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Molecular detection of *Bartonella rochalimae* and *Hepatozoon canis* in red foxes (*Vulpes vulpes*) from China

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ABSTRACT

Red foxes (*Vulpes vulpes*) have been recognized as natural reservoirs for multiple pathogens and a source of infection for domestic animals, wildlife and humans. To date, no reports are available on the *Bartonella rochalimae* and *Hepatozoon canis* infection in red foxes from China. In 2018–2022, a total of 16 red foxes were sampled in two counties and a city in Xinjiang Uygur Autonomous Region (XUAR) in northwest China. Subsequently analyzed by DNA extraction amplified by polymerase chain reaction (PCR). In the present study, based on nucleotide sequence and phylogenetic tree analyses, *B. rochalimae* and *H. canis* were molecularly identified in red foxes. Our findings provide the first molecular evidence of *B. rochalimae* and *H. canis* in red foxes from China.

1. Introduction

Canine vector-borne diseases (CVBD) are caused by numerous viral, bacterial and protozoal pathogens, which are transmitted by blood-sucking arthropods including ticks, fleas, mosquitos, and flies and pose considerable public health challenges in animals and humans globally (Otranto et al., 2009). As is well known, the majority of CVBD are closely associated with human population growth, global warming, illicit trade exchange, and habitat destruction, and these factors may have an impact on their prevalence and distribution (Kilpatrick et al., 2012; Wilke et al., 2021). However, there has been epidemiological research on CVBD in companion animals mainly focused on dogs (Hamel et al., 2012; Otranto and Dantas-Torres, 2010; Xu et al., 2015), data on the occurrence and prevalence of CVBD in red foxes in China remain scarce.

Recently, multiple fox species were reported to harbor vector-borne pathogens of public health concern, including spotted fever group rickettsiae (Dall'Agnol et al., 2018), *Borrelia burgdorferi* (Lledó et al., 2016), *Ehrlichia canis* (Fishman et al., 2004), *Leishmania infantum* (Davoust et al., 2014), and *Babesia vulpes* (Checa et al., 2018). In China, the red fox (*Vulpes vulpes*) can transmit classical zoonotic diseases, such as rabies (Yakovchits et al., 2021), echinococcosis (Jiang et al., 2012), and rickettsioses (Liu et al., 2021). In this study, we aimed to investigate the molecular characterization of *Bartonella* and *Hepatozoon* in red foxes from China.

2. Materials and methods

In 2018–2022, a total of 16 red foxes (five adults and eleven pups) were found dead as a result of road kills and poaching, and subsequently sampled in two counties and a city (Manas County, Nilka County, and Alataw City) in Xinjiang Uygur Autonomous Region (XUAR) in northwest China. The red foxes were morphologically identified by an experienced zoologist. The study had formal ethical approval from Medical College of Shihezi University.

All fox organs, including the hearts, lungs, livers, spleens, and kidneys, were removed. The spleen DNA were extracted using the TIANamp Genomic DNA Kit (TIANGEN, Beijing, China). After the DNA concentration were qualified, and subsequent polymerase chain reaction (PCR) experiments were carried out. Two genes, *gltA* and *rpoB*, were used to detect *Bartonella*, whereas one gene, *18S rRNA*, was used to detect *Hepatozoon*. The primer sequences were as follows: gltA-F: 5′-GCTTTA-CAAAATTCTAAAAACCATATA-3′, and gltA-R: 5′-TGTCTATCAATTCA-CAACTTGCCGTT-3'. The PCR cycling conditions for *Bartonella* detection

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consisted of an initial 5-min denaturation at 95 °C, followed by 35 cycles at 95 °C for 30 s, 52 °C for 30s, and 72 °C for 30s, with a final extension at 72 °C for 8 min. To confirm the positivity of this PCR, it was attempted to amplify an additional genetic market, an approximately 330-bp-long fragment of the *rpoB* gene primers rpoB-F: 5′-GCTCTTGCAACTTC-TATGTT-3′, and rpoB-R: 5′- CATTGTTCGTCAGGTTGGCG-3’. The PCR conditions were the same as in the *gltA*, except that the annealing temperature was 53 °C. The primer sequences of *18S rRNA:* 18S rRNA-F5′-ATGGCTCATTACAACAGT-3′, and 18S rRNA-R: 5′-AAGTTT-CAGCCTTGCGACCATACTC-3’. The PCR cycling conditions for *Hepatozoon* detection consisted of an initial 5-min denaturation at 95 °C, followed by 37 cycles at 95 °C for 40 s, 50 °C for 40s, and 72 °C for 50s, with a final extension at 72 °C for 8 min. The negative control was double distilled water, and the positive controls were *Bartonella elizabethae* DNA from fleas and *Hepatozoon* sp. DNA from great gerbils obtained in our previous works (Ji et al., 2021; Yin et al., 2019). Purification and sequencing of the PCR products were performed as described previously (Farkas et al., 2014).

