Inspection of sheep is labour intensive and has low sensitivity, particularly early in an infestation when only low numbers of lice are present (James et al., 2002b). For this reason producers often rely on the observation of pruritic behaviour or resultant deranged (rubbed or chewed) fleece as indicators of infestation (Pearse and Baldock, 1994). However, there have been few structured observations of the relationship between pruritis and lice infestation, particularly in the early stages of an infestation, and some preliminary observations suggest that the relationship may not be strong (Sinclair et al., 1989).

Use of fleece derangement as an indicator of infestation also has potential application in two other areas of lice control. Development of economic thresholds for the application of long wool lousicide treatments requires a rapid means of assessing the extent of an infestation, but direct census of lice numbers is impractical. The use of fleece derangement offers a possible alternative. In addition, it is often recommended that newly purchased sheep be quarantined on introduction to a property until there is a high degree of certainty that they are free of lice. However, the period of quarantine required to achieve reasonable certainty of louse freedom is unclear. Knowledge of the time for infested sheep to begin to exhibit signs of lice would assist the development of recommendations for quarantine period.

This study was carried out to determine how soon after the initiation of an infestation sheep begin to exhibit pruritic behaviour and fleece derangement, at what level of lice these signs become apparent, and to provide further information on the value of fleece derangement as an indicator of level of infestation.

2. Materials and methods

2.1. Pen studies

2.1.1. Sheep

Twenty-four castrate Merino sheep with 4 months wool and no known previous exposure to lice were assigned randomly to six groups of four. Groups were

penned separately in 3 m by 4 m pens on each side of a central raceway. Sheep in three groups were infested on the right side with 10 lice (five males and five females) while sheep in the other groups remained without lice. Pens were enclosed by 1.2 m high iron panels and an empty pen left between the experimental groups to avoid contact between sheep in different pens. The sheep were held in their pens for a week prior to the commencement of the experiment during which time handlers frequently walked up and down the race so that the animals became accustomed to human presence.

2.1.2. Fleece derangement score and pruritic behaviour

All animals were assessed for fleece derangement and pruritic behaviour at weekly intervals up to week 17, then at fortnightly periods to week 32 with a final observation at 38 weeks. Scores for fleece derangement were assigned for both the left and right side of each sheep. The scores used were: 0, no rub; 1, suspect, but not sure; 2, light but obvious rub, fluffy tip or definite pulled fibres at some sites; 3, distinct but dispersed pulled strands (thicker than fluffy); 4, definite patches or areas of pulled strands, less than 20% of the fleece affected, no bare areas; 5, definite patches or areas of pulled strands, greater than 20% of the fleece affected; 6, grossly matted fleece, often with some areas rubbed bare. Assessors were initially unaware of the treatment to which each group was assigned, but the practicalities of sheep handling and the need to prevent spread of lice to uninfested sheep made it difficult to maintain this blinding.

Pruritic behaviour was assessed in four 10-min observation periods for each group on each observation day. Observations were made from the central raceway taking care to avoid behaviours that disturbed the sheep. When sheep were disturbed by external factors observations ceased and recommenced only once the sheep were again quiet. A matrix was used to record pruritis with each discrete pruritic event recorded as rubbing, biting or scratching (with the feet). The body region to which each behaviour was directed was recorded as neck, front leg or shoulder, side/flank, back leg or rump and left or right side.

2.1.3. Louse counts

The number of adult and nymphal lice was counted in twenty-four 10 cm wool parts disposed in three lines along both sides of each sheep (James et al., 2002a) at 6, 12, 18, 24 and 38 weeks after infestation. To estimate number of lice present at times between inspections a logistic function was fitted to the data.

2.2. Paddock study

A single infested sheep with louse density of 4.1 per part was introduced to a flock of 32 uninfested Merino ewes that had been shorn 10 weeks previously. The infested sheep was run with the ewes in a 20 ha paddock for 15 weeks at which time it was removed and the infestation allowed to develop in the remaining ewes. At 6, 9, 15, 22, 28, 37 and 41 weeks after introduction of the lousy sheep the ewes were mustered, louse numbers counted and fleece derangement assessed according to the methods described above. The donor sheep was removed from the flock before the sheep were yarded for inspection and penned separately until return of the sheep to the paddock.

