

SHORT REPORT

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Molecular detection of *Babesia capreoli* and *Babesia venatorum* in wild Swedish roe deer, *Capreolus capreolus*

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Abstract

Background: The epidemiology of the zoonotic tick-transmitted parasite *Babesia* spp. and its occurrence in wild reservoir hosts in Sweden is unclear. In European deer, several parasite species, including *Babesia capreoli* and the zoonotic *B. venatorum* and *B. divergens* has been reported previously. The European roe deer, *Capreolus capreolus*, is an important and common part of the indigenous fauna in Europe, as well as an important host for *Ixodes ricinus* ticks, the vector of several *Babesia* spp. in Europe. Here, we aimed to investigate the occurrence of *Babesia* spp. in roe deer in Sweden.

Findings: Roe deer ($n = 77$) were caught and sampled for blood. *Babesia* spp. was detected with a PCR assay targeting the 18S rRNA gene. The prevalence of *Babesia* spp. was 52 %, and two species were detected; *B. capreoli* and *B. venatorum* in 44 and 7.8 % of the individuals, respectively. Infection occurred both in summer and winter.

Conclusions: We showed that roe deer in Sweden, close to the edge of their northern inland distributional range, are infected with *Babesia* spp. The occurrence of *B. venatorum* in roe deer imply that it is established in Sweden and the zoonotic implication of this finding should be regarded to a greater extent in future.

Findings

Background

The tick-transmitted intraerythrocytic parasite *Babesia* is maintained in zoonotic cycles between vertebrate hosts and tick vectors [1] and most zoonotic species are maintained in wildlife reservoirs. Various *Babesia* species have been detected in a wide range of different mammal species [1]. However, the occurrence in natural mammal hosts is still incompletely known for several zoonotic species [1]. The most prevalent zoonotic species, *Babesia microti*, is mainly reported from USA, and is maintained in various rodent reservoir hosts. In Europe, most human cases are attributed to the species *B. divergens* that is mainly associated with cattle. Moreover, also *B. venatorum* is known to infect humans in Europe [2, 3]. This species mainly

utilizes roe deer as reservoir hosts [4]. Primarily *Babesia* spp. are of veterinary importance and cause severe economic losses in cattle and other domestic animals worldwide [5–8]. However since several species are also known to infect humans, babesiosis is considered as an emerging zoonosis in parts of the world [1, 9–11].

In European deer several *Babesia* spp. has been reported, including *B. capreoli*, *B. venatorum* and *B. divergens* [4, 12, 13]. There are some uncertainties as to what extent *B. divergens* is found in deer. Several samples have previously been sequenced and published on public databases as *B. divergens* or “*B. divergens*-like”. Recent re-sequencing of such samples have however convincingly identified them as the closely related *B. capreoli* [13]. However, actual *B. divergens* is found in red deer from Ireland [12]. *Babesia capreoli* is highly similar to *B. divergens* and the two species only differ at three nucleotide positions at the 18S rRNA gene (99.83 % nucleotide similarity) [13]. The two species are considered as indistinguishable based on morphological characteristics, sequencing is therefore necessary to identify these species [12, 13].

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The roe deer (*Capreolus capreolus*) is the most common deer species in Sweden and occur in moderate to high population densities in the southern third of the country while population density gradually declines along a northern and western gradient to become completely absent in the north-western part of the mountain range [14]. *Babesia capreoli* has previously been reported from Swedish roe deer during the 1970's based on microscopic findings in blood samples [15]. However, no confirmation with molecular methods of these findings has been performed.

In the present study we investigated the prevalence of *Babesia* and diversity of species in roe deer in two sites in south-central Sweden by using molecular tools.

Sampling areas

Blood samples were taken from trapped roe deer at two different study sites, 150 km apart in southern Sweden; Bogesund (59°24'N, 18°12'E) is located at the inner reaches of the Stockholm Archipelago, surrounded by water and covered by highly productive mixed coniferous and deciduous forest and farmlands with high deer densities [16]. Grimsö Wildlife Research Area (59°60'N, 15°16'E) has a roe deer population with much lower density, and colder and longer winters due to its inland location. The area consists primarily of coniferous forest interspersed with bogs, mires and fens [17].

Roe deer capture

A total of 48 adult and juvenile roe deer (> 7 months old) were captured in box-traps from January to March 2014 and blood samples were taken from the jugular vein. Captured deer were marked using ear-tags with unique ID numbers and colours to keep track of individuals. In addition to the adult and juvenile animals a total of 38 neonate roe deer fawns (1–40 days old) were sampled from May 15th to July 3rd during 2013. The blood was collected from the fawns' tarsal vein.

Ethical approval

The marking and handling of roe deer in this study were approved by the Ethical Committee on Animal Experiments, Uppsala, Sweden (Approval Dnr: C302/2012).

