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

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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 hepatitis.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. tamae,13 B. elizabethae, B. rattimassiliensis, and B. tribocorum have been isolated from febrile patients.15 B. henselae and B. claridgeiae have been reported in cats,11 and B. claridgeiae, 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. coopersplainingensis, B. phocoensis, 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. claridgeiae and B. koehlerae were detected in Cienocephalides felis fleas removed from cats18–20 and B. henselae was identified in two C. canis19 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 tamae DNA has also been found in chigger mites (genera Leptotrombidium, Schoengastia, and Blankarritia) and in a tick (genus Haemaphysalis) collected from rodents in Thailand, suggesting a potential role for these arthropods in the transmission of B. tamae.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 (gltA)-specific primers.25 Bartonella doshiiae DNA was used as a positive control, and nuclease-free water was used as a negative control.

GltA amplicons were purified by using the Qiagen 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).
All gltA 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 gltA fragment was ≥ 96%. 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. GltA 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 gltA primers (113 fleas ITS positive and 107 fleas gltA positive). A total of 80 gltA 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) (genotype 3 with sequence similarity of 95.5%, GenBank accession no. JX123018), *B. rattimassiliensis* (EU014266) (genotypes 4 and 5 with sequence similarity of 99.7%, GenBank accession no. JX123020), *B. rochalimae* 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 genotype was also 99.5–99.9% similar to a *Bartonella* sp. detected in rodents from Nepal (GU143516) and Yunnan, China (FJ589051).

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 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. An additional study was conducted in rural Thailand to screen febrile and non-febrile patients who came to local hospitals for *Bartonella*-specific antibodies. 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*. 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 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*. 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.

### Table 1

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

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

### Table 2

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

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

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

This group was also similar to a *Bartonella* sp. detected in *R. norvegicus* from Beijing, China (EF213769) and *Praomys decoratorum* 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. rattimassiliensis* 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 99.5–99.9% similar to a *Bartonella* sp. detected in rodents from Nepal (GU143516) and Yunnan, China (FJ589051).
studies are being performed in our laboratory to determine if *X. cheopis* are competent vectors of *Bartonella* species.

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