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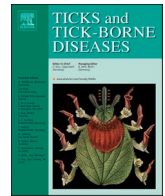
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## Original article

# Low prevalence of *Babesia hongkongensis* infection in community and privately-owned cats in Hong Kong

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## ABSTRACT

Domestic cats are susceptible to infection with at least 11 species of *Babesia*. In Hong Kong, where dogs are commonly infected with *B. gibsoni*, a single infection in a cat by a novel species, *B. hongkongensis*, was reported previously. The aim of this study was to investigate the frequency of *Babesia* spp. detection in cats in Hong Kong. Residual blood-derived DNA from healthy free-roaming community cats ( $n = 239$ ), and privately-owned cats with and without anaemia undergoing diagnostic investigations ( $n = 125$ ) was tested for *Babesia* spp. DNA using a pan-*Babesia* PCR targeting mitochondrial *Cytochrome B*, and a *B. hongkongensis* specific PCR targeting 18S rRNA. Positive samples were confirmed by sequencing and comparative sequence analysis against the GenBank nucleotide database. *Babesia hongkongensis* was detected in 4/239 (1.7 %) community cats, and 0/125 (0.0 %) privately-owned cats. *Babesia gibsoni* was detected in 0/239 community cats and 1/125 (0.8 %) privately-owned cats. Cats infected with *B. hongkongensis* were clinically healthy at the time of sampling. The *B. gibsoni*-infected cat was anaemic and thrombocytopenic. Cats in Hong Kong can be infected with *B. hongkongensis* and *B. gibsoni*, albeit at low frequency. The tick vector for *B. hongkongensis* is yet to be identified.

## 1. Introduction

*Babesia* are vector-borne apicomplexan intraerythrocytic parasites that infect a wide range of animals including mammals, birds and reptiles. Natural transmission mainly occurs via ticks from the *Ixodidae* family (Solano-Gallego and Baneth, 2011). In dogs, horizontal transmission via bites, blood transfusion or transplacental transmission, has also been reported (Birkenheuer et al., 2005; Fukumoto et al., 2005; Matsuu et al., 2004; Stegeman et al., 2003). Most infections in dogs are subclinical and are diagnosed incidentally (Almendros et al., 2020). In dogs with clinical babesiosis, the major clinicopathological abnormalities are anaemia and thrombocytopenia (Birkenheuer et al., 1999; Conrad et al., 1991; Muguero et al., 2023).

Babesiosis in cats is uncommon and is of major concern in only a few regions including South Africa, where four species of *Babesia* in three well defined clades, are responsible for most clinical presentations, including *B. felis* s.s. (clade I), *Babesia leo* (clade I), *Babesia lengau* (clade

II), and *Babesia* species cat Western Cape (clade VI) (Penzhorn and Oosthuizen, 2020; Penzhorn et al., 2004). Clade VI includes *B. canis presentii* and *B. hongkongensis*, the latter reported in a single feline case in Hong Kong (Baneth et al., 2004; Wong et al., 2012). The canine pathogens *B. canis* s.s., *B. gibsoni*, and *B. vogeli*, as well as *B. lohiae*, *B. microti*, and *B. vulpes* have also been detected in cats (Almendros et al., 2023; Colella et al., 2020; Kelly et al., 2017; Penzhorn and Oosthuizen, 2020; Wong et al., 2012; Yin et al., 2022).

Pet ownership is reported in 9.4 % of households of Hong Kong, with cats comprising 42.6 % of owned pets (HKSAR, 2019). *Babesia* infection of dogs in Hong Kong has a higher prevalence in the stray population compared to owned dogs (Muguero et al., 2023; Wong et al., 2011). To the best of our knowledge, molecular evidence that *Babesia* species are circulating in the cat population in Hong Kong has been reported in only two cases to date (Almendros et al., 2023; Wong et al., 2012). The objectives of this study were to determine the prevalence and species of *Babesia* detected in healthy community-owned cats and in

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privately-owned cats with and without anaemia in Hong Kong.

## 2. Materials and Methods

### 2.1. Group selection and distribution

Ethical approval for this study was granted by the Animal Ethics Committee of City University of Hong Kong (approvals A-0478 and AN-STA-150 0000015). Two groups of cats were investigated in this study. All data collected was treated confidentially and was deidentified.

