First detection and molecular identification of *Babesia* sp. from the giant panda, *Ailuropoda melanoleuca*, in China

Chanjuan Yue†, Zeshuai Deng†, Dunwu Qi¹, Yunli Li¹, Wenlei Bi¹, Rui Ma¹, Guangyou Yang², Xue Luo², Rong Hou¹* and Songrui Liu¹*

Abstract

**Background:** Parasitic infections are among the important causes of death of giant pandas (*Ailuropoda melanoleuca*) that hamper their survival in the wild. There are about 35 species of parasites which have been identified in giant pandas, but no information is currently available regarding the infection of *Babesia* in giant pandas. *Babesia* spp. are common intraerythrocytic parasite in wildlife, transmitted by ixodid ticks, which cause babesiosis. Clinical signs of babesiosis include fever, hemolysis, anemia, jaundice and death.

**Methods:** A species of *Babesia* was detected in the blood of a giant panda based on morphology and PCR amplification of the 18S rRNA gene. The phylogenetic relationship of *Babesia* sp. infecting giant panda was assessed by gene sequence alignment and phylogenetic analysis.

**Results:** Our analysis revealed that the *Babesia* isolate detected was most similar to an unidentified species of *Babesia* identified in black bears (*Ursus thibetanus japonicus*) from Japan (*Babesia* sp. Iwate, AB586027.1) with a 99.56% sequence similarity, followed by *Babesia* sp. EBB (AB566229.1, 99.50%) and *Babesia* sp. Akita (AB566229.1, 99.07%).

**Conclusions:** To our knowledge, this is the first report of *Babesia* detected in the giant panda. The results indicate that this *Babesia* sp. may be a novel species, currently named *Babesia* sp. strain EBP01.

**Keywords:** *Babesia*, Giant panda, ailuropoda melanoleuca, Phylogenetic tree, 18S rRNA gene

Background

*Babesia* spp. are intraerythrocytic apicomplexan protozoan parasites known to infect wild and domestic animals and humans [1]. With a near global distribution, over 120 species of *Babesia* have been identified, most of which are transmitted by ixodid ticks [2]. These protozoan parasites are not strictly host-specific and this has contributed to the growing list of new species, with several *Babesia* species having been found to infect both animals and humans. Most cases of human babesiosis are caused by *B. microti*, *B. divergens*, *B. venatorum* and *B. duncanii* and several unnamed species [3]. Geographically, *Babesia* spp. are widely distributed on all continents except Antartica, especially in tropical and subtropical temperate zones.

The clinical presentation of a *Babesia* infection is termed ‘babesiosis,’ with signs consistent with parasite entry into and destruction of erythrocytes. Typical clinical signs of babesiosis include fever, hemolysis, anemia, jaundice and even MODS [4]. Babesiosis may be clinically characterized as asymptomatic to severe morbidity.
and death. Whether the host will be infected and suffer clinical disease also depends on the specific species [5–7]. Susceptibility to a Babesia infection often depends on the age and immune resistance of the host; older and low-immunity hosts are more susceptible to infection [8]. It seems that infection with B. microti in elderly, immunosuppressed and splenectomized patients have more severe and protracted illness [9].

Various Babesia species have been sporadically identified in wildlife, while the existence of the chronic asymptomatic carrier state in babesial infections of domestic and wild animals has been recognized for many years [10, 11]; as a result, babesiosis in wildlife has not received much attention. Despite ixodid ticks are implicated as the primary vector for Babesia spp., wildlife species are considered to be the reservoir, with several recent cases of fatal babesiosis were recorded in wildlife [12–14]. There remains a paucity in the literature for what species may be affected and what factors may be caused the clinical signs, and therefore, babesiosis in wildlife deserves further attention.

