Molecular evidence of zoonotic Babesia species, other than B. microti, in ixodid ticks collected from small mammals in the Republic of Korea

Tae Yun Kim | Seong Yoon Kim | Tae-Kyu Kim | Hee IL Lee | Shin-Hyeong Cho | Wook-Gyo Lee | Hyunwoo Kim

Division of Vectors and Parasitic Disease, Korea Disease Control and Prevention Agency, Cheongju-Si, Chungcheongbuk-Do, the Republic of Korea

Correspondence
Hyunwoo Kim, Division of Vectors and Parasitic Diseases, Korea Disease Control and Prevention Agency, Cheongju-Si, Chungcheongbuk-Do, The Republic of Korea.
Email: hyunwookim@korea.kr

Funding information
Korea Disease Control and Prevention Agency.
Grant/Award Number: 2020-NI-032-00

Abstract
The occurrence of tick-borne infectious diseases, including zoonotic babesiosis, has become a serious concern in recent years. In this study, we detected Babesia spp. using polymerase chain reaction (PCR) amplification of the 18S rRNA of the parasites isolated from ixodid ticks collected from small mammals in the Republic of Korea (ROK). Sequence analysis of the PCR amplicon revealed the presence of B. duncani, B. venatorum, B. capreoli/divergens, and, the most prevalent, B. microti in the ticks. The molecular phylogenetic analysis showed that the four species-specific 18S rRNA sequences clustered in four distinct clades. This is the first study to provide molecular evidence for the presence of zoonotic Babesia spp. other than B. microti in ticks in the ROK.

KEYWORDS
babesiosis, Ixodidae, ribosomal RNA, the Republic of Korea, tick

1 | INTRODUCTION

The incidence of tick-borne diseases is increasing worldwide, and this is attributed to the growth and geographical expansion of the tick populations. Considering the effect of tick-borne infections on human health, investigations of the geographical and seasonal distribution of ticks and the epidemiology of the associated pathogens are of importance (Gratz, 2006).

Human babesiosis is a zoonotic tick-borne disease caused by protozoan parasites belonging to the genus Babesia, which infect and destroy erythrocytes (Gray et al., 2010). This disease can also be transmitted through blood transfusion and organ transplantation, and even congenitally (Herwaldt et al., 2011; Vannier & Krause, 2012). The most common symptoms of human babesiosis are hemolysis, hemoglobinuria, fever, and hypoxia, which could be severe, moderate, or mild depending on the causative species and immunological status of the patient (Kirtz et al., 2012; Michel et al., 2014). Although more than 100 Babesia spp. have been shown to cause infections in animals (Gray et al., 2010; Vannier & Krause, 2012; Yabsley & Shuck, 2013), only a few have been shown to be pathogenic to humans; among them, B. microti is the most prevalent followed by B. divergens, B. duncani, and B. venatorum (Fang et al., 2015; Leiby, 2011). Ticks of the genus Ixodes are the primary vectors of human babesiosis agents. Ixodes scapularis is the primary vector of B. microti in the United States (Hunfeld et al., 2008), whereas I. spinipalpis, I. angustus, I. muris, and I. ricinus are vectors in other parts of the world. Ixodes ricinus is the primary vector of B. divergens and B. venatorum, causative agents of human babesiosis mainly in Europe, while I. persulcatus is the most frequently encountered tick that transmits human Babesia parasites in Asia (Zamoto et al., 2004).

In the ROK, cases of human babesiosis have been sporadically reported, but most of them were imported (Kwon et al., 2018), while only two were endemic (Kim et al., 2007; Hong et al., 2019); however,
TABLE 1  Primers and PCR conditions for the detection of *Babesia* spp

