PREVALENCE OF BARTONELLA SPECIES AND 16S rRNA GENE TYPES OF BARTONELLA HENSELAE FROM DOMESTIC CATS IN THAILAND

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Abstract. Prevalence of Bartonella infection among 275 cats in 9 sites from 4 geographical regions (northern area: Chiang Mai; central area: Kanchanaburi, Ratchaburi, and Bangkok; northeastern area: Khon Kaen, Roi Et, Ubon Ratcharthani, and Nakhonratchasima; southern area: Songkhla) of Thailand was investigated. Overall, Bartonella species were isolated from 27.6% (76 of 275) of the cats. The isolation rate varied from 12.8% (5 of 39) in Songkhla (southern area) to 50.0% (26 of 52) in Khon Kaen (northeastern area). Bartonella henselae and B. clarridgeiae were isolated from 82.9% (63 of 76) and 11.8% (9 of 76) of the Bartonella-positive cats, respectively. Coinfection with both species was found in 5.3% (4 of 76) of the bacteremic cats. Of the 67 bacteremic cats from which B. henselae was isolated, 48 (71.6%) and 13 (19.4%) were infected with only Type I and Type II, respectively. Coinfection with both types was observed in 9.0% (6 of 67) of the B. henselae-positive cats. To our knowledge, this is the first report on the presence of Bartonella infection in domestic cats from Thailand, which constitute a large reservoir of Bartonella infection in this country.

INTRODUCTION

Cat-scratch disease (CSD) was first described by Debré and others in France.1 In the past decade, Bartonella henselae has been identified as the causative agent of CSD, bacillary angiomatosis and bacillary peliosis.1–4 Cats play a significant role in the epidemiology of CSD as the animal reservoir, as they harbor Bartonella species in their blood5–9 for prolonged periods of time.10

By use of polymerase chain reaction (PCR), Bergmans and others11 showed that B. henselae can be differentiated into two 16S rRNA gene types, Types I and II. Furthermore, a new Bartonella species, B. clarridgeiae, was isolated from a cat kept by a patient infected with human immunodeficiency virus,12 and the organism was suspected to cause CSD in a veterinarian bitten by a cat infected with B. clarridgeiae.8,11 Cats have also been found to harbor 2 new Bartonella species, B. koehlerae14 and B. weissii.15

Although serological and bacteriological surveys of Bartonella species in cats have been reported from many countries,3,5,7,16–22 only a few reports are available concerning Bartonella infection in cats in Asian countries, such as Japan,9,21–23 Indonesia,4 and the Philippines.6 Because Thailand is a country of devout Hinayana Buddhism, there are many temples that keep free-living dogs and cats in their precincts. Furthermore, many Thai people keep free-roaming cats in their house and yard. It is estimated that the population of Thailand is ～2 million. Although Maruyama and others14 showed the presence of B. henselae antibodies in healthy people in Thailand, there are no reports yet on the prevalence of Bartonella species in domestic cats from this country and on the 16S rRNA gene types of B. henselae.

The present study aimed at establishing the prevalence of Bartonella bacteremia in domestic cats from 9 sites located in various geographical locations in Thailand and sought to determine the 16S rRNA gene type for the feline B. henselae isolates.

MATERIALS AND METHODS

Cat samples. A total of 275 cat blood samples were collected in 9 sites from 4 geographical regions (northern area: Chiang Mai; central area: Kanchanaburi, Ratchaburi, and Bangkok; northeastern area: Khon Kaen, Roi Et, Ubon Ratcharthani, and Nakhonratchasima; southern area: Songkhla) of Thailand in August 1997 and in August 1998 (Figure 1). Cat blood samples in Bangkok were collected from pet cats at the Veterinary Teaching Hospitals of Kasetsart University (6 samples) and Chulalongkorn University (22 samples). The other samples were collected, after receiving the permission of the Buddhist monks and cat owners, from 230 free-roaming cats in the precincts of 55 Buddhist temples and from 17 pet cats from 4 households. Before collection of a blood sample for each cat, flea infestation status and general body condition were recorded. The age of cats was estimated by the appearance of the cats and the level of erosion of the teeth. A volume of 2.0 mL of blood was aseptically taken from the jugular or femoral vein and dispensed into 2-mL tubes with ethylenediamine-tetraacetic acid (Venoject II, Terumo, Tokyo, Japan). The blood samples were sent to the Laboratory of Veterinary Public Health, College of Bioresource Sciences, Nihon University, frozen with dry ice and were stored at −85°C until used.

Isolation and identification of Bartonella species. The isolation and identification of Bartonella species were performed by standard procedures, as previously reported.22

After plating the cat blood on 5% rabbit blood agar plates and incubation at 35°C in a 5% CO2 atmosphere, 3–5 colonies suspected to be Bartonella species were selected from the agar plates and subcultured on 5% rabbit blood agar plates. After Gram stain and microscopic examination, the strains were subjected to the identification of Bartonella species by PCR. The set of primers BhCS.781p (5'-GGG GAC TCG AGA GAA GAC GAC-3') and BhCS.1137n (5'-AAT CGA AAA AGA ACA GTA AAC A-3') was used to amplify a
FIGURE 1. Prevalence of Bartonella species among domestic cats in Thailand. *Statistically significant from the rate of Songkhla site (P < 0.05)

part of the citrate synthase (gltA) gene. Restriction fragment length polymorphism analysis was performed by the digestion of the amplified gltA gene with TaqI and HhaI restriction endonucleases (Takara Biomedicals, Kyoto, Japan), as described previously.

