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Anschrift des Verfassers: Dr. L. BRITZ, Bezirks-Hygienieinspektion und -institut Leipzig, Abt. Med. Parasitologie, Referenzlaboratorium für Medizinische Arachno-Entomologie der DDR, DDR - 7010 Leipzig, Beethovenstraße 25.

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Connections between poultry biting lice and microflora

By E. LONC and J. ZŁOTORZYCKA

From Department of Parasitology, Microbiological Institute, Wrocław University, Poland

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Introduction. Up to now microbiological studies of biting lice have aimed mainly at the determination of their role in the transmission of epizootia in farm animals. SIEMIENOW & KOZŁOWA (1936) proved experimentally that lice *Werneckiella equi* may transmit the virus of anaemia in horses. Lice, *Eomenacanthus stramineus*, on the other hand, are the carriers of the virus of eastern encephalomyelitis in horses (HOWIT et al. 1948). The role of haematophagous lice *E. stramineus* and *Menopon gallinae* in the transmission of pasteurellosis in hens was experimentally determined by DERYŁO (1967, 1969, 1970). It was found that *E. stramineus* parasitizing on the typhoid infected poultry may contract the strain *Salmonella gallinarum* (DERYŁO 1975), whereas negative results were achieved in experiments on the role of *E. stramineus* in the transmission of toxoplasmosis in poultry (DERYŁO 1977).

There have also been a few attempts to utilize *Bacillus thuringiensis* in biological control of ectoparasites of poultry (HOFFMAN 1966; ANONYMUS 1969). According to DERYŁO & JAROSZ (1972) studies dealing with that issue are difficult because the autochthonic microflora of biting lice is insufficiently known. Those authors isolated merely three genera of bacteria, mainly the greening streptococci. Other authors report the occurrence in biting lice of unidentified symbiotic microorganisms of bacteria and rickettsia kind (SIKORA 1922; RIES 1931, 1932; HAUG 1952; EICHLER 1963) as well as the occurrence of pathogenic fungi (CHATTON & PICARD 1909; TRINCHIERI 1910; LUNKASZU 1910). KAMYSZEK (1978) isolated *Trychophyton verrucosum* from 46 lice *Bovicola bovis* (out of 276 examined) collected from mycosis infected cattle.

So far no studies have been made that take into account external and internal microflora commonly occurring in poultry lice as well as no comparisons have been drawn between microflora on the external body covers of hosts in places where parasites occur. The purpose of the present research studies is the examination of microflora of haematophagous poultry lice (*Menopon gallinae*, *Eomenacanthus stramineus*) and lice feeding on ceratine (*Goniocotes gallinae*).

Materials and methods. Lice to be examined (several hundred specimens) were collected gradually from 18 hens chosen at random in a small private farm and a state farm near Wrocław. All hens, except one infected with Marek's disease, were clinically in good health. For the purpose of microbiological examinations swabs were also used of those parts of the bodies of 6 hens where lice were determined to occur. The following experiments were conducted:

1. Swabs were taken from 20 specimens of *Menopon gallinae* and 20 specimens of *Goniocotes gallinae* sterilized externally with 0.5% chloramine and were immediately stained with Gram's method.

2. Examination of external microflora of 109 specimens of *M. gallinae*, 5 specimens of *E. stramineus*, 80 mature specimens and 26 nymphs of *G. gallinae* was made in the following way. Single insects were dipped in 2.5 ml of sterile sugar broth, shaken several times and then incubated. Internal microflora was discovered in 148 specimens of *M. gallinae*, 100 of *G. gallinae*, 5 of *E. stramineus*, including those from which cultures of external microflora were made. In order to make them lice bodies were sterilized before the inoculation. Incubation period varied from 48 to 72 h in temperature 37 °C. In cases of growths in cultures the inoculations on the following media were made: blood agar, McConkey's, Chapman's, Levin's, and YPG media. The incubation lasted 24 h at 37 °C. Slides stained with Gram's method were made of morphologically different colonies. Detailed identification of microorganisms were made on the basis of biochemical and biological properties according to generally applied principles (KĘDZIA & KONIAR 1980).

3. 18 *M. gallinae* and 20 *G. gallinae* were placed directly on agar plates with blood agar. The same specimens taken from that were externally sterilised, crushed in saline and inoculated directly on next blood agar. Identification of cultured strains was made in the above mentioned way.

4. A culture of anaerobic bacteria from the inside of 22 specimens of *M. gallinae* and 20 *G. gallinae* was produced on thioglycolate medium according to Forhter's method (TRUSZCZYŃSKI 1978). Diagnostics of isolated strains was made on the basis of general aspect of colonies and microscopic microbiology of samples stained with Gram's method.

