

## Prevalence of *Bartonella* infection in cats and dogs in a metropolitan area, Thailand

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### SUMMARY

The prevalence of *Bartonella* infection was studied in 312 cats and 350 dogs in the Bangkok metropolitan areas, Thailand, between June 2001 and February 2003. *Bartonella* was isolated from 47 (16·3%) of 288 stray cats, but from none of the 24 pet cats studied. Of the 47 *Bartonella*-positive cats, 45 animals were infected with only *B. henselae*, one was infected with only *B. clarridgeiae*, and one with both *B. henselae* and *B. clarridgeiae*. 16S rRNA typing showed that 40 cats were infected with *B. henselae* type I, four with *B. henselae* type II, and one with both *B. henselae* types I and II. These results indicated that *B. henselae*, especially type I, was prevalent in stray cats that constituted a large *Bartonella* reservoir in Bangkok. *B. clarridgeiae* was isolated for the first time in Asia from one of 350 dogs.

**Key words:** *Bartonella*, cat, dog, prevalence, Thailand.

### INTRODUCTION

*Bartonella henselae* is the causative agent of cat-scratch disease (CSD) [1, 2] and causes bacillary angiomatosis and bacillary peliosis hepatis in immunocompromised patients [3–5]. *B. clarridgeiae* has also been implicated as a possible aetiological agent of CSD in both immunocompromised and immunocompetent subjects [6, 7]. *B. henselae* can be transmitted to humans by a scratch or bite from an infected cat, while cat fleas (*Ctenocephalides felis*) and ticks

(*Ixodes ricinus*) also play a role in transmission of the bacteria in cats [8–11].

Domestic and/or stray cats are reported to be a major reservoir of *B. henselae* and *B. clarridgeiae* in several countries [12, 13]. Recently, *B. koehlerae* was isolated from naturally infected cats and from a human patient with endocarditis [14, 15]. Therefore, cats serve as a significant reservoir for several zoonotic *Bartonella* spp. It has been reported that *B. henselae* is highly prevalent in domestic and/or stray cats in Asian countries, e.g. the Philippines [16], Indonesia [17], Thailand [18], Taiwan [19], and Japan [20]. Several studies have shown that *B. henselae* can be classified into two types by 16S rRNA typing PCR: type I is predominant in cats in Asian countries [16, 18–20] while type II is predominantly isolated from cats in European countries [21–25].

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*B. vinsonii* subsp. *berkhoffii* causes endocarditis, cardiac arrhythmias, and myocarditis in dogs, which are a reservoir of this organism, and has been isolated from a human patient with endocarditis [26–28]. Furthermore, recent studies have suggested that other *Bartonella* spp. are able to infect dogs [29]. In particular, isolation of *B. clarridgeiae* has been reported from a dog with aortic valve endocarditis [30], and CSD osteomyelitis and lymphadenopathy can be caused by the scratch of *B. henselae*-infected dogs [31, 32]. In addition, *B. washoensis* has been isolated from a dog with endocarditis and a human patient with fever and myocarditis [33, 34]. These data suggest that dogs, like cats, can be infected by several zoonotic *Bartonella* spp. and may serve as a source of infection of the bacteria.

The prevalence of *Bartonella* spp. in cats and dogs has not been thoroughly investigated in the Bangkok metropolitan areas, Thailand. Therefore, we investigated the prevalence of *Bartonella* infection in pet and stray cats and dogs in order to identify the predominant species and 16S rRNA type of *Bartonella* in those animals in the Bangkok metropolitan areas.

## MATERIALS AND METHODS

### Animal blood sampling

From June 2001 to February 2003, blood samples from 312 cats (288 strays, 24 pets) and 350 dogs (296 strays, 54 pets) were collected in the Bangkok metropolitan areas for this study. Stray animals were captured in Buddhist monasteries by monastery caretakers and our staff after receiving permission from the Buddhist monks. The samples of pet animals were collected at the Veterinary Teaching Hospital of Kasetsart University. Before sample collection, the general condition of animals was examined thoroughly, and the sex was noted. Stray cats were restrained by administration of an intramuscular injection of ketamine (10 mg/kg) and xylazine (1–2 mg/kg). Blood samples of the animals were aseptically collected from the jugular vein of cats and saphenous vein of dogs. Stray animals were immediately released after blood collection.

Blood samples (2 ml) from each animal were immediately placed into sterile EDTA tubes (Venoject II, Terumo, Japan) and sent to the Laboratory of Veterinary Public Health, Nihon University, under frozen conditions with dry ice. The samples were kept at –80 °C until examined.

