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Short Report: Molecular Detection and Identification of *Bartonella* Species in Rat Fleas from Northeastern Thailand

Sarah A. Billeter, Leah Colton, Somboon Sangmaneedet, Fanan Suksawat, Brian P. Evans, and Michael Y. Kosoy*

Division of Vector Borne Diseases, Centers for Disease Control and Prevention, Fort Collins, Colorado; Department of Pathobiology, Faculty of Veterinary Medicine, Khon Kaen University, Khon Kaen, Thailand; Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand

Abstract. The presence of *Bartonella* species in *Xenopsylla cheopis* fleas collected from *Rattus* spp. (*R. exulans*, *R. norvegicus*, and *R. rattus*) in Khon Kaen Province, Thailand was investigated. One hundred ninety-three fleas obtained from 62 rats, were screened by polymerase chain reaction using primers specific for the 16S–23S intergenic spacer region, and the presence of *Bartonella* DNA was confirmed by using the citrate synthase gene. *Bartonella* DNA was detected in 59.1% (114 of 193) of fleas examined. Sequencing demonstrated the presence of *Bartonella* spp. similar to *B. elizabethae*, *B. rattimassiliensis*, *B. rochalimae*, and *B. tribocorum* in the samples tested with a cutoff for sequence similarity ≥ 96% and 4 clustered together with the closest match with *B. grahamii* (95.5% identity). If *X. cheopis* proves to be a competent vector of these species, our results suggest that humans and animals residing in this area may be at risk for infection by several zoonotic *Bartonella* species.

Bartonella species are small, pleomorphic, gram-negative bacteria that infect a variety of mammalian hosts, including cats, dogs, rodents, ruminants, and humans. Clinical symptoms associated with *Bartonella* range from mild, influenza-like symptoms to more severe manifestations such as endocarditis, myocarditis, uveitis, bacillary angiomatosis, and peliosis hepatis.[1] Approximately half of the 20 *Bartonella* species or subspecies identified to date are known or suspected human pathogens,[2] and most are believed to be transmitted by arthropod vectors (fleas, lice, sandflies, and ticks).[3]

Xenopsylla cheopis, the Oriental rat flea, is a suspected vector of several *Bartonella* species (*B. tribocorum*, *B. elizabethae*, *B. queenslandensis*, *B. rochalimae*, and novel *Bartonella* genotypes), and *Bartonella* DNA has been detected in these fleas from various locations worldwide.[3–8] Although generally found on rodents, *X. cheopis* have been found to parasitize humans and are known vectors of the zoonotic agents *Yersinia pestis* (plague) and *Rickettsia typhi* (murine typhus).[9]

Numerous surveys have been performed to identify the presence of *Bartonella* species affecting humans and domestic and peri-domestic animals in Thailand.[10–17] *Bartonella henselae*, (the agent of cat scratch disease),[14] *B. tamiae*,[10] *B. elizabethae*, *B. rattimassiliensis*, and *B. tribocorum* have been isolated from febrile patients,[15] *B. henselae* and *B. clarridgeiae* have been reported in cats,[11] and *B. clarridgeiae*, *B. vinsonii* subsp. *arupensis*, *B. elizabethae*, *B. grahamii*, *B. quintana, B. taylorii*, and novel *Bartonella* genotypes have been found in dogs.[11,16] In rodent species, *B. grahamii*, *B. elizabethae*, *Candidatus* Bartonella thailandensis, *B. coopersplainensis*, *B. phoceensis*, *B. rattimassiliensis*, *B. tribocorum*, and novel *Bartonella* genotypes have been detected by culture and polymerase chain reaction (PCR) analysis.[12,13,17] However, little information has been obtained to identify potential arthropod vectors of *Bartonella* species in Thailand. *Bartonella henselae*, *B. clarridgeiae* and *B. koehlerae* were detected in *Ctenocephalides felis* fleas removed from cats[18–20] and *B. henselae* was identified in two *C. canis*[19] also collected from cats. Furthermore, a *Bartonella* sp., similar to *B. grahamii*, was found in a rodent flea, *Nosopsyllus fasciatus*, obtained from *Rattus surifer*.[18] *Bartonella tamiae* DNA has also been found in chigger mites (genera *Leptotrombidium*, *Schoengastia*, and *Blankarrtia*) and in a tick (genus *Haemaphysalis*) collected from rodents in Thailand, suggesting a potential role for these arthropods in the transmission of *B. tamiae*.[21]

The aim of the current study was to investigate the prevalence of *Bartonella* species in rodent-associated fleas collected in Khon Kaen Province, Thailand, and to determine what potential role, if any, these fleas may play in the transmission of *Bartonella* species to individuals residing in this area.