5. Swabs taken from body surfaces of 6 hens where *M. gallinae* occurred and of 5 hens from which *G. gallinae* was collected were incubated in solid media and subjected to the procedure of experiment 2.

Results. Experiment 1. In all slides from *M. gallinae* and *G. gallinae* microorganisms were found. They were mainly grampositive⁽⁺⁾ cocci dispersed in the field of vision as single bacteria, double or short chainlets which were accompanied sporadically only in *G. gallinae* by rod-shaped bacteria (bacilli).

Experiment 2. In broth nutrient cultures the growth of aerobic external microorganisms was determined in 83 *M. gallinae* (76% of all the examined) and the growth of internal bacteria in 82 specimens (55% of the examined). Similarly, in *G. gallinae* more microorganisms were discovered in external samples (70% of the examined) than internal ones (only 53%).

On slides stained with Gram's method grampositive⁽⁺⁾ cocci dominated and were observed as simple bacteria, short chainlets or irregular clusters. Besides, sporogenous grampositive rod-shaped bacteria were indicated as well as sporadic *Corynebacterium* and grammegative⁽⁻⁾ bacteria.

On the basis of the aspect of a colony and its microscopic morphology bacteria were classified into the genera *Bacillus*, *Corynebacterium*, *Sarcina*. Some fungi were also found. Basing on biological and biochemical properties the following genera were distinguished among the cocci: *Streptococcus*, *Staphylococcus*, *Micrococcus* and grammegative⁽⁻⁾ *Klebsiella*, *Escherichia* and *Pseudomonas*. Individual strains, in general, occurred more rarely inside *M. gallinae* (tab. 1a) and *G. gallinae* (tab. 2a) than on their surfaces. Only part of strains were found both inside and on the surface of a louse.

During the examination of microflora of *G. gallinae* nymphs the growth of external aerobic microorganisms was determined in 24 cases (92% of examined) and the growth of internal bacteria only in 2 cases (10%). Like in mature specimens *Str. faecalis* was most often isolated from the external covers of nymphs. Whereas in external microflora of nymphs mostly *S. epidermidis* and fungi were found.

On the external covers of *E. stramineus* *Str. faecalis* (in 3 specimens) and *E. coli* (in 2 specimens) were discovered.

Experiment 3. Direct inoculation on blood agar gave positive results for external microflora of *M. gallinae* in 96% and of *G. gallinae* 92%. For the internal microflora the respective values were 54% and 79%. Like in broth cultures mainly grampositive⁽⁺⁾ cocci (*Streptococcus* and *Staphylococcus*) were found beside rod-shaped bacteria and fungi (tab. 1b, 2b).

Table 1. Characteristic of microorganisms isolated from *Menopon gallinae* and *Eomenacanthus stramineus*

a) From broth culture							
Species of lice	Microorganisms	External microflora		Internal microflora		External and internal microflora	
		Number of specimens with micro-organisms	%	Number of specimens with micro-organisms	%	Number of specimens with micro-organisms	%
1	2	3	4	5	6	7	8
<i>Menopon gallinae</i>	<i>Str. faecalis</i>	35	32.1	38	25.7	12	11
	<i>Str. viridans</i>	4	3.7	4	2.7	1	9.9
	<i>Bacillus</i>	18	16.5	32	21.6	6	5.5
	<i>S. epidermidis</i>	19	17.4	6	4.1	2	1.8
	<i>S. albus</i>	6	5.5	4	2.7	2	1.8
	<i>S. aureus</i>	5	4.6	1	0.7	0	0
	<i>Micrococcus</i>	5	4.6	3	2.0	0	0
	<i>Corynebacterium</i>	2	1.8	0	0	0	0
	<i>Sarcina</i>	0	0	2	1.4	0	0
	<i>E. coli</i>	1	0.9	1	0.7	0	0
	<i>Klebsiella</i>	0	0	1	0.7	0	0
	? <i>Pseudomonas</i>	2	1.8	0	0	0	0
	Fungi	6	5.5	4	2.7	0	0
<i>E. stramineus</i>	<i>Str. faecalis</i>	3	60	5	100	3	60
n = 5	<i>E. coli</i>	2	40	0	0	0	0

b) From direct plating							
1	2	3	4	5	6	7	8
<i>Menopon gallinae</i>	<i>Str. faecalis</i>	9	50.0	5	27.8	3	16.7
	<i>Str. viridans</i>	0	0	1	5.5	0	0
	<i>Bacillus</i>	7	38.9	2	11.1	2	11.1
	<i>S. epidermidis</i>	6	33.3	2	11.1	1	5.5
	<i>S. aureus</i>	4	22.2	1	5.5	1	5.5
	<i>S. albus</i>	2	11.1	0	0	0	0
	<i>Corynebacterium</i>	1	5.5	0	0	0	0

Experiment 4. Anaerobic bacteria were discovered in 4 cultures from *M. gallinae* (18%); similarly, for *G. gallinae* positive results constituted 20%. In slides from *M. gallinae* there were mainly grampositive (+) cocci forming short chainlets (? *Streptococcus*), whereas in *G. gallinae* there were also gramnegative (-) bacteria and fungi.

