RESEARCH NOTE

BARTONELLA SPECIES IN DOGS AND THEIR ECTOPARASITES FROM KHON KAEN PROVINCE, THAILAND

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Abstract. In order to access the prevalence of *Bartonella* species in dogs, whole blood and any associated ectoparasites were collected from 164 dogs with owners in 25 villages throughout Khon Kaen Province. DNA was extracted from dog blood, 92 ticks (*Rhipicephalus sanguineus*) and 137 fleas (*Ctenocephalides* spp) and screened by PCR using intergenic spacer region and citrate synthase gene primers. *B. clarridgeiae* DNA was detected in blood of 3 dogs, 4 *C. felis* and 1 *C. canis*; *B. rochalimae* DNA was found in 1 tick; and *B. vinsonii* subsp *vinsonii* DNA was found in 2 *C. felis*. The findings indicate that dogs residing in northeast Thailand are exposed to diverse *Bartonella* species that are also potential human pathogens.

Keywords: *Bartonella*, dog, *Rhipicephalus sanguineus*, *Ctenocephalides*, fleas, ticks, Thailand

INTRODUCTION

Bartonella species are gram-negative bacteria, which infect a variety of hosts including rodents, cats, dogs, ruminants, and humans (Chomel and Kasten, 2010). Most *Bartonella* species are believed to be transmitted by arthropod vectors, such as fleas, ticks, keds, biting flies, and other blood-sucking ectoparasites (Billeter *et al*, 2008a). It has also been suggested that transmission of these bacteria may occur through the bite or scratch of an infected host (Chomel *et al*, 2006; Breitschwerdt *et al*, 2007; Duncan *et al*, 2007a). *Bartonella* species appear to be distributed in mammals worldwide and infections by these bacteria in humans and animals can cause various clinical symptoms ranging from a mild, flu-like illness to more severe manifestations, such as endocarditis, myocarditis, arthritis, hepatitis, and arthralgia (Chomel *et al*, 2006).

Previous reports have demonstrated the presence of numerous *Bartonella* species in humans and animals residing in Thailand (Maruyama *et al*, 2001; Castle *et al*, 2004; Kosoy *et al*, 2008, 2010; Paitoonpong

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et al, 2008; Inoue *et al*, 2009; Saisongkorh *et al*, 2009; Bai *et al*, 2009, 2010, 2012; Assarasakorn *et al*, 2012). Specifically, *B. henselae* (the agent of cat scratch disease), *B. elizabethae*, *B. rattimassiliensis*, *B. tribocorum*, *B. vinsonii* subsp *arupensis*, *B. vinsonii* subsp *vinsonii*, and a newly identified bacterium, *B. tamiae*, have been detected or isolated from the blood or tissues of febrile human patients (Kosoy *et al*, 2008, 2010; Paitoonpong *et al*, 2008; Bai *et al*, 2012). In rodents, *B. grahamii*, *B. elizabethae*, *B. coopersplainsensis*, *B. phoceensis*, *B. queenslandensis*, *B. rochalimae*, *B. rattimassiliensis*, *B. tribocorum*, Candidatus *B. thailandensis*, and novel genotypes have been identified in the blood (Castle *et al*, 2004; Bai *et al*, 2009; Saisongkorh *et al*, 2009). Domestic cats have also been found to harbor *B. henselae*, *B. clarridgeiae*, and *B. koehlerae* (Maruyama *et al*, 2001; Inoue *et al*, 2009; Assarasakorn *et al*, 2012). Previous investigations by our laboratory and others have demonstrated the presence of *B. clarridgeiae*, *B. vinsonii* subsp *arupensis*, *B. elizabethae*, *B. grahamii*, *B. quintana*, *B. taylorii*, and novel genotypes in domestic dogs from Bangkok and/or Khon Kaen (Inoue *et al*, 2009; Bai *et al*, 2010).

Bartonella species have also been detected in ectoparasites recovered from cats, dogs, and rodents in Thailand. In a survey performed along the Thai-Myanmar border, *B. henselae* and *B. clarridgeiae* were identified in *Ctenocephalides felis* collected from cats and a putative novel genotype was found in a *Nosopsyllus fasciatus* collected from a *Rattus surifer* (Parola *et al*, 2003). *B. henselae* and *B. clarridgeiae* DNA also were detected in *C. canis* and/or *C. felis* removed from cats in Bangkok, though *Bartonella* DNA was not detected in fleas or ticks from dogs (Foongladda *et al*, 2011). In a more recent study, 32% of 50 *C. felis* pools were shown to harbor *Bartonella* species, specifically *B. henselae*, *B. clarridgeiae*, and *B. koehlerae* (Assarasakorn *et al*, 2012). Kabeya *et al* (2010) demonstrated the presence of *B. tamiae* DNA in chigger mites and a *Haemphysalis* tick collected from wild-caught rodents, suggesting a potential role of these arthropods in the transmission of this organism.

The purpose of the study was to investigate the prevalence of *Bartonella* species in domestic dogs and their associated ectoparasites in Khon Kaen, Thailand in order to determine the potential risk of *Bartonella* infection for individuals residing in this area.

