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SUPERFICIAL AND INTERNAL BACTERIAL MICROFLORA OF LICE AND FLEAS IN GOATS

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ABSTRACT

Lice and fleas can play the role of vectors for different infectious agents. They can carry different pathogens to sensitive hosts.

The conduction of in depth research on microbiological insemination of lice and fleas meets serious setbacks because of lack of information about their normal microflora.

The objective of this study was to determine in parallel superficial and internal bacterial insemination of the goat infesting species: *Bovicola caprae, Linognathus stenopsis, Pullex irritans* collected from naturally infested goats.

The parasites that were collected with stelirized forceps were divided into 6 groups and examined according to the classic microbiological methods.

The results received from our study showed that the bacterial composition of the surface of the insects which were examined, that is presented by *Steptococcus* spp., *Staphylococcus epidermidis, Staphylococcus aureus, Corynebacterium* spp., *Escherichia coli, Bacillus* spp. and *Clostridium perfringens* is richer than the one from the inside – *Streptococcus* spp., *Staphylococcus epidermidis, Staphylococcus aureus* and *Bacillus* spp. There are certain differences between the microflora of the separate species of insects.

Key words: goats, ectoparasites, lice, fleas, microflora.

Introduction

Lice can play the role of vectors for many infectious agents. They can carry different pathogens to sensitive hosts. Therefore the examination of the microflora of these ectoparasites in farm animals deserves special attention and has to be carefully evaluated given the broad spectrum of pathogens carried by arthropods (Hornok et al., 2010).

All species of blood sucking lice and fleas are ectoparasites in mammals that feed on blood ingested from their hosts. Besides their direct negative effects on the host such as: damage to the skin during feeding, mild to moderate anemia and weight loss (Otter et al., 2003), they can carry different pathogens (viruses, bacteria, fungi and protozoa) to sensible hosts. In that way their biological (vector) role usually means permanent development of infectious agents in their intestinal cells. After the transportation of the pathogens in the feces they don't inoculate it during the blood sucking but they rub it in the skin of the hosts involuntarily (Durden and LIoyd, 2009). When they are short term mechanical carriers the transmission is possible only in the few hours after feeding. During that time they can inoculate the agent that has made its way in their mouth apparatus (Crystal, 1958).

During the feeding the blood sucking lice and fleas puncture the skin in different places and take in blood from the lumen of the blood vessels (Lavoipierre, 1967). That way they can transmit pathogens to new sensible hosts. A predisposition for effective transmission is the movement of these parasites from one host to another which is an inseparable aspect of their behavior (Durden and Lloyd, 2009).

On the topic of the epidemiological risks which the infestation with lice and fleas hides and of the transmissive role of these ectoparasites, the opinions of different scientists vary between two opposites. It is a known fact that lice and fleas are vectors of many infectious agents including in humans. Blood sucking lice have a priority in that way (Anoplura) (Mehlhorn, 2001). According to some researchers though lice not only directly influence the livability and productivity of their hosts but they also have an important role as reservoirs and vectors for agents of transmissive diseases (Hornok et al., 2010).

For lice of the orders *Haematopinu*s and *Linognathus* it is proved that they are capable of mechanically transmitting almost all microorganisms (Raoult and Roux, 1999). Besides from that it is experimentally proven that they can be biological carriers of pathogens transmitted by lice and fleas (Houhamdi and Raoult, 2006).

The fact that some species from the order Phthiraptera are partially or mainly feeding on blood is of major importance for the ingesting and transmitting of different microorganisms and nematode larvae (filariides). Some of them are proven to be vectors for different microorganisms: *Rickettsia prowazekii*, that causes endemic typhus, virulent strains of *Pasteurella multocida*, the causative agent of cholera, the encephalomyelitis virus (Nelson, 1972).

Hornok et al. (2010) prove that *Anaplasma* spp. can be transmitted by *L. vituli*, *L. stenopsis* and *Haematopinus suis*, and they advise for greater caution towards this not so harmless infectious disease. *L. stenopsis* which is found in goats was inseminated with *Anaplasma marginale* and/or *Anaplasma ovis.* The authors also found DNA from *Rickettsia helvetica* in *Linognathus* spp., while other *Rickettsia* spp. were found in *H. eurysternus* and *L. stenopsis*. With this discovery they put lice amongst the other arthropods that are potential vectors of pathogens.

Materials and methods

For the purpose of the study we examined three goat farms. The parasites were collected with the help of sterile forceps and were divided into 6 groups-in two groups for every species – to prove the superficial and internal bacterial contamination of each species. The groups had a number of 50 exemplaries.

The contamination of the outside body surfaces was observed by placement of the separate groups of 50 insects into tubes with tryptic soy broth (TSB, HiMedia, India) and thyoglicollate medium (under a layer of liquid paraffin). The samples were incubated for 24 h at 37°C. Subsequently we did a subcultuvation of TSB on blood agar with 5% defibrinated sheep blood and on McConcey agar, and of thyoglicollate medium onto Zeisler blood agar in anaerobic conditions for 24–48 h at 37°C. After obtaining pure cultures of the isolates Gram staining was performed and they were subjected to further identification.

The Gram positive isolates were tested for the presence of catalase with 3% H_2O_2 and for oxidase with commercially available test (HiMedia, India). Also, the presence of hemolysis on blood agar and the morphology of the colonial growth were taken into consideration. The production of free coagulase was tested by tube coagulase test with liopilised rabbit plasma provided by HiMedia (India).

Individual colonies (lactose positive and negative, respectively) from McConcey agar were subcultured on polytropic Kligler agar slant for primary identification of Gram negative isolates. In addition, the fermentative-oxidative test of Hugh-Leifson with 1% glucose (NCIPD, Bulgaria) was included.

