Original Article

Prevalence of *Bartonella henselae, Bartonella clarridgeiae,* and *Bartonella vinsonii* subsp. *berkhoffii* in pet cats from four provincial communities in Thailand

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Abstract

Bartonella species are Gram-negative alpha proteobacteria and intracellular parasites of erythrocytes, endothelial and dendritic cells. Many are zoonotic pathogens of various mammalian reservoir hosts and are transmitted by blood-feeding arthropods. Recently, there have been several reports indicating that cats are important reservoir hosts for *Bartonella* pathogens, including those that cause cat scratch disease affecting humans in many countries including in Thailand. To assess more completely the role of cats as reservoir hosts for *Bartonella* pathogens in Thailand, 139 blood samples were collected from companion cats from a single community in each of four provinces (Khon Kaen, Kalasin, Nakhon Si Thammarat and Nakhon Ratchasima) from January 2014 to January 2015. The blood samples were assessed for the presence and identification of *Bartonella* spp. by molecular methods. In addition, various potential risk factors were assessed. Overall, 13 (9.4%) pet cats were found to be infected with *Bartonella* species including *Bartonella henselae*, *Bartonella clarridgeiae* and *Bartonella vinsonii* subsp. *berkhoffii*. This is the first evidence of the discovery of *Bartonella vinsonii* subsp. *berkhoffii* DNA in pet cats in Asia. Further studies of *Bartonella* spp. prevalence in other locations in Thailand should be investigated.

Keywords: *Bartonella* spp., blood, cats, PCR assay

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Introduction

Bartonellosis is a contagious disease associated with humans and animals, and caused by *Bartonella* spp. The members of *Bartonella* genus are Gram negative bacteria infecting erythrocytes, endothelial and dendritic cells of mammalian hosts. *Bartonella* spp. are emerging zoonotic pathogens in humans and animals (Guptill, 2003). Recently, there have been many reports on *Bartonella* infection in a variety of mammalian hosts worldwide. More than 47 mammalian species and subspecies have been described as reservoir hosts (Boulouis et al., 2005), including rats (Heller et al., 1998), mice (Welch et al., 1999), cats (Koehler et al., 1994), dogs (Breitchwerdt et al., 1995), voles (Birtles et al., 1995) and coyotes (Chang et al., 2000). In humans, *Bartonella* spp. cause various diseases, e.g. Carrion's disease, Trench fever, cat scratch disease; and clinical abnormalities, e.g. bacillary angiomatosis, hepatic peliosis, endocarditis, neuroretinitis and chronic bacterial infection (Jacomo et al., 2002). In addition, important vectors of bartonelliosis are blood sucking arthropods such as the sand fly (*Lutzomyia verrucarum*) for *Bartonella bacilliformis* (Carrion's disease) (Ihler, 1996), the body louse (*Pediculus humanus*) for *Bartonella quintana* (trench fever and bacillary angiomatosis) (Roux and Raoult, 1999), the rat flea (*Xenopsylla cheopis*) for *Bartonella elizabethae* (Breitschwardt and Kordick, 2000), and the cat flea (*Ctenophaledes felis*) for *B. henselae* (Chomel et al., 1996).

Cats are important reservoir hosts for *Bartonella* species such as *B. clarridgeiae* (Kordick et al., 1997), *B. henselae* (Chomel et al., 2000), *B. koehlerae* (Droz et al., 1999), and *B. bovis* (Bermond et al., 2002). Cat fleas (*C. felis*) play a major role as vectors among cats (Boulouis et al., 2005). There are three species of *Bartonella* which may cause diseases in humans including *B. clarridgeiae*, *B. henselae* and *B. koehlerae* (Chomel et al., 1995). Cat fleas are also reservoirs of *B. quintana* (Bergmans et al., 1997). Pet cats live in close association with humans and although owners normally keep their cats in the house and often in their bedrooms, pet cats usually spend considerable amount of time outdoors, where they can be infested by ectoparasites that can transmit *Bartonella* spp. to humans via bites or scratches.