As an iconic “flagship” species for wildlife conservation, the giant panda (Ailuropoda melanoleuca) is considered a national treasure with the highest legal protection afforded by China. The giant panda is currently categorized as vulnerable by the IUCN, with both the in-situ and ex-situ populations facing continued threats from low reproductive rates, parasitic infection and infectious diseases [15, 16]. Regarding parasitic infection of giant pandas, previous research has focused on intestinal parasites, however, no information is currently available regarding the infection and genetic characteristics of Babesia in the giant panda [17].

The objective of the present study is to determine the phylogenetic relationships, as well as the genetic and morphological characteristics of a Babesia species detected in a giant panda suffering from clinical babesiosis.

Methods

An intraerythrocytic hemoparasite was detected on a blood smear from one female giant panda with the associated signs of fever, anemia, jaundice and hemoglobinuria. The blood smear demonstrated intraerythrocytic parasites with a morphology similar to that of known Babesia species, then the pathogen investigation was carried out by the following methods.

Blood samples

Blood samples were collected from the basilica vein of the right or left arm of the giant panda without anesthesia, using a vacutainer containing EDTA, and then stored at 4 °C until DNA extraction.

Detecting of Babesia in thin blood smears

Morphological observation of Babesia parasites was carried out under light microscopy. Thin blood smears were prepared, fixed with methanol (100% or absolute) and dried completely before staining. Two different stains, Giemsa (Sigma-Aldrich, Burlington, USA) and Diff Quick (Labokine™, Bad Kissingen, Germany), were performed according to the manufacturer’s instructions. The slides were examined by light microscope at ×1000 under an oil immersion lens [18].

DNA extraction

DNA was extracted from 200 μl of whole blood with the QIAamp 96 DNA Blood Kit (Qiagen, Hilden, Germany). DNA was eluted in 100 μl of elution buffer and used for identification of Babesia. According to the manufacturer’s instructions, the concentration of DNA was determined with a NanoDrop 2,000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The DNA was stored at –20 °C until further analysis.

Detection of Babesia spp. by PCR

Babesia sp. was identified by using PCR with pfu DNA polymerase and primary primers F1 (5’-GTC TTG TAA TTG GAA TGA TGG-3’) and R1 (5’-TAG TTT ATG GTT AGG ACT ACG-3’) [19]. Amplification consisted of an initial denaturing at 95 °C for 5 min, followed by 40 cycles of 95 °C for 30 s, 56 °C for 30 s and 72 °C for 30 s, with a final extension step at 72 °C for 10 min. The expected size of amplicons is 465 bp which is a partial sequence of Babesia 18S rRNA gene. The targeted gene was amplified from template in 25 μl PCR mixtures containing 12.5 μl of PCR mix (Sangon Biotech®), 2 μl of template DNA, and 1 μl each of primer F1 and R1. The amplified DNA fragment was visualized with SYBR® Safe (Thermo Fisher Scientific, Waltham, USA) after electrophoresis in 2 % agarose gel, then cloned and sequenced by Sangon Biotech® (Shanghai, China). In addition, 18S rRNA synthetic plasmid of the sequence of B. canis (GenBank: KT008057.1) and distilled water were used as positive and negative controls, respectively.

Amplification and sequencing of nearly full length of Babesia 18S rRNA gene

The primer used with the Primer 5 (PREMIER Biosoft, Vancouver, BC, Canada) software was designed and the Babesia sequences came from NCBI database. The primers 18SF (5’-TCC TGC CAG TAG TCA TA-3’) and 18SR (5’-TTG TTA CGA CTT CTC CT-3’) were designed to amplify approximately 1600 bp of Babesia 18S rRNA gene sequence. According to the manufacturer’s instructions, PCR was carried out in 25 μl reaction mixture.
comprised of 0.5 μl of dNTP (Thermo Fisher®), 2.5 μl of SuperFi 10X High Fidelity PCR Buffer, 0.1 μl of Platinum Taq DNA polymerase High Fidelity (5U/μl, Thermo Fisher Scientific), 2 μl of DNA, 1 μl each of primer 18SF and 18SR, 1 μl of 50 mM MgSO₄. Amplification consisted of an initial denaturing at 95 °C for 5 min, followed by 35 cycles of 95 °C for 60 s, 52 °C for 60 s and 72 °C for 60 s, with a final extension step at 72 °C for 10 min. The negative and positive controls were the same as described above. Amplified products were visualized with SYBR® Safe (Thermo Fisher Scientific) after electrophoresis in 2% agarose gel, then cloned and sequenced by Sangon Biotech® (Shanghai, China).