| Target                | Primer sequence                      | Thermal cycles | Size |
|-----------------------|--------------------------------------|----------------|------|
| 18S rRNA (Zintl et al., 2011) | 1st PCR BTH1F: 5′-GTTGGGCTACATCCTCCTCC-3′ BTH1R: 5′-TTCCGACCATCTCCTCCCA-3′ | 94°C, 10 min; 45 cycles (95°C, 30 s; 68°C, 1 min; 72°C, 1 min), 72°C, 10 min | 561 bp |
|                       | 2nd PCR GR2F: 5′-GTTGGGCTACATCCTCCTC-3′ GR2R: 5′-TCAGCTTCTCCTC-3′ | 94°C, 10 min; 40 cycles (95°C, 30 s; 60°C, 1 min; 72°C, 1 min), 72°C, 10 min |
| β-tubulin (Zamoto et al., 2004) | 1st PCR TuBu92F: 5′-GAGAYGAYCCTTACACTAGAAGGCC-3′ TuBu897R: 5′-CGRTGAAGACATTGTTGGTCARTTC-3′ | 95°C, 10 min; 35 cycles (95°C, 30 s; 58°C, 1 min; 72°C, 1 min 30 s), 72°C, 10 min | 551 bp |
|                       | 2nd PCR TuBu192F: 5′-ACHATGGATTCTGGATATCGGCC-3′ TuBu782R: 5′-GGGADADGGDATRAGATCCAGC-3′ | 94°C, 10 min; 45 cycles (94°C, 30 s; 61°C, 30 s; 72°C, 1 min), 72°C, 10 min |
3 | RESULTS

3.1 | Classification of small mammals and ticks

In our survey, Apodemus agrarius (black-striped field mouse) was the most prevalent small mammal, followed by Crocidura lasiura (Ussuri white-toothed shrew). Among the ectoparasites collected from them, ixodid ticks were morphologically classified. Ticks in the nymph stage were identified as I. nipponensis and I. angustus, whereas unidentifiable larval stage ticks were classified only to the genus Ixodes. Overall, ticks belonging to the genus Ixodes were the most prevalent in small mammals.

3.2 | PCR and sequencing analysis

To detect Babesia spp. in ticks, PCR amplification and sequencing of the 18S rRNA hypervariable region were performed. The results of multiple sequence alignment revealed four distinct 18S rRNA sequences of Babesia spp. (Figure 1). Among the four 18S rRNA sequences, the most prevalent one was 100% identical to that of B. microti isolated worldwide; the most prevalent sequence was identified in ticks from all sites examined in the study. Besides B. microti, other known zoonotic Babesia spp., B. duncani, B. capreoli/divergens, and B. venatorum were also identified. The 18S rRNA sequences amplified from I. nipponensis and I. angustus nymphs and Ixodes sp. larvae parasitizing A. agrarius from Goheung, Donghae, and Jeju showed 100% identity to those of B. duncani WA1, WA2, CA5, and CA6 isolates (Figure 2). The sequence of Babesia spp. from an Ixodes sp. larva collected from A. agrarius in Uiseong was 99.82% identical to that of B. capreoli and B. divergens. As there was no sequence variation in the 18S rRNA hypervariable region between B. capreoli and B. divergens, we used the term “B. capreoli/divergens” in this study. Furthermore, the sequence of Babesia spp. from an Ixodes sp. larva from C. lasiura at Geoje was 99.08% identical to B. venatorum. None of the six tested samples were positive for B. microti-specific β-tubulin gene, as determined by PCR, indicating that there was no B. microti in the samples.

3.3 | Molecular phylogeny

A phylogenetic tree constructed based on multiple sequence alignment of the 18S rRNA sequences showed that four sequences of B. duncani (GH33, GH44, DH32, and JJ89) clustered in a big clade together with the sequences of B. duncani from North America (Figure 3). The 18S rRNA sequences of B. venatorum (GJ51) and B. capreoli/divergens (US67) clustered in two distinct clades. Overall, the phylogenetic analysis based on 18S rRNA sequencing revealed that Babesia spp. identified in this study belonged to four independent clades.

4 | DISCUSSION

Although seroprevalence and molecular diagnostic studies of babesiosis have been conducted in domestic and wild animals in the ROK, the identification of Babesia spp. directly from ticks inhabiting geographically isolated regions across the country has been rarely performed (Hong et al., 2019; Kang et al., 2013). To the best of our knowledge, in
FIGURE 3  Phylogeny of Babesia spp. based on the 18S rRNA sequences. The phylogenetic tree was constructed using the neighbor-joining method. Scale bar shows an evolutionary distance of 0.05 nucleotide substitutions per position in the 18S rRNA sequence and numbers show bootstrap values (1000 replicates); *sequences identified in this study
this study, we identified *B. duncani*, *B. capreoli/divergens*, and *B. venatorum* in ticks parasitizing small mammals in the ROK for the first time.

