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Short Report: Molecular Detection of *Rickettsia felis*, *Bartonella henselae*, and *B. clarridgeiae* in Fleas from Domestic Dogs and Cats in Malaysia

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Abstract. The presence of *Rickettsia felis*, *Bartonella henselae* and *B. clarridgeiae* in 209 fleas (*Ctenocephalides felis*) obtained from domestic cats and dogs in several locations in Malaysia was investigated in this study. Using a polymerase chain reaction specific for the citrate synthase (*gltA*) and 17-kD antigenic protein (*17kD*) genes of rickettsiae, we detected *R. felis* DNA in 6 (2.9%) fleas. For detection of bartonellae, amplification of the heme-binding protein (*pap31*) and riboflavin synthase (*ribC*) genes identified *B. henselae* and *B. clarridgeiae* DNA in 24 (11.5%) and 40 (19.1%) fleas, respectively. The DNA of *B. henselae* and *B. clarridgeiae* was detected in 10 (4.8%) fleas. Two *B. henselae* genogroups (Marseille and Houston-1) were detected in this study; genogroup Marseille (genotype Fizz) was found more often in the fleas. The findings in this study suggest fleas as potential vectors of rickettsioses and cat-scratch disease in this country.

Fleas are hematophagous arthropods that serve as vectors of several bacterial pathogens including *Yersinia pestis*, *Rickettsia typhi*, *R. felis*, and *Bartonella henselae*, which are the etiologic agents of plague, murine typhus, flea-borne rickettsioses, and cat-scratch disease, respectively. Murine typhus is primarily maintained by the rat flea *Xenopsylla cheopsis*.[1–3] However, the cat flea *Ctenocephalides felis* is also a competent vector.[3] *R. felis* is maintained and biologically transmitted by *C. felis*.[4] The rickettsiae were initially recognized as a member of the spotted fever group (SFG), but have been recently placed in the transitional group, a fourth phylogenetic lineage within the genus *Rickettsia*.[4,5] The clinical manifestation of *R. felis* infection in human involves an acute systemic infection that is typified by fever, maculopapular rash, and headache, similar to those of murine typhus and other febrile illnesses, such as dengue, in the tropics.[5]

In recent years, *B. henselae* and *B. clarridgeiae* have been recognized as two emerging pathogens of veterinary and medical interest. *Bartonella* organisms are parasites of mammalian erythrocytes and endothelial cells that are transmitted by ticks, fleas, lice, and flies.[6] Investigation of the population structure of these organisms is essential because of the association of the organisms with a variety of clinical syndromes and a complex host/reservoir system.[7] Based on the polymorphisms of the heme-binding protein (*pap31*) gene, *B. henselae* isolates are clustered into two genogroups: Marseille, which includes genotypes Marseille, Fizz, and CAL-1, and Houston-1, which includes genotypes Houston-1, SA-2, 90-615, and ZF-1.[8]

In Malaysia, data on the presence of human pathogens in the fleas are not available. A serosurvey demonstrated a high prevalence of antibody to SFG rickettsiae in Malaysian febrile patients.[9] However, the specific etiologic agent of spotted fever and cat scratch disease have not been reported in Malaysia.

In this study, 209 fleas were obtained from 39 healthy cats and 11 dogs from three sampling sites in Malaysia (Kuala Nerang, Pendang, and Ampang) during January–August 2010 (Table 1). Fleas were picked from animals and kept individually in microcentrifuge tubes containing 70% alcohol at –20°C. All the fleas were identified as *C. felis* on the basis of morphometric characteristics. A protocol reported by Alekseev and others[10] was used to prepare DNA template from the fleas. Briefly, each flea was immersed in 100 μL of 0.7 M ammonium hydroxide and boiled for 20 minutes. The DNA extracted was then resuspended in 10 μL of sterile distilled water prior to amplification.

Polymerase chain reactions (PCRs) specific for the citrate synthase (*gltA*)[11] and 17-kD antigenic protein (*17kD*)[12] genes of rickettsiae were performed for each flea sample. For detection of bartonellae from fleas, amplification of the heme-binding protein (*pap31*)[8] gene of *B. henselae* and riboflavin synthase (*ribC*)[13] gene of *B. clarridgeiae* was conducted. All PCRs assays (25 μL) were performed in a My Cycler™ thermal cycler (Bio-Rad Laboratories, Hercules, CA)) by adding 2 μL of DNA template to 19.55 μL of sterile distilled water, 2.5 μL of 10× DreamTaq™ buffer, 0.5 μL of dNTPs (100 μM), 0.1 μL of each primer (100 μM), and 0.25 μL of DreamTaq™ DNA Polymerase (5 U/μL). Amplicons were purified by using the LaboPass PCR Purification Kit (Cosmo Genetech, Seoul, South Korea) before sequencing in both directions by using respective PCR primers.