### Isolation and identification of *Bartonella* spp. from blood samples of cats and dogs

The frozen blood samples were thawed at room temperature and centrifuged at 3800 rpm for 70 min. After centrifugation, the supernatant was removed, 120 µl supplemented medium 199 (Gibco, USA) was added to the sediment, and the contents of the tube were mixed thoroughly [20]. A 100 µl sample of the mixture was plated on two heart infusion agar plates (Difco, USA) containing 5% defibrinated rabbit blood. The plates were incubated at 35 °C under 5% CO<sub>2</sub> for 4 weeks. The plates were checked for colony formation and fungal contamination weekly during the incubation period. After the 4 weeks incubation, 3–5 colonies with genus *Bartonella* morphology (small, round, grey, and rough colonies) were selected from each plate and subcultured using the same incubation conditions.

Genomic DNA was extracted from the isolates using the Instagene Matrix (Bio-Rad, USA). Species of the isolate was identified directly by PCR analysis of the 16S–23S rRNA inter-gene spacer region (ITS) [35]. The PCR of ITS was performed with an iCycler (Bio-Rad) using a 20 µl mixture containing 20 ng extracted DNA, 200 µM each of dATP, dGTP, dCTP and dTTP, 1.5 mM MgCl<sub>2</sub>, 0.5 U *Taq* DNA polymerase (Promega, USA), and 1 pmol of each primer. The primer pair and the PCR conditions were followed as previously described [35]. DNA from *B. henselae* ATCC 49882, *B. clarridgeiae* ATCC 51734, and *B. vinsonii* subsp. *berkhoffii* ATCC 51672 were used as positive PCR controls. Following PCR analysis, the amplicons were analysed by electrophoresis on 3% agarose gels. The samples were identified as *B. henselae*, *B. clarridgeiae*, or *B. vinsonii* subsp. *berkhoffii* if the amplified ITS DNA fragment had a size of 172, 154, or 260 bp, respectively [35].

### 16S rRNA typing of *B. henselae*

*B. henselae* 16S rRNA typing was performed as previously reported [22] with a minor modification. Briefly, the primer pair of 16SF (5'-AGAGTTT-GATCCTGGCTTCAG-3') and BH1 (5'-CCGAT-AAATCTTTCTCCCTAA-3') or BH2 (5'-CCGATAAATCTTTCTCCAAAT-3') were used for the identification of *B. henselae* types I or II, respectively. The PCR was performed with an initial denaturation at 95 °C for 3 min; 30 cycles of denaturation at 95 °C for 20 s, annealing at 56 °C for 30 s, and extension at 73 °C for 1 min; and final extension at 73 °C for

Table 1. Prevalence of *Bartonella* infection in cats and dogs in the Bangkok metropolitan areas, Thailand

Animal	No. of culture-positive animals/no. of animals tested (%)			
	Male	Female	Unknown	Total
Stray cats	25/114* (21.9)	21/170* (12.4)	1/4 (25.0)	47/288 (16.3)
Pet cats	0/2	0/6	0/16	0/24 (6.3)
Total	25/116 (21.6)	21/176 (11.9)	1/20 (5.0)	47/312 (15.1)
Stray dogs	1/120 (0.8)	0/175	0/1	1/296 (0.3)
Pet dogs	0/24	0/19	0/11	0/54 (0.0)
Total	1/144 (0.7)	0/194	0/12	1/350 (0.3)

\*  $P < 0.05$ .

Table 2. Distribution of *Bartonella* spp. or *Bartonella henselae* types in cats and dogs in the Bangkok metropolitan areas, Thailand

Animal	<i>B. henselae</i>			<i>B. clarridgeiae</i>	<i>B. henselae</i> type I and <i>B. clarridgeiae</i>	Total
	Type I	Type II	Types I and II			
Stray cats	40*	4	1	1	1	47
Stray dogs	0	0	0	1	0	1
Total	40	4	1	2	1	48

\* Number of animals from which the *B. henselae* types were isolated.

5 min. Following PCR, the amplicons were analysed by electrophoresis on 3% agarose gels.

#### DNA sequencing analysis

When an isolate was identified as *B. clarridgeiae* by species-specific PCR targeting for ITS region, the PCR amplicons were purified using a commercial kit (Quantum Prep Freeze 'N Squeeze DNA Gel Extraction Spin Columns; Bio-Rad), and subcloned using the pGEM-T Vector System (Promega) and *E. coli* strain DH5 $\alpha$  (Nippon gene, Japan). Sequence analysis of subcloned DNA fragments was performed by the dye terminator method with T7 and SP6 primers, using an Applied Biosystems Model 310 Genetic Analyzer (Applied Biosystems, USA). The data were assembled and searched for sequence similarity with the database using GENETYX-win software, version 6 (Genetix Corp., Japan.) and the BLAST service in GenBank/EMBL/DDBJ.

#### Statistical analysis

The results were analysed by  $2 \times 2$  tables and  $\chi^2$  test was used to determine statistical significance. A  $P$  value  $< 0.05$  was considered to be significant.

## RESULTS

*Bartonella* spp. were isolated from 47 (15.1%) of 312 cats examined (Table 1). The prevalence in stray cats was 16.3% (47/288), compared to no *Bartonella* being isolated from pet cats (0/24). Prevalence in males (21.6%, 25/116) was significantly higher than in females (11.9%, 21/176) ( $P < 0.05$ ).