Although *B. microti* is documented as the most widely distributed zoonotic *Babesia* sp., cases of animal infections have been reported in only small wild animals, but not domestic animals in the ROK (Hong et al., 2014; Hong et al., 2017; Hwang et al., 2017). Among the six species of small mammals captured in the country, only *A. agrarius* was positive for *B. microti*, as determined by PCR and indirect immunofluorescence assay (Hong et al., 2014). *Apodemus agrarius*, the most common wild rodent in rural areas in the country (Kim et al., 2013; Lee et al., 2009), is considered a reservoir of Hantaan virus, which causes hemorrhagic fever with renal syndrome (Lee et al., 1978; Lee et al., 1981), and *Leptospira interrogans*, which is an agent of leptospirosis (Cho et al., 1998). Furthermore, *O. tsutsugamushi* has been detected in chigger mites on *A. agrarius* in the ROK nationwide (Choi et al., 2018; Lee et al., 2009), indicating that this rodent is the dominant host of vectors transmitting zoonotic pathogens to humans. Although there is no information on the tick species associated with babesiosis in the two countries, the five Korean patients reported previously (Hong et al., 2019; Kim et al., 2007), it is most likely that they originated from ticks on *A. agrarius*.

Since the first isolation of *B. duncani* type WA1 from a patient in the USA, human cases of *B. duncani* infection have been reported across the United States and Canada, with the highest incidence along the Pacific Coast (Scott, 2017; Scott and Scott, 2018; Swei et al., 2019). In this study, hypervariable regions of the 18S rRNA gene from four *Ixodes* ticks were identical to those of the WA1, WA2, CA5, and CA6 isolates of *B. duncani*. Although one 18S rRNA sequence identified in a Chinese tick (accession no. KX008042) was identical to that of the WA1 and CA5 isolates, there is no related publication. Therefore, to the best of our knowledge, the four sequences reported here represent the first evidence of *B. duncani* in ticks outside of North America.

*Babesia divergens* was associated with the first human case of babesiosis in 1957 in Europe (Skrabalo and Deaniviu, 1957). Since then, the disease has been recorded worldwide. In this study, the 18S rRNA gene region identified in an *Ixodes* tick was similar to that in *B. capreoli* and *B. divergens*. The two *Babesia* spp. are closely related as evidenced by 99.83% identity in the 18S rRNA sequences; therefore, their identification based only on molecular analysis is challenging and should be performed considering even biological characteristics, including the spectrum of infected hosts (Malandrini et al., 2010). Recently, an 18S rRNA sequence of *Babesia* sp. was identified in a Korean water deer (*Hydropotes inermis argyropus*), and it was 92.2% identical to the sequence of *B. capreoli* and was distinct from the sequence of *B. divergens* (Shin et al., 2020).

Human infection with *B. venatorum* has been mainly reported in Europe (Häselbarth et al., 2007; Herwaldt et al., 2003). However, many cases of human babesiosis caused by *B. venatorum* have been recently described in China (Jiang et al., 2015; Sun et al., 2014), suggesting that the area of *B. venatorum* infectious to humans has expanded from Europe to Asia.

Although we detected various zoonotic *Babesia* spp. infected ticks, there is little information about autochthonous clinical babesiosis in the ROK. At least seven tick species have been documented in 38 previous reports on tick bite cases in the country, most of them related to *I. nipponensis* (Shin, 2014). In spite of the predominance of *Ixodes* spp. collected from small mammals (Kim et al., 2006; Kim et al., 2014; Shin et al., 2013), *H. longicornis* is the most commonly collected tick species in almost all areas in the ROK (Kim et al., 2014; Noh et al., 2019). Since the first case of severe fever with thrombocytopenia in the country (Kim et al., 2013), requests for the identification of ticks that have bitten humans have increased considerably in the Korea Disease Control and Prevention Agency, and over 80% of them were classified as *H. longicornis* (Yang et al., 2016). In a recent human case of babesiosis in the ROK, the tick was not specified, but *B. microti* and *B. motasi* genes were detected in *H. longicornis* and *H. ilva* collected in the area around the patient’s residence (Hong et al., 2019). In this study, *B. microti* 18S rRNA was found in one *H. longicornis* larva collected from *A. agrarius* from Jeju Island (data not shown). Therefore, detection of zoonotic *Babesia* spp. should be performed in both *Ixodes* and *Haemaphysalis* spp. in the country.

