Prevalence of *Bartonella* infection in well-cared cats in Bangkok metropolitan

Pakkawan Satranarakun¹ Soichi Maruyama² Hidori Kabeya² Shigo Sato²
Sathaporn Jitapalapong³ Saowalak Jitchum³ Tawisa Jiyipong³
Channarong Rodkhum⁴ Rosama Pusoonthornthum¹*

Abstract

The study was conducted to determine the prevalence of bartonellosis in two hundred well-cared client-owned cats that have close relationship with their owners in Bangkok metropolitan area from November 2010 to November 2011. These cats had no clinical signs at the time of presentation and no visual flea infestation. Blood collection was performed and cultured in 5% rabbit blood agar. Species of *Bartonella* spp. was identified with Polymerase Chain Reaction and gene sequencing. Results demonstrated that eleven out of the two hundred cats were positive for bartonellosis. Moreover, the prevalence was 5.5% (11/200), with *Bartonella henselae* 91% (10/11) and *Bartonella clarridgeiae* 9% (1/11). Therefore, this study indicated that the major cause of bartonellosis in well-cared cats in Bangkok metropolitan was *Bartonella henselae*. It is recommended that pet owners perform regular ectoparasite prevention. Also, knowledge of bartonellosis should be spread to both veterinarians and pet owners for effective prevention of the disease.

Keywords: Bangkok, *Bartonella*, prevalence, well-cared cats

¹Department of Veterinary Medicine, Faculty of Veterinary Science, Chulalongkorn University, Thailand
²Department of Veterinary Medicine, College of Bioresource Sciences, Nihon University, Japan
³Department of Parasitology, Faculty of Veterinary Medicine, Kasetsart University, Thailand
⁴Department of Microbiology, Faculty of Veterinary Science, Chulalongkorn University, Thailand
*Correspondence: drrosana@gmail.com

Introduction

*Bartonella* spp. are considered as vector-borne parasites (Jacomino et al., 2002; Rolain et al., 2003; Just et al., 2008). Previous studies demonstrated that *Bartonella* DNA was detected in fleas (Rolain et al., 2003; Stevenson et al., 2003), ticks (Change et al., 2001; Sanogo et al., 2003), lice (Durden et al., 2004), sand files (Jacomino et al., 2002) and biting flies (Chung et al., 2004). These are suspected vectors for bartonellosis such as *B. bacilliformis*, *B. quintana* (La et al., 2005; Garré et al., 2008), *B. vinsonii* subsp. *berkhoffii* (Kordick et al., 1996) and *B. henselae* (Jonathan et al., 2007), respectively. Several wild rodent-associated *Bartonella* spp. have been linked to human diseases including *B. elizabethae*, *B. grahamii* (Boonma et al., 1997), *B. vinsonii* subsp. *arupensis* (Fenollar et al., 2005), and *B. washoensis* (Kosoy et al., 1997).

A variety of bartonella species has been identified in Thailand including *B. vinsonii* subsp. *berkhoffii* in dogs (Sukkasawat, 2007), *B. vinsonii* subsp. *arupensis* (Bai et al., 2012) and *B. thailandensis* in rats (Saisongkor et al., 2008), *B. bovis* (Boonmar et al., 2012), *B. henselae* and *B. clarridgeiae* in cats (Maruyama et al., 1997, Jitchum et al., 2009), and *B. kohlerae* (Sukkullaya et al., 2012). According to zoonotic concern about bartonellosis, there have been many reports of bartonellosis in Thai patients such as *B. henselae* (Paitoonpong et al., 2008), *B. tammiae* (Kosoy et al., 2008), and *B. visonii* var *berkoffii* (Sukkasawat et al., 2002). Vectors of most concern for the transmission of bartonellosis to human are fleas (Rolain et al., 2003; Stevenson et al., 2003), ticks (Change et al., 2001; Sanogo et al., 2003), lice (Durden et al., 2004), sand files (Jacomino et al., 2002) and biting flies (Chung et al., 2004).