Of *Bartonella* bacteraemic cats, 45 (95.7%) of 47 cats were infected with only *B. henselae*. Of these 45 cats, 40 (88.9%) were infected only with *B. henselae* type I and four (8.9%) were infected only with *B. henselae* type II. Only one cat (2.2%) was infected with both types. Of the remaining two cats, one was only infected with *B. clarridgeiae* and the other was infected by *B. henselae* type I and *B. clarridgeiae* (Table 2).

In this study, one (0.3%) out of 296 stray dogs was culture-positive for *Bartonella*, although the level of bacteraemia was low at 82 c.f.u./ml. This dog had no obvious clinical symptoms at sampling. No *Bartonella* was detected in the 54 pet dogs studied (Table 1). Species-specific PCR identified the dog isolates as *B. clarridgeiae* (Table 2). The isolates were confirmed to be *B. clarridgeiae* by DNA sequencing with 100% similarity of the 114 bp ITS region.

## DISCUSSION

There have been several reports of *Bartonella* infection in cats in Asian countries, with the bacteraemia prevalence varying by country: 64.3% (9/14) in Indonesia [17], 61.3% (19/31) in the Philippines [16], 27.6% (76/275) in Thailand [18], 19.1% (25/131) in Taiwan [19], and 7.2% (50/690) in Japan [20]. In the present study, the *Bartonella* bacteraemia prevalence in cats was found to be 15.1% (47/312) in the Bangkok metropolitan areas. Collectively, these data indicate that *Bartonella* is widely distributed throughout Asia, with a higher prevalence especially in southern Asian countries than in northern temperate countries. In agreement with this observation, it was suggested that cats in a warm humid environment had a higher seroprevalence of *B. henselae* than those in a cold dry environment [36].

Of the 47 *Bartonella*-positive stray cats, 45 (95.7%) were only infected with *B. henselae*, one was only infected with *B. clarridgeiae*, and the other was infected with both *B. henselae* and *B. clarridgeiae*. These data suggest that *B. henselae* is the predominant *Bartonella* spp. in cats in Thailand. Of the 45 stray cats with only *B. henselae*, 16S rRNA typing showed that 40 (88.9%) had type I, four (8.9%) had type II, and one (2.2%) had both types I and II (Table 2). One stray cat was infected with both *B. henselae* type I and *B. clarridgeiae*. Thus, *B. henselae* type I was the predominant type in stray cats in the Bangkok metropolitan areas. *B. henselae* type I is reported to be the predominant type in cats in other Asian countries, e.g. the Philippines (100%, 17/17) [16], Taiwan (76.0%, 19/25) [19], and Japan (97.8%, 44/45) [20]. On the other hand, type II is the prevalent type in European countries, such as Denmark (95.2%, 20/21) [24], Germany (94.7%, 18/19) [23], The Netherlands (66.7%, 14/21) [22], Italy (61.1%, 80/131) [25], and France (51.4%, 18/35) [21]. These results indicate that the distribution of *B. henselae* 16S rRNA types I and II is considerably different between Asian and European countries.

It has been reported that there was no significant difference in the prevalence of *Bartonella* between male and female cats in Thailand [18]. However, in the present study, the prevalence in male cats (21.6%, 25/116) was significantly higher than in female cats (11.9%, 21/176) in the Bangkok metropolitan areas ( $P < 0.05$ ). It may be speculated that the differences between these results is due to male cats having more opportunities to be scratched or bitten by other

cats while protecting their territories in the limited Bangkok metropolitan areas compared to other rural areas in Thailand.

In this study, *B. clarridgeiae* was isolated from only one out of 350 dog samples (0.3%). This is the first report of *B. clarridgeiae* isolation from a dog in Thailand, although the number of bacteria in the blood was low at 82 c.f.u./ml. Although *B. clarridgeiae* has been mainly isolated from cats, it has been isolated from the blood of a dog with aortic valve endocarditis in the USA [30] and from the blood of dogs in Gabon [29]. In the present study, the dog infected with *B. clarridgeiae* appeared healthy on physical examination. In addition, the prevalence of *B. clarridgeiae* bacteraemia in dogs was very low suggesting that dogs are more likely to be accidental hosts of *B. clarridgeiae* in Thailand.

In a previous report, 38% of sick dogs in Thailand, with fever, anaemia, or thrombocytopenia, tested seropositive for *B. vinsonii* subsp. *berkhoffii* [37]. Although *B. vinsonii* subsp. *berkhoffii* seroprevalence in dogs was not examined in this study, no dogs were found to harbour the species in those blood samples. Therefore, the prevalence of *B. vinsonii* subsp. *berkhoffii* in dogs should be relatively low in the Bangkok metropolitan areas.

Further studies are required to clarify the detailed prevalence and distribution of *Bartonella* spp. in cats and dogs in Asian countries, and to provide data for the prevention of bartonellosis from these animals.

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## DECLARATION OF INTEREST

None.

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