2.3. Analysis

Difference between infested and non-infested sheep in derangement score, pruritic behaviour and louse numbers was analysed using a repeated measures model in Genstat 8[®] with sheep blocked for pen. In both studies logistic models were fitted to the increase in louse numbers over time and, for the pen study, this curve was used to estimate louse density at times between inspections. For the analysis of the effect of side of the sheep on louse density, fleece derangement and pruritic behaviour a repeated measures model was also used. Louse numbers and number of pruritic events were log transformed to stabilise variance, side was treated as a random variable and sheep within pens were treated as split plots. Louse numbers were regressed against fleece derangement score to assess the relationship between the two parameters at different times in the paddock studies.

3. Results

3.1. Pen study

3.1.1. Lice numbers

Fig. 1 shows the build up in louse numbers to a maximum mean level of 9.2 per part over the period of the study. The logistic model fitted to the data was of the form $A + C/(1 + \exp(-B(x - M)))$, where x = number of days from initial infestation, $A = 0.0315$, $B = 0.05431$, $C = 9.2019$ and $M = 164.819$.

3.1.2. Pruritic behaviour

Differences between groups in the observed frequency of pruritic behaviour (Fig. 2) did not become significant until day 98 ($p < 0.05$) but were significant

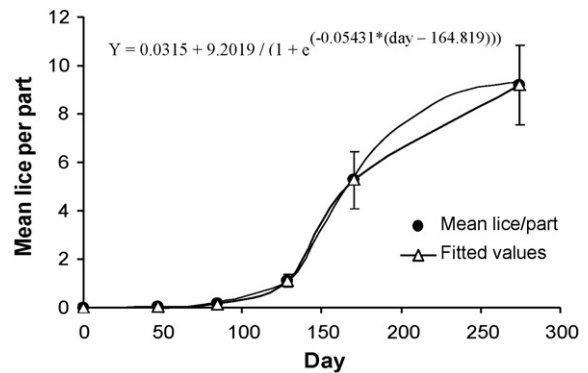


Fig. 1. Increase in mean louse counts per part (\pm S.E.) over the period of the pen study and fitted equation used to estimate louse densities at dates between inspections.

at all observations after that time. The mean louse count for all sheep at day 98, estimated from the fitted logistic equation, was 0.27 per part. The maximum number of pruritic events observed in any sheep over the four 10 min observation periods was 28 on day 197. This animal had a mean count of two lice per part on day 170, the closest inspection date.

A detailed breakdown of the numbers and proportion of pruritic behaviours directed to different body regions in infested and non-infested sheep is given in Fig. 3. The number of pruritic behaviours directed to most body sites was markedly higher in infested sheep than in those without lice. The one exception was in 'other' areas for which the numbers of pruritic events were similar. When the proportion of behaviours in different sites was considered, more rubbing was directed to the neck, front leg and sides and more biting directed to the sides in infested than uninfested sheep. However, the proportion of scratching and biting directed to other areas was higher in uninfested sheep. 'Other' areas included the belly and head where lice are generally absent or present in low numbers (James and Moon,

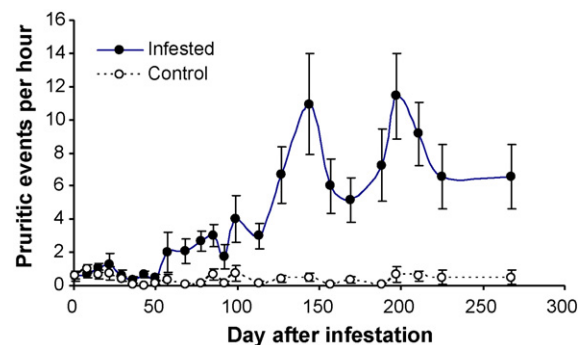


Fig. 2. Pruritic behaviour (mean pruritic events per hour \pm S.E.) in *Bovicola ovis* infested and non-infested sheep in the pen study.