Experiment 5. Microflora of the body surfaces of the examined hens varied in particular samples but was generally similar to the bacterial flora of the inside of *M. gallinae* and *G. gallinae* (tab. 3, 4). Particularly, rod-shaped bacteria were present in all samples from *G. gallinae* as well as from numerous parts of hens' bodies. However, *Str. viridans*, *Pseudomonas* found in lice were absent from swabs from hen's bodies.

Discussion. Our own studies have indicated a common occurrence of microorganisms in *Menopon gallinae*, *Goniocotes gallinae* and probably also in *Eomenacanthus stramineus*.

The results of the examinations of external and internal microflora of *G. gallinae* basically do not differ from the results achieved in experiments with *M. gallinae*. Thus, discovered strains probably contacted *G. gallinae* in the same way as *M. gallinae*. External microflora of nymphs of *G. gallinae* did no differ from the microflora of mature lice. Only the cul-

Table 2. Characteristic of microorganisms isolated from *Goniocotes gallinae*

a) From broth culture

Microorganisms	External microflora		Internal microflora		External and internal microflora	
	Number of specimens with micro-organisms	% n = 80	Number of specimens with micro-organisms	% n = 101	Number of specimens with micro-organisms	% n = 80
1	2	3	4	5	6	7
<i>Streptococcus faecalis</i>	16	28.6	19.0	35.8	5.0	9.0
<i>Streptococcus viridans</i>	9.0	16.1	9.0	17.0	4.0	7.2
<i>Staphylococcus aureus</i>	5.0	8.9	8.0	15.1	2.0	3.6
<i>Staphylococcus epidermidis</i>	13.0	23.2	8.0	15.1	4.0	7.2
<i>Micrococcus</i>	3.0	5.3	1.0	1.9	0.0	0.0
<i>Klebsiella oxytoca</i>	1.0	1.8	0.0	0.0	0.0	0.0
<i>Klebsiella</i> sp.	1.0	1.8	0.0	0.0	0.0	0.0
<i>Escherichia coli</i> 1	1.0	1.8	0.0	0.0	0.0	0.0
<i>Escherichia coli</i> 2	0.0	0.0	1.0	1.9	0.0	0.0
<i>Pseudomonas</i>	2.0	3.6	1.0	1.9	1.0	1.8
<i>Bacillus</i>	12.0	21.4	8.0	15.1	1.0	1.8
<i>Corynebacterium</i>	1.0	1.8	0.0	0.0	0.0	0.0
Fungi	2.0	3.6	4.0	7.5	0.0	0.0

b) From direct plating

1	2	3	4	5	6	7
<i>Streptococcus faecalis</i>	7.0	38.9	2.0	11.0	1.0	5.5
<i>Streptococcus viridans</i>	1.0	5.5	0.0	0.0	0.0	0.0
<i>Staphylococcus aureus</i>	12.0	66.7	3.0	16.5	2.0	11.0
<i>Staphylococcus epidermidis</i>	17.0	94.4	8.0	44.0	8.0	44.0
<i>Micrococcus</i>	1.0	5.5	1.0	5.5	0.0	0.0
<i>Bacillus</i>	9.0	50.0	4.0	22.0	3.0	16.5
<i>Klebsiella</i>	1.0	5.5	0.0	0.0	0.0	0.0
<i>Pseudomonas</i>	1.0	5.5	1.0	5.5	0.0	0.0
<i>Escherichia coli</i>	2.0	11.0	0.0	0.0	0.0	0.0
Fungi	1.0	5.5	3.0	16.5	0.0	0.0

tures of internal microflora of nymphs gave modest results since only in 2 specimens (10%) the growth of bacteria and fungi was observed. However, on all slides from swabs of the contents of nymphs some bacteria were present. It seems that strains occurring in the majority of nymphs were different from those cultured according to generally applied methods. Therefore, it is assumed that during its younger stages of development microorganisms peculiar mainly to insects occur in *G. gallinae*, whereas in mature specimens its microflora is enriched by strains coming from the external environment.

