PHYLOGENETIC ANALYSIS OF BARTONELLA DETECTED IN RODENT FLEAS IN YUNNAN, CHINA

Authors: Li, Dong Mei, Liu, Qi Yong, Yu, Dong Zheng, Zhang, Jian Zhong, Gong, Zheng Da, et al.

Source: Journal of Wildlife Diseases, 43(4) : 609-617

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-43.4.609

Journal of Wildlife Diseases, 43(4), 2007, pp. 609–617

PHYLOGENETIC ANALYSIS OF *BARTONELLA* DETECTED IN RODENT FLEAS IN YUNNAN, CHINA

Dong Mei Li,[1] Qi Yong Liu,[1,3] Dong Zheng Yu,[1] Jian Zhong Zhang,[1] Zheng Da Gong,[2] and Xiu Ping Song[1]
[1] Department of Vector Biology and Control, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention P.O. Box 5, Changping, Beijing 102206, China
[2] Yunnan Institute of Endemic Diseases Control and Prevention, Dali City, Yunnan, China
[3] Corresponding author (email: Liuqiyong@icdc.cn)

ABSTRACT: Previous studies have demonstrated a diversity of *Bartonella* spp. in rodent populations in Yunnan Province, China. Although *Bartonella* spp. have been isolated from cat fleas and cattle ticks collected from their animal hosts, little is known about *Bartonella* carried by rodent fleas. In this study, *Bartonella* DNA was detected by polymerase chain reaction (PCR) in two of five species of rodent fleas. These included *Xenopsylla cheopis* and *Ctenophthalmus lushuiensis*, which were collected from *Rattus tanezumi flavipectus* and from the nests of voles, respectively, during 1997 from two sites in western Yunnan Province, China. Sequence analysis of the *Bartonella* citrate synthase gene (*gltA*) amplicons obtained from six of 65 grouped flea samples showed that *Bartonella* genetic variants were clustered in four groups. One from *Xenopsylla cheopis* was identical to *Bartonella tribocorum*, whereas the other three genotypes from *Ctenophthalmus lushuiensis* were related to the vole-associated *Bartonella* isolates and cat-associated *Bartonella clarridgeiae*. This is the first detection of this *Bartonella* variant from fleas in China. Therefore, further investigations are needed to clarify the distribution of *Bartonella* in rodents and their ectoparasites in China to define the role of these arthropods in the transmission routes of *Bartonella*.

Key words: *Bartonella*, *Ctenophthalmus lushuiensis*, genetic diversity, phylogenetic analysis, rodent flea, *Xenopsylla cheopis*.

INTRODUCTION

The genus *Bartonella* is an oxidase-negative, fastidious, gram-negative hemotropic bacillus. These diverse species infect a variety of mammalian hosts and arthropod vectors, causing a broad spectrum of diseases. In addition to animal hosts in close contact with humans such as cats, dogs, and cattle, rodents are one of the most important reservoirs. Many *Bartonella* species have been identified in rodents, and there is an increasing number of human and canine infections reportedly caused by rodent-borne *Bartonella* (Daly et al., 1993; O'Halloran et al., 1998; Kerkhoff et al., 1999; Welch et al., 1999; Kosoy et al., 2003; Serratrice et al., 2003; Fenollar et al., 2005). Bartonellae are transmitted by variety of bloodsucking arthropods; identified vectors include the sand fly (*Lutzomyia verrucarum*) as a vector for *Bartonella bacilliformis* (Alexander, 1995), the body louse (*Pediculus humanus*) for *Bartonella quintana* (Maurin and Raoult, 1996), and the cat flea (*Ctenocephalides felis*) for *Bartonella henselae* (Chomel et al., 1996). For rodent Bartonellae, two vectors have been suggested: The oriental rat flea (*Xenopsylla cheopis*) has been demonstrated to be a competent vector of an unidentified *Bartonella* species that infects bank voles (*Clethrionomys glareolus*) (Krampitz, 1962), and the vole ear mite (*Trombicula microti*) has been proposed as the vector of *Bartonella vinsonii vinsonii* (Baker, 1946). Several researchers have suggested a role for rodent fleas as the vector of *Bartonella* species based on detection of *Bartonella* DNA from fleas collected worldwide (Parola et al., 2003; Rolain et al., 2003; Stevenson et al., 2003; Reeves et al., 2005; Loftis et al., 2006; Marie et al., 2006). In experimental transmission studies, Bown et al. (2004) demonstrated that the rodent flea *Ctenophthalmus nobilis* is a competent vector of at least two *Bartonella* species: *Bartonella grahamii*, which has previously been associated with human infection (Kerkhoff et al., 1999; Serratrice et al., 2003), and *Bartonella taylorii*.

TABLE 1. The geographic information of the sampling sites.

