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Leptospira in Bats from Hubei Province, China, 2018

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ABSTRACT: To understand the potential role of bats in the epidemiology of *Leptospira*, we investigated the frequency of *Leptospira* in bats from Central China in 2018. Sixty bats belonging to four species of two families were captured from rural areas of Xianning City, Hubei Province, China. We extracted DNA from the kidneys of bats, and *Leptospira* spp. were determined by PCR amplification of the *rrs2*, *flaB*, *lipL32*, and *secY* genes. We found that 57% (34/60) of bats were positive for *Leptospira* with at least one of the four genes, and the positive rate of *Leptospira* in bats was 45% (27/60) with *rrs2*, 50% (30/60) with *flaB*, 15% (9/60) with *lipL32*, and 27% (16/60) with *secY*. Phylogenetic analysis indicated that bats were infected with two species of *Leptospira*, including *Leptospira interrogans* and *Leptospira borgpetersenii*. A high prevalence of *Leptospira* spp. in bats suggested that bats were important carriers of *Leptospira* in China.

Key words: Bat, China, *Leptospira*, *lipL32*.

Leptospirosis is a global zoonotic disease and is caused by pathogenic spirochetes of the genus *Leptospira* (Adler 2015). The disease is prevalent in most tropical and sub-tropical regions of the world including Middle East, Europe, South and Central America (Bharti et al. 2003; Bessa et al. 2010), Asia (Victoriano et al. 2009), Australia (Tulsiani et al. 2011), and Africa (Ogawa et al. 2015). Over half a million human cases of leptospirosis are thought to occur each year worldwide with a mortality rate of more than 10% (Dietrich et al. 2015). In China, leptospirosis is caused predominantly by *Leptospira interrogans* and *Leptospira borgpetersenii* (Zhang et al. 2012). Leptospirosis is common and widespread in China, and it has been reported in 29 of 34 provinces (Zhang et al. 2012). Previous investigations of animal hosts of *Leptospira* in China focused on rodents and pigs (Hu et al. 2014). Recently, a growing number of studies highlighted the potential role of bats in the epidemiology of *Leptospira* (Dietrich et al. 2015). Moreover, a recent study reported a diversity of novel pathogenic *Leptospira* species in bats from Northern China (Han et al. 2018). However, the ecology of *Leptospira* in bats from China remains unclear. Therefore, we investigated the prevalence and genetic diversity of *Leptospira* in bats collected from Central China.

Sixty bats were collected from a karst cave in Xianning City, Hubei Province, China (29°53′N, 114°13′E) from March to July in 2018. We extracted DNA from bat kidney samples using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. By sequencing the PCR product of the cytochrome B (*cytb*) gene amplified from bat kidney DNA (Ishii et al. 2014), the 60 captured bats were classified into four species of two families: the Vespertilionidae family (32 *Myotis davidii*, 17 *Myotis adversus*, and two *Myotis altarium*) and the Miniopterus family (nine *Miniopterus schreibersii*; Table 1).

To identify the species of *Leptospira* in bats, bat kidney DNA was used as template to amplify the 16S ribosomal RNA gene (*rrs2*), flagellin B gene (*flaB*), the outer membrane lipoprotein gene (*lipL32*), and preprotein translocase gene (*secY*) as previously described (Ahmed et al. 2006). We analyzed the PCR products by 1.2% agarose gel electrophoresis, stained with ethidium bromide and observed under ultraviolet light. We excised PCR products with expected sizes from the gels and extracted them using a Gel Extraction Kit (Promega, Madison, Wisconsin, USA), and then we inserted them into the pMD 19-T vectors (TaKaRa, Shiga, Japan) for

TABLE 1. Detection of *Leptospira* spp. by PCR amplification of *rrs2*, *flaB*, *lipL32*, and *secY* genes in bats from Hubei Province in Central China in 2018.

Bat species	Percent positive (no. positive/no. tested)				
	rrs2	*flaB*	*lipL32*	*secY*	Total
Miniopterus schrebersii	0 (0/9)	33 (3/9)	0 (0/9)	0 (0/9)	33 (3/9)
Myotis adversus	82 (14/17)	77 (13/17)	29 (5/17)	65 (11/17)	82 (14/17)
Myotis davidii	41 (13/32)	44 (14/32)	13 (4/32)	16 (5/32)	53 (17/32)
Myotis altarium	0 (0/2)	0 (0/2)	0 (0/2)	0 (0/2)	0 (0/2)
Totals	45 (27/60)	50 (30/60)	15 (9/60)	27 (16/60)	57 (34/60)

cloning. One colony for each bat sample was selected randomly for Sanger sequencing using M13 universal primers on both DNA strands. Sequences of *rrs2*, *flaB*, *lipL32*, and *secY* genes were searched using BLAST in the GenBank database (National Center for Biotechnology Information 2018). The *rrs2*, *flaB*, *lipL32*, and *secY* sequences from this study were aligned with reference *Leptospira* spp. downloaded from GenBank with ClustalW and phylogenetic trees were constructed using maximum likelihood method of MEGA7.0 (Kumar et al. 2016).

