Molecular detection of *Bartonella* in ixodid ticks and plateau pika (*Ochotona curzoniae*) in Shiqu County, China

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Abstract

Background: *Bartonella* bacteria have been associated with an increasingly wide range of human and animal diseases. They were identified as being globally dispersed as emerging pathogens. Ticks and small rodents are known as **hosts** of human and animal bartonellosis. They play a significant role in the preservation and circulation of bartonellae in nature. This study investigates the occurrence of *Bartonella* spp. in ticks and plateau pika in Shiqu county which is located on the Eastern Qinghai-Tibetan plateau in China. Shiqu county is spread over approximately 26,000 square kilometers, with an average altitude of above 4,200 meters and vast area of pastureland.

Results: A total of 818 ticks (*Dermacentor everestianus*, 79.0%, 646/818; *Haemphysalis qinghaiensis*, 21.0%, 172/818) were collected in 4 villages of Shiqu county. Only *Bartonella melophagi* was detected in tick samples with a total prevalence of 30.1% (246/818). The infection rates of *Bartonella* spp. in ticks from Arizha, Maga, Derongma, and Changxgma were 4.8%, 76.8%, 12.5%, and 18.0% respectively. The infection rate of *Bartonella* spp. in Maga was higher ($p < 0.01$) than in other villages. Regarding plateau pika, the total infection rate of *Bartonella* spp was 24.1%, with 20.8% (15/72), 30.9% (25/81), 13.8% (9/65), and 29.4% (20/68) in Arizha, Maga, Derongma, and Changxgma respectively. Finally, *B. queenslandensis*, *B. grahamii*, and two unvalidated *Bartonella* species were detected. No significant difference was observed ($p > 0.05$) in the infection rates between these study sites.

Conclusion: To date, only *D. everestianus* and *H. qinghaiensis* were found in Shiqu county with high infection of *Bartonella* spp. in the ticks and plateau pika. The threats of *Bartonella* species to the public health should be closely monitored.

Background

The *Bartonella* genus currently includes 36 named and 17 Candidatus species [1] which can be found in a wide range of mammalian hosts and arthropod vectors. Some of these species are zoonoses, including *B. alsatica*, *B. bacilliformis*, *B. elizabethae*, *B. henselae*, *B. koehlerae*, *B. melophagi, B. quintana*, *B. rochalimae*, *B. tamiae, B. vinsonii* subsp. *berkhoffii*, *B. vinsonii* subsp. *arupensis*, and *B. washoensis* [2-8]. Ticks and small rodents are known as vectors and **reservoir hosts** of human bartonellosis, respectively. They play an essential role in the preservation and movement of bartonellae in nature within arthropod-mammal systems. Shiqu county spreads over approximately 26,000 square kilometers, with an average altitude of above 4,200 meters and vast area of pastureland on the Eastern Qinghai-Tibetan plateau. Its population was estimated at 97,000, consisting of individuals with low education and poor health. Yak is the largest population of local livestock (about 600,000), where severe tick infestation is often observed. Apart from yak, plateau pika (*Ochotona curzoniae*) is the largest population of local small rodents with close interaction with local people and livestock. The significance of ticks (Acari: Ixodida) has long been recognized due to their ability to feed on a large range of host species and to transmit *Bartonella* pathogens that can infect a variety of vertebrate hosts, including humans. However, little information exists on bartonellae and their **hosts and vectors** in Shiqu county. This study aims to prove the presence

of *Bartonella* spp. in plateau pika and ticks and to provide preliminary results in view of establishing prevention and control measures for this tick-borne disease.

Results

A total of 818 ticks were collected from 4 villages in Shiqu county (Fig.1). Through morphological and molecular identification using the16S rRNA gene, the presence of two different tick species was confirmed, namely *Dermacentor everestianus* (79.0%, 646/818) and *Haemphysalis qinghaiensis* (21.0%, 172/818). Information of ticks and sequences of 16S rRNA are included in Supplementary file 3 - 7.

For ticks, they were first screened using the *Bartonella* spp. *gltA* gene and *gltA*-positive samples were then screened with *rpoB*, showing a total prevalence of 30.1% (246/818, **both *gltA* and *rpoB* were positive**). The infection rates of *Bartonella* spp. in Arizha, Maga, Derongma, and Changxgma were 4.8%, 76.8%, 12.5%, and 18.0% respectively (Table2). The infection rate of *Bartonella* spp. in ticks was higher in Maga ($p < 0.01$) (marked with "*" in Table 2) than in other villages. In Maga, no significant difference was observed ($p > 0.05$), although the infection rate of *Bartonella* in *H. qinghaiensis* (79.1%) was higher than in *D. everestianus* (69.2%).

With regard to plateau pika, spleen samples were first screened using the *gltA* gene, and *gltA*-positive samples were then screened with *rpoB*. Total infection rates of *Bartonella* spp. in plateau pika were 24.1% (**both *gltA* and *rpoB* were positive**), with 20.8% (15/72), 30.9% (25/81), 13.8% (9/65), and 29.4% (20/68) in Arizha, Maga, Derongma, and Changxgma respectively. No significant difference in infection rates was observed ($p > 0.05$) between these study sites.