County	Latitude (°N)	Longitude (°E)	Mean altitude (m)	Mean annual air temperature (C)	Climate type
Longling	24.36	98.41	1,527	14.9	Subtropical plateau with monsoon climate
Longchuan	24.22	97.58	1,600	18.9	Subtropical monsoon climate
Yingjiang	24.43	97.56	827	19.9	Subtropical monsoon climate
Lincang	23.53	100.05	1,464	17.2	Subtropical monsoon climate
Gengma	23.33	99.24	1,104	19.0	Subtropical monsoon climate

Areas with a high prevalence of *Bartonella* infections in rodents are often characterized by the presence of a diversity of *Bartonella* species and strains (Birtles et al., 1994, 2001; Kosoy et al., 1997). During the period 2001–2005, a high prevalence of *Bartonella* was demonstrated through isolation from rodents (*Apodemus* species, *Eothenomys* species, and *Rattus* species) from the west, northwest, southwest, south, and middle of Yunnan Province, China; isolates typically clustered according to the genus of rodent hosts (Ying et al., 2002; Li et al., 2004a; Bai et al., 2005). Although no evidence of vector involvement was provided in these studies, we have isolated *Bartonella* from cat fleas (*Ctenocephalides felis*) collected from dogs, and ticks (*Boophilus microplus*) collected from cattle in northwestern Yunnan Province (Li et al., 2005). Based on the host range of fleas and the high prevalence of *Bartonella* spp. in rodents in Yunnan Province, we hypothesized that rodent fleas may represent an important vector of *Bartonella* in rodent populations. In this study, we report molecular evidence for the presence of a diversity of *Bartonella* genotypes from the rodent fleas collected in Yunnan Province, China, during 1997.

MATERIALS AND METHODS

Flea samples

Fleas were collected from rodents and burrows at five sites (Table 1) in Longling, Longchuan, Yingjiang, Lincang, and Gengma counties of southwestern Yunnan Province, China, during March to November of 1997. Sites were located in residential and farmland mountainous areas around the towns. All fleas were classified to species level by an entomologist, and species pools were stored in 70% ethanol.

Preparation of DNA extracts from fleas

Eight to 11 fleas of the same species from the same sites were grouped and washed three times with sterile deionized water for surface decontamination. Each group of fleas was placed in a sterilized mortar and crushed using a sterile pestle in liquid nitrogen. DNA was prepared from the crushed fleas using a QIAamp Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

PCR amplification

Polymerase chain reaction (PCR) amplification was performed using a MyCycler™ Thermal Cycler (Bio-Rad, Hercules, CA, USA). Three pairs of primers were used for the amplification of *Bartonella* DNA. The previously described PCR primers: BhCS.781p-BhCS.1137n (5′-GGGGACCAGCTC ATGGT GG-3′, 5′-AATGCAAA AAGAACAGTAAACA-3′) described by Norman et al. (1995) targets a 379 base-pair (bp) fragment of the citrate synthase gene (*gltA*); Bh.311p-Bh.452n (5′-CTCTTTCTTCAGATGATGATCC-3′, 5′-AA CCAACTGAGCT ACAAGCCCT-3′) described by Jensen et al. (2000) targets the 5′-teminal 200 bp partial sequence of the 16S-23S rRNA intergenic spacer region (ITS); and TIle.455p-TAla.885n (5′-GCTTGTA GCTCAGTTGGT-TAG-3′, 5′-TGCTTGCAAAGCAGGTGC TCT -3′) described by Li et al. (2004b) amplifies about 200–400 bp of $tRNA^{Ile}$-$tRNA^{Ala}$ ITS in the 16S-23S rRNA ITS. DNA amplification was performed using a 25 μl reaction volume. Each reaction mixture contained 1 μl of 10 pmol/μl of each primer, 1.5 U *Taq* DNA polymerase (Sino-American Biotechnology Co., Beijing, China), 2 μl of 2.5 mM deoxynucleosides triphosphate mixture, 2.5 μl of 10× PCR buffer, 1.5 μl of 20 mM $MgCl_2$, and 5 μl of DNA template. Each PCR analysis was performed using

TABLE 2. Numbers and geographic distribution of five species of fleas from Yunnan, China.

Flea species	Location					Total
	Longling	Longchuan	Yingjiang	Lincang	Gengma	
Xenopsylla cheopis	50	19	50	99	99	317
Leptopsylla segnis	58	50	48	18	0	174
Ctenophthalmus lushuiensis	49	0	0	0	0	49
Nosopsyllus elongatus	0	50	0	0	0	50
Palaeopsylla remota	0	0	51	0	0	51
Total	157	119	149	117	99	641

a negative control (molecular-grade water) and positive control (DNA from *B. henselae* strain Houston-1 [ATCC49882]). The extraction, creation of the reaction mixture, sample addition, PCR amplification, and product analysis were performed in different rooms to minimize cross contamination.

