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Anaplasma bovis and *Bartonella* spp. in Libyan Jirds (*Meriones libycus*) from China

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ABSTRACT: *Anaplasma bovis* (1), *Bartonella krasnovii* (3), and *Bartonella* sp. (17) were detected in 80 Libyan jirds (*Meriones libycus*) from China. These findings extend the known host and geographic ranges of these pathogens, with neither *A. bovis* nor *B. krasnovii* previously confirmed in Libyan jirds.

The Libyan jird (*Meriones libycus*) is found in desert, semidesert, and cultivated lands in the Central Asia, Western Asia, and Northern Africa regions (Granjon 2016). This jird may carry a variety of pathogens, such as *Yersinia pestis* and *Leishmania major* (Samy et al. 2016; Yu et al. 2020), that cause human diseases. *Anaplasma* and *Bartonella* spp. are intracellular microorganisms mainly transmitted by blood-sucking arthropods. *Anaplasma bovis* is mainly found in wild ruminants (Rjeibi et al. 2018). *Bartonella krasnovii* has been reported in the midday jird (*Meriones meridianus*), Anderson's gerbil (*Gerbillus andersoni*), black rat (*Rattus rattus*), and fleas (Rao et al. 2021; de Sousa et al. 2022). We conducted a study of *Anaplasma* and *Bartonella* spp. in Libyan jirds in the Kuitun River Wetland Nature Reserve (KRWNR; elevation, 322 m; 84°20′43E, 44°32′07N) in northwestern China.

In total, 80 Libyan jirds were captured at five survey sites in KRWNR in August 2023. We used wire-mesh live traps (30 × 15 × 15 cm) that were baited with tomato, walnut, or cucumber and placed near entrances of occupied burrows (Ji et al. 2021). Traps were set before nightfall and checked twice daily; each site was trapped for one full day. Captured rodents were killed by cervical dislocation (Elangbam et al. 1990), and necropsy was performed. The livers and spleens from 80 jirds were weighed, examined, and stored at −80 C. This study was approved by the Animal Ethics Committee of Shihezi University (approval no. A2021-053-01).

We extracted genomic DNA from a sample (~2.0 g) of livers and spleens by using a TIANamp genomic DNA kit (TIANGEN, Beijing, China), following the manufacturer's instructions. Molecular identification results of the cytochrome c oxidase subunit I (*COX1*) gene showed that the captured individuals had 100% similarity to Libyan jirds from Xinjiang, China (GenBank accession no. KU182937.1).

The 160 organ samples were subjected to specific PCR for the presence of *Anaplasma* by using the *16S rRNA* gene (Inokuma et al. 2001) and for *Bartonella* by using the citrate synthase A (*gltA*) gene (Rao et al. 2021). The products were inserted into the pMD-18T vector (Takara, Beijing, China) by using TA cloning to generate recombinant plasmids that were then used as templates for sequencing. Sequences were compared with reference GenBank (Song et al. 2018) sequences. Phylogenetic trees were constructed using the neighbor joining method with MEGA 7.0 software (Kumar et al. 2016).

Among the spleens from 80 Libyan jirds, one (1.25%) was positive for fragments of *A. bovis* 16S rRNA. The BLASTn analysis (National Center for Biotechnology Information 2023) showed that DNA sequences obtained shared 99.22% (639/644) identity to *A. bovis* (accession no. OK560164) detected in the greater bandicoot rat (*Bandicota indica*) in Taiwan, China (Fig. 1). In addition, *B. krasnovii* was positively detected in 3/80 (3.75%) of spleens; this shared 98.37% (363/369) identity to *B. krasnovii* from

