การตรวจหาการติดเชื้อ Bartonella ในแมวจรจัดในวัดเขตกรุงเทพมหานครโดยเทคนิคปฏิกิริยาลูกโซ่โพลีเมอร์

Detection of Bartonella spp. Infection of Stray Cats Resided in Monasteries of Bangkok Metropolitan areas by Polymerase Chain Reaction Technique

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บทคัดย่อ
แมวเป็นสัตว์เลี้ยงที่มีความใกล้ชิดกับคน และเป็นแหล่งเก็บเชื้อที่มีอาการภายในและอาการภายนอกที่สามารถแพร่กระจายมาสู่คนได้ โดยเฉพาะอย่างยิ่ง แมวจรจัด โรคสัตว์สูญจากแมวมีหลายโรค รวมถึง โรคแมวขวน (Cat Scratch Disease-CSD) ที่มีรายงานการระบาดทั่วโลก โดยเชื้อสามารถติดมาจากทางกัดหรือช่วนหรือติดต่อระหว่างแมวโดยหมัดแมว (Ctenocephalides felis) โรคแมวขวนมีউื่นแบคทีเรีย ที่เรียกว่า Bartonella วัตถุประสงค์ของการศึกษาเพื่อตรวจหาการติดเชื้อ Bartonella ในแมวจรจัดในเขตกรุงเทพมหานคร และจัดเจาะในแมวที่มีผลต่อการติดเชื้อ โดยทำการเก็บตัวอย่างเลือด ทั้งหมด 1,490 ตัวอย่าง ตรวจโดยเทคนิคปฏิกิริยาลูกโซ่โพลีเมอร์ (PCR) พบ 3 ประเภทการติดเชื้อ Bartonella ในแมวจรจัด ทั้งหมด ร้อยละ 53.7 โดยสามารถแบ่งเป็นการติดเชื้อ Bartonella henselae ร้อยละ 34.9 และ B. clarridgeiae ร้อยละ 16 โดยเขตที่มีการติดเชื้อกลุ่มนี้มากที่สุด คือ แขวงภาษีเจริญ ร้อยละ 90 ในเขตบางกอกนัด ร้อยละ 100 (50/50) และพบว่าเพศและอายุของแมวมีผลต่อการติดเชื้อ Bartonella ในแมว จากผลการศึกษา แสดงให้เห็นว่าแมวจรจัดที่อาศัยอยู่ในวัดอาจเป็นแหล่งโรคแมวขวนที่สำคัญ และอาจแพร่กระจายมาสู่แมวเลี้ยงและคนที่อยู่ในสภาพแวดล้อมใกล้เคียงได้

ABSTRACT
Cats, as pets, are close to humans and can serve as a reservoir of internal and external parasites capable of being transmitted to humans. Stray cats are the important sources of many zoonotic diseases including cat scratch disease (CSD). CSD caused by Bartonella, is a Gram-negative bacteria, reported worldwide. CSD is transmitted to humans via cat scratches or bites and to cats via cat fleas (Ctenocephalides felis). The objective of this study was to determine the spatial

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distribution of Bartonella infections among stray cats residing in monasteries of Bangkok Metropolitan areas by using polymerase chain reaction (PCR) and risk factors. A total of 1,490 blood samples were collected from jugular vein of stray cats residing in monasteries of 50 districts of Bangkok areas. The spatial distribution of Bartonella infections among stray cats was 53.7%. B. henselae was found at 34.9%, while B. clarridgeiae was 16%. Phasi Charoen district had the highest spatial distribution (90%) of infections. One hundred percent (50/50) of Bangkok areas were endemic for CSD. No significant differences were associated with sex and age of these cats as well as cat flea infestation. The results showed that stray cats were crucial reservoirs, and can transmit the pathogen to housed cats and humans who live in the same environment.

Keyword: cat scratch disease, Bartonella, cats, PCR, Bangkok

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INTRODUCTION

Bartonella is the Gram negative bacteria that can be transmitted to wild and domestic animals also to humans. There are many studies worldwide reported reservoirs of Bartonella including rodents (Heller et al., 1998; Bermond et al., 2000), rabbits (Heller et al., 1999), cats (Koehler et al., 1994; Kelly et al., 1998; Droz et al., 1999), dogs (Breitschwerdt et al., 1995), and coyotes (Chang et al., 2000). Most species of Bartonella in cats were B. henselae and B. clarridgeiae. CSD clinical signs contain papular lesion of the skin and tender, enlarged lymph glands, intermittent fever, bacillary angiomatosis, hepatic peliosis, endocarditis bacteremia, osteolytic lesions, pulmonary nodules, neuroretinitis and neurologic disease (Adal, 1995; Margileth, 1995; Maurin et al., 1997; Jacomo et al., 2002; Hansmann et al., 2005). Some of these manifestations may be fatal, especially in immunodeficiency patients (Cockerell et al., 1987). Some infected cats with Bartonella were asymptomatic and will be reservoirs hosts. Cat fleas (Ctenocephalides felis) play a role as a vector to spread disease to other cats (Chomel et al., 1996; Rolain et al., 2001).