In this research, all amplicons of *gltA* and *rpoB* from ticks and pikas were sequenced and compared to each other. A total of seven **unique** sequences of *gltA* (Supplementary file1) and nine **unique** sequences of *rpoB* (Supplementary file 2) were obtained and deposited in GenBank with ID numbers (*gltA*: MN056882-MN056888; *rpoB*:MN296286-MN296294). For *gltA* gene, sequence (MN056882) from ticks was completely identical to ***B. melophagi*** (AY724768), with 100% coverage; Sequences (MN056883 and MN05688) from plateau pika were 97.03%-100% identical to *B. queenslandensis* (MH748120), with 99%-100% coverage; Sequences (MN056884, MN05686, and MN05687) from plateau pika were 100%, 97.61%, and 96.73% identical to *B. grahamii* (KT445918 and CP001562), with 100% coverage; Sequence (MN056885) from plateau pika was 98.81% homologous to *B. rochalimae* (KU292571), with 100% coverage. For rpoB gene, sequences (MN296287-MN296291) from ticks were 99.12-99.71% identical to *B. melophagi* (EF605288), with 99-100% coverage; Sequences (MN296286 and MN296294) from plateau pika were 95.65-97.86% identical to *B. grahamii* (AB426697 and JN810811), with 100% coverage; Sequence (MN296292) from plateau pika was 99.69% homologous to *B. queenslandensis* (MH748136), with 100% coverage. However, sequence (MN296293) from plateau pika was only 92.28% and 92.58% similar to *Bartonella* sp. (AB529489) and *B. grahamii* (AB426696) respectively, with 100% coverage.

According to criteria (*Bartonella* spp. species thresholds: *gltA* ≥ 96.0% and *rpoB* ≥ 95.4%) proposed by La Scola, et al [13], only *B. melophagi* was detected for tick samples (Table 2), whereas for plateau pika, as

shown in Table 3, *B. grahamii* was the predominantly identified specie in the four villages, with *B. queenslandensis* detected only in Maga and 2 unvalidated *Bartonella* species (*Bartonella* sp.* and *Bartonella* sp.**) found in Ariza and Changxgma respectively. Furthermore, *gltA* and *rpoB* based phylogenetic analysis supported the classification of *Bartonella* spp. detected in the current study (Fig. 2 and Fig. 3).

Discussion

Two tick species were identified in this study: *H. qinghaiensis* (in Maga only) and *D. everestianus* (in all four sites). *D. everestianus* was reported only in Northwestern China and Nepal [15] with an altitude of 2,600 - 4,700 m [16]. Larvae and nymphs of this tick specie often infest lagomorphs and rodents, while adult ticks usually utilize medium-large sized, modest and wild mammals as hosts, including hares, sheep, yaks, and horses [15, 16]. However, *H. qinghaiensis* is only reported in China [17-21], particularly prevalent in the western plateau, including the provinces of Qinghai, Gansu, Sichuan, and Tibet [21]. Its natural hosts include sheep, goats, yaks, cattle, and hares (*Lepus oiostolus*). All life stages of the tick could develop in sheep, goats, yaks, and cattle [21-27]. Contrary to ticks, *H. qinghaiensis* mostly performs its activity at low altitude. Arizha, Changxgma, and Derongma belong to the sub-frigid zone, whereas Maga village is located in the cold temperate zone. Due to the significant difference in altitude between Maga and the other three villages, *H. qinghaiensis* was only found in Maga.

All types of ticks were found to contain *Bartonella* DNA, although in varying percentages and locations. A survey regarding ticks from 16 states of the United States revealed that the overall prevalence of *B. henselae* in Ixodes ticks was 2.5% [28]. In Austria, *Bartonella* spp. (*B. henselae, B. doshiae, and B. grahamii*) were detected in 2.1% of *I. ricinus,* with the highest rate in ticks from Vienna (with an infection rate of 7.5%) and higher prevalence in adult ticks than other life stages [29]. Furthermore, a recent One Health perspective review on *Bartonella* indicated that the overall presence of *Bartonella* in ticks (combining evidence from multiple surveillance studies) was about 15% [30]. In our results, a total prevalence of 30.1% in ticks (especially in Maga, 76.8%) indicated the severity in Shiqu county.

B. melophagi, a human bacterial pathogen, was first isolated from sheep blood in 2007 [31], and the same bacteria were then isolated from the blood of two female patients with pericarditis and skin lesions in the United States of America [32]. As a result, the first report of DNA of *B. melophagi* detected in *D. everestianus* and *H. qinghaiensis* was obtained, which was the first molecular evidence of *B. melophagi* in Shiqu county. However, there is no current evidence supporting the ability of these ticks to transmit *B. melophagi* to livestock or human. To address this issue, experiments should be performed to assess the vector competency of *D. everestianus* and *H. qinghaiensis* to transmit *B. melophagi* in the future.

Bartonella infection has been mostly reported in *Rodentia* [33-42], with few cases reported in *Lagomorpha*. Until now, there has been only one report of *Bartonella* infection in plateau pika with a positive rate of 18.99% [43]. A total of 15 *Bartonella* strains were obtained, and most of them were closely related to *B. taylorii* and *B. grahamii* [43]. Based on our research, *B. grahamii*, a pathogenic strain in

humans, was detected in all four villages, while *B. queenslandensis* was detected only in Maga. Nevertheless, similar to *B. coopersplainsensis*, the zoonotic potential of *B. queenslandensis* has not been reported. Additionally, for two unvalidated *Bartonella* species (*Bartonella*.sp* and *Bartonella*.sp**) found in Ariza and Changxgma respectively, sequences analysis showed that: 1) based on *gltA* gene, they were clustered with *B. rochalimae* and *B. queenslandensis* respectively and 2) based on *rpoB*, however, they were clustered with *B. melophagi.* There are possible explanations for why this conflicting result may occur. For instance, the potential presence of multiple *Bartonella* species in the sample although it is not commonly based on culturing. Secondly, different primer sets may also have amplification bias towards particular species based on the annealing affinity, which may cause the observed *Bartonella* diversity to differ, depending on the primer sets used for amplification. Lastly, homologous recombination, a specific form of LGT (lateral gene transfer) among *Bartonella* spp., have been reported in other studies based on sequencing multiple protein-coding loci [12, 44-48]. However, culturing, sequencing multiple loci (including 16S rRNA, *ftsZ*, *gltA*, *groEL*, *ribC* and *rpoB,* and ITS), cloning sequences into vectors before sequencing, or implementing deep sequencing approaches may discover a potentially novel *Bartonella* specie or subspecies and may differentiate these possible scenarios.

