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Tick-borne pathogens in the European polecat, *Mustela putorius* and in attached *Ixodes hexagonus* ticks from Germany

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ABSTRACT

The European polecat, *Mustela putorius*, occurs almost throughout Europe. However, there is a lack of data on the ectoparasite fauna and the potential role in the circulation of tick-borne pathogens (TBP) of this mustelid species. The aim of this study was to investigate whether *M. putorius* contributes to the maintenance of TBP in Germany. DNA samples extracted from spleen tissue of 117 *M. putorius*, which had been collected mainly in North-western Germany from 2012 to 2015, were tested by real-time and conventional PCRs for *Anaplasma phagocytophilum*, *Babesia* spp., *Bartonella* spp., *Candidatus* Neoehrlichia mikurensis (CNM) and *Hepatozoon* spp. In addition, 200 randomly selected engorged *Ixodes hexagonus* ticks (100 females and 100 nymphs) collected from 39 of the 88 *M. putorius* were tested for these TBPs, except for *Hepatozoon* spp., and additionally for *Borrelia* spp. and *Rickettsia* spp. Three of six pathogens were detected in the spleen tissue of the 117 *M. putorius*: *A. phagocytophilum* (n = 5; 4.3%), *Babesia* cf. *odocoilei* (n = 1; 0.9%) and CNM (n = 1; 0.9%), including one case of co-infection (*A. phagocytophilum* and CNM). *Ixodes hexagonus* ticks tested positive only for *Bartonella* spp. (26/200 ticks; 13.0%) which were detected exclusively in adult female ticks. Sequencing revealed the presence of *Bartonella taylorii* and uncultured *Bartonella* spp. The results suggest that *M. putorius* neither seems to serve as the main reservoir nor plays an important role in maintaining TBPs in Germany but may rather contribute to the dilution of these pathogens. However, *M. putorius* contributes to the maintenance of tick populations, especially of *I. hexagonus*. The high prevalence of *Bartonella* spp. in *I. hexagonus* ticks may suggest a certain importance of this tick species in the maintenance of these bacteria in nature.

1. Introduction

The European polecat, *Mustela putorius,* also known as the Western polecat, is a carnivore belonging to the family Mustelidae. It has a widespread distribution range and occurs across Europe up to the Ural Mountains, except for Ireland, northern Scandinavia and most of the Adriatic coast. The population in the eastern part of Europe is believed to be large and stable; however, new data show that in most of its distribution range, including Germany, the population appears to be declining (Croose et al., 2018).

The European polecat is found in a wide variety of habitats including rural areas, agricultural land, woods in riparian zones, forest edge, and mosaic environments (Rondinini et al., 2006). It is a generalist and opportunistic carnivore which consumes a wide spectrum of both terrestrial and aquatic preys depending on the habitat and availability of prey (Birks and Kitchener, 1999). An important component of the European polecat's diet are mouse-like rodents, known to be reservoirs for several tick-borne pathogens (TBPs); the European polecats hunt for them also around their nests which, additionally, can be used as resting places during the day, which thus creates possibility of being exposed to nidicolous ticks and TBPs (Gorelova et al., 1995; Silaghi et al., 2012a). Additionally, the European polecats are hosts for ticks, mainly for *Ixodes hexagonus* but also for *I. ricinus* (Arthur, 1953; Santos-Silva et al., 2011; Mihalca et al., 2012) and the latter are significant vectors for many pathogens of medical importance, e.g. *Borrelia burgdorferi* s.l., *Anaplasma phagocytophilum, Rickettsia* spp., *Babesia* spp. and *Candidatus* Neoehrlichia mikurensis (CNM) (Estrada-Peña et al., 2017). Recently, also *Bartonella* spp. were detected in ticks collected from wild mammals (Silaghi et al., 2016). *Hepatozoon* spp. infect a wide range of vertebrate hosts, including mustelids (Hodžić et al., 2018).

Research on TBPs in mustelids have been conducted in Europe before (e.g. Gherman et al., 2012), however, there is a general lack of

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Table 1
The prevalence of tick-borne microorganisms in the European polecats and ticks collected from them.

