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Short communication

# Molecular survey of *Babesia* spp. in red foxes (*Vulpes Vulpes*), Asian badgers (*Meles leucurus*) and their ticks in China

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ABSTRACT

*Babesia* species (Apicomplexa: Piroplasmorida) are tick-borne protozoan hemoparasites, which pose a significant threat to domestic animals, wildlife and humans. This study aimed to determine and characterize *Babesia* species in red foxes (*Vulpes vulpes*), Asian badgers (*Meles leucurus*) and their ticks. Blood, heart, liver, spleen, lung, kidney, large intestine and small intestine were collected from 19 wild carnivores (12 red foxes and 7 Asian badgers). All ticks were removed from these animals and identified according to morphological and molecular characteristics. The samples were tested for the presence of *Babesia* species using the *18S rRNA* gene. Molecular analyses showed that the DNA of *Babesia vogeli* and *Babesia vulpes* was present in red fox organs/tissues and blood samples. A total of 54 hard ticks (38 *Ixodes canisuga*, 6 *Haemaphysalis erinacei*, 9 *Ixodes kaiseri* and 1 *Dermacentor marginatus*) were collected from red foxes and 12 (*I. kaiseri*) from Asian badgers. All ticks were adults. Among them, one *I. kaiseri* parasiting a red fox contained the DNA of *B. vulpes* while one *I. canisuga* was positive for *Babesia* sp. belonging to the clade "*Babesia* sensu stricto". Molecular and phylogenetic analyses indicated the presence of a novel genotype, *Babesia* sp. "badger China". *Babesia* sp. badger type A and type B from Asian badgers were different from those in European badgers. Co-infection with three *Babesia* genotypes was found in one Asian badger. This study provides the first data on *Babesia* infection in red foxes, Asian badgers and their ticks in China. *Babesia vogeli* was detected for the first time in red foxes in Asia. Co-infection and genetic diversity of *Babesia* genotypes in Asian badgers were also demonstrated.

## 1. Introduction

Babesiosis is a disease caused by tick-borne intra-erythrocytic protozoan parasites of the genus *Babesia*, which may affect wild and domestic animals, even humans. Clinical manifestations of acute *Babesia*-infection include fever, anemia, hemoglobinuria, jaundice, malaise, lethargy and anorexia, while the chronic status is generally asymptomatic (Schnittger et al., 2012).

The red fox (*Vulpes vulpes*), inhabiting regions of Europe, Asia, Africa, North America and Australia, is the most geographically widespread wild carnivore species (Larivière and Pasitschniak-Arts, 1996; Schipper et al., 2008). These foxes represent an excellent sentinel species and a

*Abbreviations: 18S rRNA*, *18S* ribosomal RNA; *16S rDNA*, *16S* ribosomal DNA; XUAR, Xinjiang Uygur Autonomous Region.