In Shiqu, plateau pika, the largest population of local small rodents, is in close contact with local people and livestock and can be infested with fleas and ticks, implicating them in transmission cycles of *Bartonella* spp. In China, *Bartonella* infections among humans have been mainly reported in the central plain area such as Jiangsu, Zhejiang, Anhui, and Hubei province. No cases or suspected cases have been reported in the Qinghai-Tibetan plateau. Therefore, the relationship of plateau pika and the transmission of *Bartonella* should be further studied. A thorough analysis with controlled experiments should be conducted to determine the exact routes of transmission between plateau pika, the transmission between plateau pika and their vectors, as well as the transmission from plateau pika to humans and livestock.

Conclusion

To date, only *D. everestianus* and *H. qinghaiensis* were found in Shiqu county with high infection of *Bartonella* spp. in ticks and plateau pika. Further research should be conducted to determine the risk of *Bartonella* infections to humans and livestock.

Methods

Study sites

The study was conducted in Shiqu county (longitude: 98.102, latitude: 32.978), Sichuan province, China (Fig. 1). Ticks and pikas were collected from the following villages: Arizha (longitude: 98.532, latitude: 32.995, altitude: 4,010 m), Maga (longitude: 98.138, latitude: 32.419, altitude: 3,799 m), Derongma (longitude: 97.972, latitude: 33.069, altitude: 4,182 m), and Changxgma (longitude: 99.006, latitude: 32.754, altitude: 3,814 m). All locations of samples collection were deep into the grasslands and at least

5,000 m away from the nearest settlements. During samples collection, people and livestock did not travel through these areas.

Samples collection

A total of 818 ticks were collected by blanket dragging between June and August 2018, among which, 168, 224,192, and 234 were collected from Arizha, Maga, Derongma, and Changxgma respectively (Fig.1 C). In the same period, a total of 286 pikas were captured; 72 in Arizha, 81 in Maga, 65 in Derongma, and 68 in Changxgma. Plateau pikas were captured using mouse snap traps. Then, spleens were collected under sterile conditions and stored in liquid nitrogen until use. Each pika's body was deeply buried to avoid being eaten by dogs, cats, and other wild carnivores.

Identification of tick species

Ticks were carefully removed from blanket and stored in 70% of ethanol at 4°C. The specimens were morphologically identified according to the guidelines for tick identification [9]. Then, molecular identification of tick species was performed, targeting the mitochondrial 16S rRNA gene [10].

DNA extraction, PCR, and sequences analysis

Ticks were sectioned longitudinally, and one half per each tick was used for DNA extraction. For all spleen samples, an average of 30 mg of tissue was used. The total DNA of all samples were extracted using the TIANamp Genomic DNA Kit (TIANGEN Biotech Co., Ltd, Beijing, China; Cat No: DP304) for tick molecular identification and characterization of Bartonella spp. All samples were submitted to previously described PCR assays targeting *gltA* (379 bp) [11]. All *gltA*-positive samples were further analyzed with PCR targeting *rpoB* (379 bp) [12]. All primers were listed in Table 1. PCR amplifications were conducted in a 25µl reaction mixture consisting of 1µl of genomic DNA (2 - 3 ng), 1µl of each primer (10 µM), 12.5µl of PCR Supermix (Transgen Co., Ltd, Beijing, China; Cat No: AS111-11), and 9.5 µl of nuclease-free water. Each PCR reaction included a positive control (DNA of *B. henselae* preserved in laboratory) and a negative control (nuclease-free water). Observed bands were purified using the QIAquick Gel Extraction Kit and sent for sequencing (Sangon Biotech Shanghai Co., Ltd). Obtained sequences were analyzed by employing Bioedit v.7.0.2 and were submitted for nucleotide BLAST search through the NCBI database. Sequences with ≥ 95% quality cover and identity were considered as positive for *Bartonella* spp. and were compared with validated *Bartonella* species in GenBank/EMBL/DDBJ through the Clustal X program (http://www.clustal.org/clustal2/). Clones that share ≥ 96.0% and ≥ 95.4% similarity in *gltA* and *rpoB* sequences with the validated species, respectively, can be considered as the same species [13].

Phylogenetic analysis and statistics

For phylogenetic analysis, Neighbor-Joining phylogenetic trees were constructed based on *Bartonella gltA* and *rpoB* sequences, using the Kimura two-parameter model with partial gap deletion and a cutoff of 95% site coverage respectively. The evolutionary distance was calculated, and bootstrap analysis with 1,000 iterations was carried out with the MEGA6 [14]. SPSS19.0 (Pearson Chi-square test) was applied to

compare the difference in *Bartonella* spp. prevalence between different sampling locations, plateau pika, and tick species. A *p*-value of < 0.05 was considered significant.

Abbreviations

gltA: Citrate synthase-encoding gene; *rpoB*: beta subunit of RNA polymerase; LGT (lateral gene transfer)

Declarations

Ethics approval and consent to participate

This study was carried out in full compliance with the framework for the collection of wild species of biological diversity for purposes of non-commercial scientific research, authorized by the Sichuan's Department of Agriculture and Rural Affairs. The study received approval from the Animal Ethics Committee of Southwest Minzhu University Plateau pikas were collected during inspection by qualified veterinary officers. In this study, no experiment was conducted on live animals.