For this study, 62 rats (10 *R. norvegicus*, 9 *R. rattus*, and 43 *R. exulans*) were trapped in and around homes in 4 villages, 1 market, and on farm land (a pig farm and 2 rice fields) in Khon Kaen Province, Thailand during May–June 2011 (Table 1). Fleas were collected from rats and placed in tubes containing isopropanol. Samples were shipped to Bartonella Laboratory at the Centers for Disease Control and Prevention (Fort Collins, CO) on dry ice and stored at –20°C until further analysis. All fleas were subsequently identified as *X. cheopis* by using a taxonomic key.[22] Work involving rodents was conducted as outlined in our approved animal use protocol (#11-003), under the supervision of the Institutional Animal Care and Use Committee of the Division of Vector Borne Diseases.

Individual fleas were triturated by using a bead beater protocol,[23] and DNA was extracted by using a Qiagen QIAamp tissue kit (QIAGEN, Valencia, CA) according to the manufacturer's instruction. DNA was extracted from 1–5 fleas/rat (depending upon the number of fleas collected: in most cases, > 5 fleas per rat were recovered); a total of 193 fleas were examined. Fleas were initially screened by conventional PCR using primers specific for the 16S–23S intergenic spacer region (ITS),[24] and the presence of *Bartonella* DNA was confirmed by using citrate synthase gene (*glt*A)–specific primers.[8] *Bartonella doshiae* DNA was used as a positive control, and nuclease-free water was used as a negative control.

*Glt*A amplicons were purified by using the QIAquick PCR purification kit (QIAGEN) and sequenced by using a Model 3130 genetic analyzer (Applied Biosystems, Foster City, CA). DNA sequences were analyzed by using the Lasergene version 8 sequence analysis software (DNASTAR, Madison, WI).

*Address correspondence to Michael Y. Kosoy, Division of Vector Borne Diseases, Centers for Disease Control and Prevention, 3150 Rampart Road, Fort Collins, CO 80521. E-mail: mck3@cdc.gov

TABLE 1

Number of rats trapped per site by species and total number of fleas examined per site, northeastern Thailand

Site designation	No. *Rattus exulans*/site	No. *R. norvegicus*/site	No. *R. rattus*/site	No. fleas examined/site*
Village 1	7	8	2	60
Village 2	1	1	0	6
Village 3	15	0	1	46
Village 4	17	0	0	60
Neighborhood market	1	0	1	5
Farmland (pig farm and rice fields)	2	1	5	16
Total	43	10	9	193

*Total number of fleas per rat was not determined. No more than five fleas/rat were screened for *Bartonella* DNA.

All *glt*A sequences for this study were shortened to ≈379 base-pairs to enable further phylogenetic analysis. Sequences obtained in this study were considered similar to validated *Bartonella* spp. if similarity over the 379-base-pairs *glt*A fragment was ≥ 96%.[25] The Clustal W program in Megalign (Lasergene) was used to compare sequences obtained from this study to *Bartonella* sequences available in GenBank. The neighbor-joining (NJ) method by Kimura's two-parameter distance method and bootstrap calculation was carried out with 1,000 resamplings. *Glt*A sequences were submitted to GenBank (accession numbers JX123018–JX123023).

