The Journal of Veterinary Medical Science

NOTE

Public Health

Prevalence of hemoplasmas and *Bartonella* species in client-owned cats in Beijing and Shanghai, China

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J. Vet. Med. Sci.
83(5): 793–797, 2021
doi: 10.1292/jvms.20-0681

Received: 30 November 2020
Accepted: 21 February 2021
Advanced Epub:
18 March 2021

ABSTRACT. A year-round molecular epidemiological survey (2017 to 2018) was conducted on three hemoplasmas and two *Bartonella* species with zoonotic potential in client-owned cats in Beijing and Shanghai. Among 668 specimens, the overall hemoplasma-positive rate was 4.9% (3.4% for *Candidatus* Mycoplasma haemominutum, 0.9% for *Mycoplasma haemofelis* and 1.2% for *Candidatus* Mycoplasma turicensis). The overall *Bartonella*-positive rate was 8.5% (4.8% for *B. henselae* and 4.3% for *B. clarridgeiae*). Age, breed, ectoparasiticide use and stray history, but not city, season and gender, were significantly associated with the positive rates of one or more pathogens. This is also the first report on the prevalence of *Candidatus* Mycoplasma turicensis in cats in China.

KEY WORDS: *Bartonella*, China, feline, hemoplasma, risk factor

Hemoplasmas (aka hemotropic mycoplasmas) and *Bartonella* are vector-transmitted gram-negative bacterial pathogens in animals. Hemoplasmas adhere to and disrupt erythrocytes, causing hemolytic anemia in animals. Cats may be infected by *Mycoplasma haemofelis* (*Mhf*), "*Candidatus* Mycoplasma haemominutum" (*CMhm*), "*Candidatus* Mycoplasma turicensis" (*CMt*), and "Candidatus Mycoplasma haematoparvum-like" (*CMhp*) species [18, 19]. Among them, *Mhf* may cause severe to fatal hemolytic anemia in cats. *CMhm* is typically low virulent, but can cause severe clinical signs when co-infected with other pathogens and/or if the animal is under stressed or immunodeficient condition [18]. *CMt* can induce mild to moderate anemia in experimentally infected cats in the acute infection phase [24], while the clinical significance of *CMhp* is not fully understood.

Bartonella species are intracellular pathogens infecting animals including cats and dogs. Cats can serve as reservoir host for *B. henselae*, *B. clarridgeiae* and *B. koehlerae*. Among them, *B. henselae* and *B. clarridgeiae* are the causative agents of cat scratch disease (CSD) in humans [15]. However, naturally infected cats usually exhibit no clinical signs even after long-term experience of bacteremia [10]. Fleas are believed to be the predominant vector responsible for the transmission of *Bartonella* species.

Despite their importance in animal health and zoonotic potential, there were limited studies on the prevalence of feline hemoplasma and *Bartonella* in China. The presence of feline *Mhf* and *CMhm* in the mainland China was first reported in 2010, in which the scale of the study was limited and the prevalence of *CMt* was not evaluated [27]. A few other studies investigated the prevalence of *Bartonella* in stray or pet cats in some regions in China [25, 26]. However, the prevalence of feline hemoplasma and *Bartonella* in Beijing, the nation's capital with high population densities of human residents and pets, has not been reported.

In the present study, we conducted a year-round molecular survey between 2017 and 2018 on the prevalence of three hemoplasmas (*Mhf*, *CMhm* and *CMt*) and two *Bartonella* species (*B. henselae* and *B. clarridgeiae*) in client-owned cats in Beijing and Shanghai, two of the most populated cities in the north and south regions in China, and analyzed associated risk factors to expand epidemiological information.

For specimen collection, a total of 668 blood samples were collected from client-owned cats at four veterinary hospitals in Beijing and one in Shanghai between March, 2017 to March, 2018. Specimens were shipped to the College of Veterinary Medicine, China Agricultural University for storage at −20°C until use. During sample collection, the following information on cats was recorded by veterinarians or collected from clients: city (Beijing, Shanghai), season (spring, summer, autumn, winter), age (≤1 year, 1–10 years, ≥10 years), gender (male, female), breed (purebreds, mixed including crossbreeds or unknown breeds), stray history, and ectoparasiticide use in the past 6 months. The animal use protocol was reviewed and approved by the Laboratory Animal Welfare and Animal Experimental Ethics Committee, China Agricultural University (permit number: AW21012020-2). Prior to specimen collection, permission was obtained from animal owners.