#### 2.1.1. Privately-owned cats

Since babesiosis in dogs is often associated with anaemia, samples were collected from anaemic and non-anaemic cats to determine if anaemia was a risk factor for *Babesia* infection. Residual diagnostic EDTA blood samples and blood smears were collected from anaemic and non-anaemic cats presented to CityU Veterinary Medical Centre (VMC) between July 2022 to March 2023. Anaemia was defined as a haematocrit (HCT)  $\leq 25\%$  (Tasker, 2012). This mixed population of cats included healthy cats presented for routine healthcare (e.g. desexing) and sick cats undergoing diagnostic investigations.

Age, breed, sex, and neuter status were recorded. Cats were categorised into two age groups ( $\leq 3$  and  $> 3$  years). Haematocrit and platelet count (PLT) were recorded for all cats. Where available, absolute reticulocyte count was also recorded.

#### 2.1.2. Community cats

Community cats are free-roaming cats living in colonies that are cared for by members of the public. Whole blood samples were collected, with consent, from community cats presented to a trap-neuter-release program at the Society for the Prevention of Cruelty to Animals (SPCA), Hong Kong between January and May 2021. Whole blood was centrifuged and separated sera and blood clot were stored separately at  $-80^\circ\text{C}$ . Sera from each community cat was tested for Feline Leukaemia Virus (FeLV) antigen and Feline Immunodeficiency Virus (FIV) antibody (SNAP Combo, IDEXX, Westbrook, ME). Age, breed, sex, and neuter status were recorded. Cats were categorised into two age groups ( $\leq 3$  and  $> 3$  years). Cats were clinically examined by a veterinarian and found to be healthy prior to surgery.

### 2.2. DNA extraction

DNA was extracted from 100  $\mu\text{L}$  of EDTA blood or blood clot using the DNeasy blood and tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA was eluted in 50  $\mu\text{L}$  and stored at  $-80^\circ\text{C}$  until analysis. Conventional PCR of feline glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was performed to confirm the integrity of extracted DNA (McLuckie et al., 2018).

**Table 1**

Primers and cycling conditions used to obtain the genomic sequence of *Babesia* spp. detected in cats in Hong Kong.

Primer target	Primer name	Sequence	Amplicon size (nucleotides)	Cycling conditions (40 cycles)		
				D	A	E
<i>cytb</i> gene of <i>Babesia</i> (pan- <i>Babesia</i> )	P_cytbF	TGTTGCTCCCAATAACTCATT	360	95°C, 30s	51°C, 30s	72°C, 1 min
	P_cytbR	AGGAATTTAAATTCCTAATTGGAATT				
18S rRNA of <i>B. hongkongensis</i>	BH_18S565F	CGTTTGGGCTTTTAGCTTT	173	95°C, 30s	55°C, 30s	72°C, 1 min
	BH_18S737R	TTAACCATTACTAAGGTTCCCA				
18S rRNA (pan- <i>Babesia</i> )	Outer F (1st round)	CATCAGCTTGACGGTAGGGTATT	489 – 518	95°C, 30s	57°C, 30s	72°C, 1 min
	Outer R (1st round)	CCCCCAACCGTTCCTATTAAC				
	Inner F (2nd round)	GAGGCAGCAACGGGTAACG				
	Inner R (2nd round)	CCAACAAAATAGAACCAAGTCCTA	421 – 447	95°C, 30s	56°C, 30s	72°C, 1 min

D - denaturation; A - annealing; E - extension temperatures.

### 2.3. PCR assays

*Babesia* spp. detection was performed using two PCRs as previously described, one targeting the mitochondrial cytochrome b gene (*cytb*) and the other targeting 18S rRNA (Wong et al., 2012). PCR primers and cycling conditions are presented in Table 1. Each reaction contained 1  $\mu\text{L}$  of template DNA, DreamTaq™ Hot Start Green DNA Polymerase (Thermo Fisher Scientific, Graciuno, Vilnius, Lithuania), and dNTP (Thermo Fisher Scientific, Graciuno, Vilnius, Lithuania) at a final concentration of 200  $\mu\text{M}$  and a final primer concentration of 0.5  $\mu\text{M}$ . For all PCRs, a positive control (synthetic DNA construct, Thermo Fisher Scientific, Graciuno, Vilnius, Lithuania) and negative control (molecular grade water) were included. For samples that were *Babesia* positive, an additional pan-*Babesia* nested PCR was performed to obtain a longer 18S rRNA sequence (Li et al., 2015).