Consent for publication

Not applicable.

Availability of data and materials

The sequences generated in this study were submitted to the GenBank database under the accession numbers MN056882- MN056888 and MN296286- MN296294

(see Supplementary files).

Competing interests

The authors declare that there is no conflict of interest in this study.

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Authors’ contributions

HLL and YD performed the experiments. LR and HLL designed the project, analyzed the data and drafted the manuscript together. YD, GL and YA collected the tick samples. HW, MX and YJ collected the pika samples. All authors read and approved the final manuscript.

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References

1. Breitschwerdt EB. Bartonellosis. One Health and all creatures great and small. Vet Dermatol.2017;28:96-e21.
2. Breitschwerdt EB, Maggi RG, Duncan AW, Nicholson WL, Hegarty BC, Woods CW. Bartonella species in blood of immunocompetent persons with animal and arthropod contact. Emerg Infect Dis.2007;13:938-941.
3. Chomel BB, Boulouis HJ, Maruyama S. Breitschwerdt EB: Bartonella spp. in pets and effect on human health. Emerg Infect Dis. 2006;12:389-394.
4. Eremeeva ME, Gerns HL, Lydy SL, Goo JS, Ryan ET, Mathew SS, Ferraro MJ, Holden JM, Nicholson WL, Dasch GA,et al. Bacteremia, fever, and splenomegaly caused by a newly recognized bartonella species. N Engl J Med.2007;356:2381-2387.
5. Fenollar F, Sire S, Raoult D: Bartonella vinsonii subsp. arupensis as an agent of blood culture-negative endocarditis in a human. J Clin Microbiol. 2005;43:945-947.
6. Kosoy M, Morway C, Sheff KW, Bai Y, Colborn J, Chalcraft L, Dowell SF, Peruski LF, Maloney SA, Baggett H,et al. Bartonella tamiae sp. nov., a newly recognized pathogen isolated from three human patients from Thailand. J Clin Microbiol . 2008;46:772-775.
7. Kosoy M, Murray M, Gilmore RD, Jr., Bai Y, Gage KL. Bartonella strains from ground squirrels are identical to Bartonella washoensis isolated from a human patient. J Clin Microbiol. 2003;41:645-650.
8. Raoult D, Roblot F, Rolain JM, Besnier JM, Loulergue J, Bastides F, Choutet P. First isolation of Bartonella alsatica from a valve of a patient with endocarditis. J Clin Microbiol. 2006;44:278-279.
9. Deng, G.F., Jiang, Z.J., 1991. Economic Insect Fauna of China. Ixodes [M]. Beijing: Science Press
10. Black WCt, Piesman J. Phylogeny of hard- and soft-tick taxa (Acari: Ixodida) based on mitochondrial 16S rDNA sequences. Proc Natl Acad Sci U S A.1994;91:10034-10038.

11. Norman AF, Regnery R, Jameson P, Greene C, Krause DC. Differentiation of Bartonella-like isolates at the species level by PCR-restriction fragment length polymorphism in the citrate synthase gene. J Clin Microbiol. 1995;33:1797-1803.
12. Paziewska A, Harris PD, Zwolinska L, Bajer A, Sinski E. Recombination within and between species of the alpha proteobacterium Bartonella infecting rodents. Microb Ecol.2011;61:134-145.
13. La Scola B, Zeaiter Z, Khamis A, Raoult D. Gene-sequence-based criteria for species definition in bacteriology: the Bartonella paradigm. Trends Microbiol.2003;11:318-321.
14. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol.2013;30:2725-2729.
15. Chen Z, Li Y, Ren Q, Luo J, Liu Z, Zhou X, Liu G, Luo J, Yin H. Dermacentor everestianus Hirst, 1926 (Acari: Ixodidae): phylogenetic status inferred from molecular characteristics. Parasitol Res. 2014;113:3773-3779.
16. Apanaskevich DA, Duan W, Apanaskevich MA, Filippova NA, Chen J. Redescription of Dermacentor everestianus Hirst (Acari: Ixodidae), a parasite of mammals in mountains of China and Nepal with synonymization of D. abaensis Teng and D. birulai Olenev.J Parasitol. 2014;100:268-278.
17. Gao J, Luo J, Fan R, Fingerle V, Guan G, Liu Z, Li Y, Zhao H, Ma M, Liu J.Cloning and characterization of a cDNA clone encoding calreticulin from Haemaphysalis qinghaiensis (Acari: Ixodidae). Parasitol Res. 2008;102:737-746.
18. Gao J, Luo J, Fan R, Guan G, Ren Q, Ma M, Sugimoto C, Bai Q, Yin H. Molecular characterization of a myosin alkali light chain-like protein, a "concealed" antigen from the hard tick Haemaphysalis qinghaiensis. Vet Parasitol.2007;147:140-149.
19. Gao J, Luo J, Fan R, Schulte-Spechtel UC, Fingerle V, Guan G, Zhao H, Li Y, Ren Q, Ma M,et al. Characterization of a concealed antigen Hq05 from the hard tick Haemaphysalis qinghaiensis and its effect as a vaccine against tick infestation in sheep. Vaccine. 2009;27:483-490.
20. Gao J, Luo J, Li Y, Fan R, Zhao H, Guan G, Liu J, Wiske B, Sugimoto C, Yin H. Cloning and characterization of a ribosomal protein L23a from Haemaphysalis qinghaiensis eggs by immuno screening of a cDNA expression library. Exp Appl Acarol. 2007;41:289-303.
21. Li Y, Luo J, Liu Z, Guan G, Gao J, Ma M, Dang Z, Liu A, Ren Q, Lu B, et al. Experimental transmission of Theileria sp. (China 1) infective for small ruminants by Haemaphysalis longicornis and Haemaphysalis qinghaiensis. Parasitol Res. 2007;101:533-538.
22. Yin H, Luo J, Guan G, Lu B, Ma M, Zhang Q, Lu W, Lu C, Ahmed J. Experiments on transmission of an unidentified Theileria sp. to small ruminants with Haemaphysalis qinghaiensis and Hyalomma anatolicum anatolicum. Vet Parasitol.2002;108:21-30.
23. Yin H, Luo J, Guan G, Gao Y, Lu B, Zhang Q, Ma M, Lu W, Lu C, Yuan Z, et al. Transmission of an unidentified Theileria species to small ruminants by Haemaphysalis qinghaiensis ticks collected in the field. Parasitol Res. 2002;88:S25-27.
24. Yin H, Guan G, Ma M, Luo J, Lu B, Yuan G, Bai Q, Lu C, Yuan Z, Preston P. Haemaphysalis qinghaiensis ticks transmit at least two different Theileria species: one is infective to yaks, one is