Cats are carriers of *B. henselae*, as limited pathology has been associated with natural infection. It has been difficult to specify clinical signs of naturally infected cats with *B. henselae*. Based on serologic results, naturally *B. henselae*-infected cats have lymphadenitis and gingivitis, especially those co-infected with the feline immunodeficiency virus (Ueno et al., 1996). In experimental conditions, cats infected with *B. henselae*, mainly type II, have developed various clinical signs including fever, local inflammation (erythema, swelling) at the site of inoculation and localized or generalized lymphadenopathy (Yamamoto et al., 2003).

Cat scratch disease caused by *B. henselae* is presented worldwide, especially in Asian countries. Bartonellosis has been reported in Japan (Maruyama et al., 2007), Korea (Kim et al., 2009), Indonesia (Marston et al., 1999), the Philippines (Chomel et al., 2001) and Taiwan (Lee et al., 1998). In Thailand, the first report of *Bartonella* spp. was done by a serosurvey in cats (Boonma et al., 2000; Maruyama et al., 2007; Boonma et al., 2007). These findings suggested that Thailand might be an endemic area of *Bartonella* spp.

Up to the present time, there has been a prevalence of *Bartonella* spp. reported in stray and domestic cats in Bangkok (Jitchum et al., 2009; Maruyama et al., 2007; Maruyama et al., 2008; Boonma et al., 1997) but not in well-cared client-owned cats. Therefore, the purpose of the present study was to find the prevalence of *Bartonella* spp. in well-cared cats without visible flea infestation to investigate possible transmission to cats’ owners.

Materials and Methods

**Animals:** Two hundred healthy client-owned cats presented to the Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University and other private animal hospitals in Bangkok metropolitan with these criteria: 1) normal physical examination 2) any age, sex and breed 3) full vaccination record 4) no fleas 5) no history of previous illness and treatment with antibiotic within three months were included into this study. Unhealthy cats with antibiotic treatment were excluded from the study. The protocol was approved by the Chulalongkorn University Institutional Animal Care and Use Committee.

**Sample collection:** Blood samples were collected from all cats. An amount of 2.5 ml of blood was collected by a sterile technique from the saphenous vein. One ml of the blood sample was placed into 2 EDTA tubes (Appendorf, USA), 0.5 ml each. The first tube was for complete blood count (CBC) and the second tube was stored at -80°C for bacterial culture (Breitschwerdt et al., 2000). Sera prepared from the remaining blood sample (1.5 ml) were stored at -80°C for further analysis.

**Bartonella spp. culture and identification**

**Bacterial culture (Clarridge et al., 1999):** All blood sample from the second tube was used for *Bartonella* spp. identification by bacterial culture which was the gold standard method. Thawed blood samples were centrifuged and supernatant was drawn off and discarded. Subsequently, pellets were resuspended into 125 μl of isolation medium-199 (Koehler et al., 1992). The resuspended pellets were streaked onto a Brain-heart infusion agar plate supplemented with 5% sheep red blood cells and incubated in a humidified chamber at 35-37°C with 5% CO₂ condition.

The cultured plates were examined for bacterial growth and *Bartonella* spp. were identified based on Gram’s staining, morphology, growth characteristic and standard biochemical test. The bacterium was biochemically inert with oxidase, catalase, urease and nitrate reductase negative except for the production of peptidase (Breitschwerdt et al., 2000).

After bacteria were initially identified, *Bartonella* positive samples were tested with polymerase chain reaction (PCR) (Norman et al., 1995). DNA sequencing was employed for *Bartonella* spp. confirmation (Sanger et al., 1977).