Sequence analysis
The obtained sequences were aligned with full length sequences of 18S rRNA gene for related Babesia spp. by MegAlign component of the DNAStar software program. A phylogenetic analysis of the 18S rRNA gene was performed by the neighbour-joining (NJ) method to estimate the phylogeny of the entire alignment using MEGA 7.0 software [2]. For this study, all 29 sequences for the other species were obtained from GenBank [20]. The phylogenetic tree was constructed and performed with 1000 bootstrap replications to evaluate the reliability of the constructions [21]. The distance matrices for the aligned sequences were calculated by the Kimura 2-parameter method, and there were a total of 1102 positions in the final dataset.

Results
Morphological observation
Babesia-like parasites were observed from in the blood smear of the giant panda under the magnification of ×1000 after staining with Giemsa and Diff Quick (Fig. 1). The form of parasite was round to oval (or ring-shaped) and elongated with a pointed end (Fig. 1). These single forms were usually present close to the center of erythrocytes. The nuclei appeared as rounded forms along the outer limits. In addition, we have found paired pyriform merozoites of Babesia, but no typical cruciform merozoites. The mean size of the merozoite was 1.97 ± 0.35 μm by 1.29 ± 0.11 μm (range 1.54–3.19 μm by 0.93–1.63 μm; n = 20).

Detection of Babesia spp. by PCR
PCR amplified the target sequence for Babesia species in the positive control, but the sample of nuclease-free water (negative control) did not show any bands. The collected sample from the giant panda was subjected to PCR. The results showed a 450 bp specific band which is consistent with the predicted size (Fig. 2). The amplified fragments were cloned and sequenced. Blast in NCBI demonstrated that the cloned sequence is most closed to a species of Babesia identified from bears (GenBank: AB566229.1).

Amplification and sequencing of full length of Babesia 18S rRNA gene
One Babesia specific 18S rRNA PCR band was successfully amplified (Fig. 3). An amplicon of 1604 bp comprising the majority of the 18S rRNA gene was sequenced from the blood of the giant panda (GenBank: MT256300.1). The sequence of Babesia 18S rRNA from giant panda differed from other Babesia species, with the most similar species being Babesia sp. EBB (GenBank: AB566229.1) and Babesia sp. Iwate (GenBank: AB586027.1) which infect Japanese black and brown bears. The Babesia 18S rRNA from the panda showed 99.56% and 99.07% similarity and 7–8 bp difference compared with Babesia sp. EBB (GenBank: AB566229.1) and Babesia sp. Iwate (GenBank: AB586027.1), and a 93.15% similarity and 110 bp difference compared with B. canis; compared with B. divergens, there was 96.69% similarity and 53 bp difference (Table 1).

Molecular phylogeny
The nucleotide sequences of the 18S rRNA gene were used to reveal the phylogenetic relationship of the Babesia sp. from the giant panda with other Babesia species. The sequences identified in this study were aligned to known sequences of 18S rRNA (1604 nt) of 29 Babesia spp. in the GenBank database. For the phylogenetic tree of the 18S rRNA gene (Fig. 4), T. gondii (L24381.1) was used as the outgroup. Babesia strains formed two clades. One clade represented Babesia (sensu lato) and included B. microti, B. rodhaini and B. duncani which was close to the outgroup. The second clade represented Babesia (sensu stricto) and included B. orientalis, B. canis, B. gibsoni and other Babesia species which was far from the outgroup. The clade of Theileriidae was in the middle of Babesia (s.s.) and Babesia (s.l.). Babesia sp. detected in the giant panda is similar to the species which infects Ursus thibetanus japonicus (GenBank: AB586027.1) and Ursus arctos yesoensis (GenBank: AB566229.1) in Japan.