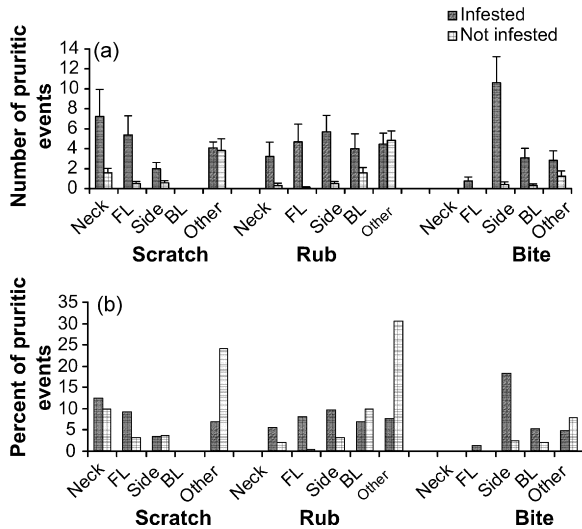


Fig. 3. Number (a) and percentage of total (b) pruritic behaviours (biting at the fleece, rubbing and scratching with the back legs) directed to different body regions in louse infested and non-infested sheep (FL, front leg and shoulder; BL, back leg and rump).

1999). The high proportion of total pruritic behaviours directed to these sites in sheep without lice was primarily a reflection of the relatively low overall number of pruritic behaviours in the uninfested sheep.

3.1.3. Fleece derangement score

Difference in fleece derangement score between the uninfested and infested groups (Fig. 4) first became significant at day 22 after infestation ($p < 0.05$). This was the fourth inspection after infestation and as it was not followed by similar differences at the four subsequent inspections, may have been a sampling aberration. The difference again became significant at day 57 ($p < 0.05$) and remained significant after this time. The mean louse density estimated from the fitted logistic equation at day 57 was 0.057 lice per part.

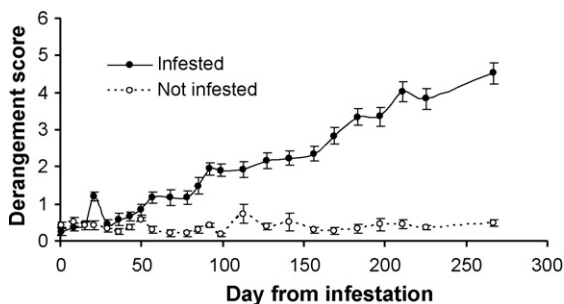


Fig. 4. Fleece derangement score (mean ± S.E.) in *B. ovis* infested and non-infested sheep in the pen study.

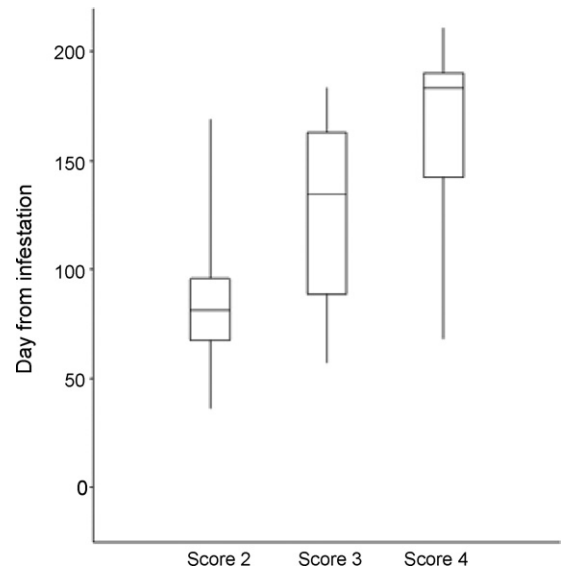


Fig. 5. Box and whisker plots for the distribution of times from initial infestation of sheep in the pen study until observation of the first definite score 2–4 for fleece derangement. Score 2 was only designated definite when followed by another score of 2 or greater at the next inspection.