Bands of the expected target size obtained on gel-electrophoresis after PCR were sequenced by Sanger sequencing (BGI Genomics, Hong Kong). Geneious software (version 2023.1.1) was used to assemble the consensus sequence. The nucleotide homology and coverage of sequences generated were compared by Basic Local Alignment Search Tool (BLAST) against sequences in the NCBI database in GenBank.

### 2.4. Microscopic examination

Blood smears from samples testing *Babesia* positive on PCR from privately-owned cats were fixed with methanol and stained with modified Giemsa prior to examination under a light microscope to identify piroplasms or intraerythrocytic inclusions. Blood smears were not available from community cats.

### 2.5. Data analysis

The proportion of the total number of cats testing positive for *Babesia* spp. (frequency) was calculated. Descriptive analyses of age, breed, sex, neuter status and haematological values (range and mean) were performed. Associations between FIV and FELV status and a positive *Babesia* result were tested by Fisher's exact test.

## 3. Results

The sample population comprised 364 cats, of which 125 were privately-owned and 239 were community cats.

### 3.1. Privately-owned cats

Amongst the 125 privately-owned cats, age ranged from 9 weeks to 19.5 years (mean 7.8 years, median 8 years). Sex, neuter status, breed and numbers of cats with and without anaemia are listed in Table 2.

The platelet count ranged from 0 to  $1304 \times 10^9/\text{L}$  (mean  $252 \times 10^9/\text{L}$ , median  $237 \times 10^9/\text{L}$ ) and was confirmed by blood smear examination in 33 cats. A total of 29/125 (23 %) cats had a platelet count below the

**Table 2**

Signalment, anaemia status and FIV status of privately-owned cats and community cats, respectively, tested for *Babesia* spp. in Hong Kong.

Parameters privately-owned cats	Number sampled (%)
<b>Sex</b>	
Male	74 (59.2)
Female	51 (40.8)
<b>Age (years)</b>	
≤3	36 (32.0)
> 3	89 (68.0)
<b>Neuter status</b>	
Intact	26 (20.8)
Desexed	99 (79.2)
<b>Breed</b>	
Domestic Shorthair (DSH)	64 (51.2)
British Shorthair (BSH)	31 (24.8)
Exotic Shorthair (ESH)	6 (4.8)
Persian	5 (4.0)
Ragdoll	5 (4.0)
Other	14 (11.2)
<b>Anaemic status</b>	
Anaemic	66 (52.8)
Non-anaemic	59 (47.2)
<b>Total</b>	125 (100)
Parameters community cats	Number sampled (%)
<b>Sex</b>	
Female	107 (44.8)
Male	132 (55.2)
<b>Age (years)</b>	
≤ 3	198 (82.8)
> 3	41 (17.2)
<b>Neuter status</b>	
Intact	233 (97.5)
Desexed	6 (2.5)
<b>Breed</b>	
Domestic shorthair	237 (99.2)
Domestic longhair	1 (0.4)
Exotic shorthair	1 (0.4)
<b>FIV/FelV status</b>	
FIV positive	42 (17.6)
FelV positive	0 (0.0)
<b>Total</b>	239 (100)

established reference range (296 - 850×10<sup>9</sup>/L) (Weiser and Kociba, 1984), and 16/125 (13 %) cats had concurrent anaemia. Haematocrit values ranged from 8.1 % to 56.0 % (mean 26.7 %, median 25 %).

Amongst the 66 anaemic cats, anaemia was regenerative (absolute reticulocyte count >50 K/μL) in 9/46 cats with available data. Underlying conditions in these cats included neoplasia (*n* = 3), inflammatory disease (*n* = 2), pyothorax (*n* = 1), hepatic lipidosis (*n* = 1), trauma (*n* =

**Table 3**

Percentage of nucleotide identity and coverage using the highest-match on the NCBI GenBank database in positive *Babesia* PCR samples identified in 5 cats in Hong Kong.