Samples	Tick-borne microorganisms Number of positive (prevalence in %, 95%CI[a])						
	Anaplasma phagocytophilum	*Candidatus* Neoehrlichia mikurensis	*Babesia* spp.	*Bartonella* spp.	*Hepatozoon* spp.	*Borrelia* spp.	*Rickettsia* spp.
Mustela putorius spleen (n = 117)	5 (4.27%, 1.58–9.87)	1 (0.85%, 0.01–5.16)	1 (0.85%, 0.01–5.16)	0	0	not tested	not tested
Ixodes hexagonus							
only females (n = 100)	0	0	0	26 (26.0%, 18.36–35.42)		0	0
only nymphs (n = 100)	0	0	0	0	not tested	0	0
ticks in total (n = 200)	0	0	0	26 (13.0%, 8.98–18.42)		0	0

[a] Confidence interval.

knowledge about the role of the European polecat, *Mustela putorius* in the circulation of TBPs and, to the best of our knowledge, there are no previous data on TBPs from these carnivores from Germany. Therefore, the aim of this study was to examine the European polecats and their ticks in order to generate information on the potential role of this mustelid in the maintenance of selected TBPs.

2. Materials and methods

In the course of a study on the parasite fauna of *M. putorius* in Germany (Kretschmar, 2016), 118 polecats originating in six federal states in Germany were examined for external and internal parasites between 2012 and 2015, and spleen tissue samples from all animals were collected and preserved in 70% ethanol for later analyses. The dominant tick species (> 97%) parasitizing *M. putorius* was *I. hexagonus* and a random selection of collected ticks was chosen for further investigations (Kretschmar, 2016).

For this study, spleen tissue of 117 of the 118 polecats (one sample was excluded due to insufficient condition) and 200 engorged *I. hexagonus* ticks (100 adult female ticks and 100 nymphs, recovered from 39 of 88 *I. hexagonus*-infested polecats) were analyzed for the presence of TBPs.

2.1. DNA extraction

Both types of samples were preserved in 70% ethanol. Prior to DNA extraction, samples were washed twice for 30 min in PBS and dried on air. All samples were homogenized individually at 5000 rpm for 2 × 30 s in the Precellys®24 tissue homogenizer (Bertin Technologies, Montigny-le-Bretonneux, France) with 0.6 g of sterile ceramic beads (Peqlab Biotechnologie, Erlangen, Germany) and 600 µl of PBS (for spleen samples) or 1.0 g sterile steel beads (Peqlab Biotechnologie) and 500 µl of PBS (for tick samples). DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. All samples were processed separately. Quality of the DNA was measured with NanoDrop® 2000c (Peqlab Biotechnologie).

2.2. PCR screening for TBP

All spleen samples were tested individually. Tick samples were tested first in pools of three specimens, and individuals from positive pools were subsequently examined separately. Both types of samples were screened by a real-time PCR (qPCR) for *A. phagocytophilum* targeting the *msp2* gene with a product size of 77 bp (Courtney et al., 2004), for CNM by amplifying a 99 bp fragment of the *groEL* gene (Silaghi et al., 2012b), by conventional PCR for *Babesia* spp. targeting the *18S rRNA* gene (411–452 bp) (Casati et al., 2006), and for *Bartonella* spp. amplifying part of the *16S-23S rRNA* ITS (453–780 bp) (Maggi et al., 2006). In addition, spleen tissues were analyzed by conventional PCR for *Hepatozoon* spp. targeting the *18S rRNA* gene (660 bp; Criado-Fornelio et al., 2006; Inokuma et al., 2001), and ticks were examined by qPCR for *Borrelia* spp. targeting the *p41 flagellin* gene (96 bp) (Schwaiger et al., 2001) and for *Rickettsia* spp. targeting the *gltA* gene (70 bp) (Wölfel et al., 2008).

The products of the conventional PCRs were visualized by electrophoresis on agarose gels stained with HD Green® Safe DNA Dye (Intas Science Imaging, Göttingen, Germany). Randomly selected *Bartonella* spp. (n = 11) and all *Babesia* sp. (n = 1) products were purified using the NucleoSpin® and PCR Clean-up Kit (MACHEREY-NAGEL, Düren, Germany) according to the instruction of the manufacturer and sequenced commercially (Interdisziplinäres Zentrum für Klinische Forschung, Leipzig, Germany) with forward and reverse primers, and were further analyzed with the Bionumerics Software (Version 7.6.1. Applied Maths, Inc., Austin, TX, USA). Nucleotide sequences were compared with GenBank entries using BLASTn and deposited in GenBank under following accession numbers: MH477603–MH477610.