DNA sequencing and data analyses

The citrate synthase sequence (*gltA*) is acknowledged as the phylogenetic marker for *Bartonella* (Birtles and Raoult, 1996; La Scola et al., 2003). To detect and identify *Bartonella* DNA, we used PCR assays and sequencing of the amplicons derived from the *gltA* gene of *Bartonella* species and compared the *Bartonella* species from the rodent fleas with the available *Bartonella* strains in GenBank. The PCR amplicons from the *gltA* fragments amplified using BhCS.781p-BhCS.1137n primers were purified using the Promega Wizard PCR Preps Kit (Promega, Madison, Wisconsin, USA); the purified amplicons were cloned into pGEM-T Easy vector system (Promega) according to the manufacturer's protocol; and the white-colored recombinant colonies were selected. Sequencing was performed using a BigDye® Terminator v3.1 Cycle Sequencing Kit and an ABI PRISM 3730xl DNA analyzer (Applied Biosystems, Foster City, California, USA). New sequences were compared with published sequences using the BLAST program from the National Center for Biotechnology Information Website (available from: http://www.ncbi.nlm.nih.gov/BLAST/). Values of similarity between the sample sequences and published *Bartonella* sequences were calculated using the clustalV analysis with the Megalign module of the DNAStar5 Package (DNASTAR, Inc., Madison, Wisconsin, USA). The sequences were aligned using ClustalX version 1.81 (Thompson et al., 1997). Phylogenetic analysis was performed using MEGA3.1 (Kumar et al., 2004) and the PAUP* 4.0 beta version programs (Sinauer Associates, Inc., Sunderland, Massachusetts, USA).

Nucleotide sequence accession numbers

The *gltA* gene partial sequences of the *Bartonella* spp. detected in the different groups of rodent fleas in this study are available in the GenBank database under accession numbers DQ884382 (F12YN), DQ884378 (F13YN), DQ884379 (F14YN), DQ884380 (F15YN), DQ884381 (F16YN), and DQ884377 (F47YN).

RESULTS

Flea sampling

A total of 641 rodent fleas comprising five species was found: 317 *X. cheopis*, 174 *Leptopsylla segnis*, 49 *Ctenophthalmus lushuiensis*, 50 *Nosopsyllus elongatus*, and 51 *Palaeopsylla remota* (Table 2). *Xenopsylla cheopis*, *L. segnis*, and *N. elongates* were collected from the bodies of *Rattus tanezumi flavipectus*. *Ctenophthalmus lushuiensis* specimens were collected in three nests of voles (*Eothenomys* spp. based on range and habitat). Because all the *C. lushuiensis* samples were mixed, we have no detailed information to infer about the origin of the four positive samples and if they came from different nests of voles. *Palaeopsylla remota* specimens were collected from the bodies of *Anourosorex squamipes*. Fleas were divided into 65 groups as follows: 33 groups of *X*, 17 groups of *L. segnis*, five groups of *C. lushuiensis*, five groups of *N. elongatus*, five groups of *P. remota*.

PCR detection of *Bartonella* DNA in rodent fleas

Six of the 65 groups were PCR positive (F12YN, F13YN, F14YN, F15YN, F16YN, and F47YN); PCR products were the

TABLE 3. Epidemiological characteristics of the polymerase chain reaction (PCR) positive groups of flea samples.

Group no.	Quantity of fleas	Flea species	Sampling site	Sampling date	Environment	Altitude (m)
F12YN	10	*Ctenophthalmus lushuiensis*	Longling	10 March 1997	Suburb	1,527
F13YN	10	*Ctenophthalmus lushuiensis*	Longling	10 March 1997	Suburb	1,527
F14YN	10	*Ctenophthalmus lushuiensis*	Longling	10 March 1997	Suburb	1,527
F15YN	10	*Ctenophthalmus lushuiensis*	Longling	10 March 1997	Suburb	1,527
F16YN	9	*Ctenophthalmus lushuiensis*	Longling	10 March 1997	Suburb	1,527
F47YN	10	*Xenopsylla cheopis*	Lincang	19–24 November 1997	Residential area in town	1,464

expected size, using all three primer pairs. Among the six groups collected from two sites, five groups were *C. lushuiensis* collected from nests of voles and the other was *X. cheopis* collected from *Rattus tanezumi flavipectus* (Table 3). Negative controls failed to yield detectable PCR products, whereas the positive controls gave consistent expected PCR products.

Comparison of the sequences from the flea samples and the *Bartonella* deposited in GenBank