**PCR for specific identification of Bartonella spp.**

**Preparing DNA for PCR:** DNA was extracted from each positive sample using Nucleospin blood kit. The sample was mixed with 100 μl of sterile water in a sterile test tube, then 20 μl of proteinase K and 200 μl of lysis buffer were added. The sample was incubated at 56°C for 10 min. Two hundred μl of absolute ethanol was added and transferred into the DNeasy Mini spin column. The DNA sequencing was employed for *Bartonella* spp. identification.
column was then centrifuged and washed twice with provided washing buffer. Subsequently, 100 μl of provided elution buffer was added into the column to elute the DNA. Finally, extract DNA samples were kept at -80°C until used for other purposes.

PCR amplification of gltA gene: PCR was used for detection of Bartonella spp. in cat blood as previously described (Norman et al., 1995) Primer of citrate synthase gene (gltA)

All PCR mixture was performed in 20 μl containing 0.2 mM each of dNTP, 0.5 pmol/μl each of forward-BhCs. 781p; 5'-GGGGACACGCTCATGGTG GGG-3', and Reverse: BhCs.1137n; 5'-AAATGCAAAAAG AACAGTAAAACA-3', 3 mM MgCl2 reaction buffer, 0.4 pmol/μl primer, 0.5 units Taq polymerase (Invitrogen, USA) and DNA template 2 μl in primary reaction. The nested reaction comprised 0.2 mM each of dNTP, 0.5 pmol/μl each of forward and reverse primers, 1.5 mM MgCl2 reaction buffer, 0.5 units Taq polymerase and DNA template 2 μl. PCR amplification was performed in a MyCycle™ Thermal Cycler (BioRad Laboratories, USA). PCR cycle conditions were optimized at 95°C for 10 min, 95°C for 30 s, 57°C for 1 min and 72°C for 2 min for 45 cycles, then at 72°C for 5 min.

After PCR processing, the PCR products were analyzed on agarose gel and identified by 1% agarose gel in 1X Tris-borate-EDTA (TBE). The PCR amplification product was visualized under an ultra-violet transluminator. The positive Bartonella samples demonstrated a 380-bp.

Sequencing of PCR amplification products: Positive DNA fragment was extracted and purified from agarose gel by Gene JET® Gel Extraction Kit (Fermentas, USA) following the manufacturer’s instructions for elimination of excess primers, nucleotides, polymerase and salts. The purified DNA products were submitted for sequencing at the 1st Base Sequence Company in Singapore.

Results

The blood samples from two hundred healthy well-cared client-owned cats were included. All cats were between 5 months to 15 years old. The selected cats had close relationship with their owners and lived mostly indoors. Results from 11 Bartonella-positive samples indicated the presence of Bartonella spp. All positive bartonellosis cats in the present study were Siamese-mixed breed (100%). Mean age of the positive cats was 5.0±3.7 years. Mean weight of the positive cats was 3.5± 0.4 kg. The positive cats were neutered male (36.3%) and neutered female (63.7%). The positive samples were identified by colony identification, Gram’s staining, morphology, growth characteristic and standard biochemical test. The bacterium was biochemically inert with oxidase, catalase, urease and nitrate reductase negative. All positive samples were further identified for Bartonella spp. by using the PCR technique with gltA gene and gene sequencing (Tables 1 and 2). Results indicated that the prevalence of bartonellosis in well-cared client-owned cats in Bangkok metropolitan area from November 2010 to November 2011 was 5.5%. Among the 11 Bartonella-positive cats, 10 (91.9%) were infected with B. henselae and 1 (9.1%) with B. clarridgeiae.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Bartonella species</th>
<th>% Similarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.03</td>
<td>B. henselae</td>
<td>99.69%</td>
</tr>
<tr>
<td>No.12</td>
<td>B. henselae</td>
<td>100%</td>
</tr>
<tr>
<td>No.27</td>
<td>B. henselae</td>
<td>100%</td>
</tr>
<tr>
<td>No.53</td>
<td>B. henselae</td>
<td>100%</td>
</tr>
<tr>
<td>No.60</td>
<td>B. clarridgeiae</td>
<td>100%</td>
</tr>
<tr>
<td>No.62</td>
<td>B. henselae</td>
<td>100%</td>
</tr>
<tr>
<td>No.76</td>
<td>B. henselae</td>
<td>99.69%</td>
</tr>
<tr>
<td>No.77</td>
<td>B. henselae</td>
<td>99.69%</td>
</tr>
<tr>
<td>No.78</td>
<td>B. henselae</td>
<td>100%</td>
</tr>
<tr>
<td>No.79</td>
<td>B. henselae</td>
<td>99.69%</td>
</tr>
<tr>
<td>No.83</td>
<td>B. henselae</td>
<td>100%</td>
</tr>
</tbody>
</table>

