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Short communication

Survey on blood-sucking lice (Phthiraptera: Anoplura) of ruminants and pigs with molecular detection of *Anaplasma* and *Rickettsia* spp

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ABSTRACT

Lice may serve as biological or mechanical vectors for various infectious agents. To investigate louse infestation of ruminants and pigs, and pathogens potentially transmitted by them, anopluran lice ($n = 1182$) were collected in Hungary, and evaluated for the presence of anaplasma, rickettsia and haemotropic mycoplasma DNA. On cattle the following species were found: *Linognathus vituli* (57%), *Haematopinus eurysternus* (38%) and *Solenopotes capillatus* (5%). *L. vituli* had a lower mean individual count/host when compared to *H. eurysternus*. On calves only *L. vituli* was observed, with a higher louse burden than on full-grown cattle. *H. eurysternus* and *S. capillatus* were more likely to occur simultaneously with another species on the same host, than *L. vituli*. Goats infested with *Linognathus stenopsis* had the overall highest prevalence (68%), while pigs harbouring *Haematopinus suis* showed the lowest (<1%). *Anaplasma* DNA was detected in 50% of pools analysed. In *L. vituli Anaplasma ovis* (or a closely related novel *Anaplasma marginale* genotype) was identified. *Anaplasma*-positivity of *H. suis* suggests that pigs may extend the reservoir and/or host spectrum of relevant species. *Anaplasma*-infected *L. stenopsis* pools show for the first time that caprine anaplasmosis is endemic in Hungary. *Rickettsia* spp. were demonstrated from *Linognathus* spp. and *H. eurysternus*. No haemotropic mycoplasmas were detected in any samples. In conclusion, this is the first molecularly confirmed report of bovine and ovine *Anaplasma* spp. in *L. vituli*, *L. stenopsis* and *H. suis*. The present results suggest that phthirapterosis of domestic animals deserves more attention, and lice should be evaluated among the broad range of potential vectors of arthropod-borne pathogens.

1. Introduction

The blood-sucking lice (Phthiraptera: Anoplura) are permanent, host-specific ectoparasites of mammals. Their veterinary significance may be twofold. First, they can be responsible for economical losses by inducing pathophysiological changes in their hosts, i.e. weight loss, hide damage and mild to severe anaemia – the latter especially in cattle (Nelson et al., 1970; Gibney et al., 1985; Otter et al., 2003). Secondly, as vectors, they can transmit louse-borne pathogens (viruses, bacteria, fungi and protozoa) to susceptible hosts. In this respect, their biological vector role usually means persistent development of disease agents in their gut cells, with or without consequent passing in their faeces. In either case, they do not inoculate the pathogen during a next blood-sucking, but it is rubbed into the host's

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skin inadvertently (Durden and Lloyd, 2009). Alternatively, if lice are short-term mechanical vectors, it becomes possible that within hours of their former feeding they inoculate disease agents attached to their mouthparts (Shope, 1940; Crystal, 1958). A prerequisite for both types of transmission is that lice transfer between hosts, which is an integral aspect of their behaviour (Durden and Lloyd, 2009).

Reports on pediculosis of domestic animals are scarce, and prevalence data from several parts of Europe are lacking or outdated. Correspondingly, in veterinary medicine the epidemiological significance and vector potential of anopluran lice also appears to be underestimated or insufficiently evaluated. In part, this may be due to the regular and widespread use of broad spectrum endectocides that are given to kill parasites other than lice. However, it must be kept in mind that in areas with lower standards of livestock management lice and their vector role should not be neglected or discounted: on the contrary, they may represent emerging problems (Otter et al., 2003; Bisdorff et al., 2006). Therefore this study was undertaken to elucidate the occurrence of sucking lice of ruminants and pigs in Hungary, and to contribute to the general knowledge on the potential vector role of relevant species.

2. Materials and methods

2.1. Sample collection

Adults and nymphs of blood-sucking lice have been removed from domestic animals kept indoors during the winter time. Sampling was performed from cattle on four farms, from goats on three farms, and from pigs in one farm, during March of 2009 in central and north-eastern Hungary. A total of 173 cattle, 50 goats and 350 pigs were included in the study. All animals were immobilized and their whole body surface scrutinized for the presence of lice. During sampling the predilection sites for macroscopically distinguishable lice on cattle (*Haematopinus* and *Linognathus* sp.) and goats (*Linognathus* sp.) were noted. The age of cattle was also recorded. Specimens collected from the same animal were stored in one vial. Species identification was completed under stereomicroscope according to Zlotorzycka et al. (1974) and Kim et al. (1986). Lice belonging to one species and removed from the same host individual were stored together in 70% ethanol until evaluation.

2.2. Pooling and DNA extraction

A maximum of ten lice per animal belonging to the same species were pooled together. All individuals in the pool were washed sequentially in detergent-containing water, in tapwater and in distilled water. Air-dried lice were subsequently minced with pointed scissors at the bottom of Eppendorf-tubes, in 100 μl of phosphate-buffered saline (PBS). DNA was extracted using the QIAamp DNA blood mini kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions and including an overnight digestion step (incubation at 56 °C for at least 8 h) with tissue lysis buffer and Proteinase-K (QIAGEN, Hilden, Germany).