It seems, however, that frequent positive results in cultures were determined by media. It has turned out that microflora was qualitatively similar in lice coming from different sources. The microflora of lice collected from the infected hen did not differ either, although the hen was distinguished by considerable losses in plumage and damaged cuticle (probably because of high intensity of lice infestation).

Grampositive (+) cocci constituted the majority of isolated strains coming both from the external covers and the inside of lice. These results correspond with the results of DERYŁO & JAROSZ (1972). When they found qualitatively poor microflora — only in 60% of inoculation — they expressed a conviction that blood feeding of *M. gallinae* and *E. stramineus* and autosterilisation in their alimentary tracts do not favour the occurrence of bacteria.

Table 3. Microflora from external surface of poultry, to compare with microflora of *Menopon gallinae*

Number of experiment	Microorganisms		
	from external body of poultry	from external surface of lice n = 10—15	inside of lice n = 10—15
1	<i>Bacillus</i> <i>Str. faecalis</i>	no probe	<i>Bacillus</i> <i>Str. faecalis</i>
2	<i>Klebsiella oxytoca</i> <i>Bacillus</i>	? <i>Pseudomonas</i> <i>Bacillus</i> <i>S. epidermidis</i> <i>S. albus</i> <i>Str. faecalis</i>	<i>Klebsiella</i> <i>Bacillus</i> <i>S. albus</i> <i>Sarcina</i> <i>Str. faecalis</i>
3	<i>Bacillus</i> <i>Str. faecalis</i> <i>E. coli</i>	<i>Bacillus</i> <i>Str. faecalis</i> <i>E. coli</i> <i>S. albus</i>	<i>Bacillus</i> <i>Str. faecalis</i> <i>E. coli</i> <i>S. albus</i> <i>S. epidermidis</i>
4	<i>Bacillus</i> <i>S. aureus</i> <i>Micrococcus</i> <i>E. coli</i> Fungi	<i>S. aureus</i> <i>S. epidermidis</i> <i>Micrococcus</i> <i>Str. faecalis</i> <i>Str. viridans</i>	<i>S. aureus</i> <i>Str. faecalis</i> <i>Micrococcus</i>
5	<i>Bacillus</i> Fungi <i>S. epidermidis</i>	<i>Bacillus</i> <i>Str. faecalis</i> <i>S. epidermidis</i>	<i>Bacillus</i> <i>Str. faecalis</i> <i>S. epidermidis</i> <i>S. albus</i> <i>Corynebacterium</i>
6	<i>E. coli</i> Fungi	no probe	<i>S. albus</i> <i>Str. faecalis</i> <i>Bacillus</i> Fungi

Table 4. Microflora from external surface of poultry, to compare with microflora of *Goniocotes gallinae*

Number of experiment	Microorganisms		
	from external body of poultry	from external surface of lice	inside of lice
1	<i>Bacillus</i> <i>E. coli</i> Fungi	<i>Bacillus</i> <i>E. coli</i> Fungi <i>Str. faecalis</i>	<i>Bacillus</i> Fungi <i>St. aureus</i>
2	<i>Bacillus</i> <i>Str. faecalis</i>	<i>Bacillus</i> <i>Str. faecalis</i>	<i>Str. faecalis</i>
3	<i>Bacillus</i> <i>Str. faecalis</i> <i>Staph. aureus</i> <i>Micrococcus</i> <i>E. coli</i> Fungi	<i>Str. faecalis</i> <i>Staph. aureus</i> <i>Micrococcus</i> <i>E. coli</i> Fungi <i>Str. viridans</i> <i>Staph. epidermidis</i> <i>Klebsiella oxytoca</i> <i>Pseudomonas</i>	<i>Str. faecalis</i> <i>Staph. aureus</i> <i>Micrococcus</i> <i>E. coli</i> Fungi
4	<i>Bacillus</i> <i>Klebsiella</i>	<i>Bacillus</i> <i>Str. faecalis</i>	<i>Str. faecalis</i>
5	<i>Bacillus</i> Fungi	<i>Bacillus</i> Fungi	<i>Str. faecalis</i>

However, it is known (EICHLER 1963) that haematophagous lice feed also on feathers. So these seems to be a possibility of getting microorganisms together with the food. Even if the food is blood, it may contain microorganisms from the body surface of the host. To collect the effused blood, biting lice like *M. gallinae* and *E. stramineus*, with their mandibles set flat at the bottoms of their heads, may damage the skin less deeply though on a larger surface than common lice. The high possibility of collecting microorganisms by contact or by alimentary tracts has been testified by our studies. E.g. *Bacillus* and *Str. faecalis* were indicated inside and on the surface of lice and on hen's bodies.