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(Supplementary material: refer to PMC https://www.ncbi.nlm.nih.gov/pmc/journals/2350/)

Table 1. PCR results for hemoplasma and *Bartonella* infections in 668 cat samples

Nested-PCR result (n=668)	No. of positive cats	Positive rate (%)
Hemoplasmas	33	4.9
CMhm	23	3.4
Mhf	6	0.9
CMt	8	1.2
CMhm only	20	3.0
Mhf only	2	0.3
CMt only	7	1.0
CMhm + *Mhf*	3	0.4
Mhf + *CMt*	1	0.1
CMhm + *Mhf* + *CMt*	0	0
Bartonella species	57	8.5
B. henselae	32	4.8
B. clarridgeiae	29	4.3
B. henselae only	28	4.2
B. clarridgeiae only	25	3.7
B. henselae + *B. clarridgeiae*	4	0.6

For molecular detection by PCR, genomic DNA was extracted from 200 µl of each blood sample using a QIAamp DNA Blood Mini Kit according to the manufacturer's instructions (Qiagen, Hilden, Germany), eluted in 100 µl elution buffer and stored at −20°C until use. Hemoplasmas and *Bartonella* species were detected by nested PCR that amplified a 16S-23S rRNA intergenic transcribed spacer (ITS). Primary PCR used genus-specific primers, while secondary PCR used species-specific primers as described (Supplementary Table 1) [12, 16, 22, 23]. PCR was performed in 25 µl volume containing 12.5 µl of 2 × PCR Starmix (GenStar BioSolutions, Beijing), 1.0 µM each of specified primers and 2.0 µl sample DNA for primary PCR (or 1.0 µl primary PCR product for secondary PCR), using thermal cycling conditions described in Supplementary Table 1. DNA elution buffer and hemoplasma or *Bartonella* DNA samples were used for negative and positive controls, respectively. PCR products were electrophoresed in 2% agarose gels. All samples were tested at least twice. PCR products were extracted from gels and submitted to Beijing Majorbio Sanger Bio-pharm Technology for bi-directional automated sequencing using ABI Prism 3730XL DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Hemoplasmas and *Bartonella* DNAs detected in the present study were compared with genomic sequences in the GenBank by BLAST search.

The association between individual risk factors and infections of hemoplasma or *Bartonella* was evaluated by Chi-square (χ^2) test or by a two-tailed Fisher's exact test when expected numbers of observations were less than five using SPSS (version 20). Variables with *P*-values <0.05 in the univariant analysis were further tested using a multivariable logistic regression model. A *P*-value <0.05 was considered statistically significant in both univariate and multivariate analyses.

The results showed that the overall positive detection rate by nested PCR for hemoplasmas was 4.9% (n=33 of the total 668 specimens) (Table 1). Among them, *CMhm* was the most prevalent species (n=23; 3.4%), followed by *CMt* (n=8; 1.2%) and *Mhf* (n=6; 0.9%). Coinfections by two hemoplasma species were observed in some cats, including three specimens with *CMhm* and *Mhf* (0.4%) and one with *Mhf* and *CMt* (0.1%), but none with three hemoplasma species (i.e., *CMhm, Mhf* and *CMt*). The positive rate of hemoplasmas (i.e., 4.9%) is much lower than an earlier study conducted in a southern city Guangzhou, China, (i.e., 41.4%) [27], and those reported in other countries, including Japan (26.4%) [21], Thailand (38.1%) [3], South Korea (47.9%) [6], Iran (22%) [4], the United States (18% to 27%) [19, 20], and the United Kingdom (18% to 27%) [14].