The 16S rRNA gene typing of B. henselae. The 16S rRNA gene typing of B. henselae was performed by PCR by the method previously reported. Briefly, 5 μL of the extracted DNA sample was added to 45 μL of reaction mixture (10 mM Tris, 50 mM KCl, 1.5 mM MgCl₂) containing 0.5 μM of each set of 16SF and BH1 or 16SF and BH2 primers, 0.8 mM dNTP, and 2.5U of Taq polymerase. The DNA amplification was performed with Zymoreactor II AB-1820 (Atto, Tokyo, Japan) with initial denaturation (95°C, 3 min), followed by 30 cycles of denaturation (95°C, 20 sec), annealing (56°C, 30 sec), and extension (73°C, 1 min), with a single final extension step (73°C, 5 min). The amplified PCR product was subjected to electrophoresis in a 4% agarose (NuSieve GTG agarose, FMC BioProducts, Rockland, ME). When a specific band of 185 bp was detected with each specific primer set, the strain was identified as Type I or Type II.

Statistical analysis. The results obtained were analyzed by chi-square test.

RESULTS

Bartonella species, mainly B. henselae or B. clarridgeiae, were isolated from 76 (27.6%) of the 275 cats from all 9 sites examined in Thailand. The isolation rate varied from 12.8% (5 of 39) in Songkhla to 50.0% (26 of 52) in Khon Kaen (Figure 1). The bacteremia prevalence in Khon Kaen, Roi Et, and Racha Bri was significantly higher than in Songkhla (P < 0.05). The Bartonella positive rate was found to be 24.8% in male and 30.4% in female cats. There was no statistical difference in the positive rate between both sexes. Unfortunately, we could not examine the flea infestation of all the cats in Thailand; 84 (91.3%) of 92 cats examined were infested with fleas.

In relation to the age of the cats, the prevalence of infection with Bartonella species was 9.2% (6 of 65) in cats aged < 1 year, 31.7% (20 of 63) in those aged 1 to < 2 years, 42.6% (26 of 61) in those aged 2 to < 3 years, 33.3% (17 of 51) in those aged 3 to < 4 years, and 24.1% (7 of 29) in those aged ≥ 4 years. The positive rates in cats 1 to < 2 years old, 2 to < 3 years old, and 3 to < 4 years old were significantly higher than those in cats < 1 year old (P < 0.01).

Bartonella clarridgeiae was isolated from 13 cats in 4 of the 9 sites tested: Khon Kaen, Roi Et, Nakhonratchasima, and Ratchaburi. Among the 76 Bartonella-positive cats, 63 (82.9%) cats were infected with B. henselae only, 9 (11.8%) cats were infected with B. clarridgeiae, and coinfection with both species was detected in 4 (5.3%) of the 76 positive cats (Table 1). Among the 67 cats from which B. henselae was isolated, 48 (71.6%) and 13 (19.4%) were infected with only Type I or Type II, respectively. Six (9.0%) of these 67 B. henselae-positive cats were infected with both types (Table 2). Bartonella henselae Type I was identified in cats from all 9 sites tested. Bartonella henselae Type II was also identified in almost all sites, with the exception of Nakhonratchasima.
BARTONELLA SPECIES IN CATS

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Table 1

Distribution of Bartonella species in domestic cats in Thailand

<table>
<thead>
<tr>
<th>Site</th>
<th>No. examined</th>
<th>B. henselae</th>
<th>B. clarridgeiae</th>
<th>B. henselae and B. clarridgeiae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chiang Mai*</td>
<td>43</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Khon Kaen†</td>
<td>52</td>
<td>21</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Roi Et†</td>
<td>19</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Ubon Racharathani*</td>
<td>37</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nakhonratchasima†</td>
<td>10</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Bangkok†</td>
<td>24</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ratchaburi†</td>
<td>23</td>
<td>5</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Kanchanaburi*</td>
<td>28</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Songkhla*</td>
<td>39</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total (%)</td>
<td>275</td>
<td>63 (82.9)</td>
<td>9 (11.8)</td>
<td>4 (5.3)</td>
</tr>
</tbody>
</table>

* Only B. henselae was isolated.† Both B. henselae and B. clarridgeiae were isolated.

Table 2

Distribution of B. henselae 16S rRNA types in domestic cats in Thailand

<table>
<thead>
<tr>
<th>Site</th>
<th>No. examined</th>
<th>Type I</th>
<th>Type II</th>
<th>Both I and II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chiang Mai*</td>
<td>43</td>
<td>8</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Khon Kaen*</td>
<td>52</td>
<td>14</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Roi Et†</td>
<td>19</td>
<td>4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Ubon Racharathani†</td>
<td>37</td>
<td>7</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Nakhonratchasima†</td>
<td>10</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bangkok†</td>
<td>24</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Ratchaburi†</td>
<td>23</td>
<td>6</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Kanchanaburi*</td>
<td>28</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Songkhla*</td>
<td>39</td>
<td>4</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total (%)</td>
<td>275</td>
<td>48 (71.6)</td>
<td>13 (19.4)</td>
<td>6 (9.0)</td>
</tr>
</tbody>
</table>

* The area where Type I and Type II were isolated.† The area where only Type I was isolated.

