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Feline bartonellosis associated with some clinico-pathological conditions in a veterinary hospital in Selangor, Malaysia

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Abstract. A cross-sectional study was conducted to determine the prevalence of feline bartonellosis and the associated clinicopathological findings in cats presented to the University Veterinary Hospital, Selangor, Malaysia from 2013–2014. Out of 284 cats examined, *Bartonella* DNA was detected in 48 (16.9%) cats using a specific polymerase chain reaction (PCR) assay targeting the internal transcribed spacer of *Bartonella* species. *Bartonella henselae* strain Houston was identified through BLAST analyses of randomly selected amplicons. Univariable analysis showed significant association of feline bartonellosis with cats < 2 years of age (OR 1.37, 95% CI 0.982–1.927, p = 0.036) and those presenting with ocular discharge (OR 3.211, 95% CI 1.422–7.248, p = 0.003). Significant associations of neutrophilia (OR 2.244, 95% CI 1.131–4.452, p = 0.019) and monocytosis (OR 2.476, 95% CI 1.154–5.312, p = 0.017) with bartonella infection in cats were observed. This study reports for the first time the prevalence (approximately 17%) of feline bartonellosis in Malaysia and highlights several clinicopathological factors associated with the disease.

INTRODUCTION

Feline bartonellosis is regarded as a mild and often asymptomatic disease of cats caused by bacteria of the genus *Bartonella*. The disease can be transmitted to humans through scratches or bites of infected cats, resulting in a wide spectrum of disease conditions namely, cat-scratch disease, endocarditis, lymphadenopathy and bacillary angiomatosis (Breitschwerdt, 2014; Harms & Dehio, 2012; Breitschwerdt, 2008; Breitschwerdt & Kordick, 2000). Among the pathogenic species of *Bartonella* spp., *Bartonella henselae* is the most frequently reported species in feline bartonellosis (Breitschwerdt, 2014; Harms & Dehio, 2012). Cat fleas are the natural vector of *B. henselae*, and their potential to transmit the pathogen in cats has been well established (Chomel *et al.*, 1996). Cats are popular pets in many households in Malaysia. They are mostly kept indoor but avoiding contact with other cats within the neighborhood is highly difficult. Hence, they could readily acquire infections such as bartonellosis from other cats and potentially transmit to their owners.

Despite reports of several human cases associated with lymphadenopathy and neuroretinitis (Kiu *et al.*, 2015; Raihan *et al.*, 2014; Lina *et al.*, 2010, there is little information available on bartonellosis and its transmission in Malaysia. The isolation of *Bartonella* spp. (not including *B. henselae*) from the kidney and spleen homogenates of 13.7% *Rattus* spp. in a previous investigation suggests wild rats as one of

the animal reservoirs for the bacteria (Tay *et al.*, 2014). *Bartonella bovis* was also detected in 4.5% cattle blood samples collected across Peninsular Malaysia (Kho *et al.*, 2015). However, there is a paucity of information regarding presence of *Bartonella* species in cats in the study area. Hence, this study was conducted to determine the prevalence and clinicopathological findings associated with feline bartonellosis in cats presented to a veterinary hospital in Malaysia.

MATERIALS AND METHODS

Sample collection

This study was approved by the Institutional Animal Care and Use Committee (IACUC) Universiti Putra Malaysia (UPM/IACUC/AUP- R061/2013). A total of 284 pet cats presented to the University Veterinary Hospital (UVH), Selangor, were recruited between December, 2013 and June, 2014. Data on age, sex, breed, clinical signs, and blood profiles of the cats were recorded. Whole blood sample (1-2 ml) was collected from each cat by jugular venipuncture.

DNA extraction and molecular detection

DNA was extracted from blood samples using G–Spin™ Total DNA extraction kit (iNtRON Biotechnology, Korea) as in accordance to the manufacturer's instructions. Poly-merase chain reaction (PCR) was performed in a 20µl reaction mixture containing 4µl of 5X HOT FIREPol Blend Master Mix (Solis BioDyne, Estonia), 10.8µl of Milli-Q water (ultra-pure water), 5µl of DNA template and 0.1µM of each primer (321s forward: 5'- AGA TGA TGA TCC CAA GCC TTC TGG- 3' and 983as reverse: 5'- TGT TCT YAC AAC AAT GAT GAT G- 3'), targeting the internal transcribed spacer region of *Bartonella* species (Maggi & Breitschwerdt, 2005). The PCR reaction was subjected to a denaturation step at 95°C for 12 min followed by 40 cycles of denaturation at 94°C for 45s, annealing at 58°C for 45s, extension at 72°C for 45s and a final extension step at 72°C for 5 mins in a Mycycler thermal cycler (Biorad, USA). Sterile milli-Q water and DNA extract obtained from a *B. elizabethae* strain (BeUM) (Tay *et al.*, 2016) were used as negative and positive controls, respectively. A sample was considered positive when an amplified DNA fragment (approximately 600 bp) was detected upon agarose gel electrophoresis.

