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Identification of *Babesia capreoli* in an *Ixodes nipponensis* tick obtained from a Korean water deer

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Not applicable.

Abstract

Babesiosis is a tick-borne disease caused by intraerythrocytic protozoa. Despite the increasing acknowledgement that babesiosis represents a threat to animal and human health, to date there have been few studies focusing on the disease in the Republic of Korea (ROK). In the present study, we report a *Babesia capreoli* infection in an *Ixodes nipponensis* tick obtained from a Korean water deer (*Hydropotes inermis argyropus*). The tick was identified with polymerase chain reaction analysis as *I. nipponensis* (Japanese hard tick). A phylogenetic analysis based on the 18S rRNA gene sequences revealed that the isolate found in *I. nipponensis* belonged to the *B. capreoli* lineage and was distinct from the Asian, European, and North American lineages of *Babesia divergens*. Although our isolate belonged to the *B. capreoli* lineage it did not form a cluster with others isolates in the same lineage; this may be due to differences in the tick species that transmit *B. capreoli* or in the host species. We were unable to identify the reservoir host for our case of *B. capreoli* transmission, though regional ticks may be the primary vector. This study confirms the presence of *B. capreoli* in the ROK, and its presence suggests that further study is warranted to determine its prevalence and pathogenicity in wild and domesticated animals.

Keywords: *Babesia capreoli*; *Ixodes nipponensis*; Korean water deer; RNA, ribosomal, 18S; phylogeny

INTRODUCTION

Babesiosis is a tick-borne disease caused by intraerythrocytic protozoa belonging to the genus *Babesia* transmitted by genera *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes* and *Rhipicephalus* ticks [1]. The disease is highly pathogenic to ruminants, horses, pigs, dogs, cats, and in some cases, even humans [2]. A babesiosis infection may be asymptomatic, but symptoms may also be acute, including fever, anemia, hemoglobinuria, and potentially death in severe cases. The severity of the illness depends on the patient's immune status and the species of *Babesia* implicated [3, 4].

There are more than 100 different species of *Babesia*, some of which are zoonotic pathogens [5]. *Babesia microti* and *Babesia divergens* are known to infect humans as well as rodents and cattle [6, 7]. Wild animals are also known vectors of *Ixodes* spp. ticks [5, 8].

Wild cervids are often infected with some variety of *Babesia* spp., among which are some species that can infect humans. Reports from Europe indicate that *B. divergens*, *Babesia capreoli* and *Babesia venatorum* have been transmitted through wild cervids [9, 10]. *B. capreoli* is so similar to *B. divergens* that it was previously identified as *B. divergens* or *B. divergens*-like [11, 12]. Phylogenetic analysis is required to identify *B. capreoli*; in 2010 Malandrin et al. identified *B. capreoli* using three nucleotide difference positions at the 18S rRNA gene [13].

Despite its threat to animal and public health, babesiosis has rarely been studied in the Republic of Korea (ROK). In the present study, we report the identification of *B. capreoli* in engorged *Ixodes nipponensis* obtained from a Korean water deer (*Hydropotes inermis argyropus*).

MATERIALS AND METHODS

In the fall of 2018 a Korean water deer killed by a moving vehicle on a road in Jeollabuk-do was brought to the Jeonbuk Wildlife Rescue Center. An engorged female tick obtained from the deer was sent to the Animal Immunology Laboratory. The tick species was determined using primers for tick identification (Table 1). Blood and tissue samples were not collected from the Korean water deer.

The engorged frozen tick was cut into pieces and the DNA was isolated using DNeasy Blood & Tissue Kits (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions. Tick-borne pathogens, including species of *Anaplasma*, *Babesia*, *Ehrlichia*, *Rickettsia*, and *Theileria*, were screened via polymerase chain reaction (PCR) amplification and partial 18S rRNA gene sequence comparisons (Table 1). A negative control was included in the PCR assay in all experiments. PCR products were separated by electrophoresis on 1.5% agarose gel and visualized after ethidium bromide staining. The PCR products were purified using an AccuPrep® PCR Purification Kit (Bioneer, Daejeon, Korea) in accordance with the manufacturer's instructions, after which they were directly sequenced (Macrogen, Seoul, Korea).