infective to sheep. Vet Parasitol.2002;107:29-35.

25. Li Y, Luo J, Guan G, Ma M, Liu A, Liu J, Ren Q, Niu Q, Lu B, Gao J, et al. Experimental transmission of Theileria uilenbergi infective for small ruminants by Haemaphysalis longicornis and Haemaphysalis qinghaiensis. Parasitol Res. 2009;104:1227-1231.
26. Guan GQ, Yin H, Luo JX, Lu WS, Zhang QC, Gao YL, Lu BY. Transmission of Babesia sp to sheep with field-collected Haemaphysalis qinghaiensis. Parasitol Res. 2002;88:S22-24.
27. Guan G, Moreau E, Liu J, Hao X, Ma M, Luo J, Chauvin A, Yin H. Babesia sp. BQ1 (Lintan): molecular evidence of experimental transmission to sheep by Haemaphysalis qinghaiensis and Haemaphysalis longicornis. Parasitol Int. 2010;59:265-267.
28. Maggi RG, Toliver M, Richardson T, Mather T, Breitschwerdt EB. Regional prevalences of Borrelia burgdorferi, Borrelia bissettiae, and Bartonella henselae in Ixodes affinis, Ixodes pacificus and Ixodes scapularis in the USA. Ticks Tick Borne Dis. 2019;10:360-364.
29. Muller A, Reiter M, Schotta AM, Stockinger H, Stanek G. Detection of Bartonella spp. in Ixodes ricinus ticks and Bartonella seroprevalence in human populations. Ticks Tick Borne Dis.2016;7:763-767.
30. Regier Y, F OR, Kempf VA. Bartonella spp. - a chance to establish One Health concepts in veterinary and human medicine. Parasit Vectors. 2016;9:261.
31. Bemis DA, Kania SA. Isolation of Bartonella sp. from sheep blood. Emerg Infect Dis.2007;13:1565-1567.
32. Maggi RG, Kosoy M, Mintzer M, Breitschwerdt EB. Isolation of Candidatus Bartonella melophagi from human blood. Emerg Infect Dis. 2009;15:66-68.
33. Inoue K, Maruyama S, Kabeya H, Yamada N, Ohashi N, Sato Y, Yukawa M, Masuzawa T, Kawamori F, Kadosaka T, et al. Prevalence and genetic diversity of Bartonella species isolated from wild rodents in Japan. Appl Environ Microbiol. 2008;74:5086-5092.
34. Kamani J, Morick D, Mumcuoglu KY, Harrus S. Prevalence and diversity of Bartonella species in commensal rodents and ectoparasites from Nigeria, West Africa.PLoS Negl Trop Dis. 2013;7:e2246.
35. Liu Q, Sun J, Lu L, Fu G, Ding G, Song X, Meng F, Wu H, Yang T, Ren Z, et al. Detection of bartonella species in small mammals from Zhejiang Province, China. J Wildl Dis.2010;46:179-185.
36. Malania L, Bai Y, Osikowicz LM, Tsertsvadze N, Katsitadze G, Imnadze P, Kosoy M. Prevalence and Diversity of Bartonella Species in Rodents from Georgia (Caucasus). Am J Trop Med Hyg. 2016;95:466-471.
37. Spitalska E, Minichova L, Kocianova E, Skultety L, Mahrikova L, Hamsikova Z, Slovak M, Kazimirova M. Diversity and prevalence of Bartonella species in small mammals from Slovakia, Central Europe. Parasitol Res. 2017;116:3087-3095.
38. Blasdell KR, Perera D, Firth C. High Prevalence of Rodent-Borne Bartonella spp. in Urbanizing Environments in Sarawak, Malaysian Borneo. Am J Trop Med Hyg. 2019;100:506-509.
39. Dybing NA, Jacobson C, Irwin P, Algar D, Adams PJ. Bartonella Species Identified in Rodent and Feline Hosts from Island and Mainland Western Australia. Vector Borne Zoonotic Dis. 2016;16:238-244.

