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Original paper

Rickettsiae among mustelids – new data from south-west Poland

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ABSTRACT. Information on the prevalence on *Rickettsia* spp. in free-ranging mustelids and their specific ectoparasites is scarce. However, stone martens (*Martes foina*), pine martens (*Martes martes*) and European badgers (*Meles meles*) are common predators in many regions of Poland. In the present study we used tissue fragments to determine *Rickettsia* prevalence in these carnivores by molecular biology techniques. In addition, we included a data on several species of invertebrates that commonly feed on badgers.

Keywords: pine marten, stone marten, European badger, rickettsiae

Introduction

Mammalian mesocarnivores play an essential role in a variety of ecosystems [1], for instance, as predators [2], scavengers [3], pray [4], ecosystems engineers [5] or reservoir of pathogens and parasites. They are involved in the circulation of viruses [6], bacteria [7], protozoans [8], fungi [9] and helminths [10,11] in wild and urban environments. It seems important to note that some species of Caniformia, such as stone marten (*Martes foina*), pine marten (*Martes martes*) and red fox (*Vulpes vulpes*), often appear in the vicinity of human settlements [12–14]. Also, the urbanization of European badgers (*Meles meles*) is still progressing [15,16]. Mustelids (Carnivora; Mustelidae), due to their hidden lifestyle, mainly nocturnal and unusual elusiveness, are relatively rarely the subject of eco-epidemiological research [17–19]. These carnivores are hosts for many tick [20,21] and flea species [20,22] that can act as vectors of arthropod-borne diseases, including rickettsiosis [23].

Notably, reports of *Rickettsia* infections among European badgers, stone martens or pine martens are very scarce, although, this bacteria has been studied in martens in some European countries such as Hungary [24] and Romania [25]. Therefore, the main aim of our study was to investigate the prevalence of *Rickettsia* spp. in three species of mustelids in southwestern Poland and assess the potential risk of their transmission, also to humans.

Materials and Methods

Sample collection

The animals obtained from Ruszów Forest District (51°24’00.1”N, 15°10’12.2”E) were shot during selective hunting expeditions between 2016 and 2020 (the predator control operation conducted as a part of the program to re-introduce the *Tetrao urogallus* – project LIFE11 NAT/PL/428). The district is located in the northwestern part of the Lower Silesia and is a part of the large, compact forest complex of the Bory Dolnośląskie Forest.

The tissue skin samples were obtained during post-mortem examination of stone marten (n=53), pine marten (n=27) and badger (n=53) and stored at −20°C until further analysis. Ectoparasites were obtained exclusively from a small number of studied badgers. Taxonomic determination of martens was performed based on characteristic

Table 1. Primers used for detection of *Rickettsia* spp. DNA

Step	Primers	Primer sequence	Product size (bp)
I	RpCs.877p	5' GGGGACCTGCCTGCTCACGGCGG 3'	381
	RpCs.1258n	5' ATTGCAAAAAGTACAGTGAACA 3'	
II	RpCS.896p	5' GGCTAATGAAGCAGTGATAA 3'	338
	RpCS.1233n	5' GCGACGGTATACCCATAGC 3'	

morphological features and confirmed by molecular analysis [27]. Ticks, fleas and chewing lice were identified using literature [28–30] and molecular biology techniques [31].

DNA extraction and PCR amplification

DNA was extracted using the GeneMATRIX Bio-Trace DNA Purification Kit (EURx, Poland) for skin samples and DNeasy Blood & Tissue Kit (Qiagen) for ectoparasites according to the manufacturers' instructions. DNA concentrations was determined with a NanoPhotometer series NP80 (A-BioTech, Poland).

The presence of *Rickettsia* spp. DNA was screened through amplification of a fragment of the *gltA* gene, which has conserved regions shared by all known *Rickettsia* species in nested PCR, using two primer sets, RpCS.877p-RpCS.1258n and RpCS.896p-RpCS.1233n [26] (Tab. 1). Each PCR reaction for *Rickettsia* was performed with 2× PCR Mix Plus (A&A Biotechnology, Poland) in a total reaction volume of 12.5 µl containing 0.6 µl of each primer (10 µM) and 2 µl (first reaction) or 1 µl (second reaction) of the DNA sample. DNA of *Rickettsia helvetica* isolated from *Ixodes ricinus* was used as positive control. In addition, negative control with nuclease-free distilled water, in the absence of template DNA, was included for each PCR reaction. The PCR products were subjected to electrophoresis on a 1.5% agarose gel, stained with SimplySafe stain (EURx, Poland), and visualized by UV transillumination.

The selected amplicons were purified using Exo-BAP (EURx, Poland) and directly sequenced in both directions by Macrogen (Amsterdam, the Netherlands) with the primers used for DNA amplification. Finally, the nucleotide sequences obtained in this study were edited using DNA Baser Sequence Assembly software (Heracle BioSoft SRL, Romania) and compared with each other and with corresponding sequences registered in GenBank using the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) program.