Sequence determination and analysis

The amplicons were purified and sequenced on an ABI PRISM 377 Genetic Analyzer (Applied Biosystems, USA), using the forward and reverse primers of the PCR assay. The sequences obtained were searched for homologous sequences in the GenBank database by using the Basic Local Alignment Search Tool (BLAST) program (National Center for Biotechnology Information, Bethesda, MD).

Statistical analysis

Statistical analysis was carried out using Statistical Package for the Social Sciences software programme (SPSS, version 22, Chicago, USA). A univariate statistical model was used to assess potential association between cats with positive PCR findings with the age, sex, breed, and clinicopathological findings. Odds ratio (OR) and 95% confidence interval (CI) were used to explore the strength of the association. A *P*-value of ≤ 0.05 was considered statistically significant.

RESULTS

Analysis of demographic data and clinicopathological findings

Table 1 summarizes the demographic data (age, sex and breed) and clinicopathological findings of the cats investigated in this study. The median age of the cats was 2 years 10 months. Almost half of the cats (42.6%) were less than 2 years old and 57.4% were older than 2 years. A majority (62.3%) of the cats were males. Intact males and females comprised 43.3% and 31.3%, respectively, of the recruited cat population. Most (73.2%) of the cats were domestic short hair cats.

Table 1. Demographic and clinic-pathological findings associated with molecular detection of *Bartonella* DNA in 284 cat blood samples obtained from a Malaysian veterinary hospital

Variable	Variable levels	No. (%) positive	OR (95% CI)	*P* value
Age	< 2 years, n=121, 42.6%	27(22.3)	1.37(0.982-1.927)	0.036*
	≥ 2 years, n=163, 57.4%	21(12.9)	1	
Sex	Castrated male, n=54, 19.0%	10(18.5)	1.104(0.480-2.536)	0.816
	Intact female, n=89, 31.3%	16(18.0)	1.065(0.520-2.179)	0.864
	Spayed female, n=18, 6.3%	1(5.6)	0.286(0.036-2.266)	0.236
	Intact male, n=123, 43.3%	21(17.1)	1	
Breed	Persian, n=58, 20.4%	9(15.5)	0.878(0.396-1.946)	0.748
	Others, n=18, 6.3%	3(16.7)	0.956(0.263-3.478)	0.945
	Domestic short hair, n=208, 73.2%	36(17.3)	1	
Pale mucous membranes	Yes, n=80, 28.2%	16(20.0)	1.344(0.691-2.613)	0.383
	No, n=204, 71.8%	32(15.7)	1	
Lymphadenopathy	Yes, n=24, 8.5%	3(12.5)	0.683(0.195-2.386)	0.548
	No, n=260, 91.5%	45(17.3)	1	
Ocular discharge	Yes, n=31, 10.9%	11(35.5)	3.211(1.422-7.248)	0.003*
	No, n=253, 89.1%	37(14.6)	1	
Respiratory-associated problems	Yes, n=50, 17.6%	12(24.0)	1.737(0.829-3.640)	0.140
	No, n=234, 82.4%	36(15.4)	1	
Gingivitis	Yes, n=19, 6.7%	5(26.3)	1.844(0.631-5.387)	0.257
	No, n=265, 93.3%	43(16.2)	1	
Flea infestation	Yes, n=30, 10.6%	5(16.7)	0.981(0.356-2.707)	0.971
	No, n=254, 89.4%	43(16.9)	1	
Thrombocytopenia	Yes, n=159, 56.0%	29(18.2)	1.245(0.661-2.343)	0.498
	No, n=125, 44.0%	19(15.2)	1	
Neutrophilia	Yes, n=59, 20.8%	16(27.1)	2.244(1.131-4.452)	0.019*
	No, n=225, 79.2%	32(14.2)	1	
Monocytosis	Yes, n=40, 14.1%	12(30.0)	2.476(1.154-5.312)	0.017*
	No, n=244, 85.9%	36(14.8)	1	
Eosinophilia	Yes, n=23, 8.1%	4(17.4)	1.038(0.337-3.201)	0.948
	No, n=261, 91.9%	44(16.9)	1	
Reticulocytosis	Yes, n=6, 2.1%	2(33.3)	2.522(0.449-14.176)	0.278
	No, n=278, 97.9%	46(16.5)	1	

*Significant level; Ref, Reference category; OR, Odds ratio; CI, Confidence interval.

A variety of health issues affecting the respiratory, gastrointestinal, urinary and reproductive systems of the cats were recorded. Respiratory-associated problems were observed in 17.6% of the cats. Other signs and symptoms included pale mucous membranes (28.2%), ocular discharge (10.9%), lymphadenopathy (8.5%), and gingivitis (6.7%). Laboratory findings demonstrated thrombocytopenia, neutrophilia, and monocytosis in 56.0, 20.8 and 14.1% of the cats, respectively. Eosinophilia and reticulocytosis were noted in less than 10% of the cats (Table 1).