In Thailand, the prevalence of *Bartonella* infection in stray and pet cats averaged 27.6% (76/275) in nine geographical regions, including Khon Kaen (50.1%), Roi Et (36.8%), Ratchaburi (34.8%), Chiang Mai (23.3%), Kanchanaburi (21.4%), Nakhon Ratchasima (20%), Songkhla (12.8%), Bangkok (5.6%) and Ubon Ratchathani (11.8%) (Maruyama et al., 2001). *Bartonella* spp. infection prevalence was also reported at 5.5% (9/163) and 1.2% (2/163) in Thai people by serosurvey and occasionally by culture and PCR (Maruyama et al., 2000). Awareness of the risk of *Bartonella* infection in Thailand needs to be increased since it is emerging everywhere in Southeast Asia as well as the rest of the world (Bai et al., 2010).

The aim of this study was to investigate the prevalence of *Bartonella* spp. in pet cats from Thailand. Blood samples were collected from cats in a single community in each of 4 provinces: Khon Kaen, Kalasin, Nakhon Si Thammarat and Nakhon Ratchasima. The *ssrA* gene based quantitative real-time PCR (qPCR) assay was chosen to detect and identify *Bartonella* species DNA (Diaz et al., 2012). The *gltA* and 16S-23S rRNA intergenic spacer genes (ITS) were used to further identify *Bartonella* spp. as previously described (Kosoy et al., 2010). Risk factors associated with *Bartonella* infection in pet cats were investigated. The goal of this study was to determine the prevalence, distribution and risk factors associated with *Bartonella* infected companion cats in Thailand to assist in the prevention and control of *Bartonella* infection in animals and humans.

Materials and Methods

Study areas: During August 2015 and March 2016, 139 pet cat blood samples were collected from one community in each of four provinces: Khon Kaen, Kalasin, Nakhon Si Thammarat and Nakhon Ratchasima.

Blood collection: One to three milliliters of blood were collected from a jugular vein from each cat by sterile technique into ethylene diamine tetra-acetic acid (EDTA) tubes and stored at -20^{0}C until used for DNA extraction and PCR analysis. The pet cats were thoroughly examined and records of age (less than 3 years and more than 3 years, based on physical examination and chief complaint from veterinarians), sex, breed (pure and mixed history taking by veterinarians), health status (healthy and unhealthy classified by medical diagnosis from veterinarians), living area, living condition, geographical area and ectoparasite prevention control were made using a standardize questionnaire. This project's protocols were approved by the Animal Ethics Committee of Khon Kaen University (protocol number: AEKKU12/2558).

Molecular detection of bartonellae: DNA was extracted from 200 µl of EDTA blood samples using QIAamp DNA blood and tissue kit (QIAGEN, Germany). All DNA samples were stored at -80^{0}C until used. Two µl of the extracted DNA was used for PCR amplification. Primers for the *Bartonella* genus- and species-specific PCR assays were designed based on the study of Diaz et al. (2012). To confirm the presence of *Bartonella* DNA in the blood sample and to identify the responsible *Bartonella* species a 767 bp fragment of the citrate synthase gene (*gltA*) was used following a previous report (Birtles and Raoult, 1996). In addition, the ITS gene (280 bp) was used for species identification following a previous report (Billeter et al., 2008). Each set of experiments included negative and positive controls. Nuclease-free water replaced DNA templates as negative controls. Template DNA from reference species of *Bartonella* spp. were used as positive controls. A specific band of each DNA sample was cut for species confirmation using DNA sequencing (AIT BIotech, Singapore). The DNA sequences were analyzed for species by using BLAST programs (http://blast.ncbi.nlm.nih.gov/Blast.cgi). GenBank accession numbers for these sequences were KX001761-KX001769.