MATERIALS AND METHODS

Whole blood and any associated ectoparasites (ticks and fleas) were collected from a total of 164 dogs with owners in 25 villages throughout Khon Kaen Province, Thailand. Information on animal sex and age was recorded. Blood samples were aseptically collected from the cephalic vein and stored on dry ice in 5 ml EDTA tubes. Fleas and ticks were also collected manually and frozen on dry ice in 1.5 ml tubes. Blood and ectoparasites were then shipped to the Bartonella Laboratory at the Centers for Disease Control and Prevention, Fort Collins, Colorado, USA for analysis.

Genomic DNA was extracted from 200 µl of blood using a Qiagen QIAamp extraction kit (Qiagen, Valencia, CA) following the manufacturer's protocol. Ticks (a pool of 2 larvae, 15 individual nymphs, and 76 individual adult *Rhipicephalus sanguineus*; 1 - 6 ticks from 55 dogs) and fleas (86 individual *C. felis* and 51 individual *C. canis*; 1 - 7 fleas from 73 dogs) were triturated using a sterile needle and DNA was extracted following the Qiagen QIAamp extraction kit tissue

Table 1
Detection of *Bartonella* DNA in Thai dogs and their associated ectoparasites (*Rhipicephalus sanguineus* or *Ctenocephalides* spp) using genus-specific PCR targeting the citrate synthase gene (*glt*A) and/or 16S-23S interegenic spacer region (ITS).

Sample ID	Sample type	Detected using *glt*A primers: Y/N[a]	Detected using ITS primers: Y/N	Sequencing results	Host PCR positive: Y/N
Dog 40	Whole blood	N	Y	*B. clarridgeiae*	N/A[b]
Dog 81	Whole blood	N	Y	*B. clarridgeiae*	N/A
Dog 98	Whole blood	N	Y	*B. clarridgeiae*	N/A
Tick 18	*R. sanguineus* (adult, male)	N	Y	*B. rochalimae*	N
Flea 16-3-8, 1	*C. felis*	Y	N	*B. clarridgeiae*	N
Flea 16-1-5, 1	*C. felis*	Y	Y	*B. vinsonii* subsp *vinsonii*	N
Flea 11-4-3, 1	*C. felis*	Y	Y	*B. vinsonii* subsp *vinsonii*	N
Flea 12-3-3, 1[c]	*C. felis*	Y	N	*B. clarridgeiae*	N
Flea 12-3-3, 2[c]	*C. felis*	N	Y	*B. clarridgeiae*	N
Flea 13-2-4, 1	*C. canis*	Y	Y	*B. clarridgeiae*	Y (Dog 40)
Flea 15-4-4, 2	*C. felis*	Y	Y	*B. clarridgeiae*	N

[a]Y/N, Yes or No; [b]N/A, Not applicable; [c]Fleas were recovered from the same host.

protocol. Samples were screened using two different conventional PCR protocols targeting the *Bartonella* 16S-23S intergenic spacer region (ITS) and citrate synthase gene (*glt*A). Primers and thermocycling conditions were described by Billeter *et al* (2008b, 2011). The positive control contained *B. doshiae* DNA and nuclease-free water was utilized as a negative control.

Amplicons were purified using QIAquick PCR purification kit (Qiagen) and sequenced using an Applied Biosystems Model 3130 genetic analyzer (Applied Biosystems, Foster City, CA). DNA sequences were analyzed using the Lasergene version 8 sequence analysis software (DNASTAR, Madison, WI). For *glt*A PCR products, sequences of ~379 bp were employed for further phylogenetic analysis. Sequences obtained were considered similar to validated *Bartonella* species if a similarity over the 379 bp *glt*A fragment was ≥ 96% (La Scola *et al*, 2003).

RESULTS

Bartonella DNA was detected in the blood of 3 of 164 dogs (2%) using the ITS primer set with sequences 99.8-100% identical to *B. clarridgeiae* (GenBank accession number: FN645454) (Table 1). Furthermore, a *Bartonella* sp, 99% identical to *Bartonella* strain 1-1C (GenBank accession number: FN645504) isolated from the blood of *Rattus norvegicus* from Taiwan (Lin *et al*, 2008) and related to *B. rochalimae* (95.7% identical to type strain; GenBank accession number: DQ683199), was detected in one adult male tick using the same primer set (Table 1). These

samples were negative, however, when screened using *glt*A primers.

The presence of *Bartonella* DNA in 7 of 137 fleas (5%) was confirmed using ITS and/or *glt*A primers (Table 1). *Bartonella* DNA (100% identical to *B. clarridgeiae*, GenBank accession number: U84386) was detected in 2 fleas (*C. felis)* recovered from 2 dogs using the *glt*A primers, though no positive results were found in these fleas using ITS primers. An additional *C. felis* was demonstrated to harbor *B. clarridgeiae* DNA (100% identical; GenBank accession number: DQ683194) when screened using the ITS PCR protocol, although this flea was negative when screened using the *glt*A specific PCR. The *B. clarridgeiae* -ITS positive flea was collected from a dog that harbored one of the *B. clarridgeiae-glt*A positive fleas. The presence of *B. clarridgeiae* was also confirmed in a *C. canis*, collected from one of the *B. clarridgeiae*-positive dogs (dog 40), and in a *C. felis* (host was PCR negative) using both primer sets. Finally, *Bartonella* DNA, 100% identical to *B. vinsonii* subsp *vinsonii* (GenBank accession number: U28074 for *glt*A and GenBank accession number: L35102 for ITS), was identified in 2 *C. felis* collected from 2 dogs using both *glt*A and ITS primers, though dog hosts for these fleas were PCR negative.