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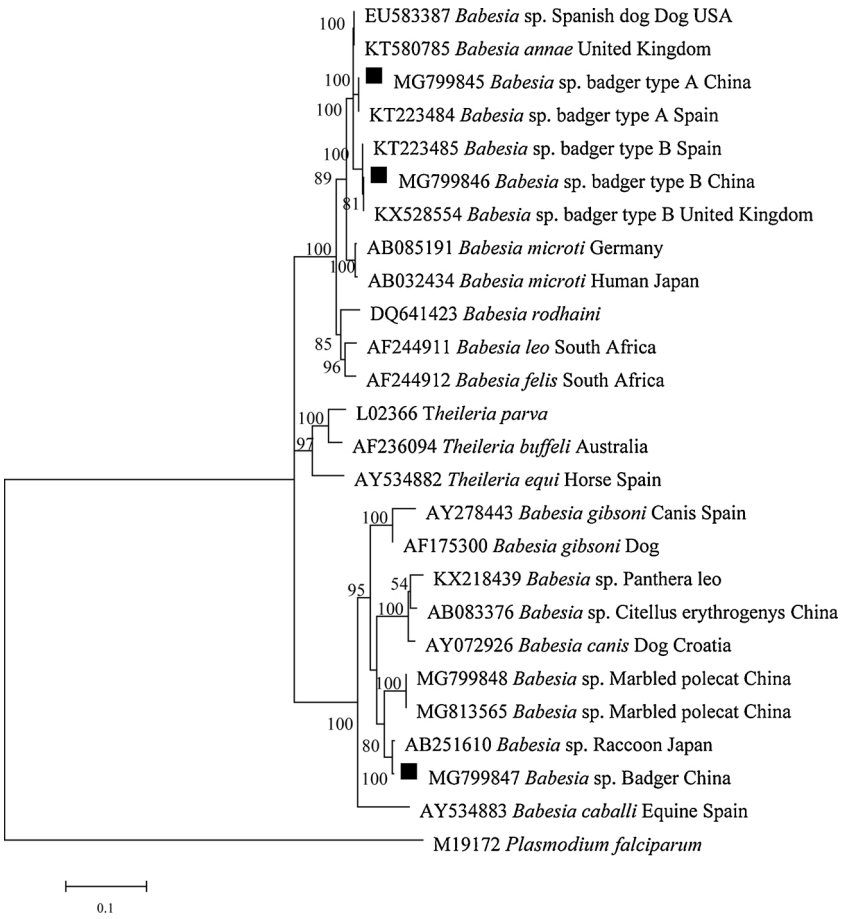


**Fig. 1.** Phylogenetic analysis of *18S rRNA* gene sequences of *Babesia* sp. badger type A China, *Babesia* sp. badger type B China and *Babesia* sp. "badger China" (■) identified in Asian badger and relevant sequences from GenBank. The tree was constructed with the Maximum Likelihood method (ML; bootstrap replicates: 1000). Branch lengths correlate to the number of substitutions inferred according to the scale shown.

possible source of several vector-borne diseases (VBDs) to domestic animals and humans, mostly due to their close proximity to urban or agricultural areas and frequent exposure to different arthropod vectors (Torina et al., 2013). The Asian badger (*Meles leucurus*) is now described as a distinct species from the European badger (*Meles meles*) and the Japanese badger (*Meles sanakuma*) (Roca et al., 2014). *Babesia* spp. have been recorded in European badgers (Bartley et al., 2017; Gimenez et al., 2009), but to the best of our knowledge, not in Asian badgers.

Typically, *Babesia* are transmitted by ixodid ticks, which acquire these piroplasms during their blood meal (Schnittger et al., 2012). *Ixodes canisuga* is found in nests and burrows (Beaucournu and Matile, 1963), and have been found in Bosnia and Herzegovina, Croatia, Denmark, France, Germany, Great Britain, Hungary, Ireland, Latvia, Lithuania, Poland, Portugal, Romania, Serbia, Spain, Sweden and Switzerland (Hornok et al., 2017; Jaenson et al., 1994; Petney et al., 2012) Several wildlife species including badgers, polecats, weasels, Eurasian owls, red foxes can be the host of *I. canisuga* (Gilot and Aubert, 1985; Hornok et al., 2018; Sandor et al., 2017; Santos-Silva et al., 2011). In addition, *Borrelia burgdorferi* sensu lato, *Ehrlichia* sp. have been detected in *I. canisuga* (A. Estrada-Peña et al., 1995; Hornok et al., 2018). *Ixodes kaiseri* is a nest-dwelling tick species and a typical parasite of hedgehogs (*Erinaceus* spp.), red foxes and ground long-tailed squirrels (Agustín Estrada-Peña et al., 2017; Zhao et al., 2019).

The objectives of the present study were to investigate and to characterize *Babesia* infection in red foxes, Asian badgers and their ticks in China using molecular techniques.