Discussion
Babesia spp. are apicomplexans that live in erythrocytes similar to Plasmodium and Theileria, and have a wide host range, including hundreds of mammal species and a limited number of bird species [22–26]. Babesia parasites are maintained in a complex system of tick vectors and animal reservoirs [27]. Although there are many kinds of animals infected with Babesia spp., up to now, there is no
report about the infection of *Babesia* and resulting babesiosis in the giant panda.

To our knowledge, this study is the first to confirm that the giant panda can be infected with *Babesia* spp. causing babesiosis. Usually, babesiosis in wildlife is mostly asymptomatic [28], but the panda in this study showed some clinical signs like fever, anemia, jaundice and hemoglobinuria. There are also some reports about babesiosis with clinical signs in wildlife. Hoby et al. [12] reported that five cases of fatal babesiosis in free-ranging chamois (*Rupicapra r. rupicapra*) attributed to infections with *Babesia capreoli*. Until now, we do not know which of the signs of babesiosis showed immediately after the giant panda was infected with *Babesia* or how the giant panda presented a chronic asymptomatic carrier state and then showed signs under specific circumstances. Further investigation is needed.

The common etiological diagnosis of babesiosis is established by the microscopic identification of the organism on stained blood smears [29]. However, if the number of parasites in the blood is low, it is difficult to detect them in the blood under the light microscope during the different stages of infection, and also detection is affected by the conditions and time of blood preservation. Therefore, a sensitive and specific diagnosis method is needed for early diagnosis of *Babesia* in the giant panda. A variety of immunological methods have been reported for the diagnosis of babesiosis in animals and humans [30]. However, due to the lack of specific giant panda antibodies against the disease, diagnostic methods still need to be developed. PCR amplification of DNA of

---

Fig. 1  
\(a, b\) Giemsa stained thin blood smear of *Babesia* from the giant panda. Final magnification of 1000 x. 
\(c, d\) Diff Quick stained thin blood smear of *Babesia* from the giant panda. Final magnification of 1000 x. Scale-bars: 5 μm
Babesia is a more sensitive detection method, especially when Babesia infection is subclinical or parasitemia level is relatively low. Therefore, we used the combination of microscopy and PCR for the detection of Babesia infection in the giant panda [31].

Morphologically, Babesia is generally divided into two forms: large form (2.5–5.0 μm long) which include B. bigemina, B. caballi and B. canis, etc.; and small from (1.0–2.5 μm long) which include B. bovis, B. gibsoni, B. microti, B. rodhaini, etc. The orientation of the parasite in the red blood cells (RBCs) depends on its size, large pyriform parasites meet each other in an acute angle at the tips while small parasites make an obtuse angle to each other [32]. Babesia are polymorphic intraerythrocytic parasites and appear under light microscopy as ring forms, pear-shaped forms, paired pyriforms, and pleomorphic ring forms [33]. In this study, under a microscope, we found round to ring-shaped, paired pyriforms and other shapes indicating Babesia, but no typical cruciform merozoites. According to aforementioned size of the Babesia infecting the giant panda should belong to the group of small Babesia, but the sample is from just one panda, therefore we need more data to prove it.

We used 18S rRNA gene of Babesia as a conserved identification sequence. In addition, 18S rRNA gene is widely used in Babesia research, making it easier to compare this undetermined species with other known Babesia species. The 18S rRNA gene, with conservative regions provide a sufficient template DNA for PCR, and is widely used as a target for Babesia species identification. Although it has been reported that there is a high degree of conservation of the 18S rRNA sequences in Babesia and Theileria species, it has been suggested to use the full 18S rRNA gene, especially when dealing with new organisms, to ensure that genetic variation is not overlooked [34].