Of the 193 *X. cheopis* fleas examined, 59.1% (114) were positive for *Bartonella* DNA by using ITS and *glt*A primers (113 fleas ITS positive and 107 fleas *glt*A positive). A total of 80 *glt*A amplicons were sequenced. Six genotypes, with at least one nucleotide difference, were found and sequence similarity between genotypes ranged between 87.6% and 99.5% (Table 2). These six genotypes were clustered around *B. elizabethae* (U28072) (genotypes 1 and 2 with sequence similarity of 96.2%, GenBank accession nos. JX123021 and JX123022), *B. grahamii* (EU014266) (genotypes 3 with sequence similarity of 95.5%, GenBank accession no. JX123018), *B. rattimassiliensis* 15908^T (AY515124) (genotype 4 with sequence similarity of 96.6%, GenBank accession no. JX123023), *B. rochalimae* BMGH (DQ683195) (genotype 5 with sequence similarity of 98.8%, GenBank accession no. JX123020), or *B. tribocorum* IBS506^T (AJ005494) (genotypes 6 with sequence similarity of 99.7%, GenBank accession no. JX123019) (Figure 1).

The *B. elizabethae* group (genotypes 1 and 2), detected in fleas recovered from 18 rats (12 *R. exulans* and 6 *R. norvegicus*), contained 36 identical sequences and a distinct sequence, respectively. This group was also similar to a *Bartonella* sp. detected in *R. norvegicus* from Beijing, China (EF213769) and *Praomys delectorum* from Tanzania (FJ851115) with 98.9–99.5% and 99.2% sequence similarity, respectively. Genotype 3, most closely related to *B. grahamii* with 95.5% similarity and a *Bartonella* sp. detected in stray animals from Taiwan (GU056195) with 99.2% similarity, contained 4 identical sequences and was detected in fleas collected from 3 rats (1 *R. exulans*, 1 *R. norvegicus*, and 1 *R. rattus*). The *B. rattimassilienis* sequence (genotype 4) was detected in a flea collected from a *R. rattus* and was also 98.9% similar to a bartonellae isolated from the blood of a *R. argentiventer* from Thailand (FJ655402). The *B. rochalimae* group (genotype 5) contained 24 identical sequences found in fleas removed from 15 rats (5 *R. exulans*, 8 *R. norvegicus*, and 2 *R. rattus*). This genogroup was also 100% identical to *Bartonella* sp. 1-1C detected in a *R. norvegicus* from Taiwan (FN545495). The *B. tribocorum* group (genotype 6) contained 14 identical sequences and was detected in fleas recovered from 10 rats (5 *R. exulans*, 4 *R. norvegicus*, and 1 *R. rattus*). This group was also 99.5–99.9% similar to a *Bartonella* sp. detected in rodents from Nepal (GU143516) and Yunnan, China (FJ589051).

TABLE 2

Bartonella citrate synthase A genotypes detected in *Xenopsylla cheopis*, number of sequences of each genotype, and flea rodent host, northeastern Thailand*

GenBank accession no.	*Bartonella* genotype	No. sequences/genotype	Flea rodent host*
JX123018	Xc61-5tl	4	RE (1), RN (1), RR (1)
JX123019	Xc70-3tl	14	RE (5), RN (4), RR (1)
JX123020	Xc70-5tl	24	RE (5), RN (8), RR (2)
JX123021	Xc101-1tl	1	RN (1)
JX123022	Xc127-2tl	36	RE (12), RN (5)
JX123023	Xc142-1tl	1	RR (1)

*RE = *Rattus exulans*; RN = *Rattus norvegicus*; RR = *Rattus rattus*.

Humans and animals residing in this area commonly come into contact with rodents and are potentially at risk for infection with rodent-borne diseases. A large percentage of rodents in this study were trapped either in or around homes or in food storage areas, increasing the likelihood of disease transmission. In a separate survey, Kosoy and others[15] screened the blood of 261 patients to identify what role *Bartonella* species play in acute febrile illness in Thailand; *Bartonella* spp. were detected in 7.7% (20) of these samples. Sequencing demonstrated the presence of rodent-borne *Bartonella* species in half of these samples, specifically *B. rattimassiliensis*, *B. vinsonii* subsp. *arupensis*, *B. vinsonii* subsp. *vinsonii*, *B. tribocorum*, and *B. elizabethae*, and 71% of patients reported exposure to rats during the two weeks before the onset of illness.[15] An additional study was conducted in rural Thailand to screen febrile and non-febrile patients who came to local hospitals for *Bartonella*-specific antibodies.[26] Of the 521 serum samples screened, 9.8% (51) were seropositive for *B. elizabethae* and 3.6% (19) for *B. vinsonii* subsp. *vinsonii*. Interestingly, 18 patients were seroreactive against *B. elizabethae* and *B. vinsonii* subsp. *vinsonii*, 1 patient was seroreactive against *B. elizabethae*, *B. henselae*, and *B. quintana*, 4 patients were seroreactive against *B. elizabethae*, *B. vinsonii* subsp. *vinsonii*, and *B. quintana*, and 6 patients harbored antibodies against *B. elizabethae*, *B. vinsonii* subsp. *vinsonii*, *B. henselae*, and *B. quintana*.[26] These results further strengthen the supposition that contact with rodents is quite common in Thailand and rodents might serve as reservoirs for human *Bartonella* infections.

