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SHORT COMMUNICATION

Bacteriological study of the feathers and lice of captive common buzzards (*Buteo buteo*)

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SUMMARY

We have investigated the external and internal bacterial flora associated with several species of lice parasites of captive common buzzards (*Buteo buteo*) and studied the bacterial flora on the host feathers. The bacterial flora inside the ectoparasites was significantly different from that of their surface. Bacteria of genus *Staphylococcus*, especially *S. aureus*, predominated, being more abundant on lice surface. *Bacillus* was another Gram-positive bacteria frequently isolated, although it was more abundant as a component of the internal flora. A very low number of enterobacteria and strict anaerobes was obtained. Bacteria isolated from bird feathers reveal some contamination from humans.

Many species of bacteria can be involved in raptor disease. Nevertheless, data on the 'normal' bacterial flora of these birds are scarce. Many organisms can be isolated from the feet, pharynx and cloaca of apparently healthy birds and it is possible that some of these may, under certain conditions, be pathogenic. The beak and talons are particularly susceptible to traumatic damage, but may also become infected. Secondary bacterial infections of the cere and digits may follow injury, and a variety of organisms have been isolated, including *Staphylococcus aureus*, *Escherichia coli* and *Proteus* spp. Inflammatory conditions of the foot are one of the most important clinical problems in captive raptors, *S. aureus* being the predominant organism in bumblefoot cases. Enteric bacteria such as *E. coli* or *Proteus* spp. may also be isolated (Cooper, 1978).

Data available on bacterial flora associated with lice often concern species parasitizing animals of economic importance (Derylo, 1969, 1970; Derylo & Jarosz, 1972; Lonc & Zlotorzyczna, 1984). Nevertheless, lice have proved to serve as vectors in bacteria and bacterial disease transmission.

In this paper we report a study of the bacterial flora associated with some lice

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species which are usually found in the common buzzard (*Buteo buteo*). Results from both internal and external isolates are compared, as well as data obtained for ischnoceran and amblyceran species, which show different feeding habits.

We have analysed both the external and internal bacterial flora from 34 lice specimens belonging to species frequently found on *Buteo buteo*: two ischnoceran (*Degeeriella fulva*, 10 specimens, and *Craspedorrhynchus platystomus*, 9) and two amblyceran (*Laemobothrion maximum*, 7, and *Colpocephalum meridionale*, 8). Samples were obtained from five birds and the bacterial flora present on feathers of different host body regions (nape, breast, axilla, remiges, dorsal coverts and cloaca) of these five, and three further buzzards was also studied. All birds were individually maintained in protected and clean rooms. Buzzards came from the area surrounding the Doñana National Park, Spain. Six of them were immature birds and two adults. Handling of birds was reduced to a minimum and latex gloves were always used. Time between capture of birds in nature and sampling ranged from 6 to 24 h.

Feathers containing lice were removed from raptors using sterile pincers and scissors, and placed in sterile tubes. Lice were removed from the feathers into a laminar-flow cabinet, and were identified and grouped according to Pérez *et al.* (1988).

Each group of lice (containing specimens belonging to a single species) was introduced into a sterile tube to which 2 ml of sterile saline solution (0.85% NaCl in distilled water) was added. Tubes were vigorously shaken for 5 min and the saline solution, containing the external micro-organisms, removed. Bacteria on feathers were obtained in the same way.

After this process, specimens were washed again three or four times in 10 ml sterile 0.85% NaCl, followed by vigorous shaking to remove the remaining external bacteria. Complete elimination of bacterial cells from the surface was achieved by washing each louse in 70% ethanol and 1:4 dilute bleach, and again, three or four times with saline solution as above. Finally, 2 ml sterile 0.85% NaCl and the samples were introduced into a glass homogenizer in which the lice were homogenized. The saline extracts (0.2 ml) containing bacterial flora from feathers or lice in suspension were used to inoculate two sets of the following media (Difco): trypticase soy agar, blood agar, chocolate agar, McConkey agar and mannitol salt agar. One set of plates was incubated under aerobic conditions at 37°C for 2 to 4 days, while the other was maintained at this temperature in an atmosphere of 5% CO₂ + 65% H₂ for the same period.

Standard methods were used to isolate and identify the bacteria (Gerhardt *et al.*, 1981; Starr *et al.*, 1981; Balows *et al.*, 1991). In the case of *Staphylococcus aureus*, the identification was completed by coagulase test.