To our knowledge, our study is the first to provide information of a Babesia species infecting the giant panda. This Babesia sp. is likely to be a novel species based on the phylogenetic analysis which has been widely used to study the genetic diversity of Babesia [35–37]. This clearly suggests that this novel Babesia sp. is separated from the other types. The phylogenetic tree (Fig. 4) showed that B. odocoilei and B. divergens are highly similar with the Babesia sp. strain EBP01. It is important to note that B. divergens will cause amphixenosis and has the risk of infecting humans [38, 39].

Nonetheless, Babesia sp. strain EBP01 is closely related with B. odocoilei and B. divergens which is closely linked to other undetermined Babesia species which were found in both the black and brown bear, dog, raccoon and wolf [40, 41]. They formed a distinct clade which was separated from the clade consisting of B. divergens and B.
odocoilei. These clades are clearly separated with a bootstrap value of 99% (Fig. 4). These Babesia species have about 99.7% similar gene sequences associated with the Babesia spp. infecting the bears and are significantly different from several common species of Babesia such as B. divergens, B. canis, B. bovis, B. ovata and B. gibsoni (Table 1). It is hypothesized that the Babesia sp. strain EBP01 is possibly a novel Babesia species unique to A. melanoleuca, which is close to the species of Babesia which infect other ursids; however, we need more evidence and investigation to verify this.

The threat of domestic dogs to biodiversity conservation is increasingly addressed in the literature, but is often overlooked and underestimated in conservation programmes worldwide [42, 43]. It is well known that domestic dogs and giant pandas share many common diseases, such as canine distemper, parvovirus and parasitic infections. As a result, it is very important to carry out and strengthen management of free-ranging dogs around the nature reserves to better protect local wildlife, such as giant pandas [44, 45]. Interestingly, our results showed that a Babesia species Akita610, collected from dogs in Japan, is closely related to the Babesia sp. strain EBP01 (99.07%). Although the above findings are not sufficient to indicate that dogs may also be infected with Babesia sp. strain EBP01, this result will help us try to investigate the Babesia infection in sympatric species of the giant panda, especially canids.

Babesia spp. are tick-borne parasites, thus it is important to find the specific definitive tick host, for the continued study of Babesia sp. in the giant panda [46, 47]. It is worth noting that captive giant pandas have a history of tick infestation. Obviously, ticks are the main threat of babesiosis in the giant panda; however, the specific species of tick that infect giant pandas and transmit Babesia need further study.

Conclusions
To our knowledge, this study found the first case of Babesia infection in the giant panda. We successfully detected the pathogen of Babesia in the blood from a giant panda by microscopic examination and PCR method, and found evidence using molecular biology to show the Babesia sp. is more closely linked to unidentified Babesia species found in bears in Japan. We think that this Babesia sp. may be a novel species, currently named Babesia sp. strain EBP01.

Fig. 4 Rooted 50% majority-rule consensus cladogram of the open reading frame of the 18S rRNA from piroplasms. Calculated by neighbor-joining and bootstrap estimates from 1000 replicates, with T. gondii as the outgroup. The bootstrap values based on 1000 replicates are displayed next to the branches, the hosts are listed in parentheses.
Abbreviations
PCR: Polymerase chain reaction; 18S rRNA: 18 Svedberg ribosomal RNA; MODS: Multiple organ dysfunction syndrome; IUCN: International Union for Conservation of Nature; EDTA: Ethylene diamine tetraacetic acid.

Acknowledgements
We would like to thank the veterinary staff of the Chengdu Panda Base for collecting blood samples, James Edward Ayala and Dr. Marc Valitutto for reviewing the manuscript.

Authors’ contributions
WLB and RM collected the blood of the giant panda. CJY and DWQ established research methodology. CJY, ZSD and YLL analyzed the infection of the panda and built the phylogenetic tree based on Babesia 18S rRNA gene. XL, CJY and ZSD were responsible for the experimental operations. RH and GYY conceived, designed, reviewed and edited the manuscript. All authors read and approved the final manuscript.