Sequences were manually edited, aligned and compared with GenBank sequences (https://blast.ncbi.nlm.nih.gov). Two phylogenetic trees were constructed using the neighbor-joining method, bootstrap analyses 1000 replicates were conducted to determine the relative support for clades in the consensus trees in MEGA 7.0 software.

3. Results

Out of the 16 fox specimens collected from the XUAR, after molecular detection, *Bartonella* was found in two juvenile red foxes, whereas *Hepatozoon* was found in one adult red fox. Based on sequencing and phylogenetic analysis, the *Bartonella* that was identified in the red foxes was most closely related to *B. rochalimae*, which was from grey foxes (*Urocyon cinereoargenteus*) (GenBank accession No. FN645459), with a 99.40% (352/354) shared sequence identity in the *gltA* gene. Meanwhile, its *rpoB* gene was most closely related to *B. rochalimae* (DQ676489) from a dog sampled in USA, with 99.71% (345/346) shared sequence identity. The phylogenetic tree of the *Bartonella gltA-rpoB* concatenated sequences provide additional support for this conclusion (shown in Fig. 1). In addition, the *Hepatozoon* sp. identified in the red foxes was most closely related to *H. canis* from a golden jackal (*Canis aureus*) sampled in the Czech Republic (KX712124), with 98.55% (1085/1101) shared sequence identity in the *18S rRNA* gene (shown in Fig. 2). All obtained sequences from this study were deposited in the GenBank (*B. rochalimae gltA*: OQ834668, *B. rochalimae rpoB*: OQ868002 and *H. canis 18S rRNA*: OQ859899).

4. Discussion

Wild animals are important indicators of vector-borne diseases. Red foxes live near ruminants (cattle, sheep, and goats), companion animals

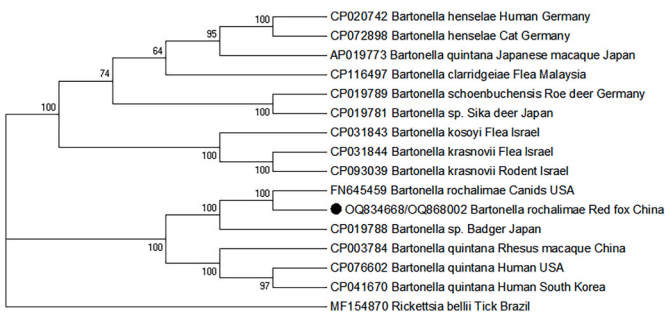

Fig. 1. The NJ phylogenetic tree of the *gltA-rpoB* concatenated sequences of *Bartonella* spp. (NJ; bootstrap replicates: 1000). Branch lengths correlate to the number of substitutions inferred according to the scale shown. Sequences of obtained in this study are indicated by solid cycle (●).

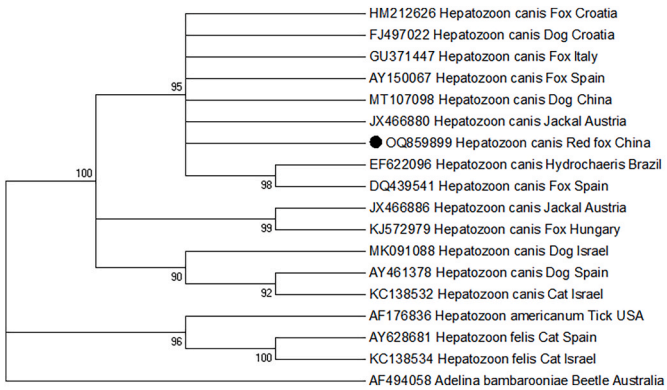

Fig. 2. The NJ phylogenetic tree of the 18S rRNA of *Hepatozoon* spp. (NJ; bootstrap replicates: 1000). Branch lengths correlate to the number of substitutions inferred according to the scale shown. Sequences of obtained in this study are indicated by solid cycle (●).