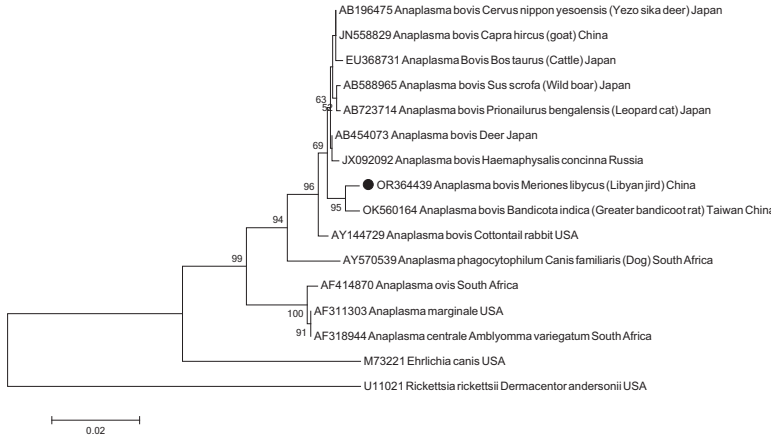

FIGURE 1. Phylogenetic tree of the *16S rRNA* of *Anaplasma bovis* in Libyan jird (*Meriones libycus*) produced using MEGA 7.0 (Kumar et al. 2016). The tree was constructed with the neighbor joining method and 1,000 bootstrap replicates. Branch lengths correlate to the number of substitutions inferred according to the scale shown. The sequences OR364439 *Anaplasma bovis Meriones libycus* (Libyan jird) China obtained in this study is indicated by the black dot (●).

an Egyptian gerbil (*Gerbillus gerbillus*) in Israel (accession no. MH618795; Fig 2). Another *Bartonella* sp. was positively detected in 17/80 (21.25%) of the livers and corresponding spleens; this shared 99.73% (364/365) identity to *Bartonella* sp. (accession no. KT327028) found in a Libyan jird from the Republic of Georgia (Fig. 2). Voucher specimens of PCR-positive samples were retained.

Previously, *A. bovis* has only been detected in Muridae species, such as the striped field mouse (*Apodemus agrarius*), Greater bandicoot rat (*Bandicota indica*), Ryukyu mouse (*Mus caroli*), and rice field rat (*Rattus losea*; Masuzawa et al. 2014; Panthawong et al. 2020). Our finding of *A. bovis* in a Libyan jird from China, sharing 99.37% identity to an *A. bovis* (OK560164) found in the greater bandicoot rat in Taiwan,

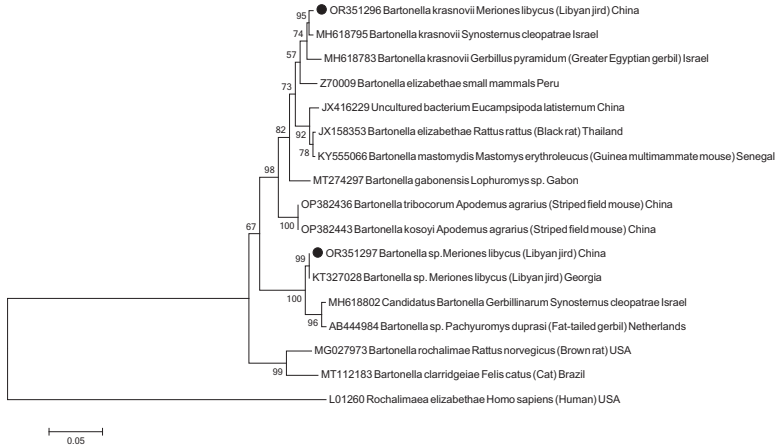

FIGURE 2. Phylogenetic tree of the *gltA* of *Bartonella krasnovii* and *Bartonella* sp. in Libyan jirds (*Meriones libycus*) constructed using MEGA 7.0 (Kumar et al. 2016). The tree was constructed with the neighbor joining method and 1,000 bootstrap replicates. Branch lengths correlate to the number of substitutions inferred according to the scale shown. The sequences OR351296 *Bartonella krasnovii Meriones libycus* (Libyan jird) China and OR351297 *Bartonella* sp. *Meriones libycus* (Libyan jird) China obtained in this study are indicated by the black dots (●).

China, extends the known host range and geographic range of *A. bovis*.

Bartonella krasnovii has previously been reported to circulate among gerbils (e.g., Anderson's gerbil and midday jird) and their associated fleas (de Sousa et al. 2022). The *B. krasnovii* that we found in a Libyan jird from northwestern China is highly similar to *B. elizabethae*, which is pathogenic to humans (Gutiérrez et al. 2020). The *Bartonella* sp. that we sequenced from the Libyan jird showed genetic diversity (99.73% similarity) from sequences from the Republic of Georgia in GenBank (KT327028). As with *A. bovis*, our findings extend the known host and geographic ranges of *B. krasnovii*.

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