Discussion

There are many reports of the prevalence and possible carriers of Bartonella, particularly cats. Most reports focused on stray cats because they are mostly infested with fleas. The present study reported the prevalence of bartonellosis in healthy well-cared client-owned cats. The prevalence of the infection was different from a previous report by Jitchum and colleagues in 2009. In that study, the authors studied stray cats and found high prevalence of B. henselae and B. clarridgeiae in stray cats but low prevalence in pet cats. Both studies used the PCR technique (16S rRNA gene), but the present study included the gltA gene.

Table 1 Sequencing result of 11 positive samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>Bartonella species</th>
<th>% Similarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.03</td>
<td>B. henselae</td>
<td>99.69%</td>
</tr>
<tr>
<td>No.12</td>
<td>B. henselae</td>
<td>100%</td>
</tr>
<tr>
<td>No.27</td>
<td>B. henselae</td>
<td>100%</td>
</tr>
<tr>
<td>No.53</td>
<td>B. henselae</td>
<td>100%</td>
</tr>
<tr>
<td>No.60</td>
<td>B. clarridgeiae</td>
<td>100%</td>
</tr>
<tr>
<td>No.62</td>
<td>B. henselae</td>
<td>100%</td>
</tr>
<tr>
<td>No.76</td>
<td>B. henselae</td>
<td>99.69%</td>
</tr>
<tr>
<td>No.77</td>
<td>B. henselae</td>
<td>99.69%</td>
</tr>
<tr>
<td>No.78</td>
<td>B. henselae</td>
<td>100%</td>
</tr>
<tr>
<td>No.79</td>
<td>B. henselae</td>
<td>99.69%</td>
</tr>
<tr>
<td>No.83</td>
<td>B. henselae</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 2 Oligonucleotide citrate synthase gene (gltA) primers used in this study

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Forward-Primer</th>
<th>Reverse-Primer</th>
<th>Oligonucleotide sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>gltA gene</td>
<td>Forward-BhCS. 781p</td>
<td>5'-GGGGACACGCTCATGGTG GGG-3'</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Reverses-BhCS.1137n</td>
<td>5'-AAATGCAAAAAG AACAGT AAAACA-3'</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

There are many reports of the prevalence and possible carriers of Bartonella, particularly cats. Most reports focused on stray cats because they are mostly infested with fleas. The present study reported the prevalence of bartonellosis in healthy well-cared client-owned cats. The prevalence of the infection was different from a previous report by Jitchum and colleagues in 2009. In that study, the authors studied stray cats and found high prevalence of B. henselae and B. clarridgeiae in stray cats but low prevalence in pet cats. Both studies used the PCR technique (16S rRNA gene), but the present study included the gltA gene.
Another report of bartonellosis in four regions of Thailand showed that bartonellosis infection in domestic cats was 82.9% (63/76) with *B. henselae* and 11.8% (9/76) with *B. clarridgeiae* (Maruyama et al., 2007).

In conclusion, owners of cats should increase their awareness of the cat scratch disease, which can be transmitted from cats to humans as shown by cats in the present study. One interesting point is that all cats in the present study had no visible flea infestation. The owners reported use of heartworm and flea prevention spot-on products once a month on most of the cats (more than 80%). The difference in the results of the present study had no visible flea infestation. The owners of cats should increase their awareness of the cat scratch disease, which can be transmitted from cats to humans as shown by cats in the present study. One interesting point is that all cats in the present study had no visible flea infestation.