Findings for detection of *R. felis, B. henselae*, and *B. clarridgeiae* DNA in fleas obtained in this study are shown in Table 1. Genes encoding citrate synthase and 17-kD antigenic protein of rickettsiae were successfully amplified from six fleas obtained from two dogs and a cat from one of the sampling sites (Ampang). The *gltA* sequences obtained were similar to previously reported sequence of *R. felis* URRWCal2 (GenBank accession no. CP000053), except for three nucleotide changes. However, the sequences were identical with that of *Rickettsia* sp. RF2125 (GenBank accession no. AF516333), a genotype closely related to *R. felis*, which has been reported from different arthropod vectors in various regions: *Echidnophaga gallinacean* in Egypt,[14] *Archeopsylla erinacei* in Algeria,[15] *C. felis* at the Thailand–Myanmar border,[16] and *Pulex irritans* in Hungary.[17] In addition, the *17kD* sequences obtained in this study were identical with *Rickettsia* sp. RF2125, which was detected in fleas obtained from the United States,[18] Peru,[19] and Uruguay.[20] Although existing data suggest a worldwide distribution of *Rickettsia* sp. RF2125, the pathogenic role of this organism has yet to be determined because it has not been isolated from any human sample.

Bartonella henselae DNA was detected in 28 (13.4%) fleas in this study. Analysis of *pap31* sequences differentiates the bartonellae into two *B. henselae* genogroups. A total of

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TABLE 1
Detection of *Rickettsia felis, Bartonella henselae*, and *B. clarridgeiae* in cat fleas obtained from dogs and cats, Malaysia, 2010

Sampling sites	No. (%) fleas examined	No. (%) fleas positive for *R. felis*	No. (%) fleas positive for *B. henselae*		No. (%) fleas positive for *B. clarridgeiae*
			Genotype Houston-1	Genotype Fizz	
Kuala Nerang (6°15′0″N, 100°36′0″E)	57 (27.3)	0 (0)	5 (8.8)	10 (17.5)	12 (21.1)
Pendang (6°0′0″N, 100°28′0″E)	16 (7.6)	0 (0)	0 (0)	8 (50.0)	5 (31.3)
Ampang (3°9′0″N, 101°46′12″E)	136 (65.1)	6 (4.4)	0 (0)	1 (0.7)	23 (16.9)
Total	209 (100)	6 (2.9)	5 (2.4)	19 (9.1)	40 (19.1)

10 (35.7%) and 18 (64.3%) *B. henselae* in this study were identified as genogroup Houston-1 (all were genotype Houston-1), and genogroup Marseille (all were genotype Fizz), respectively (Table 1). *Bartonella clarridgeiae* DNA was detected in 40 (19.1%) fleas. Analysis of *ribC* demonstrated matching sequences of the bartonellae with that of *B. clarridgeiae* strain 73 (GeneBank accession no. AJ236916, between positions 434 and 1185). The DNA of *B. henselae* and *B. clarridgeiae* was detected in 10 (4.8%) fleas. In addition, *B. clarridgeiae* was the predominant bartonellae detected in fleas from Ampang compared with *B. henselae*, which was detected more frequently in fleas from two other sampling sites (Table 1). Collectively, the prevalence of bartonellae (11.5%) in fleas was higher than that of *R. felis* (2.9%) in this study.

Our finding provides molecular evidence on the type of rickettsiae and bartonellae in Malaysia. Up to now, no clinical cases attributed to *R. felis* and *Bartonella* species have been reported in Malaysia. These infections may present as underrecognized causes of acute febrile illness because of their lack of clinical suspicions and appropriate laboratory tests.[5] In the past, serologic reactivity of the Malaysian population against SFG rickettsiae was assessed by using *R. honei* (previously known as TT118 strain).[9] With the detection of *R. felis* and bartonellae DNA in fleas obtained in this study, we speculate that population in Malaysia may be exposed to these flea-borne pathogens. In addition, it remains to be investigated whether the high antibody prevalence to SFG rickettsiae in our patients[9] are partly attributed to *R. felis*, in view of the fact that *R. felis* cross-reacts with most members of SFG rickettsiae.[21]

Human and animal infections of *R. felis* and bartonellae have been reported in Southeast Asia.[16,22–24] This study is the second study in Southeast Asia that investigated the prevalence of *R. felis* and bartonellae in flea samples. Compared with the previous study,[16] a larger number of fleas examined in this study were positive for *B. henselae* and *B. clarridgeiae*.

Co-infection of different *Bartonella* species in the mammals and fleas has been reported.[6,23–27] In this study, the rate of co-infection (4.8%) of *B. henselae* and *B. clarridgeiae* in our flea samples was low when compared with high rates (approaching 90%) reported in a previous study.[6]

The genogroups of *B. henselae* vary in human and animal samples in different geographic regions. For instance, most *B. henselae* cat isolates in The Netherlands and Germany are identified as genogroup Marseille, whereas human isolates are identified as genogroup Houston-1.[26,28] In this study, the genogroup Marseille (genotype Fizz) was found often in our flea samples.

The findings in this study show that control of fleas is important because of the public health significance of *R. felis* and *Bartonella* infection. This study provides baseline data useful for the surveillance, prevention and control of rickettsioses and bartonelloses in Malaysia.

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