DISCUSSION

Our study found a low prevalence of *Bartonella* species in the examined dogs. In a previous study, Bai *et al* (2010) demonstrated the presence of *Bartonella* species in 31% of 192 stray dogs examined; 28% of 111 dogs from Bangkok and 36% of 81 dogs from Khon Kaen. In that study, blood was aliquoted into a pre-enrichment culture, *Bartonella* alpha-proteobacteria growth medium, one week prior to DNA extraction and PCR examination. It is expected that there are a small number of bacteria circulating in the blood of non-reservoir hosts and use of the pre-enrichment culture, allowing the bacteria to replicate prior to testing, increases the likelihood of detection (Duncan *et al*, 2007b). By examining the blood directly and not after pre-enrichment, it is likely that *Bartonella* infection in some dogs was missed.

Bai *et al* (2010) did not detect *B. clarridgeiae* in any stray dogs from Khon Kaen Province; in fact, *B. elizabethae* was the most prevalent *Bartonella* species identified in dogs (31%; 9 of 29 positives) followed by *B. taylorii* (29%), *B. vinsonii* subsp *arupensis* (17%), and other putative novel genotypes. The reason for this discrepancy remains unclear, but is likely due to the difference in survey sites and potentially due to the usage of different gene targets. As is seen with *Bartonella* co-infections or multiple infections of a host, one gene target is more likely to detect one *Bartonella* species over another and the presence of *B. clarridgeiae* might have been confirmed in these dogs if a second gene target had been utilized (Abbot *et al*, 2007; Diniz *et al*, 2007). *B. clarridgeiae* has been isolated previously from a single stray dog from Bangkok (Inoue *et al*, 2009), but this is the first study, to our best knowledge, demonstrating the presence of this organism in dogs from Khon Kaen Province.

The low prevalence of *Bartonella* DNA in fleas (5%) and ticks (1%) is comparable to results from previous investigations undertaken in Thailand to screen domestic cat and dog ectoparasites. Parola *et al* (2003) found *Bartonella* DNA in 26% of 19 *C. felis* recovered from cats, although bacteria were not detected in the 34 *C. canis* recovered from dogs. Foongladda

et al (2011) reported that of 98 fleas (65 *C. canis* and 33 *C. felis*) and 304 *R. sanguineus* collected from dogs in Bangkok, not a single ectoparasite harbored detectable *Bartonella* DNA, although *B. henselae* and *B. clarridgeiae* were detected in 5% of 13 *C. canis* and 1% of 41 *C. felis* recovered from cats. On the other hand, Assarasakorn *et al* (2012) found *B. henselae* and *B. clarridgeiae* DNA in 10% of 50 flea pools and a single flea pool harbored both *B. henselae* and *B. koehlerae* DNA from cats in Bangkok. Most *Bartonella* species associated with illness in Thai patients appear rodent-borne, therefore contact with rodents and their ectoparasites, and not arthropods associated with dogs, is likely the most common route of *Bartonella* transmission to humans in this region (Kosoy *et al*, 2010).

The detection of *B. vinsonii* subsp *vinsonii* DNA in two fleas was an unexpected finding. *Bartonella vinsonii* subsp *vinsonii* was initially isolated from *Microtus pennsyloanicus* voles in 1946 (Baker, 1946) and, until recently, was not believed to be pathogenic to humans. During an on-going investigation to identify the cases of endocarditis in Thai patients, *B. vinsonii* subsp *vinsonii* DNA was detected in both aortic and mitral cardiac valves of an individual from Khon Kaen (Bai *et al*, unpublished). In a separate study, 19 Thai patients, 14 febrile and 5 non-febrile, from Chiang Rai and Khon Kaen Provinces were found to harbor *B. vinsonii* subsp *vinsonii* antibodies and an additional 17 were seroreactive against *B. vinsonii* subsp *vinsonii* and one or more other *Bartonella* species (Bhengsri *et al*, 2011). These results would suggest that, although not highly prevalent, this organism is present in certain areas of Thailand and is likely transmitted by fleas. Rodents are the assumed reservoir host for this organism, but further investigations are warranted to determine if dogs could also serve this role as well.

As *B. clarridgeiae*, *B. rochalimae*, and *B. vinsonii* subsp *vinsonii* are putative human pathogens, individuals residing in areas where these organisms exist are potentially at risk for exposure to these bacteria. Additional epidemiologic surveys are required, in order to further our understanding of the transmission dynamics of *Bartonella* species in Thailand.

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