Results

The skin tissue samples obtained from the examined mustelids species were analyzed for the prevalence of *Rickettsia* spp. using the nested PCR method and commonly used marker i.e. partial *glt*A gene and the overall prevalence was estimated at the level of 6% (8 /133).

The analysis of European badgers confirmed the presence of *Rickettsia* spp. DNA in examined skin biopsies with the prevalence 3.77%. The sequence

Table 2. Species and number of ectoparasites collected from badgers

Family	Species/stage	Number of specimens
Ixodidae	*Ixodes hexagonus* nymphs, males, females	17
	Ixodes ricinus larva, nymph, female	5
	Ixodes cf. rugicollis nymph	1
Trichodectidae	*Trichodectes melis* nymphs, females, males	122
Ceratophyllidae	*Paraceras melis* females, males	9
Vermipsyllidae	*Chaetopsylla trichosa* male	1

analysis revealed that one obtained sequence was identical to the sequences derived from raccoons (*Procyon lotor*) (ON157071 and ON157070) from the same area of Poland [32] and one sequence showed 98.8% homology to *Rickettsia* sp. endosymbiont isolated from silverleaf whitefly (*Bemisia tabaci*) (CP016305, KX645661). The prevalence of *Rickettsia* spp. in the pine marten was calculated on level 7.41%. All obtained sequences show the similarity above 98% to *Rickettsia* endosymbiotes detected in skin samples (ear) of raccoon from Poland (ON157074) and striped field mouse from Slovakia (KX051330). The DNA of *Rickettsia* spp. was also detected in 7.55% of the stone marten. Pathogens similar to *Rickettsia felis* (n=1; 100% homology to *R. felis* sequences: MF374381, MT460037 from cat fleas and ON053297 from *I. ricinus* tick) and *Rickettsia raoultii* (n=1; 98.9% homology to *R. raoultii* sequences: MT178338, LC229632 from ticks) were identified in the tissues of this species at the first time. The two remaining detections were rickettsial endosymbionts similar to sequences JQ925613 and MF156682 isolated from insects of Hybotidae and Chrysopidae family, respectively.

Additionally, data on species and number of collected ectoparasites obtained from 32 badgers is presented in table 2. Despite examining a large number of arthropods for *Rickettsia* infection, only a flea *Paraceras melis* collected from one badger was positive. The obtained sequence of *Rickettsia* sp. was similar in 88.2% to an endosymbiont from a parasitic fly belonging to the family Nycteribiidae (KT751160). The rest of the ectoparasites turned out to be negative.

Discussion

The role of wildlife, including synanthropic species, as a reservoir for *Rickettsia* spp. is studied extensively worldwide [33–35]. The stone marten (*M. foina*), pine marten (*M. martes*) and European badger (*M. meles*) are a widespread carnivore species in Poland. Additionally, these species can be legally hunted, e.g., badger lard is used in Polish folk medicine [36]. The impact of the mustelids on the ecology of pathogens of the *Rickettsia* genus has been unclear. This study showed the low prevalence levels of *Rickettsia* spp. in tissue samples of free-living mustelids in Poland. Our results are insufficient to state unequivocally that the studied animals are reservoirs or specific hosts to *Rickettsia*. We suggest that they are likely dead-end hosts for these bacteria. However, seems to be interesting and worth a deeper analysis, the statement of the same rickettsia endosymbionts in tissues of different wild living carnivores i.e. raccoon, badger, martens; mainly in the light of the theory according to which the endosymbionts are specialists completely adapted to ticks [32].

The detection of *Rickettsia felis* is unusual for Caniformia and has been confirmed in individual findings, e.g., in raccoons [37] or dogs [38]. Contact with animals is essential in the transmission of these pathogens [38]. However, no human infections with this species have been reported in Poland up to now. Certainly, fleas are the reservoir of this bacterium [39], especially species such as *Ctenocephalides felis*, and *Archaeopsylla erinacei* [39,40]. Nevertheless, their occurrence in wild mammals in Poland is poorly known [29,41]. *Rickettsia raoultii* is transmitted by ticks [42] as *Dermacentor reticulatus* [42,43], which was found on badgers and martens in Poland [20]: bacteria sspecies. However, it is the first detection of this bacteries species in this group of predators. It is noteworthy that information about systemic *Rickettsia* infection in wild mustelid mammals does not appear in the literature. Further research could confirm their involvement in the dilution effect [46–48] as incompetent hosts of pathogenic *Rickettsia*. Additionally, the increasing population of wild living carnivores, which act as a reservoir of vector-borne zoonotic pathogens should to be considered in future investigations, taking into account their colonization of urban areas.

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