2.3. Molecular investigations

The DNA contents of each sample were preliminarily assessed by an 18S rRNA gene TaqMan real-time PCR (Applied Biosystems, Rotkreuz, Switzerland) as described previously (Boretti et al., 2009). In order to evaluate the presence of bacterial agents (Rickettsiales: Anaplastamaceae and Rickettsiaceae; Mycoplasmatales: Mycoplasmataceae) conventional PCR was performed for the detection of *Anaplasma* spp. (screening for the 16S rRNA gene, positives evaluated for the *msp4* gene) and real-time PCR assays were run for the demonstration and quantification of *Rickettsia* (23S and gltA genes) and haemotropic *Mycoplasma* spp. (16S gene) as published (Brown et al., 2001; de la Fuente et al., 2005; Boretti et al., 2009; Willi et al., 2009). Negative control reactions were included using the same procedures and water instead of DNA to monitor contamination of the PCR. Amplified fragments of anaplasma DNA were resin purified (Wizard, Promega) and cloned into the pGEM-T vector (Promega) for sequencing both strands by double-stranded dye-termination cycle sequencing (Secugen SL, Madrid, Spain). At least two independent clones were sequenced for each PCR.

2.4. Statistical analysis

Sample prevalence data were analysed by using Fisher's exact test. Louse burdens were compared with Student's *t*-test. Differences were regarded significant when $P < 0.05$.

3. Results and discussion

Altogether 1182 sucking lice were collected (Table 1). All species occurring on relevant hosts in the temperate zone were found (Durden and Lloyd, 2009). However, this is the first report of *Solenopotes capillatus* from Hungary (Piotrowski, 1970). Comparing the anopluran species of cattle, *S. capillatus* was the rarest (Table 1), with highly overdispersed intensities of infestation (three cows with only one specimen, and one with 37). *Linognathus vituli* individuals were significantly more abundant than *Haematopinus eurysternus* and *S. capillatus* ($P < 0.001$). Cattle infested with *L. vituli* also predominated ($P < 0.001$). The order of importance for the three bovine blood-sucking louse species – based on abundance data and prevalence (extensity of infestation, Table 1) – is opposed to what was reported for Germany, where *S. capillatus* was the most frequently encountered species, followed by *H. eurysternus*, and *L. vituli* as the rarest (Matthes et al., 1991).

L. vituli was represented by a significantly lower mean individual count per host (Table 1), than *H. eurysternus* ($P = 0.027$), unlike in another study from Canada (Colwell et al., 2001). Regarding co-infestations of cattle, *H. eurysternus* and *S. capillatus* were significantly more prone to be in concurrence with another species (37.5 and 50%, respectively) than *L. vituli* (6.4%; $P = 0.019$). On calves only *L. vituli* was found. No significant difference was observed between the mean age of *L. vituli*, *H. eurysternus* and *S. capillatus*-infested animals (data not shown). However, the mean louse count of *L. vituli* was significantly higher ($P = 0.001$) on calves (18.1 ± 18.4), than on full-grown cattle (4.5 ± 3.4).

Table 1
Ecological data of blood-sucking lice from ruminants and pigs, and results of their molecular investigation.

Host	Louse sp.	Relative species abundance: individuals of one/all louse species of the same host (%)	Extensity of infestation: no. of hosts infected/all (%)	Intensity of infestation (mean ± SD lice on one host)	*Anaplasma marginale*, *A. ovis msp4* PCR positives/all tested	Sequencing (no. of samples) accession number	*Rickettsia helvetica*: 23S PCR positives/all tested (CT value; no. of copies)	Other *Rickettsia* spp.: *gltA* PCR positives/all tested (CT value)
Cattle	*Linognathus vituli*	**56.84**%[a] (440/774)	**27.17**%[a] (47/173)	8.98 ± 12.63[a]	**14/35**	*A. ovis* (4) HM063433	**1/47** (43.49; 1)	0/47
	Haematopinus eurysternus	**37.98**%[b] (294/774)	**4.62**%[b] (8/173)	36.75 ± 26.43[b]	0/7	–	0/8	**1/8** (36.56)
	Solenopotes capillatus	**5.17**%[c] (40/774)	**2.3**%[b] (4/173)	10 ± 15.56[a,b]	0/3	–	0/4	0/4
Goats	*L. stenopsis*	**100**% (375/375)	**68**% (34/50)	11.03 ± 7.17	**22/27**	nd	**1/34** (36.69; 5)	**1/34** (32.09)
Pigs	*H. suis*	**100**% (33/33)	**0.86**% (3/350)	11 ± 2.16	**1/2**	nd	0/3	0/3

Abbreviations: nd – not done; CT – threshold cycle.
[a,b,c]Values of cattle lice within a column followed by the same superscript are not statistically different ($P > 0.05$).