## 2. Materials and methods

### 2.1. Sample collection

A total of 12 red foxes and 7 Asian badgers were included in the survey in Xinjiang Uygur Autonomous Region (XUAR), northwestern China (shown in Appendix Table 1). Necropsies were performed under aseptic conditions by veterinarians, and portions of organs/tissues (blood, heart, liver, spleen, lung, kidney, large intestine and small intestine) from red foxes and Asian badgers were stored individually frozen at −20 °C until DNA extraction. Required permission to conduct research on wild animals was obtained from the Animal Ethics Committee of Shihezi University (Approval No. AECSUKJ2015−01).

Simultaneously, a total of 66 ticks (54 from red foxes and 12 from Asian badgers) were collected. The ticks were placed in tubes with 75 % ethanol and stored at −20 °C. All ticks were morphologically identified according to standard taxonomic keys (Estrada- Peña et al., 2004; Tesgera et al., 2017; Walker et al., 2003).

### 2.2. Molecular analyses

The genomic DNA was extracted from red fox and Asian badger organs and each individual tick using the TIANamp Genomic DNA Kit (TIANGEN, Beijing, China), following the manufacturer's instructions. Similarly, DNA was extracted from 200 μl of EDTA – treated blood samples obtained from red foxes and Asian badgers.

All the ticks were identified by amplifying and sequencing their *16S rDNA* (Black and Piesman, 1994). *Babesia* genus-specific PCR was

performed for ticks and red fox samples using published primers, BJ1/BN2, which amplify an approximately 500-bp-long part of the *18S* ribosomal RNA (*18S rRNA*) gene (Casati et al., 2006). Then primers BT1-F and BT-Outer-R/BT-Inner-R were used to sequence the nearly complete, 1700-bp-long fragment of *18S rRNA* gene of *Babesia* spp. from badgers (Bartley et al., 2016; Criado-Fornelio et al., 2003). These primers allowed the detection and discrimination of *Babesia* sp. badger type A and type B in European badgers (Bartley et al., 2017). Primer data are shown in Appendix Table 2. Sequence-confirmed *Babesia occultans* DNA amplified in our laboratory and double distilled water were used as positive and negative controls, respectively.

The sequences obtained here were compared to others deposited in GenBank with the BLASTn programme (http://www.ncbi.nlm.nih.gov/BLAST/). Phylogenic trees were constructed using the Maximum Likelihood method with MEGA 7.0 software (Kumar et al., 2016).

## 3. Results

### 3.1. Molecular identification of piroplasms in carnivores

In five organs of red fox #1 and blood samples of red foxes #1, #4, #5 and #6, the DNA of *B. vulpes* was present, showing 100 % (520/520 bp) and 99.81 % (520/521 bp) sequence identity to *B. vulpes* reported from Turkey (GenBank accession no. MF040153) and Slovakia (KY175165), respectively. At the same time, the DNA of *Babesia vogeli* was detected in the spleen samples of red fox#2 and #3. Its sequence had 99.79 % (485/486 bp) and 99.59 % (484/486 bp) identity to *Babesia* sp. from China (AB083376) and *B. vogeli* from Russia (MG041384), respectively.

Molecular and phylogenetic analyses indicated three distinct *Babesia 18S* rRNA genotypes in Asian badgers (Fig. 1). *Babesia* sp. "badger China" (MG799847), a novel *Babesia* genotype, was clearly distinct from *Babesia* sp. badger type A or type B reported previously from European badger or Asian badger, and clustered together with *Babesia* sp. raccoon (AB251610) from Japan, with which it shared 99.37 % (1579/1589 bp) sequence identity (Appendix Fig. 1). *Babesia* sp. badger type A from Asian badger (MG799845) showed 99.93 % (1529/1530 bp) identity with *Babesia* sp. badger type A (KT223484) from European badger, whereas *Babesia* sp. badger type B (MG799846) from Asian badger differed at 4 nucleotide positions, and shared 99.22 % (1315/1319 bp) identity with *Babesia* sp. badger type B (KT223485) from European badger.