A pair-wise comparison was performed using *Bartonella* deposited in the GenBank. The *gltA* sequences of F12YN and F14YN were identical and had 100% sequence similarity to a homologous amplicon of the *Bartonella* strain Em1712yn (AF391282) of *Eothenomys miletus* from northwestern Yunnan, China (Ying et al., 2002). There was one nucleotide mutation, G→A, at position 206 among the DNA sequences of F13YN, F12YN, and F14YN; however, the deduced amino acid composition was not affected. These *gltA* amplicon sequences differed from those described for all the known *Bartonella* variants. F15YN had the highest BLAST sequence similarity (97%) to *Bartonella* strains detected from rodents from Greece (Tea et al., 2004) and Sweden (Holmberg et al., 2003) and 95% similarity to *B. clarridgeiae*. Based on the pair-wise divergences of 338 bp *gltA* sequences, F16YN was most similar (98%) to the *Bartonella* strain Em1531yn (AF391281) (Ying et al., 2002) of *E. miletus* from the middle of Yunnan among all available sequences of *Bartonella* deposited in the GenBank at 97.4–97.6% (with nine nucleotide mutations) and similar to F13YN, F12YN, and F14YN, which had no variation in amino acid sequences. DNA similarity values based on the 379 bp of the *gltA* gene from F47YN compared to other *Bartonella* strains show that F47YN was more than 99% similar to *Bartonella* strains from *Rattus norvegicus* from Beijing (GenBank accession numbers DQ 884392, DQ884391, DQ884389, DQ 884388, DQ884387, and DQ884383), *Bartonella* strains from *R. tanezumi flavipectus* and *R. norvegicus*, fleas from *X. cheopis* from Indonesia (GenBank accession numbers AY902189, AY902191, AY902190, and AY902188), and the *Bartonella* strains from *Rattus rattus* from Portugal (GenBank accession no. AF 086636). All of these *Bartonella* are almost identical to *Bartonella tribocorum*, but there are two different amino acids found at two different positions (I→T, I→V) in F47YN compared to *B. tribocorum* and the other *Bartonella* strains, which had the

same amino acid (I) at these two positions based on 109 amino acids.

The *gltA*-based phylogeny of *Bartonella*

The sequence alignment of *gltA* of the *Bartonella* was 338 base pairs. The phylogenetic tree derived from the *gltA* data set using distance methods with MEGA3.1 showed four clusters of *Bartonella* strains identified within the flea samples (Fig. 1). The clusters between the flea sample sequences and published *Bartonella* sequences derived from interpretations of the parsimony analysis with the PAUP* 4.0 beta version were identical (data not shown). The genotype of F15YN formed a clade with the Grecian (strain af102nev, AY435103) and Swedish (strain af82up, AF391788) *Bartonella* isolates and was related to *Bartonella clarridgeiae*. Another two groups were the *E. miletus*–associated *Bartonella* isolates originating from different geographic areas of Yunnan Province. One group consisted of F12YN, F13YN, F14YN, and Em1712yn (AF391282) using supporting significant bootstrap values (99%). This genotype was similar to another *E. miletus*–associated group, which included F16YN and Em1531yn (AF391281). These two vole-associated *Bartonella* spp. represent a genotype that is related to *Bartonella taylorii* (from *Apodemus* spp. or voles). The genotype of F47YN, the five Beijing's *R. norvegicus* isolates (DQ884392, DQ884391, DQ 884389, DQ884388, DQ884387, and DQ 884383), the four Indonesian isolates (AY902189, AY902191, AY902190, and AY902188), and the one Portuguese *Rattus rattus* isolate (AF086636) formed a well-supported cluster (94% bootstrap support) and were closely related to *B. tribocorum*.

DISCUSSION

Using PCR assay, we found *Bartonella* DNA in two flea species, *C. lushuiensis* from Longling county, and *X. cheopis* from Lincang county of Yunnan Province, China. The phylogenetic tree of these *gltA* sequences showed that there were divergent *Bartonella* groups from the two flea species. In this study, F47YN represented the genotype *B. tribocorum* based on the phylogenetic analysis; however, there are several mutations in the *gltA* gene among the *Bartonella* (F47YN) from *X. cheopis* from Lincang, the Portuguese *Bartonella* isolate from *R. rattus*, the Indonesian isolates (*R. norvegicus*, *R. tanezumi flavipectus,* and *X. cheopis*), and the Beijing *R. norvegicus* isolates. We infer that these mutations occurred among the different original isolates that are hypothetically attributed to the variants in host and geographic factors. *B. tribocorum* is a rodent-associated species that is distributed widely and that has been isolated from *R. norvegicus* in Europe and North America (Heller et al., 1998; Ellis et al., 1999). Ying et al. (2002) demonstrated a *B. tribocorum* bacteremia in *R. norvegicus* and *R. tanezumi flavipectus* in western Yunnan, China. Li et al. (2004) reported a high prevalence of *Bartonella* (42.0%) from *R. tanezumi flavipectus*, with *X. cheopis* as dominant species (Guo et al., 2000), which inhabits indoor areas and courtyards, usually in south China, and is in close contact with human beings.

The *Bartonella* DNA detected in pools of *C. lushuiensis* from three nests of voles showed genotypic diversity into three clusters according to the *gltA* gene phylogenetic analysis: F12YN, F14YN, F13YN, and *E. miletus Bartonella* strain Em-1712yn (AF391282) from the west of Yunnan were the same genotype and were different from the F16YN genotype and another *E. miletus Bartonella* strain Em1531yn (AF391281) from the middle of Yunnan. Even though they are branched together, there was no solid evidence to indicate that these *Bartonella* spp. are *B. taylorii* based on the similarity value (86.5%) of the *gltA* gene. Based on La Scola et al. (2003), who proposed that newly encountered *Bartonella* isolates

