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BACTERIAL MICROFLORA OF BITING LICE IN AN EXPERIMENTAL MIXED INVASION IN CHICKENS

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Summary

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The study aimed to obtain data about present the bacterial microflora, both superficial and internal, of four biting lice species, being the commonnest *Mallophaga* species in chickens in Bulgaria: *Menopon gallinae* L. (1758), *Eomenacanthus stramineus* Nitzsch (1818), *Menacanthus cornutus* Schömmer (1913) and *Goniocotes gallinae* DeGeer (1778). The experimental invasion was performed in 2-month old chickens using the four *Mallophaga* species obtained from healthy donors. From each species, 100 biting lice were collected.

It was observed that bacterial isolates on lice's body surface belonged to 5 while those in lice's viscera - to 3 genera. No obligate avian pathogens were isolated. There were some differences in the composition between the external and the inner bacterial microflora of each *Mallophaga* species as well as between the various species in chickens. A correlation between the characteristics of *Mallophaga* bacterial contamination and these related to mobility, location and feeding mode of each *Mallophaga* species was determined.

Key words: bacteria, biting lice, chickens, *Mallophaga*, microflora

INTRODUCTION

During the last three decades, the prevailing opinions in the literature established the belief that *Mallophaga*, considered up to then as harmless ectoparasites (*insecta epizoa*), have a complex pathogenic effect. They damage the intactness of hair, feathers and skin, irritate the nervous ends, disturb the animals and sensibilize the organism. It was proved (Derylo and Gogacz, 1974; Zlotorzycka et al., 1974; Agarwal et al., 1983; Ribbeck, 1992; Arends, 1997) that *Mallophaga* from the *Amblycera* suborder are haematophages.

With regard to *Mallophaga's* haematophagy, the problem of the possible involvement of those parasites in the carriership and the transmission of microorganisms, causing systemic or cutaneous infections becomes actual.

It is known that the species *Menopon gallinae*, *Eomenacanthus stramineus* and *Goniocotes gallinae*, parasitizing on chickens, are carriers of 7 bacterial genera and various fungi (Lonc and Zlotorzycka, 1984). A big group of insects including *Mallophaga*, host several *Ascomyces* species (Weir, 1996). It is reported that *Eomenacanthus stramineus* and *Menopon gallinae* are carriers of *Pasteurella multocida* when isolated from birds with fowl cholera (Derylo, 1970). A carriership of *Salmonella Gallinarum* in chicken-specific *Eomenacanthus stramineus* is established (Derylo, 1975). There are few data about the role of *Menopon gallinae* in the transmission of avian ornithosis

(Arends, 1997). The species composition of bacterial microflora of *Mallophaga*, its dependence upon the insect species as well as the localization of bacteria in the organism of parasites are however inadequately studied.

The present experiment aimed to obtain more information about all those questions in an experimenal model of mixed *Mallophaga* invasion of chickens.

MATERIALS AND METHODS

The bacterial microflora of biting lice was determined in 10 experimentally invaded 2-month-old chickens. The invasive material was obtained from 4 clinically healthy donor birds with a high degree of mixed invasion. The differentiation of biting lice on experimental birds was done according to the manuals of Zunker (1928), Neveu-Lemaire (1938), Zlotorzycka et al. (1974) and Furman and Catts (1982) after a prior conservation for 24 h in 70^o ethanol and embedment in Canada balsam on slides.

The lice used for the experimental invasion were collected from all donors by putting the body of birds (without the head) in polyethylene bags with a cotton swab impregnated with diethyl ether. The insects were collected in a Petri dish after removal of the bag and vigorous shaking of feathers on a white surface. The invasive material was divided into10 equal parts and after a 1 hour stay for evaporation of diethyl ether, was spread over the back of each experimental bird.

The intensity of invasion and the microbial contamination of lice were determined 30 days after the infection.

The lice, collected with sterile forceps were divided into 8 groups of 50 depending on their species affiliation. From each *Mallophaga* species, 2 groups were formed – one for the study of the superficial microflora and another for the study of interior microflora.

The contamination of external body surfaces of lice was studied by placement of collected insects in tubes with tripticase soy broth (TSB) and incubation at 37 ^{o}C for 24-48 h. Subsequently, a subcultivation on blood agar with 5% defibrinated sheep blood and McConkey's agar was performed.

The pure cultures obtained from isolates were stained according to Gram and identified using routine methods (Holt et al., 1994).

The microbial contamination in parasite's viscera was studied similarly, but after a preliminary triple decontamination of their surface by washing in a sterile saline followed by three washings in 70^o ethanol for 10, 20 and 30 min respectively. The decontamination ended by a thorough washing with sterile distilled water (Steinhaus, 1947). From each species, 50 insects were studied as well.

Then, the *Mallophaga* were stirred in a sterile mortar in TSB and incubated as mentioned above.