### 3.2. Molecular identification of piroplasms in ticks

A total of 54 ticks collected from 12 red foxes were identified as *I. canisuga* (n = 38), *Haemaphysalis erinacei* (n = 6), *I. kaiseri* (n = 9) and *Dermacentor marginatus* (n = 1). Twelve ticks, collected from Asian badgers, were identified as *I. kaiseri*. *Babesia vulpes* detected in *I. kaiseri* (n = 1) infesting red fox #4 shared 100 % identity with sequences of *B. vulpes* in blood and organs of its parasitized host (red fox 4#). The *Babesia* sequence from *I. canisuga* (n = 1) was detected, and showed 100 % identity with that detected in a marbled polecat in China (MG799848). No *Babesia* was detected in *I. kaiseri* (n = 12) from Asian badgers.

All sequences obtained in this study were deposited in the GenBank. Accession numbers are included in Appendix Table 3.

## 4. Discussion

To date, information regarding the prevalence of piroplasmids in wild animals in China is limited, and no studies on the presence of *Babesia* spp. in red foxes and their ticks have been published. Infection of red foxes with *B. vulpes* (at that time called *Theileria annae*) was first reported from Spain (Criado-Fornelio et al., 2003). The pathogenicity of *B. vulpes* in dogs has been proved (Garcia, 2006; Miro et al., 2015) and

**Table 1**
The type of *Babesia* positive-tissues/organs excised from Red foxes and Asian badgers in this study.

| Host ID | Blood | Heart | Liver | Spleen | Lung | Kidney | Small intestine | Large intestine |
|---|---|---|---|---|---|---|---|---|
| Red Fox#1 | *Babesia vulpes* | *Babesia vulpes* | *Babesia vulpes* | *Babesia vulpes* | *Babesia vulpes* | *Babesia vulpes* | – | – |
| Red Fox#2 | – | – | – | *Babesia vogeli* | – | – | – | – |
| Red Fox#3 | – | – | – | *Babesia vogeli* | – | – | – | – |
| Red Fox#4 | *Babesia vulpes* | – | – | – | – | – | – | – |
| Red Fox#5 | *Babesia vulpes* | – | – | – | – | – | – | – |
| Red Fox#6 | *Babesia vulpes* | – | – | – | – | – | – | – |
| Asian badger#1 | Type B | – | – | Type B and *Babesia* sp. "badger China" | – | – | – | – |
| Asian badger#2 | Type A and Type B | Type A, Type B and *Babesia* sp. "badger China" | Type A, Type B and *Babesia* sp. "badger China" | Type A, Type B and *Babesia* sp. "badger China" | Type A, Type B and *Babesia* sp. "badger China" | Type A, Type B and *Babesia* sp. "badger China" | Type A and Type B | Type A and Type B |
| Asian badger#3 | Type A | Type A and *Babesia* sp. "badger China" | Type A and *Babesia* sp. "badger China" | Type A and *Babesia* sp. "badger China" | Type A and *Babesia* sp. "badger China" | Type A and *Babesia* sp. "badger China" | Type A | Type A |


**Fig. 2.** Phylogenic analysis of *Babesia* spp. in red foxes. The tree was constructed with the Maximum Likelihood method (ML; bootstrap replicates: 1000), using concatenated sequence data of the *18S rRNA* gene with MEGA7.0. *Babesia* sequences obtained in are indicated by solid triangle (▲) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