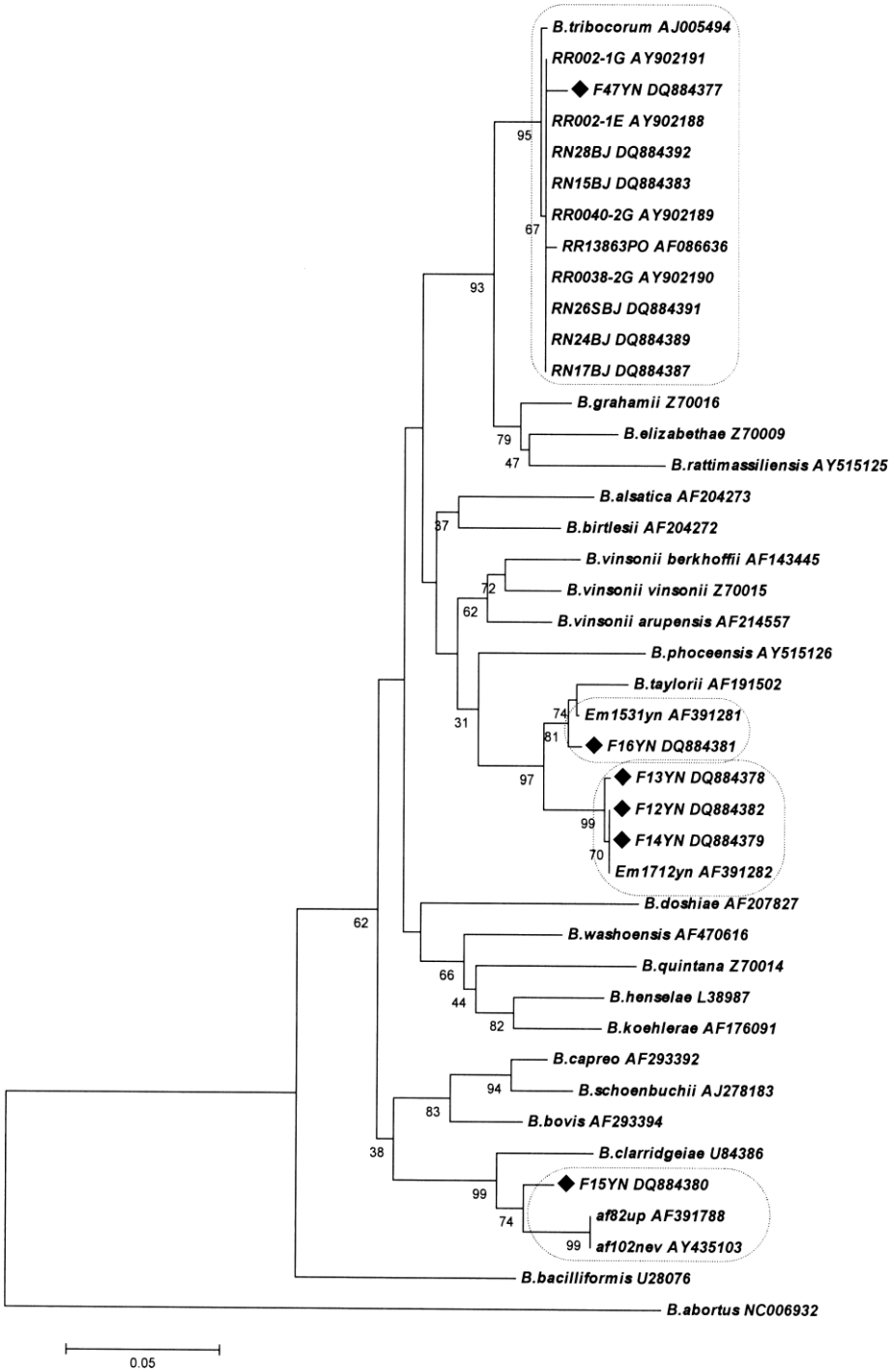

FIGURE 1. The phylogenetic tree based on 338 base pairs (bp) of *gltA* for *Bartonella* strains drawn using the MEGA3.1 software (Sinauer Associates, Inc., Sunderland, Massachusetts, USA). The distance matrix was calculated using the Kimura-2 parameter. The tree was obtained using the neighbor-joining method. The scale bar represents 5% divergence. The support of each branch was determined using 500 bootstrap samples and is indicated by the value at the node. The sequence of *Brucella abortus* was chosen as the out-group in the tree. The position of new sequences generated in this study is indicated with a black diamond.

should be considered new species if a 327 bp *gltA* fragment shares <96.0% sequence similarity with validated species, we suspect that these flea-associated *Bartonella* spp. are new genotypes. Further experiments are needed in order to prove it they are two separate new species or subspecies.

Interestingly, the F15YN genotype is more closely related to cat-associated *B. clarridgeiae* (Kordick et al., 1997) than the other recognized *Bartonella* strains. A similar report by Loftis et al. (2006) showed that the new *Bartonella* genotype from *X. cheopis* in Egypt was related to *B. clarridgeiae* based on the *groEL* gene. Within the *B. clarridgeiae* clade, there are two *Bartonella* isolates from *Apodemus flavicollis* from Greece that are 97.0% similar to the genotype of F15YN. Because *B. clarridgeiae* is a human pathogen, further studies may be warranted related to pathogenesis, range of host species (including rodents), modes of transmission, and epidemiology of human exposure to this *Bartonella* variant.