The first sheep to develop a score of 2 followed by an equivalent score or higher at the next inspection on the right hand side (the side on which the sheep were initially infested) did so at day 36 after infestation (range across sheep of 36–169 days) (Fig. 5). The first sheep to reach score 3 did so at day 57 (range of 57–183 days) and score 4 at day 68 (range of 68–211 days) (Fig. 5). The same two sheep were earliest to reach score 2–4.

3.1.4. Effect of side

There was a significant effect of side of the sheep on louse numbers ($p < 0.002$) with numbers of lice on the right side of the sheep significantly greater than on the left from the first louse census at day 47 until the penultimate inspection at day 170 ($p < 0.05$) (Fig. 6). However, there was no difference between sides at the last count at 38 weeks. Difference between sides in louse count was reflected in differences in number of pruritic events and difference in fleece derangement score ($p < 0.001$) in both cases (Fig. 6) but the interaction between side and time was not significant in either case ($p > 0.05$).

3.2. Paddock study

The pattern of increase in percent sheep in the flock detected with lice and mean lice per part, with the fitted

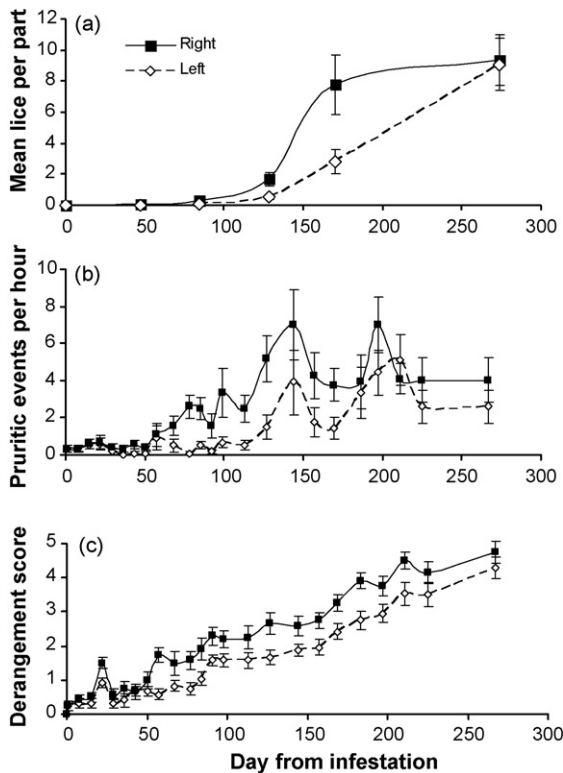


Fig. 6. Increase in (a) numbers of lice per 10 cm part, (b) number of pruritic events and (c) fleece derangement score (means \pm S.E.) on the right and left sides of sheep following initial infestation with 10 lice at the right mid-side position in the pen study.

models, are shown in Fig. 7. By 28 weeks after introduction of the infested sheep, lice were found on 97% of the ewes and the mean density of lice was 0.97 per part. At the end of the study at week 41 all sheep were infested and mean louse density had increased to 3.04 per part. Fleece derangement score also increased as the infestation developed and showed maximum correlation with louse density at the 22 week inspection at which time 84% of sheep were infested and the mean louse density on those sheep with lice was 0.29 lice per