Data obtained were grouped into bacterial genera and then compared by means of χ^2 test (ANOVA) according to the following criteria: external flora vs. internal flora and ischnoceran flora *v.* amblyceran flora. The estimator used was Snedecor's *F*.

Over 1500 cultures were isolated and approximately 95% of them identified (Table 1). Gram-positive bacteria predominated, mainly *Staphylococcus* and *Bacil-*

Table 1. Bacteria isolated from different samples

	Material examined								
	1 ^a	2 ^b		3 ^c		4 ^d		5 ^e	
		E ^f	I ^g	E	I	E	I	E	I
Number of isolates	899	287	50	22	26	74	41	10	24
Gram-positive bacteria									
Gen. <i>Staphylococcus</i>									
<i>S. aureus</i>	184	200	4	8	6	0	0	0	0
<i>S. capitis</i>	0	60	0	0	0	1	0	0	0
<i>S. hominis</i>	402	1	2	1	0	0	0	0	0
<i>S. epidermidis</i>	137	0	0	0	3	0	1	2	0
<i>S. warneri</i>	0	0	0	0	0	2	2	0	0
<i>S. haemolyticus</i>	0	0	0	0	0	0	3	0	0
<i>S. saprophyticus</i>	0	0	0	0	0	8	0	0	0
Gen. <i>Micrococcus</i>									
<i>M. luteus</i>	0	0	0	0	0	4	0	0	0
<i>M. sedentarius</i>	0	0	0	0	3	1	1	3	0
<i>M. nishinomiyaensis</i>	0	0	0	5	3	0	0	0	0
<i>M. kristinae</i>	0	0	0	3	0	0	0	0	0
<i>M. varians</i>	0	0	0	0	0	0	4	0	7
<i>M. halobius</i>	0	0	0	0	0	0	0	0	5
Gen. <i>Streptococcus</i>									
<i>S. avium</i>	0	0	0	0	0	28	8	0	0
<i>S. equinus</i>	0	0	0	0	0	1	0	0	0
Gen. <i>Corynebacterium</i>									
<i>C. pseudodiphtheriticum</i>	0	0	0	1	3	0	0	0	0
<i>C. renale</i>	0	0	0	0	0	0	0	0	2
Gen. <i>Bacillus</i>									
<i>B. coagulans</i>	87	18	1	1	8	19	2	5	7
<i>B. megaterium</i>	0	2	3	0	0	2	1	0	0
<i>B. cereus</i>	0	0	40	0	0	0	0	0	3
<i>B. lentus</i>	0	0	0	0	0	2	0	0	0
<i>B. alcalophilus</i>	0	0	0	0	0	0	14	0	0
Gram-negative bacteria									
Gen. <i>Pasteurella</i>									
<i>P. gallinarum</i>	0	6	0	0	0	1	2	0	0
<i>P. ureae</i>	0	0	0	0	0	4	1	0	0
<i>P. anatispestifer</i>	0	0	0	0	0	1	1	0	0
Gen. <i>Francisella</i>									
<i>F. novicida</i>	0	0	0	3	0	0	0	0	0
Gen. <i>Eikenella</i>									
<i>E. corrodens</i>	0	0	0	0	0	0	1	0	0
Gen. <i>Pseudomonas</i>									
<i>P. aeruginosa</i>	65	0	0	0	0	0	0	0	0
Gen. <i>Bacteroides</i>									
<i>B. melaninogenicus</i>	24	0	0	0	0	0	0	0	0

^aFeathers of *Buteo buteo*; ^b*Degeeriella fulva*; ^c*Craspedorrhynchus platystomus*; ^d*Laemobothrion maximum*; ^e*Colpocephalum meridionale*; ^fexternal bacteria and I, ^ginternal bacteria.

lus species. These comprised 90% of the bacteria found on host feathers, and a high percentage of the flora isolated from lice. *Staphylococcus*, specially *S. aureus*, is most common on the surface of ectoparasites. On the other hand, *Bacillus*, mainly *B. coagulans*, reaches a higher prevalence in the internal flora of these arthropods.

When the prevalence of different bacterial genera was compared between ischnoceran and amblyceran lice, no significant differences were observed ($\chi^2 = 9.542$; $P > 0.01$). On the other hand, the external flora of lice was significantly different from the internal one ($\chi^2 = 12.333$; $P < 0.01$).