Bartonella organisms cause a persistent, often asymptomatic intra-erythrocytic bacteremia in their mammalian hosts. These arthropod-borne pathogens depend on the ecology of the blood-sucking arthropod vector, and an intra-erythrocytic bacterium can be transmitted among natural reservoir hosts, a cycle that is difficult to interrupt in the natural environment. Various flea vectors play an important role in this infection cycle. *Xenopsylla cheopis*, which uses *R. norvegicus* and *R. tanezumi flavipectus* as its major host, is a notorious vector for transmission of plague (*Yersinia pestis*) and endemic typhus (*Rickettsia typhus*). *Ctenophthalmus lushuiensis* is endemic in Yunnan Province, and it is carried by the common flea of *Eothenomys* spp. (Xie et al., 2000). Our data demonstrate that *Bartonella* spp. detected in fleas associated with voles is similar to *Bartonella* spp. from the voles, and the *Bartonella* spp. detected in the rat fleas is identical to *Bartonella* isolates from rats. This correlation between animal hosts and arthropod vectors indirectly reflects the characteristics of host specificity of the fleas and partial host specificity of *Bartonella* (Breitschwerdt and Kordick, 2000) and suggests that the two species of fleas presumably play a role as the transmission vectors of rodent-associated *Bartonella* in their natural cycle.

Previous investigations concerning *Bartonella* infections in rodents, cat fleas, and ticks have shown a high genetic diversity in *Bartonella* in Yunnan, China (Ying et al., 2002; Bai et al., 2005; Li et al., 2005). Our findings further confirm that there are different *Bartonella* genotypes in the rodents, and the rodent fleas can carry *Bartonella* in Yunnan Province. Humans are incidental hosts for the numerous rodent-associated *Bartonella* species. An increasing number of bacteria are identical or closely related to *Bartonella* and have been obtained from rodents and are being isolated from human patients. Further studies are needed in China to clarify the characteristics of the new flea-associated *Bartonella* and the potential vectors and the role of the ectoparasites in the route of transmission.

ACKNOWLEDGMENTS

We thank S. Karpathy (Viral and Rickettsial Zoonoses Branch, Centers for Disease Control and Prevention, Atlanta, Georgia, USA) for the phylogenetic analysis using the PAUP* 4.0 beta version, C. Dehio (Division of Molecular Microbiology, Biozentrum, University of Basel, Basel, Switzerland) for critically reading the manuscript, and. M. Y. Kosoy (Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, Colorado, USA) for many helpful discussions. We also thank M. E. Eremeeva (Viral and Rickettsial Zoonoses Branch, Centers for Disease Control and Prevention) for reading and making corrections to the manuscript.

This research was supported by the National Natural Science Foundation of China (NSFC) grant 30371246 and the Project of State Scientific & Technological Development of the 10th Five-Year Plan grant 2003BA712A09-02.

LITERATURE CITED

ALEXANDER, B. 1995. A review of bartonellosis in Ecuador and Colombia. American Journal of Tropical Medicine and Hygiene 52: 354–359.

BAI, H. M., F. L. YANG, H. YANG, AND Q. ZHANG. 2005. Study on *Bartonella* in rodents in western Yunnan, China. Zhonghua Liu Xing Bing Xue Za Zhi 26: 868–870. [In Chinese.]

BAKER, J. A. 1946. A Rickettsial infection in Canadian voles. The Journal of Experimental Medicine 84: 7–51.

BIRTLES, R. J., AND D. RAOULT. 1996. Comparison of partial citrate synthase gene (*gltA*) sequences for phylogenetic analysis of *Bartonella*. International Journal of Systematic Bacteriology 46: 891–897.

———, T. G. HARRISON, AND D. H. MOLYNEUX. 1994. *Grahamella* in small woodland rodents in the U.K.: Isolation, prevalence and host specificity. Annals of Tropical Medicine & Parasitology 88: 317–327.

———, S. M. HAZEL, M. BENNETT, K. BOWN, D. RAOULT, AND M. BEGON. 2001. Longitudinal monitoring of the dynamics of infections due to *Bartonella* in UK woodland rodents. Epidemiology and Infection 126: 323–329.

BOWN, K. J., M. BENNET, AND M. BEGON. 2004. Flea-borne *Bartonella grahamii* and *Bartonella taylorii* in bank voles. Emerging Infectious Diseases 10: 684–687.

BREITSCHWERDT, E. B., AND D. L. KORDICK. 2000. *Bartonella* infection in animals: Carriership, reservoir potential, pathogenicity, and zoonotic potential for human infection. Clinical Microbiology Reviews 13: 428–438.

CHOMEL, B. B., R. W. KASTEN, K. FLOYD-HAWKINS, B. CHI, K. YAMAMOTO, J. ROBERTS-WILSON, A. N. GURFIELD, R. C. ABBOTT, N. C. PEDERSEN, AND J. K. KOELHER. Experimental transmission of *Bartonella henselae* by the cat flea. Journal of Clinical Microbiology 34: 1952–1956.