40. Li DM, Hou Y, Song XP, Fu YQ, Li GC, Li M, Eremeeva ME, Wu HX, Pang B, Yue YJ, et al. High prevalence and genetic heterogeneity of rodent-borne Bartonella species on Heixiazi Island, China. Appl Environ Microbiol. 2015;81:7981-7992.
41. Fernandez-Gonzalez AM, Kosoy MY, Rubio AV, Graham CB, Montenieri JA, Osikowicz LM, Bai Y, Acosta-Gutierrez R, Avila-Flores R, Gage KL, et a. Molecular Survey of Bartonella Species and Yersinia pestis in Rodent Fleas (Siphonaptera) From Chihuahua, Mexico. J Med Entomol. 2016;53199-205.
42. Qin XR, Liu JW, Yu H, Yu XJ. Bartonella Species Detected in Rodents from Eastern China. Vector Borne Zoonotic Dis.2019.
43. Rao HX, Yu J, Guo P, Ma YC, Liu QY, Jiao M, Ma ZW, Ge H, Wang CX, Song XP, et al. Bartonella Species Detected in the Plateau Pikas (Ochotona curzoiae) from Qinghai Plateau in China. Biomed Environ Sci.2015;28:674-678.
44. Berglund EC, Ellegaard K, Granberg F, Xie Z, Maruyama S, Kosoy MY, Birtles RJ, Andersson SG. Rapid diversification by recombination in Bartonella grahamii from wild rodents in Asia contrasts with low levels of genomic divergence in Northern Europe and America. Mol Ecol. 2010;19:2241-2255.
45. Chaloner GL, Palmira V, Birtles RJ. Multi-locus sequence analysis reveals profound genetic diversity among isolates of the human pathogen Bartonella bacilliformis. PLoS Negl Trop Dis.2011;5:e1248.
46. Bai Y, Hayman DT, McKee CD, Kosoy MY. Classification of Bartonella strains associated with straw-colored fruit bats (Eidolon helvum) across Africa using a multi-locus sequence typing platform. PLoS Negl Trop Dis.2015;9:e0003478.
47. Buffet JP, Pisanu B, Brisse S, Roussel S, Felix B, Halos L, Chapuis JL, Vayssier-Taussat M. Deciphering bartonella diversity, recombination, and host specificity in a rodent community. PLoS One.2013;8:e68956.
48. Guy L, Nystedt B, Sun Y, Naslund K, Berglund EC, Andersson SG. A genome-wide study of recombination rate variation in Bartonella henselae. BMC Evol Biol. 2012;12:65.

Tables

Table 1 Primer sequences used for ticks and *Bartonella* spp. identification

Target gene	Primer sequence（5'-3'）	Product (bp)	References
16S rRNA	16S+1: CTGCTCAATGATTTTTTAAATTGCGG 16S-1: CCGGTCTGACAGATCAAGT	460	[10]
gltA	bart781: ATGGCGAATATTTCTCCAAAA bart1137: AGTGCAGCATTCGCTCCCCCT	379	[11]
rpoB	rpoF: GCACGATTYGCATCATCATTTTCC rpoR: CGCATTATGGTCGTATTTGTCC	379	[12]

Table 2 The prevalence of *Bartonella* spp. in ticks in Shiqu county

Location	No of samples		Infection rates %		
	H.qinghaiensis	*D.everestianus*	*H.qinghaiensis*	*D.everestianus*	Total
Ariza	0/168	168/168		4.8(8/168)	4.8
Maga	172/224	52/224	79.1(136/172)	69.2 (36/52)	76.8*
Derongma	0/192	192/192		12.5 (24/192)	12.5
Changxgma	0/234	234/234		18.0(42/234)	18.0

Table 3 The prevalence of *Bartonella* spp. in plateau pika in Shiqu county

Location	Infection rates %				
	Bartonella.sp*	*B. queenslandens*	*B.grahamii*	*Bartonella*.sp**	Total
Ariza	4.2(3/72)	0	16.7(12/72)	0	20.8
Maga	0	8.6(7/81)	22.2(18/81)	0	30.9
Derongma	0	0	13.8(9/65)	0	13.8
Changxgma	0	0	23.5 (16/68)	5.9(4/68)	29.4

Supplementary Files

Supplementary file1 Sequences of *gltA* gene

Supplementary file 2 Sequences of *rpoB* gene

Supplementary file 3 Adult specimen of *H.qinghaiensis.* A Dorsal view; B. Ventral view.

Supplementary file 4 Adult specimen of *D. everestianus*. A Dorsal view; B. Ventral view.

Supplementary file 5 Sequences of 16S rRNA (*H. qinghaiensis*)

Supplementary file 6 Sequences of 16S rRNA (*D. everestianus*)