Almost 60% of fleas examined in this study harbored *Bartonella* DNA. Parola and others[18] found a much lower *Bartonella* prevalence in rodent fleas collected along the Thailand–Myanmar border. In this study, 10 *X. cheopis* and 26 *N. fasciatus* were tested and 1 flea (2.8% positivity), a *N. fasciatus* collected from a *R. surifer*, contained a species closely related to *B. grahamii*.[18] The results from our study demonstrate that a large percentage of *X. cheopis* from northeastern Thailand harbor *Bartonella* species, including known zoonotic pathogens. What role, if any, *X. cheopis* plays in the transmission of *Bartonella* species remains unclear. Currently,

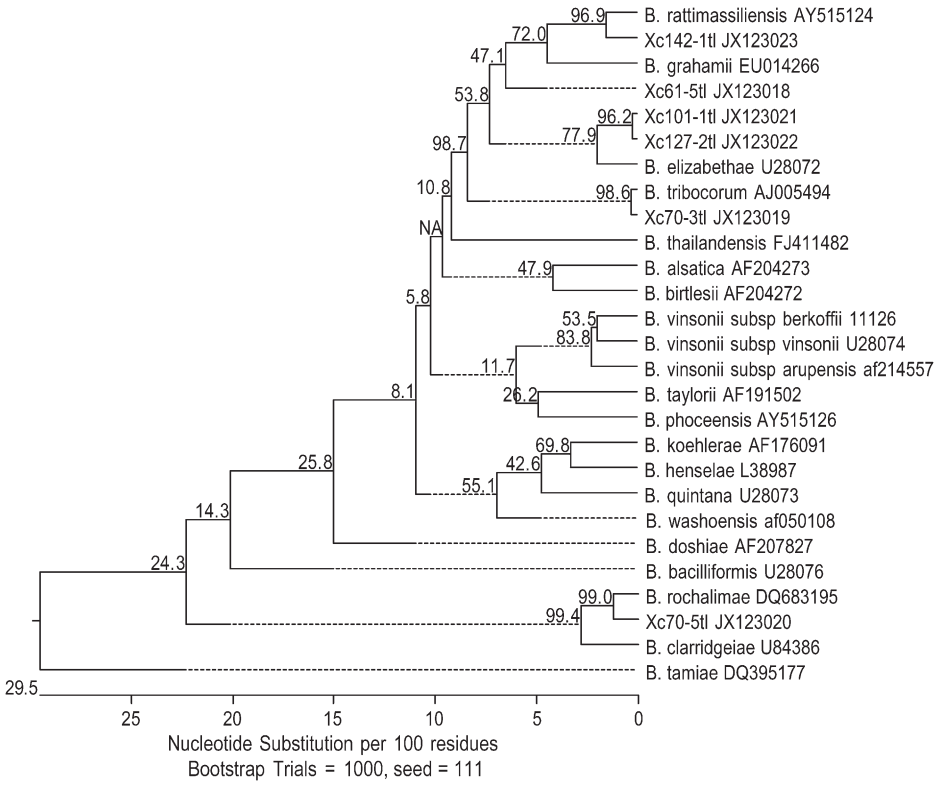

FIGURE 1. Tree topology displaying similarity of *Bartonella* DNA detected in *Xenopsylla cheopis* with known *Bartonella* sequences based upon partial citrate synthase gene (*glt*A) sequences, northeastern Thailand. *Glt*A sequences obtained from fleas are represented by GenBank Accession nos. JX123018–JX123023.

studies are being performed in our laboratory to determine if *X. cheopis* are competent vectors of *Bartonella* species.