Table 1. Primer sequences and PCR conditions used in this study

Target pathogen	Sequence (5′ to 3′)	Annealing (℃)	Amplicon size (bp)	Reference
Anaplasma spp.	TACCTCTGTGTTGTAGCTAACGC CTTGCGACATTGCAACCTATTGT	58	429	[14]
Babesia spp.	GTTTCTGMCCCATCAGCTTGAC CAAGACAAAAGTCTGCTTGAAAC	61	420–440	[15]
Ehrlichia spp.	CGGAATTCCTAGTGTAGAGG AGGAGGGATACGACCTTCAT	58	340	[14]
Rickettsia spp.	TAGGGGATGATGGAATTCCTA CCCCCGTCA ATTCCTTTGAG	58	252	[14]
Theileria orientalis	CACGCTATGTTGTCCAAGAG TGTGAGACTCAATGCGCCTA	55	830	[16]
Tick species	CTGCTCAATGATTTTTTAAATTGCTGTGG CCGGTCTGAACTCAGATCAAGTA	55	475	[17]

PCR, polymerase chain reaction.

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The nucleotide sequences thereby obtained were analyzed using BioEdit software (version 7.2.5) as well as the Basic Local Alignment Search Tool available from the National Center for Biotechnology Information database. To investigate the homologies of the *Babesia* gene, nucleotide sequences were aligned using ClustalX program and analyzed by direct comparison to reference sequences obtained from GenBank. Phylogenetic analysis based on *B. capreoli* partial18S rRNA gene sequences (356 bp). A tree was constructed using the MEGA7 software following the maximum likelihood method with Jukes-Cantor model (G+I). A bootstrap analysis was conducted with 1,000 replicates using MEGA7 software [18].

RESULTS

One adult tick obtained from the Korean water deer was identified with PCR analysis as *I. nipponensis* (Japanese hard tick). *I. nipponensis* is common throughout the ROK. Of the tick-borne pathogens tested for, only *Babesia* spp. was detected in the *I. nipponensis.* The *B. capreoli* infection was confirmed via PCR amplification and partial 18S rRNA gene sequence comparisons. The nucleotide sequence obtained in this study was ultimately submitted to the GenBank database with accession number MN170561.

Phylogenetic analysis based on the 18S rRNA gene sequences showed that the isolate found in *I. nipponensis* belonged to the *B. capreoli* lineage and was distinct from the Asian, European, and North American *B. divergens* lineages (Fig. 1). The *B. capreoli* lineage includes *B. capreoli*, *Babesia* sp., and *B. divergens*. According to a previous sequence analysis, *B. capreoli* is highly similar to *B. divergens*, with the sequence showing 99.83% homology. More specifically, the nucleotides at positions 631, 663, and 1637 in the 18S rRNA gene are different between the two species [19]; *B. capreoli* has G, T, and T, respectively, whereas *B. divergens* has A, A, and C. Knowing this, we were able to differentiate the two species. Two *B. divergens* (AY098643 and AY572456) and *Babesia* sp. (EU182596) isolates were confirmed to belong to the *B. capreoli* lineage, as opposed to *B. divergens*, as the nucleotides at these positions were identical to those of *B. capreoli*. Our sequence analysis revealed that our isolate shows 98.86%–99.47% homology with the *B. capreoli* lineage. And our sequence had 96.6%–99.6% identity to those of *B. capreoli* reported from Korea (Fig. 1). This may be due to a different tick species having transmitted the *B. capreoli* or a different host species.

DISCUSSION

We report a case of *B. capreoli* infection from an *I. nipponensis* tick in the ROK. *I. nipponensis* larvae and nymphs are found only among small mammals, whereas adults feed on larger animals; moreover, nymphs and adults frequently bite humans [20]. *I. nipponensis* is known to transmit potential zoonotic tick-borne pathogens in the ROK [20–22]. Although the present study does not confirm whether the Korean water deer was infected with *B. capreoli*, we cannot rule out this possibility. One recent study suggested that the Korean water deer as act as a reservoir host for *B. capreoli* [23]. As we were unable to identify the reservoir host for the