DALY, J. S., M. G. WORTHINGTON, D. J. BRENNER, C. W. MOSS, D. G. HOLLIS, R. S. WEYANT, A. G. STEIGERWALT, R. E. WEAVER, M. I. DANESHVAR, AND S. P. O'CONNOR. 1993. *Rochalimaea elizabethae* sp. *nov.* isolated from a patient with endocarditis. Journal of Clinical Microbiology 31: 872–881.

ELLIS, B. A., L. REGNERY, L. BEATI, F. BACELLAR, M. ROOD, G. G. GLASS, E. MARSTON, T. G. KSIAZEK, D. JONES, AND J. E. CHILDS. 1999. Rats of the genus *Rattus* are reservoir hosts for pathogenic *Bartonella*: An Old World origin for a New World disease? The Journal of Infectious Diseases 180: 220–224.

FENOLLAR, F., S. SIRE, AND D. RAOULT. 2005. *Bartonella vinsonii* subsp. *arupensis* as an agent of blood culture-negative endocarditis in a human. Journal of Clinical Microbiology 43: 945–947.

GUO, X. X., Z. D. GONG, T. J. QIAN, X. G. FENG, X. D. DUAN, W. LI, AND X. K. ZHANG. 2000. Spatial pattern analysis of *Xenopsylla cheopis* (Siphonaptera: Pulicidae) on its dominant rat host *Rattus flavipectus* in the foci of human plague in Yunnan, China. Entomologia Sinica 7: 47–52.

HELLER, R., P. RIEGEL, Y. HANSMANN, G. DELACOUR, D. BERMOND, C. DEHIO, F. LAMARQUE, H. MONTEIL, B. CHOMEL, AND Y. PIEMONT. 1998. *Bartonella tribocorum* sp. nov., a new *Bartonella* isolated from the blood of wild rats. International Journal of Systematic Bacteriology 48: 1333–1339.

HOLMBERG, M. I., J. N. MILLS, S. MCGILL, G. BENJAMIN, AND B. A. ELLIS. 2003. *Bartonella* infection in sylvatic small mammals of central Sweden. Epidemiology and Infection 130: 149–157.

JENSEN, W. A., M. Z. FALL, J. ROONEY, D. L. KORDICK, AND E. B. BREITSCHWERDT. 2000. Rapid identification and differentiation of *Bartonella* using a single-step PCR assay. Journal of Clinical Microbiology 38: 1717–1722.

KERKHOFF, F. T., A. M. BERGMANS, A. VAN DER ZEE., AND A. ROTHOVA. 1999. Demonstration of *Bartonella grahamii* DNA in ocular fluids of a patient with neuroretinitis. Journal of Clinical Microbiology 37: 4034–4038.

KORDICK, D. L., E. J. HILYARD, T. L. HADFIELD, K. H. WILSON, A. G. STEIGERWALT, D. J. BRENNER, AND E. B. BREITSCHWERDT. 1997. *Bartonella clarridgeiae*, a newly recognized zoonotic pathogen causing inoculation papules, fever, and lymphadenopathy (cat scratch disease). Journal of Clinical Microbiology 35: 1813–1818.

KOSOY, M. Y., R. L. REGNERY, T. TZIANABOS, E. L. MARSTON, D. C. JONES, D. GREEN, G. O. MAUPIN, J. G. OLSON, AND J. E. CHILDS. 1997. Distribution, diversity, and host specificity of *Bartonella* in rodents from the southeastern United States. American Journal of Tropical Medicine and Hygiene 57: 578–588.

KOSOY, M., M. MURRAY, R. D. GILMORE, Y. BAI, AND K. L. GAGE. 2003. *Bartonella* strains from ground squirrels are identical to *Bartonella washoensis* isolated from a human patient. Journal of Clinical Microbiology 41: 645–650.

KRAMPITZ, H. E. 1962. Further studies on Grahamella Brumpt 1911. Zeitschrift für Tropenmedizin und Parasitologie 13: 34–53. [In German.]

KUMAR, S., K. TAMURA, AND M. NEI. 2004. MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. Briefings in Bioinformatics 5: 150–163.

LA SCOLA, B., Z. ZEAITER, A. KHAMIS, AND D. RAOULT. 2003. Gene-sequence-based criteria for species definition in bacteriology: The *Bartonella* paradigm. Trends in Microbiology 11: 318–321.

LI, D. M., D. Z. YU, Q. Y. LIU, AND Z. D. GONG. 2004a. Study on the prevalence of *Bartonella* in

rodent hosts from different environmental areas in Yunnan. Zhonghua Liu Xing Bing Xue Za Zhi 25: 934–937. [In Chinese.]

———, ———, ———, R. Hai, and B. H. Guo. 2004b. Study on *Bartonella* infection using molecular biological diagnostic techniques from China. Zhonghua Liu Xing Bing Xue Za Zhi 25: 602–602.. [In Chinese.]

