Ticks and Tick-borne Diseases xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Short communication

High diversity of blood-associated parasites and bacteria in European wild cats in Bosnia and Herzegovina: A molecular study

Adnan Hodžić[a,1], Amer Alić[b,1], Georg Gerhard Duscher[a,*]

[a] Institute of Parasitology, Department of Pathobiology, University of Veterinary Medicine Vienna, Vienna, Austria
[b] Department of Pathology, Faculty of Veterinary Medicine, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

ARTICLE INFO

Keywords:
Babesia
Cytauxzoon
Hepatozoon
Hemotropic mycoplasma
Felis silvestris silvestris
Bosnia and Herzegovina

ABSTRACT

Wild felids may play a significant role in the transmission of various pathogens to domestic cats, in particular, but also to other animals and humans. However, since data on the occurrence of blood-borne organisms in European wild cats (*Felis silvestris silvestris*) are scarce, the present study aimed to provide an insight into the genetic diversity of the agents carried by this sylvatic species in Bosnia and Herzegovina and to elucidate their pathogenic impact. Tissue samples from 18 adult wild cats were collected and examined by PCR and histopathology. Five species of apicomplexan parasites belonging to three genera (*Babesia* sp., *Cytauxzoon* sp., *Hepatozoon silvestris*, *H. felis*, *Hepatozoon* sp.), as well as two different sequence types of undescribed hemotropic mycoplasmas (designated as type A and type B), were identified in 15 animals (83%). Histopathology revealed no relevant lesions associated with any of the agents detected. The results clearly showed that European wild cats can harbour a broad range of blood-associated parasites and bacteria. However, further studies are required to investigate the possible implication of hematophagous arthropod vectors in their transmission and to clarify the true pathogenic significance of these organisms. Direct transmission of the agents by bites should also be considered as an alternative, non-vectorial route of transmission in wild cats.

1. Introduction

The European wild cat (*Felis silvestris silvestris*) is the most abundant and widespread wild felid species in Europe, with populations living in sympatry with domestic cats (*Felis catus*) throughout the continent (Otranto et al., 2015). The sympatric lifestyle has led to various degrees of introgression (i.e. gene exchange) in different populations of wild cats in Europe, to nearly complete loss of genetic purity and cryptic extirpation in some countries (Mattucci et al., 2016). The close interface between these two genetically related feline species also results in the bidirectional exchange of arthropod vectors and the pathogens they transmit (Otranto et al., 2015). This had further been emphasized by molecular confirmation of *Hepatozoon silvestris*, a novel species primarily infecting European wild cats (Hodžić et al., 2017), in the blood of a domestic cat (Giannelli et al., 2017). In the recent past, the wild cats came into the focus of the scientific community as they have been acknowledged as potential reservoirs of different pathogenic agents (Otranto et al., 2015; Gallusová et al., 2016). However, scientific data on their blood pathogens are scanty and limited to case reports, often confined to a single parasite species. The lack of available information most probably reflects the difficulties in obtaining the samples due to the wild cat's secretive life and its small population size (Otranto et al., 2015), which highlights the importance of such studies. Therefore, reports on the parasitic spectrum affecting the wild cat population in a certain area are crucial for the estimation of potential risks of infections, not only for sympatric domestic animals but also for humans (Napoli et al., 2016).

In the present study, we investigated the occurrence and genetic diversity of blood-associated parasites and bacteria in European wild cats from Bosnia and Herzegovina. In addition, histopathology of different tissues was performed in order to elucidate the pathogenic effect of the identified agents on this sylvatic species.

2. Material and methods

2.1. Animals, sample collection and histopathology

From 2011–2017, the carcasses of 18 adult European wild cats were collected from eight municipalities in Bosnia and Herzegovina (Table 1). Apart from two animals killed by shepherd dogs, all others

* Corresponding author at: Institute of Parasitology, Department of Pathobiology, University of Veterinary Medicine, Vienna, Veterinaerplatz 1, 1210 Vienna, Austria.
E-mail address: georg.duscher@vetmeduni.ac.at (G.G. Duscher).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.ttbdis.2018.01.017
Received 7 August 2017; Received in revised form 11 December 2017; Accepted 24 January 2018

Table 1
Data on origin and sex of the European wild cats and blood-associated agents detected by PCR in different tissue samples.