**Acknowledgements**

The present study was supported by the grant from the 90 years graduate scholarship, Ratchadapisek Sompot Fund, Chulalongkorn University.

**References**


บทคัดย่อ

อุบัติการณ์การติดเชื้อبارโทเนลล่าในแมวบ้านในเขตกรุงเทพมหานคร

ภควัน ศาสตรานรากุล¹ โซอิชิ มารูยามา² ฮิโดริ คาเบยา² ชินโก ซาโต² สถาพร จิตตปาละพงษ์³ เสาวลักษณ์ จิตชุ่ม³ ทวิษา จิยี่พงษ์³ ชาญณรงค์ รอดค้ำ⁴ รสนา ภู่สุนทรธรรม¹*

ทำการศึกษาสัตวแพทย์ด้านเชื้อโรคเฉพาะกลุ่มของเชื้อبارโทเนลล่าในแมวบ้านที่มีเจ้าของและได้รับการเลี้ยงดูอย่างดีจำนวน 200 ตัว ที่มารับการบริการที่โรงพยาบาลสัตว์เลี้ยงทางธรรมนูญมหาวิทยาลัย โดยเจ้าของที่เข้าร่วมโครงการต้องไม่แสดงอาการคลาสสิกและไม่มีคนดื้อตัวทำการเก็บตัวอย่างเลือดเพื่อตรวจเชื้อตั้งแต่เดือนพฤศจิกายน 2553 ถึงเดือนพฤศจิกายน 2554 ทำการตรวจเชื้อเรียบร้อยแล้ว สำนักงานสัตวแพทย์แห่งประเทศไทย และที่มารับการบริการที่โรงพยาบาลสัตว์เลี้ยงดูดังกล่าว

ทำการตรวจเชื้อبارโทเนลล่า เฮนเซลเลในตัวอย่างเลือดด้วยวิธีการเพาะเชื้อบน 5% rabbit blood agar และวิธี PCR ตัวอย่างที่ได้ผลเป็นบวกส่งตรวจ DNA Sequencing เพื่อยืนยันชนิดของเชื้อ การศึกษาพบว่าการติดเชื้อبارโทเนลล่าคิดเป็น 5.5% (11/200) ของแมวที่ทำการศึกษาทั้งหมด โดยเป็นเชื้อبارโทเนลล่า เฮนเซลเล 91% (10/11) และเชื้อobaretrococcus clarridgeiae 9% (1/11) จากการศึกษา สรุปได้ว่าเชื้อobaretrococcus เฮนเซลเลเป็นเชื้อหลักที่พบในแมวบ้าน ดังนั้น เจ้าของแมวซึ่งมีป้องกันหมัดซึ่งเป็นพิษของโรคไว้แมวเป็นประจำ นอกจากนี้การให้ความรู้กับสัตวแพทย์และเจ้าของแมวเกี่ยวกับโรคเป็นสิ่งสำคัญที่จะช่วยในการวางแผนการป้องกันการติดต่อของโรค

คำสำคัญ: กรุงเทพมหานคร บารโทเนลล่า อุบัติการณ์ แมวบ้าน
¹ภาควิชาอายุรศาสตร์ คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ประเทศไทย
²ภาควิชาอายุรศาสตร์ที่ปรึกษาสัตวแพทย์ วิทยาลัยอุตสาหกรรมสัตวแพทย์ มหาวิทยาลัยเชียงใหม่ ประเทศไทย
³ภาควิชาปรสิต คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเกษตรศาสตร์ ประเทศไทย
⁴ภาควิชาจุลชีววิทยา คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ประเทศไทย
*ผู้รับผิดชอบบทความ E-mail: drrosama@gmail.com