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Authors' addresses: Sarah A. Billeter, Leah Colton, and Michael Y. Kosoy, Division of Vector Borne Diseases, Centers for Disease Control and Prevention, Fort Collins, CO, E-mails: ina8@cdc.gov, ant6@cdc.gov, and mck3@cdc.gov. Somboon Sangmaneedet and Fanan Suksawat, Faculty of Veterinary Medicine, Khon Kaen University, Amphur Mueng, Khon Kaen, Thailand, E-mails: sombn_sa@kku.ac.th and jsvetmed@yahoo.com. Brian P. Evans, Department of Entomology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, E-mail: brian.patrick.evans@us.army.mil.

REFERENCES

1. Chomel BB, Boulouis HJ, Maruyama S, Breitschwerdt EB, 2006. *Bartonella* spp. in pets and effect on human health. *Emerg Infect Dis 12:* 389–394.
2. Vayssier-Taussat M, Le Rhun D, Bonnet S, Cotté V, 2009. Insights in *Bartonella* host specificity. *Ann N Y Acad Sci 1166:* 127–132.
3. Billeter SA, Levy MG, Chomel BB, Breitschwerdt EB, 2008. Vector transmission of *Bartonella* species with emphasis on the potential for tick transmission. *Med Vet Entomol 22:* 1–15.
4. Loftis AD, Reeves WK, Szumlas DE, Abbassy MM, Helmy IM, Moriarity JR, Dasch GA, 2006. Surveillance of Egyptian fleas for agents of public health significance: *Anaplasma*, *Bartonella*, *Coxiella*, *Ehrlichia*, *Rickettsia*, and *Yersinia pestis*. *Am J Trop Med Hyg 75:* 41–48.
5. Li DM, Liu QY, Yu DZ, Zhang JZ, Gong ZD, Song XP, 2007. Phylogenetic analysis of *Bartonella* detected in rodent fleas in Yunnan, China. *J Wildl Dis 43:* 609–617.
6. Reeves WK, Rogers TE, Durden LA, Dasch GA, 2007. Association of *Bartonella* with the fleas (Siphonaptera) of rodents and bats using molecular techniques. *J Vector Ecol 32:* 118–122.
7. Tsai YL, Chuang ST, Chang CC, Kass PH, Chomel BB, 2010. *Bartonella* species in small mammals and their ectoparasites in Taiwan. *Am J Trop Med Hyg 83:* 917–923.
8. Billeter SA, Gundi VA, Rood MP, Kosoy MY, 2011. Molecular detection and identification of *Bartonella* species in *Xenopsylla cheopis* fleas (Siphonaptera: Pulicidae) collected from *Rattus norvegicus* rats in Los Angeles, California. *Appl Environ Microbiol 77:* 7850–7852.
9. Brouqui P, Raoult D, 2006. Arthropod-borne diseases in homeless. *Ann N Y Acad Sci 1078:* 223–235.
10. Kosoy M, Morway C, Sheff KW, Bai Y, Colborn J, Chalcraft L, Dowell SF, Peruski LF, Maloney SA, Baggett H, Sutthirattana S, Sidhirat A, Maruyama S, Kabeya H, Chomel BB, Kasten R, Popov V, Robinson J, Kruglov A, Petersen LR, 2008. *Bartonella tamiae* sp. nov., a newly recognized pathogen isolated from three human patients from Thailand. *J Clin Microbiol 46:* 772–775.
11. Inoue K, Maruyama S, Kabeya H, Kawanami K, Yanai K, Jitchum S, Jittaparapong S, 2009. Prevalence of *Bartonella* infection in cats and dogs in a metropolitan area, Thailand. *Epidemiol Infect 137:* 1568–1573.

