Original Article

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

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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

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Introduction

Bartonella spp. are considered as vector-borne parasites (Jacomo 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 (Jacomo 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[5] 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 bactonella species has been identified in Thailand including *B. vinsonii* subv *berkoffii* in dogs (Suksawat, 2007), *B. vinsonii* subv *arupensis* (Bai et al., 2012) and *B. thailandensis* in rats (Saisongkorh 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. tamiae* (Kosoy et al., 2008), and *B. visonii ver berkoffii* (Suksawat 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 (Jacomo 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*[6]. 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., 1997). In 2000, the first incidence of bartonellosis in Thailand was confirmed by Maruyama and colleagues. In 2008, the first human case was published and it was the first paper of *B. henselae* isolation in Thailand (Paitoonpong et al., 2008). Three cases of endocarditis caused by *B. tamiae* were reported in Khon Kaen, a province in the north-eastern part of Thailand (Kosoy et al., 2008). Those findings suggested that Thailand might be an endemic area of *Bartonella* spp.

Up to the present time, there has been prevalence of *Bartonella* spp. reported in stray and domestic cats in Bangkok (Jitchum et al., 2009; Maruyama et al., 2007; Maruyama et al., 2000; 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^{o}C for bacterial culture (Breitschwerdt et al., 2000). Sera prepared from the remaining blood sample (1.5 ml) were stored at -80^{o}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^{o}C with 5% CO_2 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^{o}C for 10 min. Two hundred µl of absolute ethanol was added and transferred into the DNeasy Mini spin column. The

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 (*glt*A)

All PCR mixture was performed in 20 µl containing 0.2 mM each of dNTP, 0.5 pmoles/µl each of Forward-BhCS. 781p; 5'-GGGGACCAGCTCATGGT GG-3', and Reverse: BhCS.1137n; 5'-AATGCAAAAAG AACAGTAAACA-3', 3 mM $MgCl_2$ reaction buffer, 0.4 pmoles/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 pmoles/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 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 1 Sequencing result of 11 positive samples

Samples	*Bartonella* species	% Similarity
No.3	*B.henselae*	99.69%
No.12	*B.henselae*	100%
No.27	*B. henselae*	100%
No.53	*B. henselae*	100%
No.60	*B. clarridgeiae*	100%
No.62	*B. henselae*	100%
No.76	*B. henselae*	99.69%
No.77	*B. henselae*	99.69%
No.78	*B. henselae*	100%
No.79	*B. henselae*	99.69%
No.83	*B. henselae*	100%

Table 2 Oligonucleotide citrate synthase gene (*glt*A) primers used in this study

Target gene	Primers	Oligonucleotide sequences
gltA gene	Forward-BhCS. 781p	5'-GGGGACCAGCTCATGGTGG-3
	Reveres-BhCS.1137n	5'-AATGCAAAAAGAACAGTAAACA-3'

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. clarridgieae* 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. clarridgieae* (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 *Bartonella* prevalence among studies might be caused by the difference in vectors such as wood louse hunter spider (Mascarelli et al., 2012) or chigger mite (Kabeya et al., 2010; Kosoy et al., 2010), which should be further investigated in future studies of bartonellosis.

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บทคัดย่อ

อุบัติการณ์การติดเชื้อบาร์โทเนลลาในแมวบ้านในเขตกรุงเทพมหานคร

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ทำการศึกษาลักษณะทางด้านชีวโมเลกุลของเชื้อบาร์โทเนลลาในแมวบ้านที่มีเจ้าของและได้รับการเลี้ยงดูอย่างดีจำนวน 200 ตัว ที่มารับการบริการที่โรงพยาบาลสัตว์เล็กจุฬาลงกรณ์มหาวิทยาลัย โดยแมวที่เข้าร่วมโครงการต้องไม่แสดงอาการผิดปกติและไม่มีหมัดบนตัว ทำการเก็บตัวอย่างเลือดเพื่อตรวจหาเชื้อตั้งแต่เดือนพฤศจิกายน 2553 ถึงเดือนพฤศจิกายน 2554 ทำการตรวจหาเชื้อบาร์โทเนลลา เฮนเซลเล ในตัวอย่างเลือดด้วยวิธีการเพาะเชื้อบน 5% rabbit blood agar และวิธี PCR ตัวอย่างที่ได้ผลเป็นบวกถูกส่งตรวจ DNA Sequencing เพื่อยืนยันชนิดของเชื้อ การศึกษาพบว่าการติดเชื้อบาร์โทเนลลาคิดเป็น 5.5% (11/200) ของแมวที่ทำการศึกษาทั้งหมด โดยเป็นเชื้อบาร์โทเนลลา เฮนเซลเล 91% (10/11) และเชื้อบาร์โทเนลลา *clarridgeiae* 9% (1/11) จากการศึกษา สรุปได้ว่าเชื้อบาร์โทเนลลา เฮนเซลเลเป็นเชื้อหลักที่พบในแมวบ้าน ดังนั้น เจ้าของแมวจึงควรป้องกันหมัดซึ่งเป็นพาหะของโรคในแมวเป็นประจำ นอกจากนี้การให้ความรู้กับสัตวแพทย์และเจ้าของแมวเกี่ยวกับโรคเป็นสิ่งสำคัญที่จะช่วยในการวางแผนการป้องกันการติดต่อของโรค

คำสำคัญ: กรุงเทพมหานคร บาร์โทเนลลา อุบัติการณ์ แมวบ้าน

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