Bartonella Species Detected in Rodents from Eastern China

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Abstract

Bartonella are vector borne gram-negative facultative intracellular bacteria. *Bartonella* species are associated with rodents and their flea parasites worldwide. The genetic variation and distribution of *Bartonella* species in rodents are not clear in China. We investigated the presence and genetic diversity of *Bartonella* species in rodents from eastern China. We captured rodents from 2015 to 2016 in Jiaonan County, Shandong Province, and detected *Bartonella* species in the spleen of rodents by PCR amplification of the citrate synthase (*gltA*) gene and RNA polymerase beta subunit (*rpoB*) gene. We found that 8.38% (16/191) of the rodents were *Bartonella* positive by PCR for both *gltA* and *rpoB* genes; that *Bartonella* sequences from the rodents were phylogenetically divided into five clades, which were closely related to *B. tribocorum*, *B. rattimassiliensis*, *B. grahamii*, *B. fuyuanensis*, and *B. queenslandensis*, respectively; and that each *Bartonella* species is rodent species-specific with *B. rattimassiliensis* and *B. tribocorum* for *Rattus norvegicus*, *B. grahamii* for *Tscherskia triton*, *B. fuyuanensis* for *Apodemus agrarius*, and *B. queenslandensis* for *Niviventer confucianus*. This study indicated that *Bartonella* organisms have a broad distribution and a variety of genotypes in rodents in eastern China and the threats to public health by these *Bartonella* species should be monitored in China.

Keywords: *Bartonella* species, rodent, PCR, China

Introduction

BARTONELLA SPECIES ARE gram-negative facultative intracellular bacteria, which are mainly transmitted by blood-sucking arthropods (Chomel et al. 2006). They parasitize erythrocytes and endothelial cells in mammals (Anderson and Neuman 1997). The genus *Bartonella* contains >30 species or subspecies, and many human diseases are caused by a variety *Bartonella* species, including trench fever (*B. quintana*), cat scratch disease (*B. henselae*), and Carrion's disease (*B. bacilliformis*) (Jacomio et al. 2002, Saisongkroh et al. 2009, Breitschwerdt et al. 2010a, Lipatova et al. 2015). Several clinical manifestations caused by *Bartonella* such as cardiovascular, central nervous system, endocarditis, and hepatosplenic disease have also been described in humans (Daly et al. 1993, Arisoy et al. 1999, Breitschwerdt et al. 2010b, Raoult 2012).

Other species of *Bartonella* that have been reported to cause human infections include *B. elizabethae*, *B. grahamii*, *B. tribocorum*, *B. rattimassiliensis*, *B. vinsonii* subsp. *arupensis*, *B. washoensis*, and *Candidatus B. volans* (Daly et al. 1993, Kerkhoff et al. 1999, Kosoy et al. 2003, 2010, Breitschwerdt et al. 2009, Bai et al. 2012). Humans can be infected by close contact with rodents or by scratches and

bites by rodents or cats (Morick et al. 2010, Angelakis and Raoult 2014). A serosurvey showed that dog bites may be a risk factor for *Bartonella* infection (Sun et al. 2010).

Recently, several studies have reported *Bartonella* infection in rodents worldwide, including in America, Asia, Africa, and Europe (Kosoy et al. 1997, Gundi et al. 2004, Pretorius et al. 2004, Bordes et al. 2013). Moreover, an increasing number of *Bartonella* species are reported to cause human infection (Eremeeva et al. 2007). Previous studies demonstrated that a variety of *Bartonella* species existed in south and northeast of China and *Bartonella* infection has obvious regional characteristics and host specificity (Ying et al. 2002, Ye et al. 2009, Liu et al. 2010, Li et al. 2015). However, little is known of the distribution of *Bartonella* species in rodents from eastern China. The aim of this study was to investigate the presence and genetic diversity of *Bartonella* species in Jiaonan County in eastern China.

Materials and Methods

Sample collection

We collected rodents from June, 2015, to November, 2016, in Jiaonan County, Shandong Province (longitude 119°30'–

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TABLE 1. PRIMERS USED TO AMPLIFY *BARTONELLA GLTA* AND *RPOB* GENES

Target gene	Primer names and sequences (5'-3')	Product size (bp)	Reference
<i>gltA</i>	CS443f: GCTATGTCTGCATTCTATCA CS1210r: GATCYTCAATCATTCTTTCCA	790	Billeter et al. (2011)
<i>rpoB</i>	<i>rpoB</i> F: GAGCGTATGTCATCGGTTGAAATTG <i>rpoB</i> R: TGCACTTACAGCAGCTCCTGAATC	726	This study

120°30', latitude 35°35'–36°08'). The rodents were captured with snap traps baited with peanuts. The traps were placed 10 meters apart along the edges of peanut terraces in a hilly terrain. The traps were set up at dusk and collected the next morning. The rodent species were determined morphologically. The spleen was harvested from each dead rodent. The tissues were frozen in liquid nitrogen until use.