Statistical analysis: Data were analyzed by SPSS version 17 software for Windows (http://software.kku.ac.th). Pearson's chi-square test and the Fisher exact test were used to analyze relationships between *Bartonella* infection and risk factors obtained from questionnaires filled out by client owners. Significance was defined as $p < 0.05$.

Results

Prevalence of Bartonella spp. based on molecular detection: The prevalence of *Bartonella* infection among the pet cats was 9.4% (13/139). The infection prevalence was 5.7% (8/139), 2.2% (3/139) and 1.4% (2/139) for *B. henselae*, *B. clarridgeiae* and *B. vinsonii* subsp. *berkhoffii*, respectively. The percentages of PCR positive cats for *Bartonella* spp. in different geographic regions were 26.67% in Khon Kaen, 10.2% in Nakhon Si Thammarat, 6.67% in Nakhon Ratchasima and 4.44% in Kalasin (Fig. 1, Table 1).

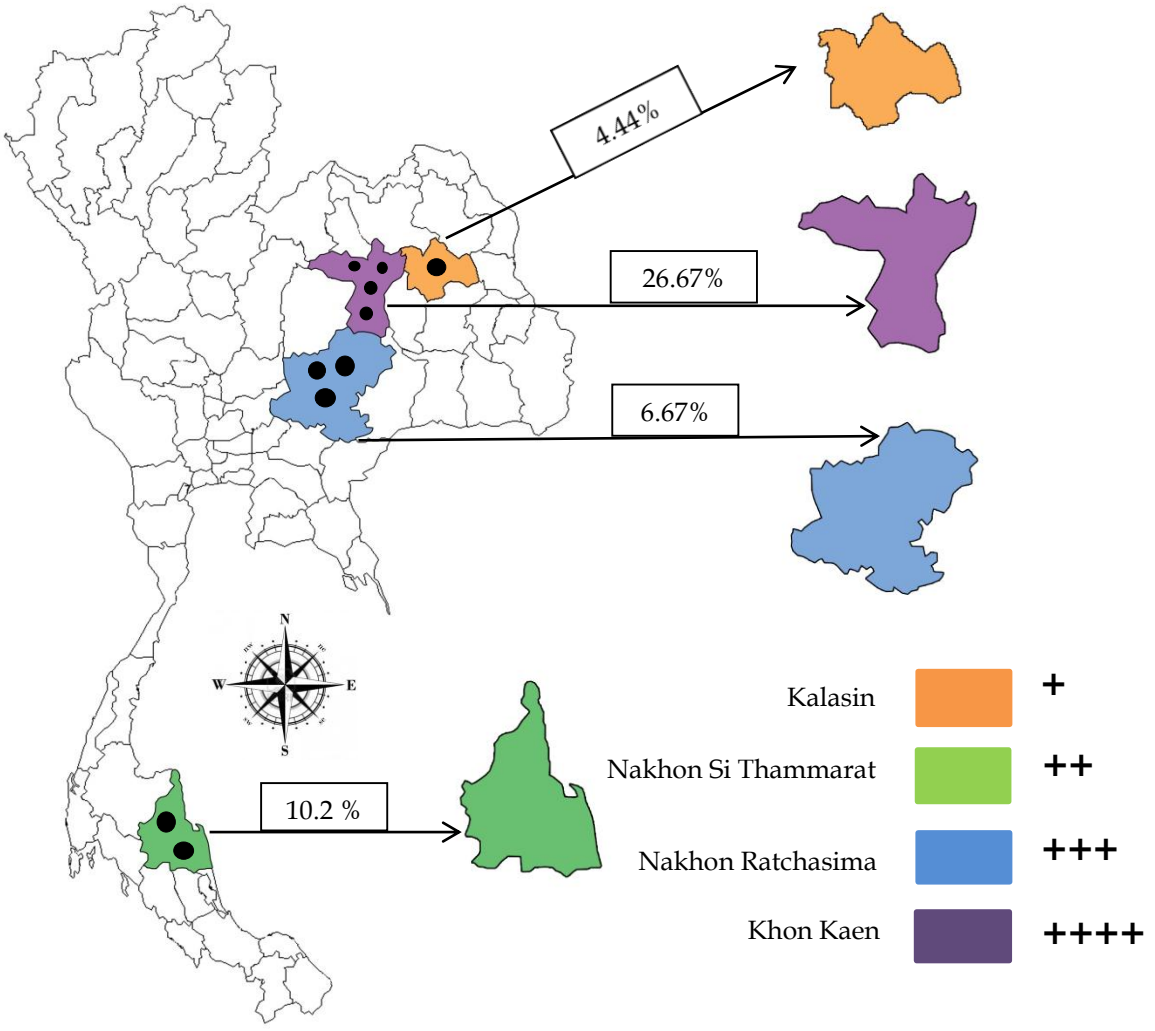

Figure 1 Presence of *Bartonella* infection in pet cats from four provinces in Thailand

Risk factors associated with Bartonella spp.: The risk factors associated with *Bartonella* spp. are presented in Table 2. For gender, the prevalence of *Bartonella* infection between the males and females was 13.5% (7/52) and 6.9% (6/87), respectively, and was not significantly different (chi-square = 4.86, df = 1, p = 0.235). The prevalence of infection with *Bartonella* between the two different age groups of cats, 0-3 years and more than 3 years, was 8.2% (9/110) and 13.8% (4/29), respectively, and was not significantly different (chi-square = 2.71, df = 1, p = 0.471). The prevalence of *Bartonella* infection based on health condition, healthy or unhealthy, was 7.6% (8/105) and 14.7% (5/34), respectively, and