———, Q. Y. Liu, D. Z. Yu, and X. Q. Dong. 2005. Isolation and molecular identification of *Bartonella* from fleas and ticks. Chinese Journal of Zoonoses 21: 49–56. [In Chinese.]

Loftis, A. D., W. K. Reeves, D. E. Szumlas, M. M. Abbassy, I. M. Helmy, J. R. Moriarity, and G. A. Dasch. 2006. Surveillance of Egyptian fleas for agents of public health significance: *Anaplasma*, *Bartonella*, *Coxiella*, *Ehrlichia*, *Rickettsia*, and *Yersinia pestis*. American Journal of Tropical Medicine and Hygiene 75: 41–48.

Marie, J. L., P. E. Fournier, J. M. Rolain, S. Briolant, B. Davoust, and D. Raoult. 2006. Molecular detection of *Bartonella quintana*, *B. elizabethae*, *B. koehlerae*, *B. doshiae*, *B. taylorii*, and *Rickettsia felis* in rodent fleas collected in Kabul, Afghanistan. American Journal of Tropical Medicine and Hygiene 74: 436–439.

Maurin, M., and D. Raoult. 1996. *Bartonella* (*Rochalimaea*) *quintana* infections. Clinical Microbiology Reviews 9: 273–292.

Norman, A. F., R. Regnery, P. Jameson, C. Greene, and D. C. Krause. 1995. Differentiation of *Bartonella*-like isolates at the species level by PCR-restriction fragment length polymorphism in the citrate synthase gene. Journal of Clinical Microbiology 33: 1797–1803.

O'Halloran, H. S., K. Draud, M. Minix, A. K. Rivard, and P. A. Pearson. 1998. Leber's neuroretinitis in a patient with serologic evidence of *Bartonella elizabethae*. Retina 18: 276–278.

Parola, P., O. Y. Sanogo, K. Lerdthusnee, Z. Zeaiter, G. Chauvancy, J. P. Gonzalez, R. S. Miller, S. R. Telford, Iii., C. Wongsrichanalai, and D. Raoult. 2003. Identification of *Rickettsia* spp. and *Bartonella* spp. in fleas from the Thai-Myanmar border. Annals of the New York Academy of Sciences 990: 173–181.

Reeves, W. K., M. P. Nelder, and J. A. Korecki. 2005. *Bartonella* and *Rickettsia* in fleas and lice from mammals in South Carolina. U. S. A. Journal of Vector Ecology 30: 310–315.

Rolain, J. M., M. Franc, B. Davoust, and D. Raoult. 2003. Molecular detection of *Bartonella quintana*, *B. koehlerae*, *B. henselae*, *B. clarridgeiae*, *Rickettsia felis*, and *Wolbachia pipientis* in cat fleas, France. Emerging Infectious Diseases 9: 338–342.

Serratrice, J., J. M. Rolain, B. Granel, N. Ene, J. Conrath, J. F. Avierinos, P. Disdier, D. Raoult, and P. J. Weiller. 2003. Bilateral retinal artery branch occlusions revealing *Bartonella grahamii* infection. Revue Roumaine de Médecine Interne 24: 629–630. [In French.]

Stevenson, H. L., Y. Bai, M. Y. Kosoy, J. A. Montenieri, J. L. Lowell, M. C. Chu, and K. L. Gage. 2003. Detection of novel *Bartonella* strains and *Yersinia pestis* in prairie dogs and their fleas (Siphonaptera: Ceratophyllidae and Pulicidae) using multiplex polymerase chain reaction. Journal of Medical Entomology 40: 329–337.

Tea, A., S. A. Daniel, A. Papoutsi, A. Papa, and A. Antoniadis. 2004. *Bartonella* isolated from rodents, Greece. Emerging Infectious Diseases 10: 963–964.

Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. G. Higgins. 1997. The ClustalX windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 24: 4876–4882.

Welch, D. F., K. C. Carroll, E. K. Hofmeister, D. H. Persing, D. A. Robison, A. G. Steigerwalt, and D. J. Brenner. 1999. Isolation of a new subspecies, *Bartonella vinsonii* subsp. *arupensis*, from a cattle rancher: Identity with isolates found in conjunction with *Borrelia burgdorferi* and *Babesia microti* among naturally infected mice. Journal of Clinical Microbiology 37: 2598–2601.

Wilbur, W. J., and O. J. Lipman. 1983. Rapid similarity searches of nucleic acid and protein data banks. Proceedings of the National Academy of Sciences of the United States of America 80: 726–730..

Xie, B. Q., and J. F. Zeng. 2000. The siphonaptera of Yunnan., Yunnan Science and Technology Press, Yunnan, China, pp. 226–228..

Ying, B., M. Y. Kosoy, G. O. Maupin, K. R. Tsuchiya, and K. L. Gage. 2002. Genetic and ecologic characteristics of *Bartonella* communities in rodents in southern China. American Journal of Tropical Medicine and Hygiene 66: 622–627.

Received for publication 24 February 2007.