Sample ID	Cytochrome B results			Accession number of sample sequence	18 s rRNA results			Accession number of sample sequence
	BLAST result top match (accession number)	% identity	% coverage		BLAST result top match (accession number)	% identity	% coverage	
BH1	<i>Babesia hongkongensis</i> (JQ867357.1)	99.72	100.00	OR230433	<i>Babesia hongkongensis</i> clone 66A (MH143397.1)	100.00	100.00	OR226548
BH2	<i>Babesia hongkongensis</i> (JQ867357.1)	100.00	100.00	OR230434	NIL			
BH3	<i>Babesia hongkongensis</i> (JQ867357.1)	99.72	100.00	OR230435	<i>Babesia hongkongensis</i> clone 53A (MH143396.1)	100.00	99.00	OR226567
BH4	<i>Babesia hongkongensis</i> (JQ867357.1)	100.00	100.00	OR230436	<i>Babesia hongkongensis</i> clone 53A (MH143396.1)	99.76	100.00	OR226558
BG1	<i>Babesia gibsoni</i> isolate WH58 (KP666169.1)	99.44	98.00	OR020936	<i>Babesia gibsoni</i> Wayanad isolate 8 (MN134517.1)	100.00	99.00	OQ981666

1) and idiopathic (*n* = 1). Amongst the 37 cats with non-regenerative anaemia the most common underlying conditions were neoplasia (*n* = 9), renal disease (*n* = 7), hepatic lipidosis (*n* = 4), idiopathic (*n* = 4), feline panleukopenia (*n* = 4), gastroenteropathy (*n* = 3) and pneumonia (*n* = 3).

Amongst privately-owned cats, 1/125 (0.8 %) tested positive for *Babesia*, identified as *B. gibsoni* and described in a separate report (Almendros et al., 2023). Briefly, the 6-year-old, male neutered DSH lived indoors with no exposure to dogs or ticks. The cat was anaemic (HCT=9.8 %) and thrombocytopenic (platelet count=0).

### 3.2. Community cats

Of the 239 community cats, age ranged from 1 to 9 years (mean 2.2 years, median 2 years). Signalment details and results of FIV and FeLV testing are presented in Table 2. Four community cats (1.7 %) tested PCR positive for *Babesia*, and all were confirmed by sequencing to be *B. hongkongensis* (Table 3). For each positive sample, a CytB sequence and a 18 s rRNA sequence was obtained except for one, for which we were unable to obtain the 18 s rRNA by PCR (Table 3).

The *B. hongkongensis* positive community cats were all sexually intact DSH including three males and one female aged 1, 2, 5 and 6 years old. At the time of blood sampling, fleas were detected on one of these cats while ticks were detected on none. One cat also tested positive for FIV, compared to 40/235 *Babesia* negative cats that also tested positive for FIV positive. No significant statistical association was found between FIV and *Babesia* infection by Fisher exact test (*p*=0.5314).

## 4. Discussion

The key findings in this study were that *Babesia* infections occur in cats in Hong Kong and are caused by *B. hongkongensis* and *B. gibsoni*. The infection rate (1.4 %) amongst cats sampled in this study was low. In dogs in Hong Kong, *B. gibsoni* is by far the most common *Babesia* species detected (Almendros et al., 2020; Hussain et al., 2023; Muguero et al., 2023). By contrast, *B. hongkongensis* was discovered serendipitously in cats in 2012 after piroplasms were identified in histologic stains of kidney tissue from a community cat in an unrelated study (Wong et al., 2012). Molecular surveillance of 300 community cats at that time failed to identify any other *Babesia*-infected cats (Wong et al., 2012). Despite molecular surveillance spanning over a decade, *B. hongkongensis* has never been detected in dogs (Almendros and Burchell, 2021; Almendros et al., 2020; Hussain et al., 2023; Muguero et al., 2023), suggesting that it has a particular tropism for cats, or that cats are more likely to come into contact with its vector.

In Hong Kong, ixodid ticks from four genera have been reported - *Rhipicephalus*, *Haemophysalis*, *Ixodes* and *Hyalomma* (Chan et al., 2011). However, surveillance to determine the relative prevalence of tick species parasitizing dogs in Hong Kong is lacking. *Rhipicephalus sanguineus* and *Haemophysalis longicornis* are the most common tick species identified on dogs in East and Southeast Asia (Cheng et al., 2018; Colella et al., 2020; Nguyen et al., 2020; Wang et al., 2020; Zhang et al., 2017). Whether these or other tick species could be the vector for *B. hongkongensis* remains to be determined.