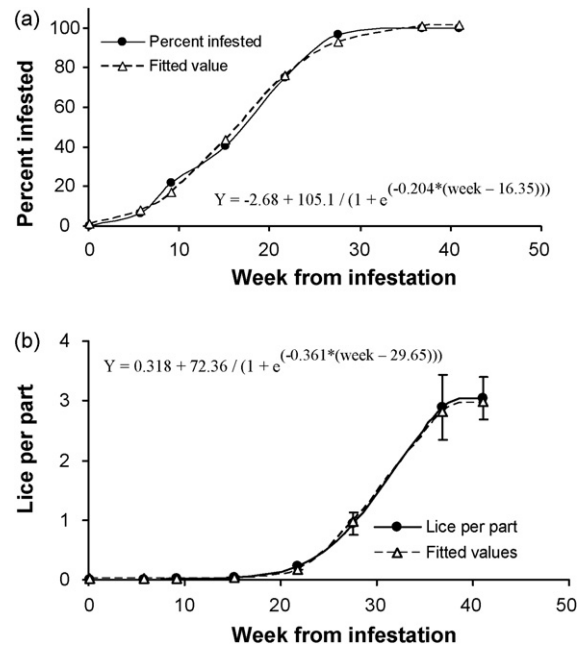


Fig. 7. Data points and fitted models for (a) increase in percent sheep infested and (b) mean louse counts per part (\pm S.E.) following the introduction of an infested donor animal for 15 weeks in the flock study.

part (Table 1). Once all sheep in the flock were infested the level of correlation between louse numbers and derangement scores declined and was not significantly different from zero ($p > 0.05$) at the final inspection at 41 weeks.

4. Discussion

Detection of low numbers of lice in sheep, as in the early stages of an infestation, is extremely difficult. From a consideration of the sensitivity of detecting lice on individual sheep and in flocks under different scenarios of infestation, James et al. (2002a,b) concluded that direct inspection of a sheep soon after

Table 1

Correlation between louse density and fleece derangement score during development of an infestation of *Bovicola ovis* in a flock of Merino ewes

Week after introduction of infestor sheep	Percent sheep with lice	Mean lice per part on infested sheep (S.E.)	Mean fleece derangement score (S.E.)	Correlation between derangement score and louse count (r)
6	6	0.05 (0.0)	0.09 (0.04)	0.26
9	25	0.06 (0.01)	0.34 (0.11)	0.44*
15	53	0.09 (0.03)	0.8 (0.15)	0.40*
22	84	0.29 (0.05)	1.75 (0.25)	0.79***
28	97	0.97 (0.20)	2.56 (0.22)	0.63**
37	100	2.90 (0.54)	3.42 (0.22)	0.49*
41	100	3.04 (0.35)	3.78 (0.17)	0.12

* r significantly different from 0, $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

contact with other infested animals was unlikely to determine with any degree of certainty whether or not the sheep had lice. In a flock situation this is compounded because early in an infestation only a few sheep in the mob will have lice and detection depends on first selecting an infested sheep and then finding lice on that animal. James et al. (2002a,b) suggested that it was likely that preliminary selection on the basis of deranged fleece or pruritic behaviour would increase sensitivity but were unable to provide an estimate of the degree of improvement because of absence of information on the level of infestation at which sheep began to exhibit pruritic behaviour.

In natural flock infestations determination of the exact relationship between infestation and the appearance of clinical signs of lice is difficult because the time at which each sheep in the flock first becomes infested is not accurately known. In our pen study both the time of infestation and level of initial inoculum were accurately known. The difference between infested and non-infested sheep in fleece derangement score became significant 57 days after initial infestation when the mean louse count was only 0.057 per part. Some sheep within the infested group showed clear signs of derangement as early as 36 days post-infestation.

These results confirm that deranged fleece is a very powerful early indicator of infestation and becomes apparent at low louse densities. James et al. (2002a,b) estimated that in a flock with 10% of sheep infested and a louse density of 0.05 per part in the infested sheep, choosing 10 sheep at random and inspecting 10 parts on each of these sheep would only give a probability of detection of approximately 0.4. However, using their estimate of the probability of detection of 0.39 on an individual sheep with 0.05 lice per part and lice distributed among parts according to a Poisson distribution, if 10 sheep were selected on the basis of wool rub each with this level of infestation, the probability of detection in the flock would be $1 - ((1 - 0.39)^{10}) = 0.99$. Thus, at low levels of infestation selection on the basis of wool rub markedly increases the probability of detection. However, many factors can cause sheep to develop deranged fleece (Johnson et al., 1993) and a definitive diagnosis of lice still requires inspection of sheep and the detection of insects.