PCR detection of *Bartonella* spp.

Bartonella DNA was detected from 48 (16.9%) of 284 cat blood samples. BLAST analysis of the sequences obtained from several randomly selected samples (GenBank accession no. KT318617-19) demonstrated the highest sequence similarity (99%) with that of *B. henselae* type strain Houston (GenBank accession no. L35101).

Association of PCR-positive cats with clinicopathological findings

Univariable analysis showed significant association between *Bartonella* detection (OR 1.37, 95% CI 0.982-1.927, $p = 0.036$) with younger cats (< 2 years of age) (Table 1). Cats presenting with ocular discharge were three times more likely to be positive for bartonella compared to the rest (OR 3.211, 95% CI 1.422-7.248, $p = 0.003$). No significant association was noted for cats with other clinical presentations. Low flea infestation (10.6%) was noted in this study. However, it was not significantly associated with cats with positive PCR findings.

Examination of the blood profiles reveals significant association of bartonella detection in cats with neutrophilia (OR 2.244, 95% CI 1.131–4.452, p=0.019) and monocytosis (OR 2.476, 95% CI 1.154–5.312, p=0.017) (Table 1). Other hematological findings such as eosinophilia, reticulocytosis and thrombocytopenia were not significantly associated with bartonella infection in cats.

DISCUSSION

The percentages of cats reported with *Bartonella* bacteremia vary in different parts of the world. This study presents for the first time the detection of *B. hensalae* in Malaysian cats. The detection rate (16.9%) of *Bartonella*-infected cats in this study is comparable to a molecular study conducted in Thailand, a neighbouring country of Malaysia, whereby 17% of client-owned cats from veterinary hospitals in Bangkok were positive for *Bartonella* DNA (Assarasakorn *et al*., 2012). However, an attempt to isolate *Bartonella* spp. from PCR-positive samples was not successful in this study, probably due to the fastidious nature of the bacteria, low levels of bacteremia, or timing of blood sampling.

A significant association was noted between younger cats (< 2 years of age) with *Bartonella* detection in this study. The finding is in agreement with a study which reported that younger kittens could easily be infected with *Bartonella henselae* in natural environment than older ones (Fleischman *et al*., 2015). In this study, the sex and breed of cats were not significantly associated with the disease, which is consistent with a study in the United States (Guptill *et al*., 2004).

Most cats investigated in this study were not infested with fleas at the time of the study, hence; it was not possible to assess the carriage of *Bartonella* spp. in cat fleas. However, the presence of *B. henselae* and *Bartonella clarridgeiae* in *Ctenocephalides* fleas infesting Malaysian stray cats has been previously reported (Mokhtar & Tay, 2011). Although feline bartonellosis is often regarded as asymptomatic and *Bartonella* spp. has been demonstrated in the blood of healthy cats, evidences supporting a pathogenic role of *Bartonella* spp. have been documented (Breitschwerdt & Kordick, 2000). For instance, there has been a significant increase in the incidence of lymphadenopathy, gingivitis, uveitis, renal and urinary tract abnormalities in cats with serologic evidence of *Bartonella* infection (Lappin *et al*., 2000; Lappin & Black, 1999; Glaus *et al*., 1997; Ueno *et al*., 1996). Nonetheless, with the exception of ocular discharge, no significant association was noted in the clinical manifestations of cats with PCR-positive findings in this study.

Hematological anomalies due to bartonella infections in cats are rarely reported. Thrombocytopenia, lymphocytosis, neutropenia, and eosinophilia have been reported in feline bartonellosis (Breitschwerdt, 2008). The findings in this study show that cats with neutrophilia and monocytosis are significantly associated with *Bartonella* infections (Table 1). In the human infection, neutrophils are recruited to fight against bacterial infection, and can be seen scattered around the skin lesion of

Bartonella-infected patients with bacillary angiomatosis (Koehler *et al*., 1992). In the other hand, monocytosis is an indicator for chronic tissue inflammation, and has been associated with infection of *B. henselae*, *B. elizabethae* and *B. vinsonii* subsp. *berkhoffii* in dogs (Mexas *et al*., 2002; Breitschwerdt *et al*., 1999). The association of neutrophilia and monocytosis with bartonella-infection in cats should be further investigated, as these hematological characteristics may be useful as biological markers in the diagnosis of feline bartonellosis.

In conclusion, this study reports for the first time the prevalence (approximately 17%) of bartonellosis in our cats and highlights several clinicopathological factors associated with the disease. In view of the significant prevalence of feline bartonellosis and its potential zoonotic transmission, public awareness and institution of prevention and control measures are necessary.

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