Total nucleic acid extraction and PCR

Total nucleic acid (DNA as well as RNA) was extracted with the PAX gene Blood RNA kit (PreAnalytix, Qiagen/BD) following the manufacturer's recommendations (without adding DNase). Subsequently, cDNA was synthesized and the total DNA concentration was diluted to 10 ng/μl. PCR detection of *Babesia* spp. was carried out with the primers BJ1 5'-GTC TTG TAA TTG GAA TGA TGG-3' and BN2 5'-TAG TTT ATG GTT AGG ACT ACG-3' [18] with the cycling conditions as described in Casati et al. [18]. These primers amplify 411–452 bp of the

18S rRNA gene. PCR was performed in a GeneAmp® PCR System 9700 (Applied Biosystems). Sanger sequencing of the purified amplicons was performed and the obtained sequences were subjected to nucleotide BLAST searches on the NCBI database (<http://www.ncbi.nlm.nih.gov>).

Results and discussion

We show with molecular methods that two *Babesia* spp. occur in wild roe deer in Sweden, *B. capreoli* and *B. venatorum*. This is, to the best of our knowledge, the first molecular detection of *Babesia* spp. in any wildlife species in Sweden. In total we obtained 86 blood samples from 77 individual roe deer. Nine individuals were re-captured on separate occasions. Most of them within a month of the first capture. Out of the recaptured individuals, two went from uninfected to infected, one individual lost the infection and two individuals went from being infected with one *Babesia* spp. to being infected with the heterologous *Babesia* spp., demonstrating the dynamic nature of *Babesia* infection in wild animals. Calculations of prevalence is based on the first capture of each individual. In total 52 % of the individuals (40 out of 77) were infected with *Babesia* spp. The prevalence of *B. capreoli* in the individuals were 44 % (34/77) and the prevalence of *B. venatorum* was 7.8 % (6/77). *Babesia capreoli* is the dominating *Babesia* species in Swedish roe deer in the investigated areas with a remarkably high prevalence, however, consistent with findings in central Europe that also reported high infection rates in roe deer [19]. Detailed information about the number of samples from animals caught in the different areas and the number of infections are presented in Table 1. The obtained sequences were all 100 % identical to the published *B. capreoli* sequence FJ944827 and clearly differed from *B. divergens* sequence U16370. *Babesia capreoli* and *B. divergens* differ from each other by only three nucleotides on the 18S rRNA gene, on positions 631, 663 and 1637 [13]. The two first positions are included in the DNA fragment amplified by the primers used in this study [16].

The *B. venatorum* sequences from the Swedish roe deer were identical to sequence KF724377 found in a human infection in China [20]. The sequences obtained

Table 1 *Babesia* spp. infection in roe deer individuals

Location	Grimsö	Bogesund	Total
Fawns			
<i>B. capreoli</i>	11 % (2/18)	27 % (4/15)	18 % (6/33)
<i>B. venatorum</i>	17 % (3/18)	13 % (2/15)	15 % (5/33)
Adult/juv.			
<i>B. capreoli</i>	87 % (13/15)	52 % (15/29)	64 % (28/44)
<i>B. venatorum</i>	6.7 % (1/15)	0	2.3 % (1/44)
Total <i>Babesia</i> spp.	58 % (19/33)	48 % (21/44)	52 % (40/77)

in this study have been deposited in GenBank with the following accession numbers: *B. capreoli* KU145465 and *B. venatorum* KU145466.

Babesia capreoli is seemingly not able to infect cattle [13], and no reports of infections in humans have been published. This species is therefore not likely to be a threat to other species than the natural hosts [13]. Infection have been reported from several different deer species ([13] and references herein). Contrastingly, *B. venatorum* apparently has a broader host range and is also capable of infecting humans, it is also known to infect chamois (*Rupicapra rupicapra*) and ibex (*Capra ibex*) in the Alpine region [21], and has also been found in a captive reindeer (*Rangifer* sp.) in the Netherlands [22]. Several human cases of *B. venatorum* have been reported from Europe and more recently from China [2, 3, 23, 24] and the zoonotic potential of this species requires further investigation to correctly estimate risks for humans and perhaps domestic animals. *Babesia venatorum* has been reported from questing ticks in Norway [20] and a recent study on *Babesia* spp. in *Ixodes ricinus* in Sweden reported that 1 % of the investigated ticks were infected with *B. venatorum* [25]. Interestingly no ticks in that study were infected with *B. capreoli*, contrasting to the high prevalence found in roe deer in the present study. To better understand the importance of *Babesia* spp. as an infectious agent in Sweden there is a need to investigate the occurrence in several wild and domestic mammal species as well as in humans.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MOA, UAB, JC, PEL and PK conceived and designed the study. MC and JN performed fieldwork. MOA performed genetic analyses and drafted the manuscript. All authors contributed to the writing of the manuscript, and approved the final version.

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