2.3. Data analyses

Confidence intervals (95%CI) for the frequency of TBPs in spleen tissue and ticks were determined by the modified Wald method using the Graph Pad Software (Graph Pad Software Inc., San Diego, CA., USA). The chi-square test was used to compare the independence of compared prevalence levels and Fisher's exact test for 1-df tests. The significance level (P) was set at 0.05.

3. Results

Three of six pathogens were detected in the spleen tissue of the 117 European polecats, *M. putorius* (Table 1): *A. phagocytophilum* (n = 5; 4.3%) and CNM (n = 1; 0.9%) of zoonotic importance (including one case of co-infection with *A. phagocytophilum* and CNM) as well as *Babesia* sp. (n = 1; 0.9%) later identified by sequencing as non-zoonotic *B.* cf. *odocoilei* (GenBank Accession Number MH477610) with 99% identity to GenBank Acc. No. KU351828. DNA of *Hepatozoon* spp. or *Bartonella* spp. was not found in any of the spleen samples. All PCR-positive mustelids originated in North Rhine-Westphalia, Germany. There was no difference in the frequency of the three pathogens in the spleen tissue of the polecats ($\chi^2 = 4.664$, df = 2, $P = 0.097$).

In *I. hexagonus* samples, only *Bartonella* spp. (Table 1) were detected in 26 of 200 (13.0%) tested ticks. *Bartonella* spp. were found only in female ticks (26/100) while all 100 tested nymphal ticks were negative ($P = 0.0002$). The *Bartonella*-positive ticks were recovered from 10 European polecats which originated from two federal states: North Rhine-Westphalia and Lower Saxony. Sequencing of 11 *Bartonella*

samples revealed the presence of non-zoonotic *Bartonella taylorii* (n = 7, GenBank Acc. No. MH477603 to MH477609) which showed 97–100% identity with GenBank Acc. No. AJ269788, and uncultured *Bartonella* spp. (n = 4) with 99–100% identity to KX267690.

4. Discussion

This study presents prevalences of arthropod-borne pathogens in European polecats and their ticks from Germany for the first time.

Mustela putorius is a small-sized Carnivora living in sylvatic surroundings as well as in urban areas. It is often found in human settlements and may even inhabit buildings (Zabala et al., 2005). Further, the European polecats are capable of migrating long distances and have a wide home range (Lodé, 2003). They are known to be parasitized by several species of ticks with *I. hexagonus* usually representing the most common one (Santos-Silva et al., 2011; Kretschmar, 2016). Thus they may function as a reservoir and may be a link to human infection for zoonotic tick-borne pathogens. *Ixodes hexagonus* is a common tick species in Central Europe, mostly parasitizing carnivores, such as foxes and dogs, and foremost hedgehogs (Dziemian et al., 2014; Najm et al., 2014; Claerebout et al., 2013).