Infestations with ticks were not identified on any of the cats in our study. In contrast to dogs, ticks are infrequently found on cats due to their fastidious self-grooming behaviours. In dogs, *B. gibsoni* is also transmitted horizontally through fights and bites, blood transfusions or by transplacental infection (Birkenheuer et al., 2005; Fukumoto et al., 2005; Matsuu et al., 2004; Stegeman et al., 2003). Horizontal transmission for *B. hongkongensis* amongst community cats may be possible too.

The infection rate of *Babesia* in cats in our study was low (1.4 %) compared to what has been reported in other regions like the Western Cape, Eastern Cape and KwaZulu-Natal provinces of South Africa, where feline babesiosis is a major concern with high infection rates (>50 %) (Bosman et al., 2007; Jacobson et al., 2000). Other regions that have reported high infection rates include St Kitts (31.0 %), Iraq (26.0 %) and Portugal (9.4 %) (Kelly et al., 2017; Maia et al., 2014; Suliman, 2009). The higher infection rates in these regions might be due to differences in exposure to and prevalence of tick vectors and the *Babesia* species they carry as well as the differences in frequency of tick prophylactic control.

The *Babesia* infection rate in cats in geographic locations closer to Hong Kong, such as mainland China and Thailand, are reported in several studies to be similarly low (Simking et al., 2010; Yin et al., 2022; Zhang et al., 2019). In mainland China, *B. gibsoni* was detected in 2.8 % of feline blood samples from four different provinces (Yin et al., 2022; Zhang et al., 2019). A previous study investigating the association between age and *Babesia* detection reported that cats less than 3 years old were more susceptible to infection (Schoeman et al., 2001). The low number of *Babesia* positive samples in our study precluded the investigation of any age association.

There were several limitations in our study including that the healthy status designation of community cats was based on clinical examination only as clinicopathological tests other than for FIV and FeLV were not performed. Also, since low volumes of residual blood were used for testing of privately-owned cats, FIV and FeLV testing could not be performed. Since *B. hongkongensis* was only detected in community cats in our study, whether it could be a risk factor for anaemia and/or thrombocytopenia in cats could not be determined and further investigations are warranted.

*Babesia gibsoni* has been reported previously to cause subclinical infections in 5/119 (4 %) healthy cats in St Kitts in the Caribbean, 12/ 429 (3 %) cats in China and in one cat in Singapore (Colella et al., 2020; Kelly et al., 2017; Yin et al., 2022). Clinical signs were identified retrospectively in the single *B. gibsoni* positive privately-owned cat in our study, including lethargy, pallor, hyporexia, fever and weakness with associated anaemia and thrombocytopenia (Almendros et al., 2023). Further surveillance is required to determine whether *B. gibsoni* has a pathogenic role in cats.

## 5. Conclusion

This study confirms the molecular presence of *B. gibsoni* and *B. hongkongensis* in cats in Hong Kong, with a low infection rate overall (1.4 %) in the studied population. The higher proportion of *Babesia*-positive community cats compared to privately-owned cats could be due to a higher risk of vector exposure or horizontal transmission during fighting, amongst community cats. Identified *B. hongkongensis* infections were subclinical. Further research on the virulence of *B. gibsoni* and *B. hongkongensis* infection in cats, as well as their potential routes of

transmission and determination of the potentially involved arthropod vector(s) is warranted.

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## Author statement

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

## CRedit authorship contribution statement

**A. Almendros:** Conceptualization, Methodology, Formal analysis, Writing – original draft. **Y.R. Choi:** Methodology, Formal analysis, Investigation, Data curation, Writing – review & editing. **T.L. Leung:** Writing – original draft. **W.Y.J. Tam:** Data curation. **D. Hernandez Muguero:** Conceptualization. **F.M. Woodhouse:** Data curation. **J.J. Gray:** Data curation. **J.A. Beatty:** Methodology, Writing – review & editing. **V.R. Barrs:** Methodology, Formal analysis, Writing – review & editing.

## Declaration of Competing Interest

The authors declare not conflict of interest.

## Data availability

Data will be made available on request.

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