Predilection sites for *L. vituli* included the lateral aspect of the neck, the scapular region and the dewlap, whereas for *H. eurysternus* the dewlap and the dorsal edge of the neck; similarly, but adding to those reported previously (Watson et al., 1997). *Linognathus stenopsis* was more evenly distributed (sides of neck, shoulders, lateral abdomen and gluteal region). Goats infested with *L. stenopsis* had the overall highest, and pigs on which *Haematopinus suis* was found the lowest prevalence (Table 1), corresponding to the higher or lower veterinary significance of pediculosis in these host species. Low extensity of *H. suis* infection was also documented in Germany (Damriyasa et al., 2004).

Blood-sucking lice frequently transfer between hosts (Durden and Lloyd, 2009) and pierce the skin at different places each time they feed from the lumen of blood vessels (Lavoipierre, 1967). During this activity they can transmit disease agents to new susceptible individuals. In this respect lice of this study were evaluated for the presence of certain bacterial pathogens, i.e. to indicate their potential as vectors. None of the samples contained *Anaplasma phagocytophilum* or haemotropic mycoplasma DNA. However, based on *msp4* PCR results, 50% of anopluran louse pools were found infected with either *Anaplasma marginale* or *Anaplasma ovis* (Table 1). To the best of our knowledge, this is the first report of molecularly confirmed infections of *L. vituli*, *L. stenopsis* and *H. suis* with bovine and/or ovine *Anaplasma* spp. On the other hand, it was unexpected to find all *H. eurysternus* pools PCR negative, because these lice were collected from cattle seropositive for *A. marginale* (Hornok et al., 2007) and they were already recognized as potential vectors for this pathogen (Hofmann-Lehmann et al., 2004). Nevertheless, another study also failed to demonstrate *A. marginale* DNA in samples of bovine *Haematopinus* spp. (Reeves et al., 2006).

Interestingly, all sequences obtained from *L. vituli* corresponded to *A. ovis* or to a novel *A. marginale* genotype closely related to *A. ovis*. Since lice are host-specific ectoparasites, this result implies that most likely relevant cattle harboured this *Anaplasma* sp. According to data in the literature, small ruminants may be subclinically infected with *A. marginale*, while intact and healthy cattle are resistant to *A. ovis* (Kuttler, 1984). Therefore this is the first report to show that cattle may be susceptible to bacteria most closely related to *A. ovis*.

L. stenopsis pools positive for *A. marginale* and/or *A. ovis* represent the first data on the endemicity of caprine anaplasmosis in Hungary, since anaplasmosis was formerly reported only from cattle and sheep in the country (Hornok et al., 2007). Additionally, although the *Anaplasma* spp. detected in *H. suis* could not be sequenced (Table 1), this is the first account of data suggesting that pigs may belong to the host/reservoir spectrum of *Anaplasma* spp. formerly known to infect only ruminants. On the other hand, *Anaplasma* DNA found in *H. suis* may also represent a new genotype of *A. phagocytophilum*, a species which can infect wild boars and pigs (Hulinska et al., 2004; Torina et al., 2008). These results suggest that genetic variants of *Anaplasma* spp. may differ not only in pathogenicity (de la Fuente et al., 2005), but also in their host tropism.

Louse pools were also evaluated for the presence of *Rickettsia* spp. These bacteria can cause disease in humans and

domestic or wild animals, depending on the geographical occurrence of vectors and reservoirs. Nowadays the veterinary significance of rickettsioses becomes increasingly recognized (Hechemy et al., 2006). Four louse pools were *Rickettsia* PCR-positive. *R. helvetica* DNA was detected in *Linognathus* spp. while other *Rickettsia* spp. were found in *H. eurysternus* and *L. stenopsis* (Table 1). As with some *Anaplasma*-positive samples, the low pathogen DNA levels precluded sequencing. At the same time, contamination of PCR reactions was ruled out by appropriate controls. Although attempts were made, another study failed to find rickettsia-positivity in *Haematopinus* and *Linognathus* spp. (Reeves et al., 2005). Therefore this is the first report of *Rickettsia* spp. in lice from livestock animals. Lice have been demonstrated to be capable mechanical transmitters of virtually all microorganisms tested, including *Rickettsia* spp. (Raoult and Roux, 1999). Furthermore, it was experimentally proved that flea-borne and tick-borne rickettsiae may be not only acquired, but maintained and inoculated by sucking lice (Houhamdi et al., 2003; Houhamdi and Raoult, 2006). According to the explanation such an alternative vector-borne transmission is based on a non-specific association (Houhamdi et al., 2003). Therefore, the finding of *Rickettsia*-positive lice in the present study confirms that synanthropic animal and vector communities may contribute to an increased risk of exposure to zoonotic pathogens.

In conclusion, these results suggest that not only does pediculosis of domestic animals deserve more attention, but lice may be biological or mechanical vectors of more bacterial agents than previously thought, i.e. they should always be considered among the broad range of potential transmitters of arthropod-borne pathogens.

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