The actual population of stray cats in Bangkok areas is still questionable particularly in monasteries since population of stray animal is continuously rising (Jittapalapong et al., 2003). Stray cats cause more public health problems, such as zoonoses. The objective of this study was to determine spatial distribution of Bartonella infections among stray cats from monasteries of Bangkok Metropolitan areas and the risk factors associated with this pathogen.
MATERIALS AND METHODS

1. Study areas

The study areas were assigned in 50 districts of Bangkok Metropolitan areas and at least 3 monasteries were randomly chosen. The sample size of stray cats was randomly selected by the simple randomization assay.

2. Blood samples

A total of 1,490 cat blood was collected from March to May 2004. In each cat, 3 – 5 ml of blood were drawn from jugular vein and preserved in sodium citrate vacuum tubes and stored at - 40°C until use. The cats were thoroughly examined and recorded for age, sex, animal health condition, environmental details and their ectoparasites.

3. Polymerase chain reaction assay (PCR)

A 100 μl of blood sample was extracted by phenol - chloroform technique (Sambrook and Russell, 2001) for DNA extraction. The extracted DNA was stored at -20°C for using as the PCR template. The primary PCR was described by Jensen et al., (2000) used P-bhenfa primer (5’-TCTTCGTTTCTCTTTCTTCA) and P-benr1 primer (5’-CAAGCGCGCCTCTCTAACC) then the nested PCR used primers N-bhenf1a (5’-GATGATC CCAAGCCTTCTGGC) and primer N-bhenr (5’-AACCAACTGAGCTACAAGGCC) were conducted for identification of Bartonella infection which resulted by the appearance of a 152 bp fragment for B. henselae and a 134 bp fragment for B. claridgeiae. The method of Nested PCR for identification of Bartonella infection followed the PCR protocol described by Rampersad et al (2005). Optimized PCR cycle conditions were 94°C 15 s, 48.2°C 30 s and 72°C 30 s for 35 cycles for the primary-PCR and 94°C 15 s, 56°C 30 s and 72°C 30 s for 35 cycles for the nested-PCR. PCR products were separated on 2% agarose gel at 50 volts for 2 hours in electrophoresis chamber, stained with ethidium bromide and visualized under ultra-violet transilluminator.

4. DNA sequence

The positive DNA fragment was extracted and purified by QIAquick® Gel Extraction Kit (QIAGEN, Germany).

5. Statistical analysis

Chi – square and number cruncher statistical system (NCSS) ver. 2000 (Kaysville, UT) were used to assess differences in the spatial distribution and intensity of infection. Analysis was also undertaken to investigate environmental variable correlated with the infection patterns, as determined by the probability (ρ) that an individual cats were infected. If ρ is less than 0.05, it indicates the significant differences.
RESULTS

The overall spatial distribution of *Bartonella* infections among stray cats was 53.7 % (800/1,490). Almost 35% (507/1490) and 16% (238/1490) were infected by *B. henselae* and *B. clarridgeiae*, respectively. Classification by sex, 55.7% (312/560) of males and 52.4% (488/930) of females were infected with no significant differences between males and females ($\chi^2 = 3.39$, df = 1, $p = 0.06$). Different age groups of cats including 0 – 24 months, 25 – 48 months, more than 49 months were infected at 56.1% (302/538), 51.8% (375/723), and 53.7% (123/229), respectively. Therefore, age was not the significant factor ($\chi^2 = 3.60$, df = 4, $p = 0.46$). By health condition, cats was infected at 53.7% (800/1,490), 53.8% (752/1,396), 51.3% (19/37) and 50.9% (29/57) in cats classified as healthy, fair and poor, respectively ($\chi^2 = 0.53$, df = 4, $p = 0.97$). Then, health condition was not the significant factor for *Bartonella* infection. For environment condition, cats was infected at 53.7% (800/1,490), 52.9% (451/853), 55% (345/627) and 40% (4/10) in good, fair and poor, respectively ($\chi^2 = 7.30$, df = 4, $p = 0.12$). Thus, environment condition was not the significant factor. In condition of flea infestations, 56 % (280/500) were infected in cats with fleas but there were no significant differences ($\chi^2 = 2.70$, df = 1, $p = 0.10$). The result of PCR examination of blood samples demonstrated that the total district (100%: 50/50) of Bangkok Metropolitan areas were infected by *B. henselae* and *B. clarridgeiae*. The highest spatial distribution (90%, 27/30) of *Bartonella* infection was found at Phasi Charoen district. In addition, Bang Sue district (40%, 12/30) and Phasi Charoen district (40%, 12/30) were the high endemic areas of *B. clarridgeiae* infections. Likewise, Bangkok Noi district (60%, 18/30) was the endemic area of *B. henselae* infection due to the high frequency of pathogens.