Further identification tests were selected according to the characteristics determined according to Bergey's Manual of Determinative Bacteriology, (Holt et al., 1994).

Analogical approach was used in the examination of endobacterial microflora of parasites but after preliminary decontamination of their surface three times with washing in sterile saline and then three times washing in 70% ethanol. The procedure of decontamination was completed with substantial washing in sterile distillated water (Steinhaus, 1947). Afterwards the parasites were crushed in tryptic soy broth or thyoglicollate medium with subsequent cultivation in the manner that was previously described. 50 exemplaries of the three species were used again.

Results

After identification of the insects we found that the animals in each farm were naturally infested with one parasitic species each.

The bacterial strains that were isolated from the surface and the inside of the body of the parasitic species infesting goats were determined into 6 orders presented in table 1.

Table 1: Bacterial isolates from sucking, biting lice and fleas in goats with natural monoinfestation.

Species	**Bacterial isolates**	
(n = 50 + 50)	**Surface**	**Inside**
Bovicola caprae	*Streptococcus* spp. *Staphylococcus epidermidis* *Staphylococcus aureus* *Corynebacterim* spp. *Bacillus* spp. *Enterobacter* spp. *Clostridium perfringens*	*Streptococcus* spp. *Staphylococcus epidermidis* *Staphylococcus aureus* *Bacillus* spp.
Linognathus africanus/ Linognathus stenopsis	*Streptococcus* spp. *Staphylococcus epidermidis* *Staphylococcus aureus* *Bacillus* spp. *Escherichia coli* *Clostridium perfringens*	*Streptococcus* spp. *Staphylococcus epidermidis* *Staphylococcus aureus* *Bacillus* spp.
Pulex irritans	*Streptococcus* spp. *Staphylococcus epidermidis* *Staphylococcus aureus* *Corynebacterim* spp. *Escherichia coli* *Bacillus* spp. *Clostridium perfringens*	*Bacillus* spp.

The results obtained from the study show that similar microbial species can be isolated from the surface of the examined species of ectoparasites– mostly Gram positive bacteria such as *Streptococcus* spp., *Staphylococcus* spp, and *Corynebacterium* spp. as well as spore-producing bacteria belonging to *Clostridium* spp, and *Bacillus* spp. Some of the coagulase-negative staphylococci were determined as *Staphylococcus epidermidis*, while others showing the presence of the enzyme coagulase were identified as *Staphylococcus aureus*. The intestinal bacteria from *Enterobacteriaceae* family were present on the surface of all ectoparasites that were studied although it was only one species – *Escherichia coli*. On the body of *B. caprae* representatives of *Enterobacter* spp. were found.

Discussion

The conduction of in depth studies of the microbial insemination of lice and fleas meets severe difficulties due to the lack of information about their normal microflora.

From the data in table 1 we saw that there was a small difference between the superficial microflora of each individual species. More significant were the differences between the bacteria inhabiting the surface of the body and the inside of the parasites. The inside microflora was significantly less rich. Clostridiums, corynebacteriums and enterobacterias were missing. In fleas from the *P. irritans* species the bacteria isolated from the inside only included examples of the genus *Bacillus*.

There was a point that some bacterial variations (*Corynebacterim* spp., *Clostridium* spp. and family *Enterobacteriaceae*), that were present on the surface were not found on the inside of the body of the insects. Most likely these parasites have systems in their intestinal tracts that eliminate some bacterial species or some bacteria do not find proper conditions for development there. Especially characteristic were the differences in *P. irritans.* On the surface of that parasite were found exemplaries of six species and on the inside of only one *(Bacillus* spp.*).*

Insignificant differences in the composition of the surface bacterial microflora between some of the ectoparasitic species according to us could be accounted for by the similar localization and behavior. For example Prelezov and Lyutskanov (2002) find that *Menacanthus cornutus*, which is very active is commonly localized on the skin around the cloaka and is contaminated with *Enterobacter cloacae*, that is produced alongside the feces. While the multi species bacterial presence on the surface of *Menopon gallinae* is explained by its good motility and the fact that it is seen on almost the whole body surface of birds. The same author discovers smaller number of bacterial species in the intestinal tract of *Eomenacanthus stramineus* and *M. cornutus*, which is contributed to the fact that both species are haemophags while the other species feed on the horneous substance of the feather and skin where the bacterial presence is more diverse and massive compared to the blood.

Although throughout the study pathogen microbial variants were not found the fleas' role in transmitting bacteria from the genus *Salmonella* is well known and the most popular is the transmission of *Salmonella* Paratyphi B (Desenclos et al., 1996). It is logical that the carriers of salmonella or goats sick with salmonella have possibly contaminated lice and fleas as well as from some ubiquitous serovars *Salmonella* Enteritidis, *S.* Typhimurium and others or with species adapted towards goats *Salmonella* Abortusovis.

There is very little data in the literature about bacterial microflora of the haemophags in small animals. The studies are mainly focused on the detection through molecular-genetic methods of *Acinetobacter* spp. (Kumsa et al., 2012).

Similar to the results that we have most scientists that had studied the problem that is discussed do not isolate obligate pathogen microorganisms from the goats on the surface and inside of the body of the lice (Pérez-Jiménez et al., 1994).

Conclusion

The results gathered from our study showed that the bacterial composition of the surface of the insects that were examined is presented by *Streptococcus* spp., *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Corynebacterim* spp., *Escherichia coli*, *Bacillus* spp. and *Clostridium perfringens*, is richer than the one from the inside - *Streptococcus* spp., *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Bacillus* spp. Certain differences between the microflora of the different species of insects were observed.

These results therefore have to be taken for reliable as they are species connected to fecal contamination of the environment (enterobacteria, bacillus) or are examples of resident skin microflora (staphylococcus).

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