DNA extraction

Spleen tissues (20 mg) of each rodent were homogenized using metal beads (Tissue Lyser; Qiagen, Germany) in the RLT buffer (Qiagen). The homogenized spleen tissues were used to extract total nucleic acids by using the AllPrep DNA/RNA Mini Kit according to the manufacturer's instructions (Qiagen). Spleen specimens were considered more suitable for DNA extraction than other tissues or blood (Ohad et al. 2010).

PCR amplification and DNA sequencing

DNA extracted from spleen tissues were used to investigate *Bartonella* species in rodents. PCR amplification was performed and targeted at two housekeeping genes, citrate synthase (*gltA*) gene and RNA polymerase beta subunit (*rpoB*) gene, with the universal primers described in Table 1. Primers of *rpoB* gene were designed in this study, since we have failed to amplify all positive sequences by using primers published in the literature. PCR conditions are as follows: 95°C for 5 min; 35 cycles of 95°C for 40 s, 48°C for 1 min, and 72°C for 1 min; and one cycle of 72°C for 10 min. To prevent false-positive results, DNA extraction, reaction mixture, PCR amplification, and product analysis were performed at separate rooms. Distilled water was used as negative control. Amplified products were visualized by electrophoresis in a 1.2% agarose gel.

The expected PCR products were extracted using a Gel Extraction Kit (Omega Bio-tek, Norcross, GA) following the manufacturer's instructions and cloned into pMD 19-T vectors (TaKaRa, Shiga, Japan). Positive clones were picked up for sequencing on both strands by Sangon Biotech Company (Shanghai, China).

Phylogenetic analysis

DNA sequences were analyzed with BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Phylogenetic tree was created by using the maximum-likelihood method with the software MEGA version 7.0, and bootstrap values were calculated with 1000 replicates. The sequence of *gltA* gene between positions 801 and 1127 was used for phylogenetic analysis because most *Bartonella* sequences in GenBank correspond to this segment (La Scola et al. 2003, Gundi et al. 2012). *Bartonella* isolates were considered similar to validated *Bartonella* species if the 327-bp *gltA* fragment shares not <96% sequence similarity (La Scola et al. 2003).

GenBank number

The *Bartonella* sequences obtained in this study were deposited in the GenBank with accession numbers for the *gltA* gene and *rpoB* gene sequences MH748108–MH748123, MH748124–MH748139, respectively.

Ethics statement

This study was performed in accordance with the Ethics Committee of Prevention Medicine of Wuhan University (No. 2018010).

Result

A total of 191 rodents were captured from four sites in Jiaonan County, Shandong Province, in East China. The captured rodents were identified morphologically into six species, including *Apodemus agrarius*, *Cricetulus barabensis*, *Mus musculus*, *Niviventer confucianus*, *Rattus norvegicus*, and *Tscherskia triton*. Overall, 8.38% (16/191) of the rodents were *Bartonella* positive by PCR for both *gltA* and *rpoB* genes. Four out of six rodent species were positive to *Bartonella* with prevalence rate as follows: 13.79% (4/29) in *R. norvegicus*, 12.50% (1/8) in *N. confucianus*, 11.11% (8/72) in *A. agrarius*, and 8.57% (3/35) in *T. triton*. *Bartonella* DNA was not detected in neither *M. musculus* (0/39) nor *C. barabensis* (0/8) (Table 2).

TABLE 2. PREVALENCE OF *BARTONELLA* SPECIES IN RODENTS FROM JIAONAN COUNTY, CHINA

Rat species	No. of rats	Prevalence (%)	<i>Bartonella</i> species
<i>Apodemus agrarius</i>	72	11.11	<i>Bartonella fuyuanensis</i>
<i>Mus musculus</i>	39	0	
<i>Tscherskia triton</i>	35	8.57	<i>Bartonella grahamii</i>
<i>Rattus norvegicus</i>	29	13.79	<i>Bartonella rattimassiliensis</i> <i>Bartonella tribocorum</i>
<i>Cricetulus barabensis</i>	8	0	
<i>Niviventer confucianus</i>	8	12.50	<i>Bartonella queenslandensis</i>
Total	191	8.38	

DNA sequence homology analysis and phylogenetic analysis indicated that five *Bartonella* species were detected in the spleens of rodents (Table 2). All eight strains from *A. agrarius* were 99.7–100% identical to *B. fuyuanensis* (KJ175033); all three strains from *T. triton* were 96.9% identical to *B. grahamii* (KT445929), three strains from *R. norvegicus* were 99.7–100% similar to *B. rattimassiliensis* (GU056199), and one strain from *R. norvegicus* was 99.4% similar to *B. tribocorum* (AM260525), and the only strain from *N. confucianus* was clustered together with *B. queenslandensis* (JX158357) with 97.2% sequence similarity (Fig. 1). The prevalence rate for *Bartonella* species was 50% (8/16) for *B. fuyuanensis*, 18.75% (3/16) for *B. grahamii* and *B. rattimassiliensis*, and 6.25% (1/16) for *B. tribocorum* and *B. queenslandensis*.