this piroplasm has also been found to be responsible for human babesiosis (Hildebrandt et al., 2007). In recent years, *B. vulpes* was detected in red foxes in new regions of Eurasia, including Italy, Turkey, Germany, Spain, Israel, Slovakia (Koneval et al., 2017; Margalit Levi et al., 2018; Millan et al., 2016; Najm et al., 2014; Orkun and Karaer, 2017; Santoro et al., 2019). In this study, *B. vulpes* was detected in six out of twelve red foxes in China. This finding extends the geographic distribution of *B. vulpes*. Although the number of red foxes was limited, the prevalence of 50 % is not negligible. *Babesia canis* is closely related to two further species: *B. rossi* and *B. vogeli*, which were formerly regarded as its subspecies (Hauschild et al., 1995; Uilenberg et al., 1989) and usually infect dogs. In this study, further *Babesia* genotypes were detected in spleen samples of two red foxes (red fox #2 and #3) and these clustered with *B. vogeli* (Table 1). In China, the current dog population is between 150 and 200 million (Ma et al., 2012). In a previous study, *B. vogeli* was detected in dogs in Jiangxi Province, eastern China (Xu et al., 2015; Zheng et al., 2017). The present finding indicates that red foxes might act as natural reservoirs for *B. vogeli*, therefore the possibility of its transmission between suburban red foxes and stray dogs deserves future attention.

Red foxes are hosts for a variety of tick species such as *Ixodes hexagonus*, *Ixodes ricinus*, *Dermacentor reticulatus*, *I. canisuga*, *D. marginatus*, and *Rh. sanguineus* sensu lato (Checa et al., 2018; Mihalca et al., 2012; Sandor et al., 2017). Previously, *B. vulpes* was detected for the first time in *I. kaiseri* parasitizing red foxes in Romania (Hornok et al., 2020). In this study, we obtained four tick species from red foxes. Interestingly, *B. vulpes*, detected in *I. kaiseri* and its respective host (red fox 4#), clustered with sequences from Slovakia and Turkey (KY175165 and MF040153, respectively) (Fig. 2). *Babeisa* (MW190076) which was detected in *I. canisuga* clustered with sequences (MG813565 and MK742775) from Marbled polecat from XUAR, and phylogenetically belonged to the "*Babesia* sensu stricto" group. These findings suggest that the potential role of *I. kaiseri* and *I. canisuga* in the transmission of *B. vulpes* and *Babesia* sp., respectively, should be evaluated further.

Previously, several new pathogens, including *Spirometra* sp., *Borrelia*

*burgdorferi* sensu lato and *Babesia* sp. have been reported in European badger (Bartley et al., 2017; Gern and Sell, 2009; Gimenez et al., 2009). To the best of our knowledge, this study is the first to demonstrate the presence of *Babesia* DNA in Asian badger. The above findings suggest that co-infection with different piroplasms in Asian badger is not rare, similarly to the mixed infection with *Babesia* sp. badger type A and type B in European badger (Bartley et al., 2017). BLASTn analysis further indicated that there are some differences between *Babesia* sp. badger type A and type B originating from Asian and European badger (Appendix Fig. 1). In addition, *Babesia* sp. "badger China", a novel *Babesia* genotype was proved to be significantly distinct from other related piroplasms in GenBank. In the future, the genetic diversity of *Babesia* sp. "badger China" should be explored further.

## 5. Conclusion

In this study, we verified the presence of *B. vulpes* and *B. vogeli* for the first time in red foxes in China. In addition, three *Babesia* genotypes including *Babesia* sp. "badger China" (a novel *Babesia* genotype), *Babesia* sp. badger type A and type B were detected in Asian badger. Co-infection with *Babesia* genotypes in Asian badger was also demonstrated. These results highlight the importance to study further vector-borne pathogens in wildlife in China.

## Author statement

Chunli Sang, Yicheng Yang, Qiaoyan Dong, and Yuanzhi Wang conceived and designed the study, and writing the manuscript. Chunli Sang, Bin Xu, Guangyuan Liu, Zhiqiang Liu and Wurelihazi Hazihan performed the experiments, analyzed the data. Sándor Hornok critically revised the manuscript. All authors read and approved the final manuscript.

## Ethics approval

This study was approved by the Animal Ethics Committee of Shihezi University (Approval No. AECSU2015−01).

## Availability of data and materials

Data supporting the conclusions of this article are included within the article. The datasets used and analyzed during the present study are available from the corresponding author upon reasonable request.

## Declaration of Competing Interest

The authors declare that they have no competing interests.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ttbdis.2021.101710.

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