was not significantly different (chi-square = 3.18, df = 1, p = 0.306). For the relationship between living area and *Bartonella* infection, it was found that the prevalence of *Bartonella* infection between cats living indoors and outdoors was 12.5% (3/24) and 8.7% (10/115), respectively, and was not significantly different (chi-square = 2.24, df = 1, p = 0.698). For the living condition, the prevalence of infection with *Bartonella* spp. between cats living singly and with other animals was 2.5% (3/121) and 55.6% (10/18), respectively, which was significantly different

(chi-square = 1.68, df = 1, p = 0.000). The use of ectoparasite product was also related to the prevalence of *bartonella* infection; the prevalence was 2.4% (3/127) in the groups that used and 83.3% (10/12) in the groups that never used (chi-square = 1.12, df = 1, p = 0.000). Moreover, the presence or absence of ectoparasite infestation at 3.7% (3/45) was not a risk factor for cats infected with *Bartonella* 10.6% (10/94) (chi-square = 4.21, df = 1, p = 0.548).

Table 1 List of matching proteins obtained by LC-MS/MS analysis from gel spot of peak fractions F1, F2, F3, F4, F5, F6 and F7

Locations	Total Number of cats(n)	Number of cats		Number of PCR positive (%)	*Bartonella* species	Numbers of PCR positve (%)	
		M	F			M	F
Nakhon Ratchasima	49	17	32	5 (10.2%)	*B. vinsonii subsp. berkhoffii*	1	1
					Bartonella spp.	2	1
Kalasin	45	15	30	2 (4.44%)	*B. clarridgeiae*	2	0
Khon Kaen	15	8	7	4 (26.67%)	*B. clarridgeiae*	1	0
					B. henselae	0	2
					Bartonella spp.	0	1
Nakhon Si Thammarat	30	12	18	2 (6.67%)	*Bartonella* spp.	1	1

Table 2 Risk factors associated with *Bartonella* infection among pet cats in four communities in Thailand