Funding
Supported by the Chengdu Research Base of Giant Panda Breeding (2020CPB C11), (Establishment and application of rapid identification technology platform for viral pathogens of endangered wildlife).

Availability of data and materials
All data generated or analyzed during this study are included in this published article. The newly generated sequence was deposited in the GenBank database under the accession number MT256300.1.

Ethics approval and consent to participate
All blood samples were collected noninvasively from a giant panda trained to volunteer for blood collection and did not require the use of anesthesia. The use of materials and all experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee of the Chengdu Research Base of Giant Panda Breeding (IACUC number 2020006).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1 Sichuan Key Laboratory of Conservation Biology for Endangered Wildlife, Chengdu Research Base of Giant Panda Breeding, Sichuan Academy of Giant Panda, 1375 Panda Road, Chenghua District 610081, Sichuan, China. 2 College of Veterinary Medicine, Sichuan Agricultural University, Chengdu 611130, China.

Received: 8 June 2020 Accepted: 21 October 2020 Published online: 29 October 2020

References
1. Niu Q, Liu Z, Yu P, Yang J, Abdallah MO, Guan G, et al. Genetic characterization and molecular survey of Babesia bovis, Babesia bigemina and Babesia ovoat in cattle, dairy cattle and yaks in China. Parasit Vectors. 2015;8:518.
2. Schmittner L, Rodriguez AE, Florin-Christensen M, Morrison DA. Babesia: a world emerging. Infect Genet Evol. 2012;12:1788–809.
3. Jiang JF, Zheng YC, Jiang JR, Li H, Hua QB, Jiang BG, et al. Epidemiological, clinical, and laboratory characteristics of 48 cases of “Babesia venatorum” infection in China: a descriptive study. Lancet Infect Dis. 2015;15:196–203.
4. Zamoto-Nikura A, Morikawa S, Hanaki K, Holman PJ, Ishihara C. iodes persultus ticks as vectors for the Babesia microti U.S. Lineage in Japan. Appl Environ Microbiol. 2016;82:6624–32.
5. Pattullo KM, Wobeser G, Lockerbie BP, Burgess HJ. Babesia odoocile infection in a Saskatchewan elk (Cervus elaphus canadensis) herd. J Vet Diagn Invest. 2013;25:335–40.
6. Anderson JF, Magnarelli LA, Sulzer AJ. Babesiosis in Connecticut, USA. Babesia tafoni sp. n. J Parasitol. 1985;67:417–25.
7. Birkenheuer AJ, Whittington J, Neel J, Large E, Barger A, Levy MG, et al. Molecular characterization of a Babesia species identified in a North American raccoon. J Wildl Dis. 2006;42:375–80.
8. Zintl A, Gray JS, Skerrett HE, Mulcahy G. Possible mechanisms underlying age-related resistance to bovine babesiosis. Parasite Immunol. 2005;27:115–20.
9. Gombert ME, Goldstein EJ, Benach JL, Tenenbaum MJ, Grunwaldt E, Kaplan MH, et al. Human babesiosis clinical and therapeutic considerations. JAMA. 1982;248:3005–7.
10. Lykins JD, Ristic M, Weisgerm RM. Babesia microti: pathogenesis of parasite of human origin in the hamster. Exp Parasitol. 1975;37:388–97.
11. Telford SR III, Gorenflot A, Braisier P, Spielman A. Babesial infections in humans and wildlife, vol. 2. San Diego: Elsevier Inc. 1993. p. 20–8.
12. Hoby S, Robert N, Mathis A, Schmid N, Meli ML, Hofmann-Lehmann R, et al. Babesiosis in free-ranging chamois (Rupicapra rupicapra) from Switzerland. Vet Parasitol. 2007;148:341–5.