Leptospira were detected in 57% (34/60) of bats with at least one of four genes found positive. The positive rate of *Leptospira* in bats was 10% (6/60) with all genes, 45% (27/60) with *rrs2*, 50% (30/60) with *flaB*, 15% (9/60) with *lipL32*, and 27% (16/60) with *secY*. Among four captured bat species, three species were positive for *Leptospira*, and the frequency of *Leptospira* was 82% (14/17) in *Myotis adversus*, 53% (17/32) in *Myotis davidii*, 33% (1/3) in *Miniopterus schreibersii*, and 0% (0/2) in *Myotis altarium* (Table 1).

Phylogenetic analysis of the sequences of *rrs2*, *flaB*, *lipL32*, and *secY* genes showed that *Leptospira* strains detected in bats in this study all belonged to the pathogenic group (Fig. 1). Phylogenetic analysis with the *rrs2* sequences indicated that *Leptospira* detected in 27 bats were classified into two clusters; 22 strains were clustered with *L. borgpetersenii* (support value 70%; Fig. 1A) and five strains were in the same clade with *L. interrogans* (support value 72%; Fig. 1A). Phylogenetic analysis with the *flaB* sequences indicated that 30 *Leptospira* from bats were classified into two clades with 14 sequences closely related to *L. borgpetersenii* (support value 92%; Fig. 1B) and 16 sequences closely related to *L. interrogans* (support value 99%; Fig. 1B). Phylogenetic analysis with the *lipL32* sequences indicated that the *Leptospira* detected in nine bats were classified into two clades: three strains clustered with *L. interrogans*, *L. noguchii*, and *L. kirshneri* group with high support value (99%) and six strains were clustered with *L. borgpetersenii* (support value 96%; Fig. 1C). Phylogenetic analysis with the *secY* sequences indicated that *Leptospira* detected in 16 bats were classified into two clades; 14 *Leptospira* sequences clustered with *L. borgpetersenii* (support value 53%; Fig. 1D) and two *Leptospira* sequences with *L. interrogans* (support value 87%; Fig. 1D). The *Leptospira* sequences of this study are available in GenBank with accession numbers MH882942–MH882987, MK291978–MK291986, and MH915392–MH915418.

Leptospira infection has been reported in over 50 bat species belonging to eight bat families in the tropics and subtropics as well as in Europe (Dietrich et al. 2015). The prevalence of *Leptospira* in bats was reported to be 35% (18/52) in Madgascar (Lagadec et al. 2012), 12% (9/77) in Comoros (Lagadec et al. 2012), 19% (7/36) in Tanzania (Mgode et al. 2014), 15% (79/529) in Zambia (Ogawa et al. 2015), 11% (19/173) in Australia (Tulsiani et al. 2011), and 2% (6/343) in Brazil (Bessa et al. 2010). In our study, the prevalence of *Leptospira* in bats from Central China was 56.7% (34/60), which was comparable to the preva-