Although clear differences in the frequency of pruritic behaviour were observed between infested and non-infested sheep, the difference between the two groups in the pen trial did not become significant until 98 days after infestation, almost twice the period for fleece derangement. In similar studies with American breeds of sheep the correlation between louse numbers

and pruritic behaviour also became significant at 14 weeks after infestation at which time mean louse density was 0.05 per part, although the association approached significance ($p = 0.056$) at the prior inspection at 7 weeks (James and Moon, 1998). As fleece derangement presumably results from pruritic behaviour, the longer time for the difference in number of pruritic events to become significant was most likely due to a sampling effect. We only monitored sheep behaviour for a total of 40 min on each sampling date. With the low number of pruritic events generally observed during these observations, particularly early in the infestation, it is not surprising that there was a higher degree of variability amongst observation periods than for fleece derangement. Although the observation of pruritic behaviour can be a good sign of the presence of lice, fleece derangement is likely to be a more reliable and more easily assessed indicator.

The largest proportion of pruritic behaviour was directed to the sides of the sheep, which is where the highest density of lice is generally found (James and Moon, 1999) and where the sheep were initially infested. The most marked difference in the pattern of behaviours between infested and non-infested sheep was the proportion of biting at the sides. This was the most common behaviour in the sheep with lice but seen only infrequently in uninfested sheep. Although clearly associated with louse infestation, the degree to which this behaviour is characteristic of lice in comparison to other causes of pruritis is unclear.

Lice were applied only to the right hand side of each sheep in this study and this provided a further opportunity to examine the association between lice and pruritis. That the significant difference between sides in louse density was maintained until the last count despite the likelihood of substantial transfer between animals suggests that, in contrast to anecdote that became common with the advent of backline lousicide treatments, lice are not highly mobile over a sheep's body. Difference between sides in number of pruritic events and fleece derangement score reflected differences in louse numbers, with clearer differences between fleece derangement score than pruritic behaviour, particularly in the later stages of the study.

A further aim of this study was to assess the potential value of fleece derangement as a basis for the development of economic thresholds for the application of long wool lousicides. In the paddock study the correlation between louse counts and derangement became significant at 9 weeks after introduction of the challenge sheep, increased to a maximum of $r = 0.79$ at week 22 at which time 84% of sheep had lice and the

mean count was 0.97 per part and then declined to be not significantly different from zero at the end of the study at 41 weeks by which time all sheep in the mob were infested. These results suggest that fleece derangement is a good indicator of the level of lice in a developing infestation but not where sheep have been infested for an extended period. This relationship may account for the unpublished observations noted by Sinclair et al. (1989) suggesting a lack of direct relationship between louse numbers and intensity or duration of pruritic behaviour in infested flocks. A similar effect may also help to explain the inconsistent results reported regarding the accuracy of scratching index for the diagnosis of *Sarcoptes scabiei* in swine (Loewenstein et al., 2006). Once sheep have been infested for some time it is likely that individual sheep sensitivity and immune responsiveness to the presence of lice may be a more important determinant of pruritic response than numbers of lice *per se* (James, 1999).

The models fitted to build up of lice in Merino sheep in these two studies may be of use in further population dynamics studies and in assessing the need for the application of mid season treatments for lice. However, many factors can influence susceptibility of sheep to lice and the functions will likely be different for different breeds and strains of sheep and in different environments (James et al., 1998, 2002a).

These studies confirm that fleece derangement is a powerful early indicator of the presence of lice infestation and that it may be useful in developing infestations as a basis for establishing economic thresholds for long wool treatments. Experiments are currently underway to provide more information to this end.

Acknowledgements

Financial support from the Western Australian Sheep Lice Research Fund and the willing assistance of staff at Turretfield Research Centre, particularly the manager Mr. Darryl Smith, is gratefully acknowledged.

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