Lab code	Origin	Sex	Sample			*Babesia*	*Cytauxzoon*	*Hepatozoon*	*Mycoplasma*
			B	S	H				
115A/16	Bosanski Petrovac	F	N/A	x	x	neg	*Cytauxzoon* sp. ^{S,H}	Already published (Hodžić et al., 2017)	neg
115B/16	Bosanski Petrovac	M	N/A	x	x	neg	*Cytauxzoon* sp. ^{S,H}		neg
115C/16	Bosanski Petrovac	M	N/A	x	x	neg	*Cytauxzoon* sp. ^{S,H}		neg
115D/16	Bosanski Petrovac	F	N/A	N/A	x	neg	*Cytauxzoon* sp. ^{H}		neg
152/16	Bihać	M	N/A	x	x	neg	*Cytauxzoon* sp. ^{S,H}		neg
382/15	Bosanski Petrovac	M	N/A	x	x	neg	neg		neg
431/15	Gornji Vakuf	F	N/A	N/A	x	neg	*Cytauxzoon* sp. ^{H}		neg
53/11	Goražde	F	N/A	N/A	x	neg	neg		neg
170/16	Odžak	F	N/A	x	x	neg	neg		neg
326/16	Maglaj	M	N/A	x	x	neg	neg	*H. felis* ^{S,H}	neg
331A/16	Bosanski Petrovac	F	x	x	x	neg	*Cytauxzoon* sp. ^{B,S,H}	*Hepatozoon* sp. ^{B,S,H}	neg
331B/16	Bosanski Petrovac	M	x	x	x	neg	neg	*H. felis* ^{B,S,H}	*Mycoplasma* sp. type A ^{B,S}
351/16	Olovo	M	x	x	x	neg	neg	*H. felis* ^{B,S,H}	*Mycoplasma* sp. type A ^{B,S}
412/16	Bosanski Petrovac	M	N/A	x	N/A	neg	neg	*H. silvestris* ^{S}	neg
53A/17	Bosanski Petrovac	M	x	x	x	neg	neg	*Hepatozoon* sp. ^{B}	*Mycoplasma* sp. type A ^{H}
53B/17	Bosanski Petrovac	F	x	x	x	neg	*Cytauxzoon* sp. ^{B,S,H}	*H. silvestris* ^{B,S,H}	neg
86/17	Bosanski Petrovac	F	N/A	x	x	*Babesia* sp. ^{S,H}	*Cytauxzoon* sp. ^{S,H}	neg	*Mycoplasma* sp. type B ^{S}
90/17	Srebrenik	F	x	x	x	neg	*Cytauxzoon* sp. ^{B,S}	neg	neg

N/A – not available, tissue was not collected; x – tissue was collected and tested by PCR; neg – all tissues collected were PCR negative.
Tissue: B – blood, S – spleen, H – heart.

were killed by licensed hunters in line with federal hunting laws and regulations. The wild cat species differentiation was done based on the morphometric and pelage characters (Kitchener et al., 2005). Different tissues were sampled at necropsy and used for histopathology and molecular analyses. If available, the blood was additionally taken from the hearts and/or large blood vessels using a syringe. Samples from nine individuals tested in our previous study, which focused solely on *Hepatozoon* species (Hodžić et al., 2017), were also included in the present one. Prior to the necropsy, all animals were checked for the presence of ectoparasites.

For histopathology, samples of spleen, heart, lungs, brain, and muscle tissues were processed by standard histological techniques and stained with haematoxylin and eosin. Stained tissue cross-sections were observed for pathologic changes by light microscopy (Olympus BX51 microscope, Japan).

2.2. Molecular and phylogenetic analyses

In the current study, blood, spleen and heart tissue samples were tested by molecular techniques. Genomic DNA was extracted with a High Pure PCR Template Preparation Kit (Roche Diagnostics, Germany) from the tissues of all 18 animals. The DNA was subjected to conventional PCR amplifications to detect vector-borne pathogens listed in Table 2. However, the samples from only nine out of 18 animals were checked for *Hepatozoon* spp. (Table 2), and the results from the remaining ones are already published (Hodžić et al., 2017). The resulting amplicons were sequenced in both directions by the commercial company (Microsynth, Austria) using the same primers as for PCRs. To investigate the phylogenetic relationships among hemotropic mycoplasmas, the 16S rRNA nucleotide sequences derived from the European wild cats were compared to those selected from GenBank® database. A Neighbor-Joining tree was calculated based on the Tamura 3-parameter (TN92 + G) model implemented in MEGA 7 (Kumar et al., 2016).

Representative nucleotide sequences of the agents found in this study have been submitted to GenBank® and are available with the following accession numbers: *Babesia* sp. (MF614153), *Cytauxzoon* sp. (MF614154), *H. silvestris* (MF614155), *H. felis* (MF614156), *Hepatozoon* sp. (MF614157), *Mycoplasma* sp. type A (MF614158) and *Mycoplasma* sp. type B (MF614159).

Table 2
Primer sets and protocols used for PCR amplification of vector-borne agents.

Target organism	Genetic marker	Primer sequences (5′→3′)	Product size (bp)	Reference
Babesia/Hepatozoon/Cytauxzoon	18S rRNA	**BTH-1F**: CCTGAGAAACGGCTACCACATCT **BTH-1R**: TTGCGACCATACTCCCCCCA	720/750	Zintl et al. (2011)
		Nested	590/610	
		GF2: GTCTTGTAATTGGAATGATGG **GR2**: CCAAAGACTTTGATTTCTCTC		
Hepatozoon spp.	18S rRNA	**H14Hepa18SFw:** GAAATAACAATACAAGGCAGTAAAATGCT **H14Hepa18SRv:** GTGCTGAAGGAGTCGTTTATAAAGA	620	Hodžić et al. (2015)
Leishmania infantum	ssu-rRNA	**Lei70L:** CGCAACCTCGGTTCGGTGTG **Lei70R:** CGCGGTGCTGGACACAGGGTA	345	Spanakos et al. (2002)
Anaplasmataceae	16S rRNA	**EHR16SD:** GGTACCYACAGAAGAAGTCC **EHR16SR**: TAGCACTCATCGTTTACAGC	345	Brown et al. (2001)
Bartonella spp.	*gltA*	**BhCS.781p**: GGGGACCAGCTCATGGTGG **BhCS.1137n**: AATGCAAAAAGAACAGTAAACA	379	Norman et al. (1995)
Rickettsia spp.	*gltA*	**RpCS.877p:** GGGGGCCTGCTCACGGCGG **RpCS.1258n:** ATTGCAAAAAGTACAGTGAACA	381	Regnery et al. (1991)
Hemotropic mycoplasma	16S rRNA	**HBT-F**: TACGGCCCATATTCCTACG **HBT-R**: TGCTCCACCACTTGTTCA	600	Criado-Fornelio et al. (2003)