RESULTS AND DISCUSSION

Thirty days after the invasion, all chickens were invaded with *Mallophaga* from the species: *Menopon gallinae* L. (1758), *Eomenacanthus stramineus* Nitzsch (1818), *Menacanthus cornutus* Schömmer (1913) (all three from the *Amblycera* suborder) and *Goniocotes gallinae* DeGeer (1778) (from the *Ischnocera* suborder of *Phthiraptera* order).

The bacterial isolates from both the body surface and the viscera of insects were determined as belonging to 5 genera. Their generic and species affiliation in the different *Mallophaga* species are presented in Table 1.

Table 1. Bacterial isolates from biting lice in chickens with experimental mixed invasion

Mallophaga species	Body surface (n=50)	Viscera (n=50)
M. gallinae	*Proteus vulgaris* *Staphylococcus epidermidis* *Bacillus spp.*	*Proteus vulgaris* *Bacillus spp.*
E. stramineus	*Escherichia coli* *Staphylococcus epidermidis*	*Escherichia coli*
M. cornutus	*Enterobacter cloacae* *Proteus vulgaris* *Staphylococcus epidermidis*	*Proteus vulgaris*
G. gallinae	*Escherichia coli* *Staphylococcus epidermidis*	*Escherichia coli*

The results of the study showed that the body surfaces of all four *Mallophaga* species were inhabited by similar microbial species. They were predominantly representatives of the *Enterobacteriaceae* family - *Escherichia coli*, *Proteus vulgaris* and *Enterobacter cloacae*. Furthermore, coagulase-negative staphylococci, determined as *Staphylococcus* epidermidis and sporulating bacteria from the *Bacillus* genera were recovered.

Those results were to be expected because the species found were related to faecal environmental pollution (enterobateriae, bacilli) or were representatives of the resident cutaneous microflora (coagulase-negative staphylococci).

The table shows that there was some difference between the surface and internal microflora of each insect species as well as between the bacterial microflora of the various *Mallophaga* species.

It could be emphasized that some bacterial species (*Staphylococcus epidermidis* and *Enterobacter cloacae*) that were present on body surfaces, were not found out within the body of insects. Probably, the intestinal tract of *Mallophaga* possesses mechanisms for elimination of some bacterial species or the conditions for the growth of some bacteria in parasite's viscera are not favourable.

Our data are partially different from those of Lonc and Zlotorzycka (1984) who evidenced particularly the presence of Gram-positive cocci in 300 insects from the *Eomenacanthus stramineus, Menopon gallinae* and *Goniocotes gallinae* species. They do not observe a significant difference between the external and internal bacterial constellation of those *Mallophaga* species in chickens while Perez et al. (1994) found out such variations in *Mallophaga* on some wild avian species.

The differences existing in the composition of superficial bacterial microflora among some *Mallophaga* species are due, in our opinion, to the difference in the localization and the behaviour of insects. Thus, *Menacanthus cornutus*, that is residing predominantly on the skin around the cloaca, is the only species, contaminated with *Enterobacter cloacae*, discharged with faeces. The multibacterial presence on the surface of *Menopon gallinae* could be explained by its mobility and the fact that it is encountered on the skin as well as the feathers on almost the entire body surface of birds.

We suppose that the poorer bacterial contamination of the intestinal tract of *Eomenacanthus stramineus* and *Menacanthus cornutus* is due to the fact that both species were haematophaguous while the others ingest predominantly horny substance from feathers and skin where the variety of bacteria is greater and they are more abundant.

Although that during the study, no pathogenic microbial isolates were found out, it could be anticipated that in birds with salmonellosis or carriers of salmonellae, the *Mallophaga* could be contaminated with some of ubiquiter serovars of *Salmonella Enteritidis*, *S. Typhimurium* etc., or with *S. Gallinarum* and *S. Pullorum*, adapted to birds. Thus, Derylo (1975) reported about a frequent isolation of *S. Gallinarum* from *Eomenacanthus stramineus* related to its haematophagy, but this was not related to the transmission of typhus infection.

The same is valid for *Pasteurella multocida* (Derylo, 1967; 1970). The author suggests that *Mallophaga* are a potential reservoir of cholera agent as well as that they are involved in the epizootology of the disease.

CONCLUSIONS

Microbial varieties from the group of enterobacteriae, coagulase-negative staphylococci and aerobic bacilli were isolated from the body surface and the viscera of chicken's biting lice, belonging to the species *Menopon gallinae* L. (1758), *Eomenacanthus stramineus* Nitzsch (1818), *Menacanthus cornutus* Schömmer (1913) and *Goniocotes gallinae* DeGeer (1778).

They were some differences between the superficial and the internal bacterial contamination of *Mallophaga*, invading chickens.

The composition of bacterial microflora, contaminating chicken's biting lice, depended on their location, mobility and the type of feeding.

During an experimental mixed invasion with *Mallophaga*, obtained from clinically healthy donors, there were no bacterial isolates, obligately pathogenic for chickens on both external and internal parts of insects.

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