Gram-positive bacteria, mainly cocci, seem to be common on the surface of lice (Lonc & Zlotorzycza, 1984; Murray & Edwards, 1987; Pérez *et al.*, 1987). Variability of this species of bacteria is higher among amblyceran species, from which certain *Micrococcus* and *Streptococcus* species were also isolated. *Streptococcus* species are commonly isolated as components of the flora associated with amblyceran lice (Derylo & Jarosz, 1972; Lonc & Zlotorzycza, 1984). Certain *Staphylococcus* species found on host feathers and lice, such as *S. hominis*, *S. capitis* or *S. warneri* (common bacteria on human skin) suggest possible contamination from humans during raptor handling.

Over 50% of the total isolates identified from lice homogenates were *Bacillus* spp. A large number of *Bacillus* have been isolated from the digestive tract of *Bovicola ovis* (Murray & Edwards, 1987). These authors considered this bacterium as a complementary food supply for lice. Data obtained may also reflect the resistance of *Bacillus* endospores to changes of pH which occur in the digestive tract of lice.

Among Gram-negative bacteria found in this study, we consider *Pasteurella* spp. one of the most important since species of this genus are usually involved in avian infectious processes (Heddleston, 1975). A special role in the transmission of diseases like avian pasteurellosis is played by haematophagous ectoparasites, which could act as passive vectors for these bacteria (Derylo, 1969, 1970). In this respect, consumption of host blood by *Laemobothrion maximum* has been previously reported (Pérez *et al.*, 1989).

The only strict anaerobe isolated from host feathers was *Bacteroides melaninogenicus*, common in human and other animal cavities, including insect cavities (Krieg & Holt, 1985). There was also a low prevalence of enterobacteria. In this respect, our results agree with those of Lonc & Zlotorzycza (1984), who postulated the existence of autosterilization mechanism in lice intestines.

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RESUME

Etude bactériologique des plumes et des poux chez des busards captifs communs

La flore bactérienne externe et interne associée à diverses espèces de poux, parasites de busards captifs (*Buteo buteo*), a été étudiée ainsi que celle des plumes de l'hôte. La flore à l'intérieur des ectoparasites était significativement différente de celle de la surface. Les bactéries appartenaient au genre *Staphylococcus*, *S. aureus* en particulier, plus abondant à la surface des poux. *Bacillus* était une autre bactérie Gram négatif fréquemment isolée, composant notamment la flore interne. Un très faible nombre d'entérobactéries et d'anaérobies stricts ont été isolés. Les bactéries mises en évidence au niveau des plumes des oiseaux ont révélé quelques contaminations d'origine humaine.

ZUSAMMENFASSUNG

Bakteriologische untersuchung der federn und läuse in gefangenschaft gehaltener mäusebussarde (*Buteo buteo*)

Die externe und interne Bakterienflora von parasitären Läusearten von in Gefangenschaft gehaltenen Mäusebussarden (*Buteo buteo*) und die Bakterienflora auf den Wirtsfedern wurden

untersucht. Die Bakterienflora innerhalb der Ektoparasiten unterschied sich erheblich von der auf ihrer Oberfläche. Bakterien der Gattung *Staphylococcus*, speziell *S. aureus*, waren vorherrschend und auf der Läuseoberfläche reichlicher vorhanden. *Bacillus* war ein weiteres, häufig isoliertes grampositives Bakterium, obgleich es als Bestandteil der internen Flora reichlicher vorhanden war. Eine sehr geringe Anzahl von Enterobakterien und strikten Anaerobiern wurde isoliert. Die Bakterienflora von Vogelfedern ließ eine gewisse Kontamination mit Bakterien vom Menschen erkennen.

RESUMEN

Estudio bacteriológico de las plumas y de los piojos del águila ratonera común (*Buteo buteo*) en cautividad

Se investigó la flora externa e interna asociada a diversas especies de piojos en el águila ratonera común (*Buteo buteo*), además de la flora bacteriana de las plumas. La flora bacteriana encontrada en el interior de los ectoparásitos fue significativamente diferente de la encontrada en su superficie. Se aislaron mayoritariamente bacterias del género *Staphylococcus*, especialmente *S. aureus*, siendo más abundantes sobre la superficie del piojo. También se aisló frecuentemente bacterias gram positivas del género *Bacillus* aunque éstas fueron más abundantes como componentes de la flora interna. Se aisló un número muy reducido de enterobacteriáceas y anaerobios estrictos. Algunas de las bacterias aisladas de las plumas de las aves revelaron cierto grado de contaminación procedente del manejo humano.