In summary, this is the first report for the molecular identification of *B. duncani*, *B. venatorum*, and *B. capreoli/divergens* in ticks of the ROK. It is possible that they are autochthonous species, as zoonotic babesiosis may have been overlooked in the country. Therefore, to prevent and prepare for the emergence of these zoonotic *Babesia* spp. in the ROK, extensive nationwide surveillance of ticks and their animal hosts should be performed.

**ACKNOWLEDGMENTS**

The authors are particularly grateful to Mr. Bong Gu Song, Mr. Won Il Park, Mr. Hak Seon Lee, and Mr. Hyung Woo Lim for their expert help in capturing wild rodents. This work was supported by a grant from the Korea Disease Control and Prevention Agency (2020-NI-032-00).

**ETHICAL STATEMENT**

The animal-handling protocol used in this study was based on the Institutional Animal Care and Use guidelines and was approved by the Ethical Committee of the Korea Centers for Disease Control and Prevention (KCDC-046-13-2A).

**CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest in this study.

**DATA AVAILABILITY STATEMENT**

Data generated specifically for this study are included within the article. Materials obtained or generated for this study are available from the corresponding author upon reasonable request.

**PEER REVIEW**

The peer review history for this article is available at https://publons.com/publon/10.1002/vms3.581.

**ORCID**

Tae Yun Kim https://orcid.org/0000-0002-3083-8424
Sun, Y., Li, S. G., Jiang, J. F., Wang, X., Zhang, Y., Wang, H., & Cao, W. C. (2014). Babesia venatorum infection in child, China. Emerging Infectious Diseases, 20, 896–897.

Swei, A., O’Connor, K. E., Couper, L. I., Thekkiniath, J., Conrad, P. A., Padgett, K. A., Burns, J., Yoshimizu, M. H., Gonzales, B., Munk, B., Shirkey, N., Konde, L., Mamoun, C. B., Lane, R. S., & Kjemtrup, A. (2019). Evidence for transmission of the zoonotic apicomplexan parasite Babesia duncanii by the tick Dermacentor albipictus. International Journal for Parasitology, 49, 95–103.

Vannier, E. & Krause, P. J. (2012). Human babesiosis. New England Journal of Medicine, 366, 2397–2407.

Yabsley, M. J. & Shock, B. C. (2013). Natural history of zoonotic Babesia: role of wildlife reservoirs. International Journal for Parasitology: Parasites and Wildlife, 2, 18–31.

Yamaguti, N., Tipton, V. J., Keegan, H. L., & Toshioka, S. (1971). Ticks of Japan, Korea, and the Ryukyu Islands. Brigham Young University Science Bulletin, Biological Series 15, 1–226.

Yang, S. C., Lee, W. G., & Ju, Y. R. (2016). Hard tick bite cases and distribution in the Republic of Korea (2013–2015) [in Korean]. PHWR, 9, 1054–1059.

Zamoto, A., Tsuji, M., Wei, Q., Cho, S. H., Shin, E. H., Kim, T. S., Leonova, G. N., Hagiwara, K., Asakawa, M., Kariwa, H., Takashima, I., & Ishihara, C. (2004). Epizootiologic survey for Babesia microti among small wild mammals in northeastern Eurasia and a geographic diversity in the beta-tubulin gene sequences. Journal of Veterinary Medical Science, 66, 785–792.

Zintl, A., Finnerty, E. J., Murphy, T. M., de Waal, T., & Gray, J. S. (2011). Babesias of red deer (Cervus elaphus) in Ireland. Veterinary Research, 42, 7.

How to cite this article: Kim, T. Y., Kim, S. Y., Kim, T.-K., Lee, H. I., Cho, S.-H., Lee, W.-G., & Kim, H. (2021). Molecular evidence of zoonotic Babesia species, other than B. microti, in ixodid ticks collected from small mammals in the Republic of Korea. Veterinary Medicine and Science, 7, 2427–2433. https://doi.org/10.1002/vms3.581