Parameters	Number of pet cats (n)	Number of *Bartonella* infected cats (%)	p-value
Sex			
female	87	6 (6.9)	0.235
male	52	7 (13.5)	
Age (years)			
0-3	110	9 (8.2)	0.471
more than 3	29	4 (13.8)	
Health status			
healthy	105	8 (7.6)	0.306
unhealthy	34	5 (14.7)	
Breed			
pured	20	4 (20)	0.094
mixed	119	9 (7.6)	
Living area			
indoor	24	3 (12.5)	0.698
outdoor	115	10 (8.7)	
Living condition			
single	121	3 (2.5)	0.00*
multi	18	10 (55.6)	
Geographical area			
NE	109	11 (10)	0.734
SOUTH	30	2 (6.7)	
Drug			
yes	127	3 (2.4)	0.00*
no	12	10 (83.3)	
Total	139	13 (9.4)	-

Discussion

The prevalence of *Bartonella* infection in cats varies among Asian countries. Previous reports on *Bartonella* bacteraemic prevalence based on conventional PCR in pet cats included 0% (0/24) in metropolitan areas of Thailand, 7.2% (50/690) in Japan, 19.1% (25/131) in Taiwan, 27.6% (76/275) from 9 rural sites in Thailand, 61% in the Philippines and 64.3% in Indonesia (Maruyama et al., 2001; Chang et al., 2006). In this study, 9.4% (13/139) of the pet cats from four communities in different provinces of Thailand were infected with *Bartonella* spp. Together, these studies indicate that *Bartonella* is widely distributed in domestic cats throughout Asia with higher prevalence in southern Asian countries than in northern temperate countries (Maruyama et al., 2001; Inoue et al., 2009). *Bartonella* seroprevalence in warm and humid environments is higher than that in cold dry environments (Inoue et al., 2009).

The overall infection rate in this study was lower than that previously reported in domestic cats in Thailand (Maruyama et al., 2001). This difference may be due to several reasons including; (1) the sampling sites (Maruyama et al., 2001), (2) the lower numbers of bacteria in blood, resulting in increased PCR negative results, (3) the blood samples not being collected during the maximum period of *Bartonella* septicemia (Rodkhum et al., 2010) and (4) the blood samples from our study collected mostly from pet cats that visited veterinary hospitals regularly, and raised by owners who often used flea preventive products. As cat fleas are the most important vector of this pathogen, and are often found on stray cats in Bangkok areas (Chomel et al., 1999), the lower prevalence in our study compared to the study by Maruyama et al. (2001) is likely due to the differences in cat population studied (feral urban vs household pets).

Our findings of *B. henselae* and *B. clarridgeiae* infection rates are consistent with previous studies in Thailand and other countries in Asia: the Philippines (Chomel et al., 1999), Indonesia (Marston et al., 1999), Singapore (Narisudeen and Thong, 1999), and Japan (Maruyama et al., 2001). However, to our knowledge, this is the first DNA evidence of *B. vinsonii* subsp. *berkhoffii* in pet cats in Thailand and Asia. Recurrent osteomyelitis in domestic cats in USA caused by *B. vinsonii* subsp. *berkhoffii* infections was reported by Breitschwerdt et al. (2009). *B. vinsonii* subsp. *berkhoffii* normally infects canine reservoir hosts and humans, causing persistent intravascular infection and endocarditis (Breitschwerdt et al., 2010). Before 2009, dogs were the only known reservoir hosts of *B. vinsonii* subsp. *berkhoffii* (Breitschwerdt et al., 1995; Chomel et al., 2006; Maggi et al., 2006) and the disease was transmitted via ticks. However, the transmission mode of this *Bartonella* species to cats has not been determined (Breitschwerdt et al., 2009). In this study, the cats infected with *B. vinsonii* subsp. *berkhoffii* were raised with other animals including dogs and therefore dog ticks or dog bits may be the cause. Therefore, pet cats may be an accidental host for *B. vinsonii* subsp. *berkhoffii* infection.

