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Babesia vesperuginis in Common Pipistrelle (*Pipistrellus pipistrellus*) and the Bat Soft Tick *Argas vespertilionis* in the People's Republic of China

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ABSTRACT: *Babesia vesperuginis* was molecularly detected in 10% (5/48) of common pipistrelle bats (*Pipistrellus pipistrellus*) in Shihezi City, Northwestern China. Interestingly, four bat ticks (*Argas vespertilionis*), from *Babesia* DNA-positive common pipistrelle bats, were also positive for *B. vesperuginis*. Our findings extend the geographic range of the common pipistrelle bat as a reservoir of *B. vesperuginis* in Asia.

The piroplasm *Babesia vesperuginis* was first discovered in the common noctule bat (*Nyctalus noctula*) in Italy (Dionisi 1898). From 1964–96, it was found in the blood in seven bat species belonging to five genera in the Netherlands, the UK, and Columbia (Goedbloed et al. 1964; Gardner and Molyneux 1987; Gardner et al. 1987; Marinkelle 1996). In 2005, *B. vesperuginis* was first detected in *Pipistrellus* spp. using molecular methods in the UK and was described as a pipistrelle-associated piroplasmida species (Concannon et al. 2005). During 2016–17, *B. vesperuginis* was found in the DNA of *Ixodes ariadnae*, *Ixodes vespertilionis*, and the soft tick (*Argas vespertilionis*) collected from bats in Hungary and the People's Republic of China (Hornok et al. 2016, 2017).

As part of a survey on bats and bat tick-borne *Babesia*, 98 bat carcasses were submitted for postmortem examination to the Xinjiang Uygur Autonomous Region Wildlife Management Office, Northwestern China, and sent to our laboratory during 2015–16. Our study was approved by the Animal Ethics Committee of Shihezi University (Approval no. AECSU2015-01). Of the bat carcasses, 48 came from an idle classroom in Shihezi University, Xinjiang Province (Shihezi, 44°18′7″N, 86°03′16″E, elevation 450.8 m) and 50 originated from bat caves in Xinyuan County, Xinjiang Province (Xinyuan, 43°25′42″N, 83°15′30″E, elevation 800 m). Carcasses were morphologically identified as the common pipistrelle bat (*Pipistrellus pipistrellus*) by an experienced zoologist and further confirmed by PCR based on the *cytB* gene (Sudman et al. 1994). Twenty-four and 21 tick larvae were picked from whole bodies of the bats from Shihezi and Xinyuan Counties, respectively. The ticks were morphologically identified as *A. vespertilionis* according to the standard taxonomic keys as previously described (Roshdy 1961). Molecular identification showed they had a similarity of 99.31% (431/434) with *A. vespertilionis* (GenBank no. HM751841) based on *16S* mitochondrial gene sequences (Black and Piesman 1994).

The heart, liver, spleen, lung, small intestine, and large bowel of the collected bats were dissected (Concannon et al. 2005), and genomic DNA was extracted from tissues and ticks using the 96 Flux Automatic Nucleic Acid Extraction Instrument (Bio Teke, Beijing, People's Republic of China) with a matching commercial kit (Cell & Tissue Kit, Bio Teke) according to the manufacturer's instructions. Two genetic markers (452 base pairs [bp] and 517 bp) targeting *18S* rRNA at different regions were employed to screen the 45 DNA extracts of bat ticks and 588 DNA extracts of bat tissues for *Babesia* spp. detection. Two pairs of primers based on different *18S* rRNA fragments were commercially synthesized (Beijing Huada Inc., Bei-

jing, China). The PCR reaction systems were used as previously described (Ano et al. 2001; Casati et al. 2006). The DNA from *Babesia bovis* (obtained from Qinghe County, Xinjiang Province) was used as the positive control and double-distilled water was used as the negative control. The PCR products were visualized in 1.5% agarose gel and were purified and sequenced (Sangon Biotech, Shanghai, China). The resulting sequences from *Babesia* were compared with the reference sequences found in the centralized databases using the BLAST tool (National Center for Biotechnology Information 2016).

Two genetic markers targeting different regions of *Babesia 18S* rRNA were both positive in 19% (4/21) of *A. vespertilionis* and 10% (5/48) of common pipistrelle bat originating from Shihezi City but were both negative in samples from Xinyuan County. The BLASTn analysis showed that one of the resulting sequences targeting 517 bp was 100% identical with that of *B. vesperuginis* in GenBank (AJ871610) that had originated from the UK. Another sequence was only 94% (427/452) identical with that of *Babesia equi* (no corresponding *18S* rRNA gene sequence targeting this region of *B. vesperuginis* exists in GenBank). The sequences of *B. vesperuginis* from our study were deposited into GenBank (MF280261 and MG356827).

To date, no published evidence indicates that the common pipistrelle bat can be infected with *B. vesperuginis* by *A. vespertilionis*. Here, our detection of *B. vesperuginis* was consistent both in bat ticks and in their corresponding host, the common pipistrelle bat. Interestingly, four organs, including heart, liver, spleen, and lung, from common pipistrelle bats were positive to *B. vesperuginis* while other two organs (small intestine and large bowel) were negative (Table 1).

Bats are susceptible to a broad range of endoparasites including trypanosomes, the piroplasm *B. vesperuginis*, and the hemosporidian *Polychromophilus murinus* (Gardner et al. 1987; Lord and Brooks 2014). Among these, only *B. vesperuginis*-infected bats show pathologic changes (Gardner et al. 1987; Lord and Brooks 2014) including anemia, splenomegaly, hemoglobinuria, and elevated reticulocyte and leukocyte counts (Gardner and Molyneux 1987). In 2005, taxon-specific PCR was first used to detect *B. vesperuginis* in heart tissues of *Pipistrellus* spp. (Concannon et al. 2005). We found three additional organs (liver, spleen, and lung) of common pipistrelle bats were also positive to *B. vesperuginis*, with a prevalence rate of 10% (5/48). All four tissues (heart, liver, spleen, and lung) were positive in five individuals. Our findings extend the geographic range of the common pipistrelle bat as a reservoir of *B. vesperuginis*.

TABLE 1. The numbers of *Babesia vesperuginis*-positive ticks (*Argas vespertilionis*) and tissues detected in carcasses of the common pipistrelle bat (*Pipistrellus pipistrellus*) collected at two locations in Northwestern China, 2015–16.

Sample	County	Total samples	Positive samples	Prevalence (%)
Heart	Shihezi	48	5	10
	Xinyuan	50	0	0
Liver	Shihezi	48	5	10
	Xinyuan	50	0	0
Spleen	Shihezi	48	5	10
	Xinyuan	50	0	0
Lung	Shihezi	48	5	10
	Xinyuan	50	0	0
Small intestine	Shihezi	48	0	0
	Xinyuan	50	0	0
Large bowel	Shihezi	48	0	0
	Xinyuan	50	0	0
Argas vespertilionis	Shihezi	21	4	19
	Xinyuan	24	0	0

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