13. Yabsley MJ, Shock BC. Natural history of zoonotic Babesia: role of wildlife reservoirs. Int J Parasitol Parasites Wildl. 2013;2:18–31.
14. Millnes EL, Thorton C, Latos J, Coutts A, Barta IR, Smith DA, et al. Babesia odoocile and zoonotic pathogens identified from ixodes scapularis ticks in southern Ontario, Canada.Ticks Tick Borne Dis. 2019;10:670–6.
15. Li G, Connor T, Bai W, Yang H, Zhang J, Q D, et al. Dynamics of the giant panda habitat suitability in response to changing anthropogenic disturbance in the Liangshang mountains. Biol Conserv. 2019;237:445–55.
16. Zhang W, Yang S, Shan T, Hou R, Liu Z, Li W, et al. Virome comparisons in mild-diseased and healthy captive giant pandas. Microbiome. 2017:9:40.
17. Kang D, Li J. Giant panda protection: challenges and hopes. Environ Sci Pollut Res Int. 2019;26:18001–2.
18. Jorgensen JH, Pfaal MA, Carroll KC. Manual of clinical microbiology. Washington: ASM Press, 2015. p. 1061–106.
19. Casati S, Sager H, Gern L, Piffaretti JC. Presence of potentially pathogenic Babesia sp. for human in ixodes ricinus in Switzerland. Ann Agric Environ Med. 2006;13:65–70.
20. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987;4:406–25.
21. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. Evolution. 1985;39:783–91.
22. Gigandet L, Stauffer E, Douet V, Rais O, Monet J, Gern L. Prevalence of three zoonotic Babesia species in Ixodes ricinus (L., 1758) nymphs in a suburban forest in Switzerland. Vector Borne Zoonotic Dis. 2011;11:563–6.
23. Zamoto-Nikura A, Tsuji M, Qiang W, Nakao M, Hirata H, Ishihara C. Detection of two zoonotic Babesia microti lineages, the Hobetsu and U.S. lineages, in two sympatric tick species, Ixodes ovatus and Ixodes incomparabilis, respectively, in Japan. Appl Environ Microbiol. 2012;78:3424–30.
24. Gray A, Capewell P, Loney C, Katzer F, Shields BR, Weir S. Sheep as host species for zoonotic Babesia venatorum, United Kingdom. Emerg Infect Dis. 2019;25:2257–60.
25. Lemperreur L, Wirtgen M, Nahayo A, Caron Y, Shields B, Saegeerman C, et al. Wild cervids are host for tick vectors of Babesia species with zoonotic capability in Belgium. Vector Borne Zoonotic Dis. 2012;12:275–80.
26. Scott JD, Clark KL, Durden LA. Presence of Babesia odocoilei and Borelia burgdorferi sensu stricto in a tick and dual parasitism of Amblyomma inornatum and Ixodes scapularis on a bird in Canada. Healthcare. 2019;7:46.
27. Young KM, Corrin T, Wilhelm B, Uhland C, Greig J, Masurenhas M, et al. Zoonotic Babesia: a scoping review of the global evidence. PLoS ONE. 2019;14:e0226781.
28. Homer ML, Aguilar-Delfin I, Telford SR 3rd, Kraje PJ, Persing DH. Babesiosis. Clin Microbiol Rev. 2000;13:451–69.
29. Kukina N, Guzeeva TM, Zelya OR, Ganushkina LA. Fatal human babesiosis caused by Babesia divergens in an asplenic host. ICDCases. 2018;1:3:e00414.
30. Ferrer D, Castella J, Gutierrez JF. Seroprevalence of Babesia ovis in sheep in Catalonia, northeastern Spain. Vet Parasitol. 1998;79:275–81.
31. Laha R, Das M, Sen A. Morphology, epidemiology, and phylogeny of Babesia: an overview. Trop Parasitol. 2015;5:94–100.
32. Inacio EL, Perez-Macchi S, Alabi A, Bittencourt P, Muller A. Prevalence and molecular characterization of piroplasmids in domestic dogs from Paraguay. Ticks Tick Borne Dis. 2019;10:321–7.