Similar to a previous study in Thailand (Maruyama et al., 2001), there was no significant difference ($p = 0.235$) in *Bartonella* infection between male and female cats. However, an association between gender and rates of infection was reported in the study of Zangmill (1993). As the presence of infection in male cats was higher than in female cats in this study, the results suggest that male cats have more opportunities to be scratched or bitten by other infected cats, while protecting their territories (Inoue et al., 2009).

In this study, *Bartonella* DNA was not detected in most cats receiving flea control products. A significant connection between the prevalence of *Bartonella* infection and the use of ectoparasite control product was evident, similar to a previous report indicating that the owners who used flea control products were able to prevent ectoparasite infection in their cats (Bradbure and Lappin, 2010; Assarasakorn et al., 2012).

In conclusion, the zoonotic *Bartonella* spp. was discovered in this study and has been increasingly discovered worldwide. Pet cats were discovered to harbor *Bartonella* spp. including *B. henselae*, *B. clarridgeiae* and *B. vinsonii* subsp. *berkhoffii*, which was formerly reported only in dogs. The three zoonotic *Bartonella* spp. may play a significant role in causing disease in Thai people. Therefore, good health care management, routine *Bartonella* detection and urgent implementation of appropriate flea control for cats and their ectoparasites are necessary. Additionally, *Bartonella* surveillance may be the approach necessary to prevent the transmission of infection from animals to humans. As Thailand has not been a *Bartonella*-endemic area in the past, further investigation is required to determine the incidence of *Bartonella* spp. infection in other animals infested with ectoparasites.

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บทคัดย่อ

การตรวจหาเชื้อบาร์โทเนลลาในแมวไทยในระดับโมเลกุล

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บาร์โทเนลลา (*Bartonella* spp.) เป็นอัลฟาโปรตีโอแบคทีเรียแกรมลบที่อาศัยอยู่ในเม็ดเลือดแดง เซลล์หลอดเลือด และเซลล์เดนไดรติก บาร์โทเนลลาหลายชนิดก่อโรคสัตว์สู่คนอยู่ในตัวถูกเบียนเก็บเชื้อหลายชนิดแพร่เชื้อโดยแมลงดูดเลือดเป็นพาหะ ไม่นานนี้เองมีรายงานแสดงให้เห็นว่าแมวเป็นตัวถูกเบียนเก็บเชื้อของโรคแมวข่วนในมนุษย์ในหลายประเทศรวมทั้งประเทศไทย เพื่อประเมินบทบาทของแมวในบทบาทการเป็นตัวถูกเบียนเก็บเชื้อของโรคอเป็นการตรวจหาเชื้อบาร์โทเนลลาในเลือดของแมวเลี้ยง 139 ตัวอย่างจากแหล่งชุมชนในสี่จังหวัด คือ ขอนแก่น กาฬสินธุ์ นครศรีธรรมราช และนครราชสีมาในช่วงเดือนมกราคม 2557 ถึงมกราคม 2558 และวิเคราะห์ความเสี่ยงของการพบเชื้อนี้ ในการตรวจหาได้ใช้จีน small stable RNA (*ssrA*) citrate sythase (*gltA*) และ the 16S- 23S rRNA intergenic spacer (ITS) เป็นจีนเป้าหมาย พบว่าร้อยละ 9.4 ของแมวเลี้ยงกลุ่มที่ศึกษามีดีเอ็นเอของเชื้อบาร์โทเนลลาสามชนิดคือ *Bartonella henselae*, *Bartonella clarridgeiae* และ *Bartonella vinsonii* subsp. *berkhoffii* (accession numbers KX001761-KX001769) การศึกษาครั้งนี้เป็นรายงานแรกที่พบดีเอ็นเอของ *Bartonella vinsonii* subsp. *berkhoffii* ในแมวเลี้ยงในทวีปเอเชีย และในอนาคตควรมีการศึกษาการติดเชื้อนี้ในแหล่งอื่นของประเทศไทย

คำสำคัญ: บาร์โทเนลลา เลือดแมว ปฏิกิริยาลูกโซ่โพลิเมอร์เรส

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