(dogs and cats), and wildlife (rodents), and in close proximity to humans. Red foxes usually display strong migratory behavior associated with habitat destruction, food loss, and climate change. Previously, red foxes were reported to harbor several vector-borne pathogens, including tick-borne encephalitis virus, *H. canis*, *Rickettsia raoultii*, *Rickettsia sibirica*, Candidatus *Rickettsia barbariae*, *Babesia vulpes*, *Babesia vogeli* and et al. (Wurm et al., 2000; Mierzejewska et al., 2021; Sang et al., 2021; Liu et al., 2021). In China and adjacent countries, some pathogens such as rabies virus, novel norovirus, *Echinococcus* spp., *Babesia* spp, and *H. canis*, were found in red foxes, corsac foxes, wolves and dogs (Wang et al., 2022; Altay et al., 2023; Xu et al., 2015). In this study, *B. rochalimae* and *H. canis* were detected for the first time in red foxes from China.

Bartonella rochalimae, a zoonotic agent, was first isolated from a patient who had a history of travel to Peru, had sustained multiple insect bites, and exhibited fever, rash, myalgia, anemia, and splenomegaly (Eremeeva et al., 2007). Subsequently, *B. rochalimae* was detected in many wild animals. In the canid red fox, *B. rochalimae* has been reported in France, Spain, and the USA (Greco et al., 2021). In this study, *B. rochalimae* were found in approximately 4-week-old juvenile red foxes in Manas County in XUAR, China. Juvenile mammals may be more susceptible to *Bartonella* transmitted through flea-biting than adult mammals, and this finding is consistent with a previous study (Chomel et al., 2006). In previous reports, *B. rochalimae* was vectored by *Pulex simulans*, *Paraceras melis*, *Xenopsylla gerbilli minax*, and *Xenopsylla conforms conforms* (Yin et al., 2019), implying that this pathogen may be vectored by flea species. Here, *B. rochalimae* was detected in red foxes in China for the first time.

Hepatozoon canis, a known veterinary parasite, has been mainly reported in canid animals, including the African wild dog (*Lycaon pictus*), red fox (*V. vulpes*) crab-eating fox (*Cerdocyon thous*), grey wolf (*Canis lupus*), golden jackal (*Canis aureus*), raccoon (*Procyon lotor*), and spotted hyena (*Crocuta crocuta*) (Gabriel et al., 2009; Allen et al., 2011; Baneth, 2011; Gerrikagoitia et al., 2012). In China, *H. canis* was previously only reported in domestic dogs (Guo et al., 2020). In the present study, *H. canis* was detected in the red fox in China for the first time. In addition, as a tick-borne pathogen, *H. canis* has also been documented in several tick species, such as *Rhipicephalus sanguineus*, *Haemaphysalis* sp., and *Dermacentor reticulatus* (Giannelli et al., 2017; Mierzejewska et al., 2021; Najm et al., 2014). In our previous research, *Ixodes canisuga*, *Ixodes kaiseri*, *Haemaphysalis erinacei*, and *Dermacentor marginatus* were detected in red foxes (Liu et al., 2021). Further research is needed to determine the distribution of this two pathogens in domestic dogs and wild canine, and it is also important to identify the transmission route.

5. Conclusion

To our best knowledge, this is the first study to report the molecular characterization of *B. rochalimae* and *H. canis* in wild red foxes from China. This work has particular importance for the continued and extended surveillance of wild red foxes as potential sources of canine vector-borne pathogens.

Authors' contributions

SW, NC and ZL conceived and designed the study, and wrote the manuscript. NW, GL, SZ and CL performed the experiments and analyzed the data. SW and YW contributed to study design and edited the manuscript. All authors have read and agreed to the published version of the manuscript

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Data availability

DNA sequences were deposited in GenBank; accession numbers: OQ834668, OQ868002, OQ859899.

Consent to participate

Informed verbal and written consent were obtained from each study participant.

Ethical approval

All procedures performed in this study involving wild animals were in accordance with the ethical standards of the Animal Ethics Committee of Shihezi University (Approval No. AECSU2015-11).

Declaration of competing interest

The authors declare that they have no conflict of interest.

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