In this study, *Bartonella* spp. were the only microorganisms found in *I. hexagonus* ticks. There is a previous report about *Bartonella schoenbuchensis* found in *I. hexagonus* from the Netherlands at low prevalence (2.2%) (Oldehinkel, 2010). To the authors' knowledge, the current study reports the occurrence of *B. taylorii* and uncultured *Bartonella* sp. in *I. hexagonus* for the first time. *Bartonella taylorii* and most uncultured Bartonellae are mainly rodent-associated and thought not to be pathogenic to humans (Harms and Dehio, 2012; Silaghi et al., 2016). In our study, only adult *I. hexagonus* ticks were positive for *Bartonella* spp., while all nymphal ticks (even though co-feeding with 23 *Bartonella*-positive females on the same 7 hosts) and spleens from the European polecats tested negative. This leads to the assumption that the *Bartonella*-positive *I. hexagonus* adults had taken up *Bartonella* in a previous life stage by feeding on an infected animal, e.g. on rodents rather than on mustelids. Transstadial survival of *Bartonella henselae* and *Bartonella birtelsii* in *I. ricinus* ticks has been previously verified (Cotté et al., 2008; Reis et al., 2011) which makes that hypothesis plausible. Known TBPs, such as *A. phagocytophilum*, CNM, *Babesia* spp., *Borrelia* spp., and *Rickettsia* spp. were not detected in *I. hexagonus* ticks in this study. A previous study from Germany reported a prevalence of 11% for tick-borne rickettsiae in *I. hexagonus* collected, however, from the European hedgehog (*Erinaceus europaeus*) (Speck et al., 2013). Another research on host-attached *I. hexagonus* from Germany reported the presence of *A. phagocytophilum* (3.9%), *Babesia* spp. (3%), *Borrelia* spp. (11.2%), CNM (5.9%) and *Rickettsia* spp. (44%) which were not detected in *I. hexagonus* from our study; however ticks were collected from dogs (Schreiber et al., 2014). High prevalence of *B. burgdorferi* s.l. (7.4–23%), *A. phagocytophilum* (26.8–37%), and *Rickettsia* spp. (18.9–41.5%) were observed also in *I. hexagonus* in Belgium and the Netherlands (Claerebout et al., 2013; Jahfari et al., 2017), however, one should take into account that those ticks were collected from hedgehogs and pets. In Great Britain, the prevalence of *B. burgdorferi* s.l. in *I. hexagonus* collected mostly from mustelids was less than 8% (Hubbard et al., 1998). In contrast, the prevalences for CNM and *Babesia/Theileria* obtained from *I. hexagonus* ticks in Europe were quite low (0.09–5.3% and 2.23–4.54%, respectively) (Najm et al., 2014; Davies et al., 2017; Jahfari et al., 2017). The reason for the absence of TBPs in *I. hexagonus* collected from mustelids in this study could be that the European polecats do not transmit TBPs to attached vectors or they could play a zooprophylactic role and thus contribute to magnify the dilution effect (Mannelli et al., 2012; Kjelland et al., 2011).

Studies on TBPs in mustelids have been carried out before in Europe (Gerrikagoitia et al., 2012; Gherman et al., 2012; García-Pérez et al., 2016; Wodecka et al., 2016; Hornok et al., 2017), however there is not much data on *M. putorius* and research has focused on this species just recently (Hofmeester et al., 2018). Three out of five TBPs were detected in the European polecats from this study, however, with very low prevalences. To our knowledge, this is the first report of *A. phagocytophilum* and CNM of zoonotic concern and non-zoonotic *B.* cf. *odocoilei*, in the European polecat from Central Europe. A previous study reported the European badger (*Meles meles*), also belonging to the family Mustelidae, positive for *Candidatus* Neoehrlichia sp. (FU98) and *Babesia* sp. Meles-Hu1 (Hornok et al., 2017). In another study from Spain, 183 specimens belonging to the family Mustelidae screened for *A. phagocytophilum* were all negative (García-Pérez et al., 2016). However most of these animals belonged to one species, *M. meles* (n = 130) and only 6 individuals were *M. putorius*. In our current study, *Bartonella* spp. DNA was not detected in the European polecats, which is in line with findings from Poland and Spain (Szewczyk et al., 2016; Gerrikagoitia et al., 2012). Interestingly, the *Babesia* species detected in our samples, *Babesia* cf. *odocoilei*, is cervid related. In this study, all the European polecats, which were either infected with TBPs or were hosts for infected ticks, originated in two federal states of Germany, North Rhine-Westphalia and Lower Saxony. However, these federal states were the dominant study areas, 106 individuals out of 118 were collected there.

5. Conclusions

This is the first report of *A. phagocytophilum*, CNM and *Babesia* cf. *odocoilei* in the European polecat, *Mustela putorius* from Germany. However, the low detection rate of TBPs found in this study suggests that European polecats in Germany do not play an important role in spreading and/or maintaining TBPs. Taking into account the pathogens screened for in this study, the low rate of detection of TBPs and the lack of evidence for transmission of pathogens (from hosts to ticks and from ticks to hosts), European polecats do not appear to serve as reservoirs for these TBPs but may rather contribute to their dilution. However, *M. putorius* appears to be an important host for *I. hexagonus* ticks, and thus may contribute to the maintenance of this tick species. The unexpectedly high frequency of *Bartonella* spp. in *I. hexagonus* ticks may suggest a certain importance of this tick species in the maintenance of these bacteria in nature.

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