Our investigation showed that there was an association between *Bartonella* genotypes and rodent species. Apparently *B. rattimassiliensis* and *B. tribocorum* were *R. norvegicus* specific, *B. grahamii* was *T. triton* specific, *B. fuyuanensis* was *A. agrarius* specific, and *B. queenslandensis* was *N. confucianus* specific.

Discussion

In this study, we investigated the prevalence and genetic diversity of *Bartonella* species in rodents in east China. To our knowledge, this is the first report of the investigation in this area. The prevalence of *Bartonella* species in infected rodents varied from 8.57% to 13.79% and the highest rate was detected in *R. norvegicus*. We obtained five *Bartonella*

FIG. 1. Phylogenetic analyses of *Bartonella* species based on citrate synthase (*gltA*) gene identified in rodents from eastern China. The maximum-likelihood method was used for constructing the phylogenetic tree. Numbers associated with internal nodes indicated the percentage of 1000 bootstrap replicates supporting the branch (only percentages >50% are shown). *Bartonella* detected in this study is shown in boldface and marked with a black dot.

species sequence in rodents collected in China and each *Bartonella* species was detected in a single rodent species except for *R. norvegicus* that contained *B. rattimassiliensis* and *B. tribocorum*. Our results demonstrated the host specificity of *Bartonella* in rodents in this area with similar phenomenon previously reported in the United States, United Kingdom, and other areas of China (Birtles et al. 1994, Kosoy et al. 1997, Ying et al. 2002, Ye et al. 2009, Li et al. 2015). The study from Heixiazi island in northeastern China reported that the extent of intraspecies variation in *Bartonella* clades was different and related to their host species; *B. doshiae* and *B. coopersplainsensis* showed identical genotype consistent with detected rodents, whereas *B. taylorii*, *B. grahamii*, and *B. washoensis*-like isolates showed intraspecies genetic diversity (Li et al., 2015). Another study showed that *B. grahamii* strains from 10 rodent species had divergent host specificity based on geographic location (Inoue et al. 2009).

Bartonella infections are widely distributed and highly prevalent in rodents. Because of close contact with humans and species diversity, rodents are the major source for these bacteria (Malania et al. 2016). *Bartonella* species are mainly transmitted through arthropod vectors, including fleas, mites, and ticks and they may play an important role in transmission of *Bartonella* among rodents (Bown et al. 2004, Kim et al. 2005, Li et al. 2007, Billeter et al. 2008). We identified *B. tribocorum*, *B. rattimassiliensis*, *B. grahamii*, *B. fuyuanensis*, and *B. queenslandensis* in rodents from eastern China. *Bartonella tribocorum*, *B. rattimassiliensis*, and *B. grahamii* have all been implicated as causative agents of human infections (Kerkhoff et al., 1999, Kosoy et al., 2010). *Bartonella fuyuanensis* has been identified in *A. agrarius* collected from northeastern China previously (Li et al., 2015).

Considering the close association of *A. agrarius* with humans in transmission of hemorrhagic fever with renal syndrome (Fang et al. 2015, Wang et al. 2016), the pathogenicity of *B. fuyuanensis* to humans needs to be investigated. *Bartonella queenslandensis* has been isolated/detected worldwide from the United States, Thailand, Australia, and other countries in Southeastern Asia (Saisongkorh et al., 2009, Jiyipong et al. 2012, Pangjai et al. 2014, Peterson et al. 2017), but its pathogenicity to humans has yet been reported.

This study provided a better understanding of the prevalence and genetic diversity of *Bartonella* species in rodents from eastern China. In our previous investigation in this area, several pathogens that caused human diseases have been detected in rodents and arthropods, including severe fever with thrombocytopenia syndrome virus, hantavirus, and *Anaplasma phagocytophilum* (Liu et al. 2014, Fang et al., 2015, Qin et al. 2018). With an increasing number of rodent-borne *Bartonella* species reported as human infectious pathogens, it has become a public health problem. Further studies are needed to determine the potential role of rodent-associated *Bartonella* species in human diseases.

Conclusions

In this study, we have detected *B. tribocorum*, *B. rattimassiliensis*, *B. grahamii*, *B. fuyuanensis*, and *B. queenslandensis* in rodents collected from eastern China. It is suggested that *Bartonella* species have a broad geographical distribution and a variety of genotypes. Since *Bartonella* is an important pathogen

to cause disease in humans and animals, it has become a public health problem in China.

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Author Disclosure Statement

No conflicting financial interests exist.

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