Supplementary file 7 Tick collection information

Figures

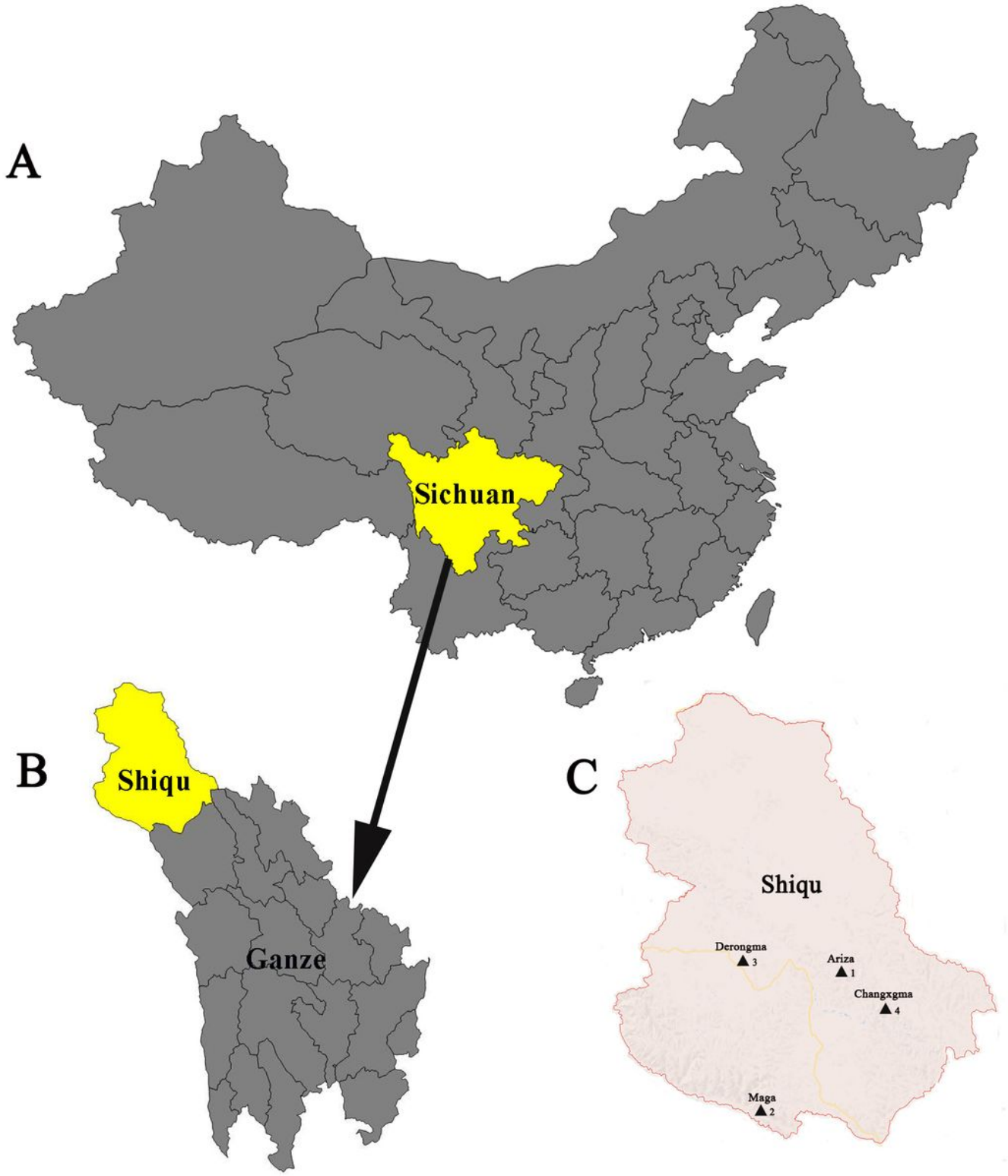

Figure 1

The map of Shiqu county. A. The map of China where Sichuan province is marked as yellow; B. The map of Ganze Tibetan autonomous prefecture where Shiqu county is marked as yellow; C. The map of Shiqu where location of samples collection is represented with black triangles (1. Ariza; 2. Maga; 3. Derongma; 4. Changxgma). Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal

status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

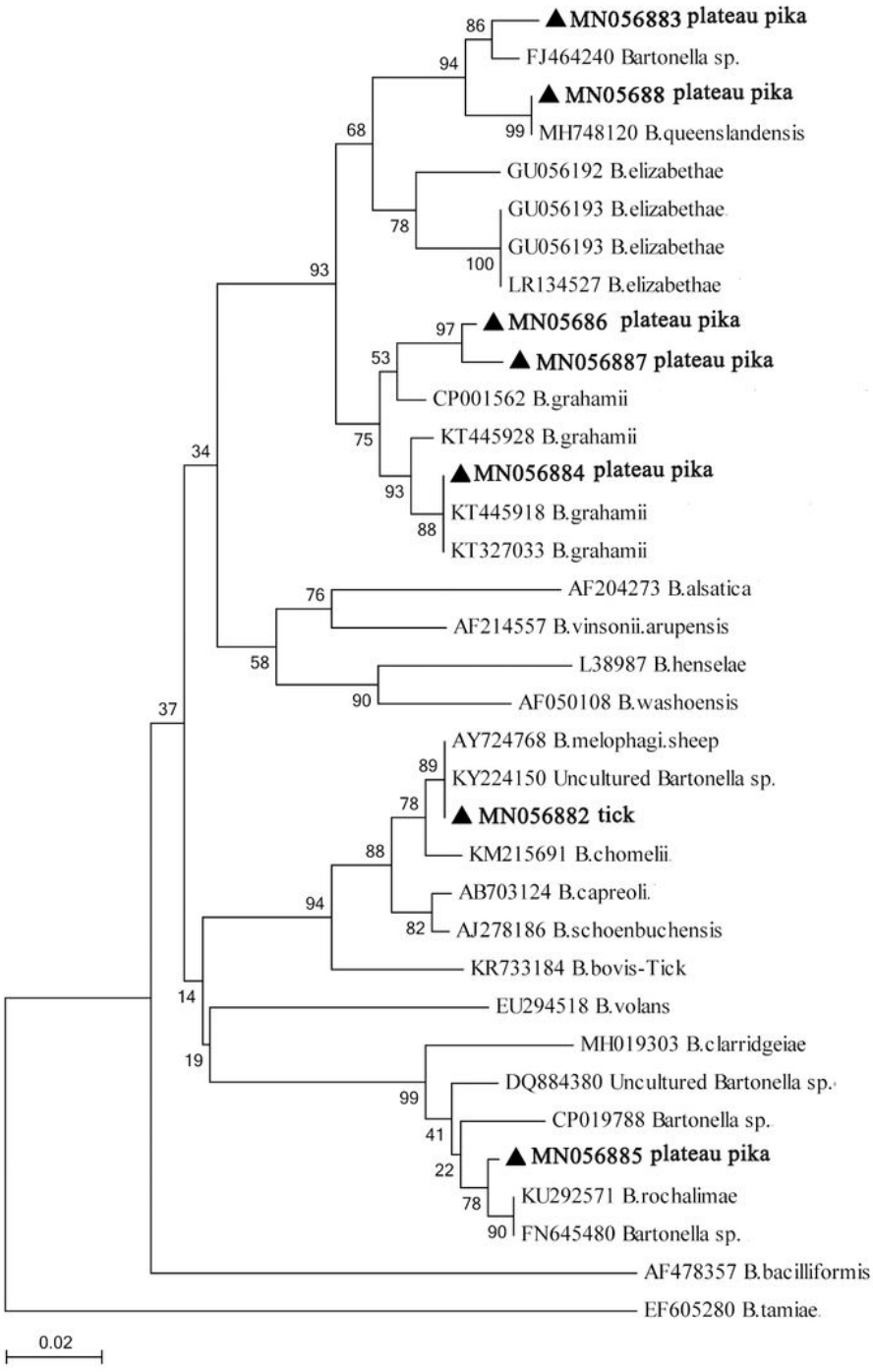

Figure 2

Neighbor joining (NJ) phylogenetic trees based on Bartonella gltA gene; Sequences obtained in this study were marked with black triangles.

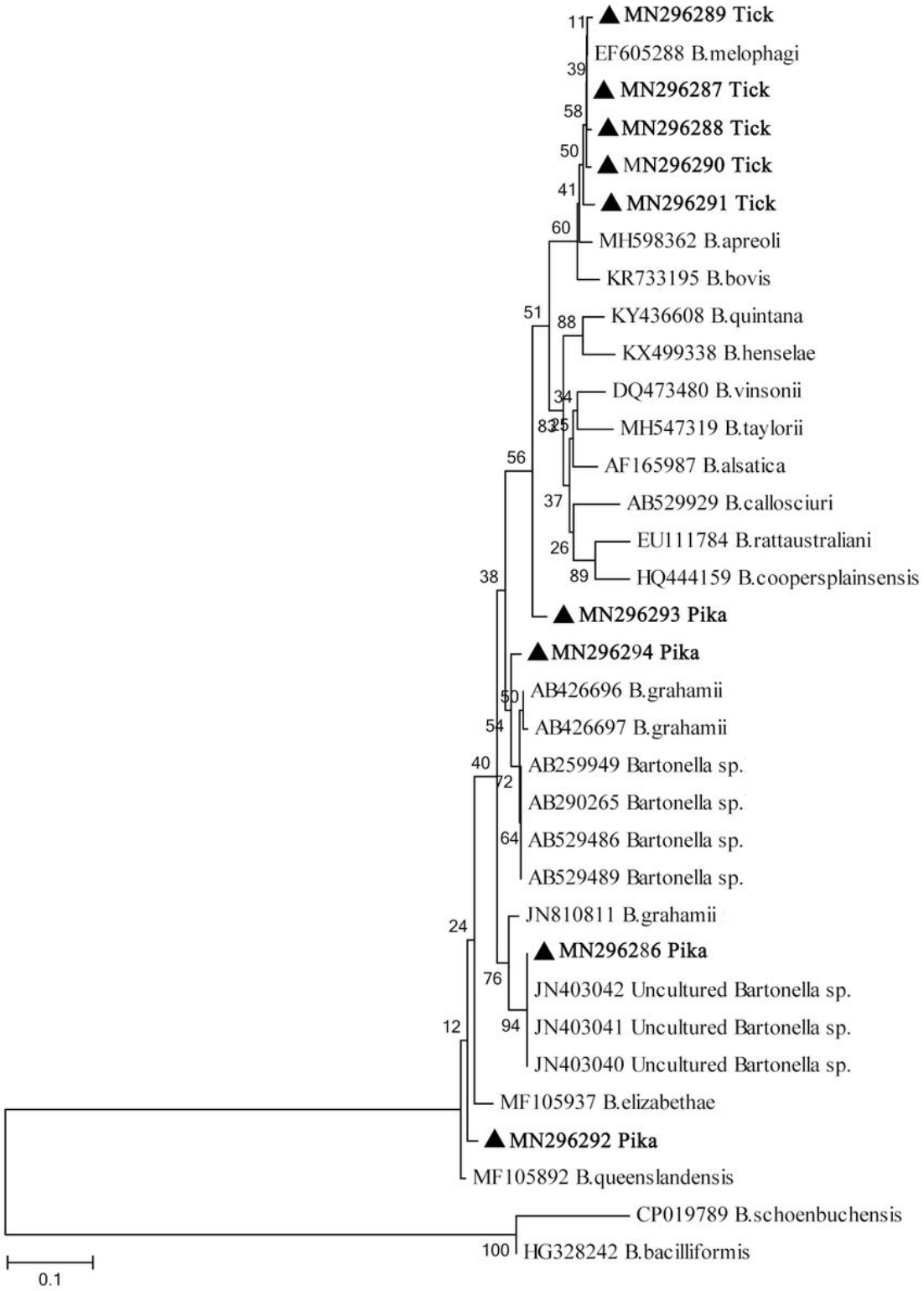

Figure 3

Neighbor joining (NJ) phylogenetic trees based on Bartonella rpoB gene; Sequences obtained in this study were marked with black triangles.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaryfile2.txt
- Supplementaryfile7.docx
- Supplementaryfile5.txt
- Supplementaryfile6.txt
- Supplementaryfile3.jpg
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