![Figure 1](https://via.placeholder.com/150)  
**Figure 1** PCR Products of Bartonella spp. A 2% of agarose gel show M = Marker, 1-9 = samples, BH = *B. henselae* (positive control), BC = *B. clarridgeiae* (positive control), N = negative control.
Table 1 Factors associated with Bartonella infection among stray cats in Bangkok Metropolitan areas

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Number of normal cats</th>
<th>Number of Bartonella infected cats (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>560</td>
<td>312 (55.4)</td>
</tr>
<tr>
<td>female</td>
<td>930</td>
<td>488 (52.6)</td>
</tr>
<tr>
<td><strong>Age (months)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 – 24</td>
<td>538</td>
<td>302 (56.1)</td>
</tr>
<tr>
<td>25 - 48</td>
<td>723</td>
<td>375 (51.8)</td>
</tr>
<tr>
<td>more than 49</td>
<td>229</td>
<td>123 (53.7)</td>
</tr>
<tr>
<td><strong>Health condition</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>healthy</td>
<td>1396</td>
<td>752 (53.8)</td>
</tr>
<tr>
<td>fair</td>
<td>37</td>
<td>19 (51.3)</td>
</tr>
<tr>
<td>poor</td>
<td>57</td>
<td>29 (50.9)</td>
</tr>
<tr>
<td><strong>Environmental condition</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>good</td>
<td>853</td>
<td>451 (52.9)</td>
</tr>
<tr>
<td>fair</td>
<td>627</td>
<td>345 (55)</td>
</tr>
<tr>
<td>poor</td>
<td>10</td>
<td>4 (40)</td>
</tr>
<tr>
<td><strong>Ectoparasites (Fleas)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>infected</td>
<td>500</td>
<td>280 (56)</td>
</tr>
<tr>
<td>non-infested</td>
<td>990</td>
<td>220 (22.2)</td>
</tr>
<tr>
<td><strong>Districts</strong></td>
<td>50 districts</td>
<td>50 districts</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Bartonella has been known as an emerging zoonotic pathogen, which causes CSD in both animals and humans especially in immunodeficiency patients. The spatial distribution of Bartonella among cats in Asian countries varied from 7.2% in Japan (Maruyama et al., 2000) to 64.3% in Indonesia (Marston et al., 1999). In Thailand, previous reports showed 19.1% (Maruyama et al., 2001) compared with 53.7% in this study.

In this study, the result had shown the spatial infection of Bartonella spp. in Bangkok areas was extremely high distribution (53.7%). Housed and stray cats share the same environment and their population are increasing annually. Stray animals are lacked of food, health care and owners. These animals are becoming the important source of many pathogens such as Bartonella spp. Stray cats
Figure 2 Map of Bangkok Metropolitan area showing districts A) *B. henselae* and *B. claridgeiae* infection B) Infection of *Bartonella claridgeiae* C) infection of *B. henselae* D) mixed infection of *B. claridgeiae* and *B. henselae* (light gray = low infection at 0-35%, dark grey = moderate infection at 36-70%, black = high infection at 71-100%)
can release the pathogen to other cats via cat fleas which were recognized as the vectors (Arvand et al., 2001). The results indicated that stray cats might be the potential reservoirs of bartonellosis.

The gold standard for diagnosis of *Bartonella* infection is based on culture technique, but it is time consuming and delivering unpredictable results due to its slow, fastidious growth characteristics (Sander, 1998). In this study, nested PCR for isolate and differentiate *Bartonella* infection in stray cats was performed because this technique has more specificity and sensitivity than the other methods such as culture and serology. The weak aspect of serological diagnosis is lack of its specificity so that it can cause the cross reaction to other members of the same bacterial group.

Through the study, *B. henselae* and *B. clarridgeiae* were found among stray cats of Bangkok Metropolitan areas, and noticed these findings were similar to the previous report by Maruyama et al. (2001). Therefore, it will confirm that the major pathogens of CSD is *B. henselae*. However, no factors associated with *Bartonella* infections among stray cats in Bangkok areas were found. The only factor that might be related was the sex of cats (*p* = 0.06).

In conclusion, *Bartonella* infections are currently existed in stray cat population and Bangkok Metropolitan areas are endemic of this infection. CSD might be a life threatening disease in immunosuppressed patients. Control and prevention program will be based on control of stray cats population and isolation of the reservoir from the population.

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