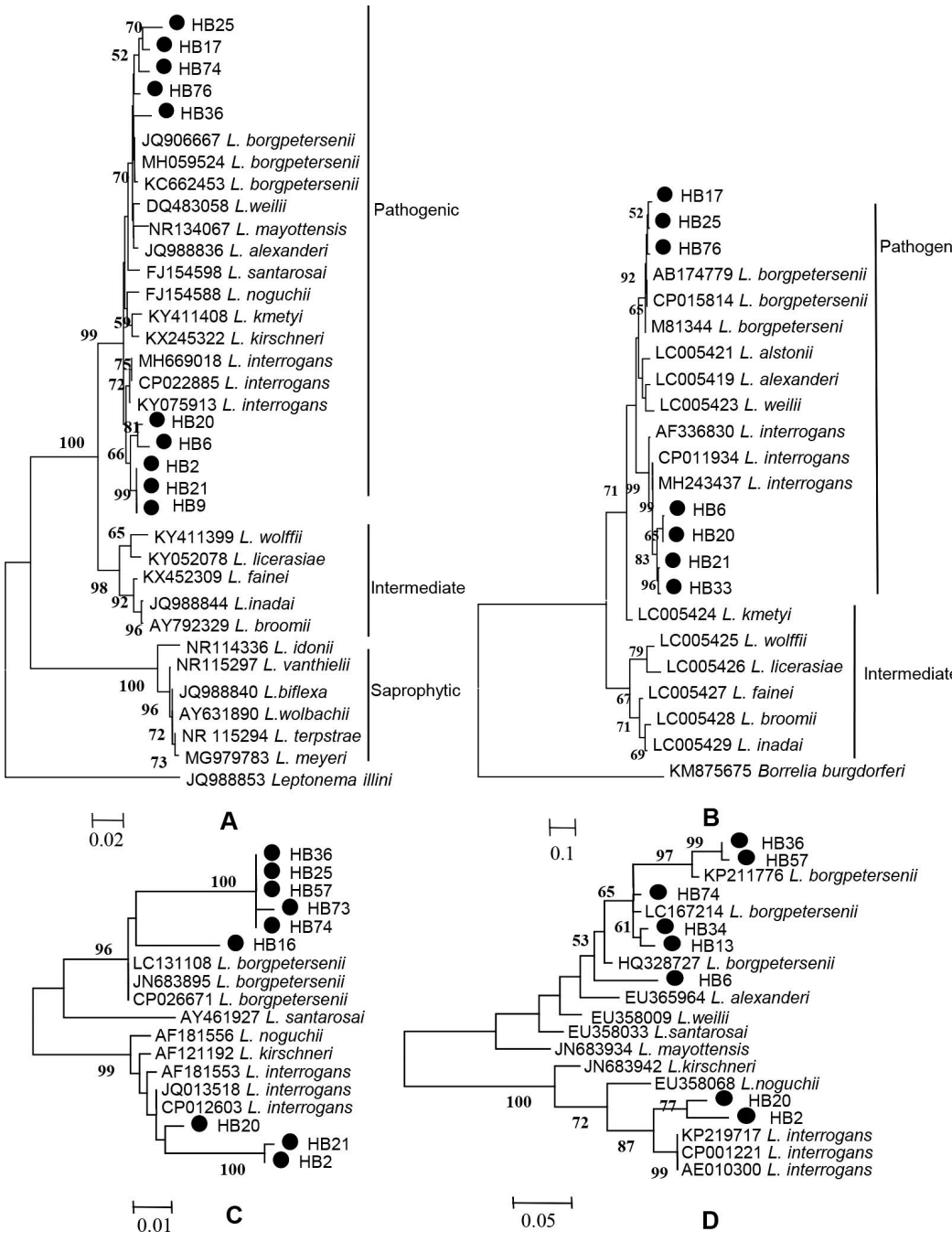

FIGURE 1. Phylogenetic trees of *Leptospira* spp. detected in bats from Hubei Province in Central China in 2018 were conducted using sequences of the (A) *rrs*2, (B) *flaB*, (C) *lipL32*, and (D) *secY* genes. The phylogenetic tree was constructed using the maximum likelihood method with MEGA 7.0; bootstrap values were calculated with 1,000 replicates. Sequences of representative *Leptospira* strains detected in bats in this study are indicated with a black dot and with a bat number. *Leptospira* reference sequences and outgroup reference sequences from GenBank are indicated with GenBank numbers. Only bootstrap values >50% are shown on the lines. For the sequences obtained in this study, which shared 99–100% homology, only one sequence is presented in the phylogenetic trees. Scale bar indicates nucleotide substitutions per site.

lence in Northern China (62%, 62/124) in a previous report (Han et al. 2018). *Myotis* spp. bats showed a high prevalence for *Leptospira* (53%) in our study as well as in a previous study (Han et al. 2018) in Northern China (63%), indicating *Myotis* bats are a major carrier of *Leptospira* in China. Our study enriched knowledge on *Leptospira* in bats from China. It is important to prevent potential spillover events of *Leptospira* between humans and bats. The positive rates of *rrs2*, *flaB*, *lipL32*, and *secY* genes were different, which may be caused by the differences of primer sensitivity of different genes and the differences in polymorphism rate of genes. The limitation of our study was that we did not culture *Leptospira* spp. from the bat kidney samples. The prevalence we determined might have been affected by the limitations of extraction, amplification, and sequencing of *Leptospira* DNA from clinical samples.

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