At the present stage of our studies it is difficult to present a definite interpretation of the results indicating the fact that the external microflora of *M. gallinae* and *G. gallinae* turned out to be in many respects more abundant than the internal one. However, numerous observations testifying to the fact of collecting the secretions of lacrimal glands of birds by lice (MEY 1978) suggest a hypothesis that lysozymes in tears reaching the alimentary tracts of lice may influence inhibitively the development of microorganisms.

Zusammenfassung. Beziehungen zwischen Hühnermallophagen und Mikroflora. Bei etwa 300 Mallophagen (*Menopon gallinae*, *Eomenacanthus stramineus*, *Goniocotes gallinae*) und 11 Wirtshühnern wurden Mikroorganismen nachgewiesen. Die Mehrzahl der isolierten Bakterien wurde als grampositive Kokken bestimmt. Die an der Oberfläche und innerhalb der Mallophagen sowie in den Proben von Hühnerhaut festgestellte Mikroflora zeigte im Durchschnitt keine bedeutenden qualitativen Unterschiede. Die interne Mikroflora der Mallophagen erschien qualitativ etwas ärmer als die äußere. Eine Züchtung von Bakterien aus inneren Geweben der Nymphen von *G. gallinae* gelang selten, obwohl dort Bakterien auf allen mikroskopischen Präparaten festgestellt wurden. Die Ergebnisse deuten auf die bakteriostatische Wirkung eines unbekannten Wirkstoffes im Verdauungstrakt der Mallophagen hin, aber auch auf das Vorhandensein von (wahrscheinlich für die untersuchten Insekten spezifischen) Mikroorganismen in den Nymphen von *G. gallinae*, die jedoch mit den üblichen Methoden nicht zu unterscheiden sind.

Резюме. Отношения между куриным малофагам и микрофлоры. Во время исследований приблизительно 300 пухоедов: *Menopon gallinae*, *Eomenacanthus stramineus*, *Goniocotes gallinae*, а также 11 кур получено микроорганизмы. Большую часть выделенных бактерий определено как грам+ микрококки. Преимущественно, микроорганизмы установлены во внутренних тканях пухоедов, а также из кожи кур не показывают значительных, качественных различий. Внутренняя микрофлора пухоедов была более убогая чем внешняя. Культивирования бактерий из внутренних тканей личинок *G. gallinae* редко были положительные. Однако бактерии обнаружено на всех этих микроскоповых препаратах. Результаты исследований показывают на бактериостатическую деятельность ближе неопределенных факторов в пищеварительном тракте пухоедов, а также на наличие в личинках *G. gallinae* этих микроорганизмов (вероятно специфических для исследованных насекомых), которых однако не возможно отличить при помощи обычных методов.

Summary. Microorganisms were found in about 300 Mallophaga (*Menopon gallinae*, *Eomenacanthus stramineus*, *Goniocotes gallinae*) and 11 host hens. The majority of isolated bacteria was determined as grampositive cocci. The microflora found on the surface of and inside the Mallophaga as well as in the samples of hen's skin did not exhibit significant qualitative differences. The internal microflora in Mallophaga appeared qualitatively a little poorer than the external. Breeding of bacteria from interior tissues of nymphs of *G. gallinae* seldom succeeded, although bacteria were found in all microscopical preparations. The results indicate the bacteriostatistical effect of an unknown agent in the digestive system of Mallophaga, as well as the presence of microorganisms (probably specific to the insects investigated) which, however, cannot be distinguished by the usual methods.

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Address of the authors: Dr. E. LONG and Prof. Dr. J. ZŁOTORZYCKA, Wrocław University, Microbiological Institute, Przybyszewskiego 63/77, PL - 51-148 Wrocław, VR Polen.

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Einige historische Bemerkungen zur Einführung des Begriffes „Parasit“ in das Schrifttum der Zoologie, Medizin und Tierheilkunde

Von B. HÖRNING

Aus dem Institut für Tierpathologie der Universität Bern

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Im 1. Band ihres gerade erscheinenden Lehrbuches befassen sich HIEPE et al. (1981) in einer kulturgechichtlichen Betrachtung (S. 9 und 10) mit der Herkunft der Bezeichnung „Parasit“. Sie schreiben dann am Schluß des Abschnittes, daß noch nicht bekannt sei, wer den Begriff im naturwissenschaftlichen Sinne zuerst geprägt hat. Die Frage wurde von ODENING (1974) bereits kurz berührt, als er darauf hinwies, daß das Wort im biologischen Sinne bereits im 17. und 18. Jahrhundert in der Botanik Verwendung fand.