DISCUSSION

In this study, it was found that overall, 27.6% of cats tested in Thailand were infected with Bartonella henselae, B. clarridgeiae, or both. To our knowledge, this is the first report of the isolation of Bartonella species from its feline reservoir in Thailand. In other tropical countries, the percentage of bacteremic cats were 61% (19 of 31) in Manila and Cebu City, the Philippines, and 43% (6 of 14) in Jakarta, Indonesia. The average percentage of bacteremic cats in Thailand was lower than those found in these Asian tropical countries. These results indicate, therefore, that the bacteremic rate in cats may depend on the sites or cities examined in Asian tropical countries. Our lower overall prevalence may also result from our larger cat population sample size coming from more sites than for the other investigations.

Bartonella species were isolated from cats in all 9 sites examined, and the infection rate in cats varied from 12.8% in Songkhla, located in the most southern area, to 50.0% in Khon Kaen, located in the northeastern area. However, no geographical gradient could be observed, as previously reported for other parts of the world. In the United States, Jameson and others suggested that cats in a warm and humid environment were associated with higher seroprevalence of B. henselae than those in a cold and dry environment. In Japan, Ueno and others also found a higher B. henselae antibody prevalence in cats from the central and southwestern areas than in the northeastern areas. Similarly, bacteremia prevalence in domestic cats was higher in southwestern than northern Japan. Thailand is a tropical country, and there is no obvious difference in the climate between the areas examined. Therefore, no relationship between the infection rate of Bartonella species in cats and the latitude in this tropical country could be found when compared with those in temperate countries.

Bartonella henselae infection was experimentally transmitted from cat to cat by fleas (Ctenocephalides felis). In this study, most cats examined were infested with fleas. Chomel and others demonstrated that 61% (19 of 31) of Filipino cats were infected with feline Bartonella species and all bacteremic cats were flea-infested outdoor cats. These facts suggest that in tropical settings, prevalence of arthropod vectors, especially fleas, may be strongly associated with a high prevalence of Bartonella species in cats.

Patients with CSD are more likely to own a kitten aged < 12 months old. Several investigations have also suggested that cats aged < 12 months are more likely to be bacteremic or seropositive for B. henselae. However, the Bartonella bacteremia prevalence in cats < 1 year old in Thailand was relatively low when compared with other age groups. Although the age of cats was estimated by the appearance of the cats and the level of erosion of the teeth, these data indicate that the prevalence of bacteremia in cats increased with age until 3 years of age, and then declines for cats > 3 years old in Thailand. Similar results were reported for cats from the Philippines on the basis of serological data.

In Thailand, 5.3% of the bacteremic cats were coinfected with both B. henselae and B. clarridgeiae, and 9% of the bacteremic cats were coinfected with B. henselae Type I and Type II. Interestingly, B. clarridgeiae was mainly isolated from the areas where the highest bacteremic rates were observed in cats. Coinfection by both B. henselae and B. clarridgeiae or both types of B. henselae has previously been reported from Europe. The prevalence of coinfection with both Bartonella species in this study is similar to the prevalence observed in European domestic cats. In Asia, Chomel and others reported a coinfection prevalence of 12.9% in domestic cats from the Philippines. By contrast, in Japan, only one (2%) cat out of 50 Bartonella bacteremic cats was coinfected with B. henselae and B. clarridgeiae. However, we report in this study the highest prevalence of coinfection with both types of B. henselae. These results suggest that higher bacteremia prevalence provide better opportunity for coinfection with different Bartonella species or strains.

Bartonella henselae Type II has been detected in 18% of the isolates from CSD patients in the Netherlands, 19% of cat isolates in France, and 94% of cat isolates in Germany. On the other hand, Type I was the predominant type among B. henselae isolates in Japan and the Philippines, and no coinfection with both types were observed in these countries. In this study, 19.4% of the bacteremic cats in Thailand were found to harbor Type II, with 9.0% of these cats being coinfected with both types. The prevalence of Type II in this country is similar to those in France and the
Netherlands. Although the distributions of Bartonella species and 16S rRNA gene type of B. henselae vary by country or region, Type II may be more prevalent in cats in the Eurasian continent than in Japan and the Philippines, suggesting the possibility of a different origin for cats between the Eurasian continent and the Asian islands.

Cats represent an important reservoir of infection for humans in Thailand. Despite the absence of any clinical report of cases of CSD in this country, we were able to document the presence of B. henselae antibodies in ~ 5.5% of healthy Thai people, a percentage very similar to what has been observed in other countries where CSD is endemic. However, further investigation is necessary to better understand the epidemiology of CSD in Asian countries.

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REFERENCES