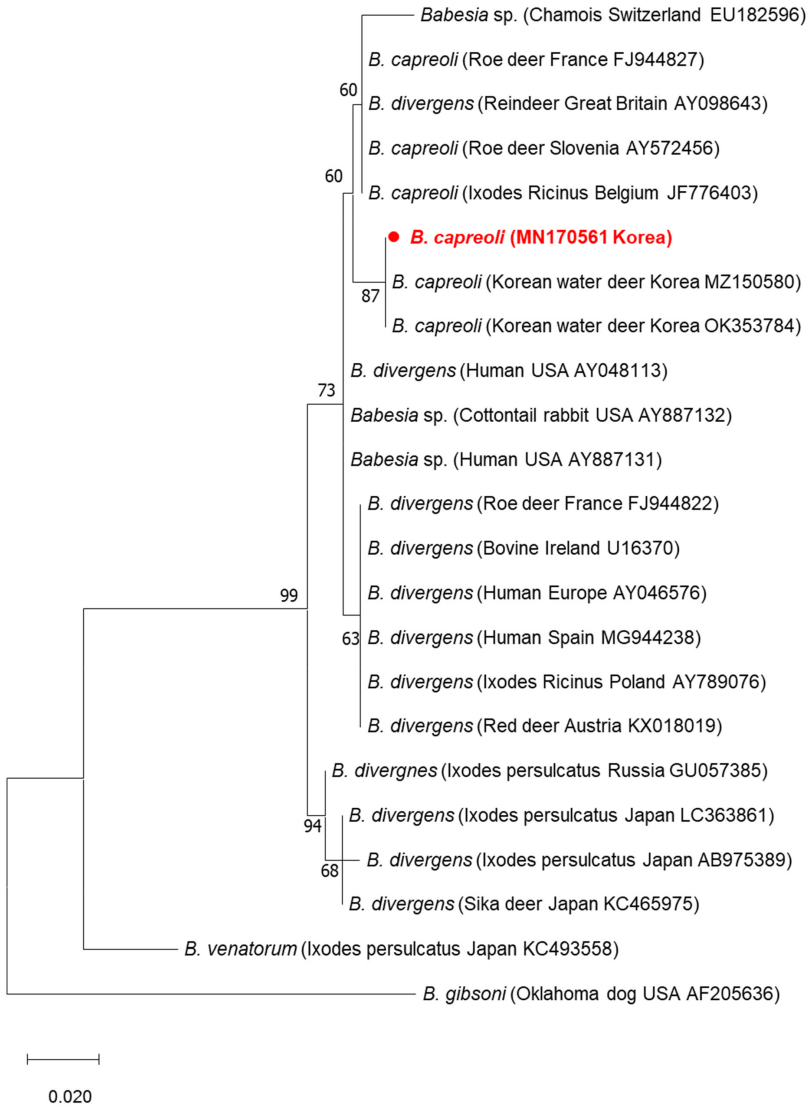

Fig. 1. Phylogenetic analysis based on partial *Babesia* 18S rRNA gene sequences. *Babesia divergens* is composed of four lineages: the *Babesia capreoli* lineage and the North American, European, and Asian lineages of *B. divergens*. The *B. capreoli* lineage includes *B. capreoli*, *Babesia* sp., and *B. divergens*. The phylogenetic tree was constructed with MEGA7 software using the maximum-likelihood method, and the numbers over the branches indicate bootstrap values as a percentage of 1,000 replicates that support each phylogenetic branch. The tick isolate identified in this study is indicated by a circle symbol and in bold.

B. capreoli transmission, the existence a of *B. capreoli* infection in the ROK warrants further study.

In Europe, *Ixodes ricinus* is the main vector for *B. microti*, *B. divergens*, *B. venatorum*, and *B. capreoli* [24], whereas in the present study, *I. nipponensis* is suggested as being responsible for the transmission of *B. capreoli* in the ROK. Although we cannot draw a definite conclusion from this study alone, it is possible that regional ticks are the primary vectors for *B. capreoli*. The most dominant tick species in Korea are known as *Haemaphysalis longicornis* and *Haemaphysalis flava*, and some reports have identified *Babesia* spp. infection by *H. longicornis* in humans and cattle [25, 26], so further studies will be needed to identify *Babesia* spp. infection in these ticks.

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In general, *Babesia* infections are usually made in Korean grazing cattle, though *Babesia* spp. are found in a wide range of hosts, such as rodents, cottontail rabbits, small mammals, cattle, and even birds [27]. We hypothesize that small rodents act as competent reservoirs, promote the maintenance of this parasite, and allow transmission to the next generation of feeding ticks. In addition, unlike domestic animals, the wide range of activity of wild animals allows the presence of diseases to spread to all regions, rather than being confined to one region. With this in mind, it may be worthwhile to further examine the life cycle and hosts of *B. capreoli* in the ROK through investigations of prevalence and pathogenicity in wildlife as a first step towards understanding the possible effects of *B. capreoli* on livestock and humans.

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