12. Saisongkorh W, Wootta W, Sawanpanyalert P, Raoult D, Rolain JM, 2009. "Candidatus *Bartonella thailandensis*": a new genotype of *Bartonella* identified from rodents. *Vet Microbiol 139:* 197–201.
13. Bai Y, Kosoy MY, Lerdthusnee K, Peruski LF, Richardson JH, 2009. Prevalence and genetic heterogeneity of *Bartonella* strains cultured from rodents from 17 provinces in Thailand. *Am J Trop Med Hyg 81:* 811–816.
14. Paitoonpong L, Chitsomkasem A, Chantrakooptungool S, Kanjanahareutai S, Tribuddharat C, Srifeungfung S, 2008. *Bartonella henselae*: first reported isolate in a human in Thailand. *Southeast Asian J Trop Med Public Health 39:* 123–129.
15. Kosoy M, Bai Y, Sheff K, Morway C, Baggett H, Maloney SA, Boonmar S, Bhengsri S, Dowell SF, Sitdhirasdr A, Lerdthusnee K, Richardson J, Peruski LF, 2010. Identification of *Bartonella* infections in febrile patients from Thailand and their potential animal reservoirs. *Am J Trop Med Hyg 82:* 1140–1145.
16. Bai Y, Kosoy MY, Boonmar S, Sawatwong P, Sangmaneedet S, Peruski LF, 2010. Enrichment culture and molecular identification of diverse *Bartonella* species in stray dogs. *Vet Microbiol 146:* 314–319.
17. Castle KT, Kosoy M, Lerdthusnee K, Phelan L, Bai Y, Gage KL, Leepitakrat W, Monkanna T, Khlaimanee N, Chandranoi K, Jones JW, Coleman RE, 2004. Prevalence and diversity of *Bartonella* in rodents of northern Thailand: a comparison with *Bartonella* in rodents from southern China. *Am J Trop Med Hyg 70:* 429–433.
18. Parola P, Sanogo OY, Lerdthusnee K, Zeaiter Z, Chauvancy G, Gonzalez JP, Miller RS, Telford SR III, Wongsrichanalai C, Raoult D, 2003. Identification of *Rickettsia* spp. and *Bartonella* spp. in fleas from the Thai–Myanmar border. *Ann N Y Acad Sci 990:* 173–181.
19. Foongladda S, Inthawong D, Kositanont U, Gaywee J, 2011. *Rickettsia*, *Ehrlichia*, *Anaplasma*, and *Bartonella* in ticks and fleas from dogs and cats in Bangkok. *Vector Borne Zoonotic Dis 11:* 1335–1341.
20. Assarasakorn S, Veir JK, Hawley JR, Brewer MM, Morris AK, Hill AE, Lappin MR, 2012. Prevalence of *Bartonella* species, hemoplasmas, and *Rickettsia felis* DNA in blood and fleas of cats in Bangkok, Thailand. *Res Vet Sci 93:* 1213–1216.
21. Kabeya H, Colborn JM, Bai Y, Lerdthusnee K, Richardson JH, Maruyama S, Kosoy MY, 2010. Detection of *Bartonella tamiae* DNA in ectoparasites from rodents in Thailand and their sequence similarity with bacterial cultures from Thai patients. *Vector Borne Zoonotic Dis 10:* 429–434.
22. Furman DP, Catts EP, 1982. *Manual of Medical Entomology*. Fourth Edition. New York: Cambridge University Press, 138–157.
23. Halos L, Jamal T, Vial L, Maillard R, Suau A, Le Menach A, Boulouis HJ, Vayssier-Taussat M, 2004. Determination of an efficient and reliable method for DNA extraction from ticks. *Vet Res 35:* 709–713.
24. Billeter SA, Miller MK, Breitschwerdt EB, Levy MG, 2008. Detection of two *Bartonella tamiae*-like sequences in *Amblyomma americanum* (Acari: Ixodidae) using 16S-23S intergenic spacer region-specific primers. *J Med Entomol 45:* 176–179.
25. La Scola B, Zeaiter Z, Khamis A, Raoult D, 2003. Gene-sequence-based criteria for species definition in bacteriology: the *Bartonella* paradigm. *Trends Microbiol 11:* 318–321.
26. Bhengsri S, Bagget HC, Peruski LF, Morway C, Bai Y, Fisk TL, Sitdhirasdr A, Maloney SA, Dowell SF, Kosoy M, 2011. *Bartonella* seroprevalence in rural Thailand. *Southeast Asian J Trop Med Public Health 42:* 687–692.