33. Hong SH, Kim SY, Song BC, Rho JR, Cho CR, Kim CN, et al. Detection and characterization of an emerging type of Babesia sp. similar to Babesia-motasi for the first case of human babesiosis and ticks in Korea. Emerg Microbes Infect. 2019;8:869–78.
34. Lempereur L, Beck R, Fonseca I, Marques C, Duarte A, Santos M, et al. Guidelines for the detection of Babesia and Theileria parasites. Vector Borne Zoonotic Dis. 2017;17:51–65.
35. Mira A, Unlu AH, Bilgic HB, Bakirci S, Hacilarlioglu S, Karagenc T, et al. High genetic diversity and differentiation of the Babesia ovis population in Turkey. Transbound Emerg Dis. 2020;67:26–35.
36. Wang T, Guan G, Korhonen PK, Koehler AV, Young ND, Hall RS, et al. Mitochondrial genomes of two Babesia taxa from sheep in China as a foundation for population genetic and epidemiological investigations. Infect Genet Evol. 2017;47:51–5.
37. Nagore D, Garcia-Sanmartin J, Garcia-Perez AL, Juste RA, Hurtado A. Identification, genetic diversity and prevalence of Theileria and Babesia species in a sheep population from Northern Spain. Int J Parasitol. 2004;34:1059–67.
38. Yamamoto-Kikura A, Hagiwara K, Imaoka K, Morikawa S, Ishihara C, Hanaki K. Epidemiological survey of Babesia divergens North America Lineage in wild sika deer (Cervus nippon) by using direct PCR in Japan. Jpn J Infect Dis. 2020;73:68–71.
39. Debierré-Grockiego F, Smith TK, Delbecq S, Ducournau C, Lantier L, Schmidt J, et al. Babesia divergens glycosylphosphatidylinositol modulate blood coagulation and induce Th2-biased cytokine profiles in antigen presenting cells. Biochimie. 2019;167:135–44.
40. Ikawa K, Aoki M, Ichikawa M, Itagaki T. The first detection of Babesia species DNA from Japanese black bears (Ursus thibetanus japonicus) in Japan. Parasitol Int. 2011;60:220–2.
41. Inokuma H, Yoshizaki Y, Shimada Y, Sakata Y, Okuda M, Onishi T. Epidemiological survey of Babesia species in Japan performed with specimens from ticks collected from dogs and detection of new Babesia DNA closely related to Babesia odocoilei and Babesia divergens DNA. J Clin Microbiol. 2003;41:3494–9.
42. Yan X, Owens JR, Chen Y, Su X, Wang Z, Liu S, et al. Dogs and disease threats to giant pandas in China. J Wildl Manag. 2019;84:268–76.
43. Jin Y, Zhang X, Ma Y, Qiao Y, Liu X, Zhao K, et al. Canine distemper viral infection threatens the giant panda population in China. Oncotarget. 2017;8:113910–9.
44. Alvarado-Rybik M, Solano-Gallego L, Millan J. A review of piroplasmid infections in wild carnivals worldwide: importance for domestic animal health and wildlife conservation. Parasit Vectors. 2016;9:538.
45. Callan R, Owens JR, Bi W, Kilham B, Yan X, Qi D, et al. Free-roaming dogs limit habitat use of giant pandas in nature reserves. Sci Rep. 2020;10:10247.
46. Boldbaatar D, Battsetseg B, Matsuo T, Hatta T, Umemiyia-Shiraufi R, Xuan X, et al. Tick vitellogenin receptor reveals critical role in oocyte development and transovarial transmission of Babesia parasite. Biochem Cell Biol. 2008;86:331–44.
47. Karbowiak G, Biernat B, Stanczak J, Werszko J, Szewczyk T, Sytykiewicz H. The role of particular ticks developmental stages in the circulation of tick-borne pathogens in central Europe. 6. Babesia. Ann Parasitol. 2018;64:265–84.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.