The overall positive detection rate for *Bartonella* spp. in this study was 8.5% (n=57 of the total 668 specimens), which was much higher than that of hemoplasmas. It included 4.8% (n=32) for *B. henselae* and 4.3% (n=29) for *B. clarridgeiae*. Among them, 0.6% (n=4) of the *Bartonella*-positive specimens were coinfected both species (Table 1). The overall positive rate of 8.5% was between the two values reported earlier for feline *Bartonella* in other regions in China, i.e., 3.9% in cats from the southern city Shenzhen [26], and 12.7% in cats from 7 provinces (Beijing not included) [25]. In comparison with studies in other regions, the overall prevalence of *Bartonella* in our study is comparable to those reported in Japan (4.6%) [16], Turkey (9.4%) [1], Greece (8.5%) [13], and Ireland (5.2%) [8], but lower than that those in Thailand (16.3%) [7], South Korea (41.8% to 44.1%) [9] Taiwan (19.1%) [2], and Israel (18.7 to 30.7%) [5].

Our results indicated that hemoplasmas and *Bartonella* species were commonly present as a potential health risk to cats in China. The presence of zoonotic *B. henselae* and *B. clarridgeiae* was also an indication of potential risk for CSD in humans. Indeed, several CSD cases have already been reported in China [11], suggesting the necessity for veterinarians to educate pet owners regarding the risk of CSD in contacting with cats.

In risk factor analysis, we observed no significant differences between Beijing and Shanghai in the overall positive detection rates of feline hemoplasmas or *Bartonella* (*P*=0.285 to 0.856 by the univariant test) (Table 2). Although both bacterial groups had the highest positive rates in the spring, season was not a significant risk factor (*P*=0.344 to 0.935). Gender was also not a significant risk factor for infections of hemoplasma or *Bartonella* species (*P*=0.690 to 0.910). However, age, breed, ectoparasiticide

Table 2. Univariate analysis of risk factors for infection of feline hemoplasmas and *Bartonella* spp. in cats

Risk factors		Total No.	Hemoplasmas		*B. henselae*		*B. clarridgeiae*	
			No. of positive cats (%)	*P*	No. of positive cats (%)	*P*	No. of positive cats (%)	*P*
City		668	33 (4.9%)	0.285	32 (4.8%)	0.580	29 (4.3%)	0.856
	Beijing	516	28 (5.4%)		26 (5.0%)		22 (4.3%)	
	Shanghai	152	5 (3.3%)		6 (3.9%)		7 (4.6%)	
Season		668		0.360		0.344		0.935
	Spring	200	13 (6.5%)		14 (7.0%)		10 (5.0%)	
	Summer	122	3 (2.5%)		5 (4.1%)		5 (4.1%)	
	Autumn	132	5 (3.8%)		4 (3.0%)		6 (4.5%)	
	Winter	214	12 (5.6%)		9 (4.2%)		8 (3.7%)	
Age		668		0.006		0.025		0.019
	≤1 year	153	4 (2.6%)		11 (7.2%)		10 (6.5%)	
	1–10 years	286	23 (8.0%)		17 (5.9%)		16 (5.6%)	
	≥10 years	229	6 (2.6%)		4 (1.7%)		3 (1.3%)	
Breed		668		0.002		0.008		0.003
	Mixed breeds	327	25 (7.6%)		22 (7.0%)		22 (6.7%)	
	Purebreds	341	8 (2.3%)		9 (2.6%)		7 (2.1%)	
Gender		668		0.690		0.910		0.848
	Male	403	21 (5.2%)		19 (4.7%)		17 (4.2%)	
	Female	265	12 (4.5%)		13 (4.9%)		12 (4.5%)	
Ectoparasiticide use		668		0.608		0.029		0.901
	Yes	292	13 (4.5%)		8 (2.7%)		13 (4.5%)	
	No	376	20 (5.3%)		24 (6.4%)		16 (4.3%)	
Former stray		668		0.002		0.015		0.001
	Yes	187	17 (9.1%)		15 (8.0%)		16 (8.6%)	
	No	481	16 (3.3%)		17 (3.5%)		13 (2.7%)	
Anemia status		224		0.372				
	Yes	131	12 (9.2%)					
	No	93	12 (12.9%)					

use and stray history were significantly associated with the positive detection rates of one or more pathogens (Table 2). For age, significantly higher hemoplasma-positive rates were observed in 1 to 10-year old cats (8.0%) than in younger (≤1 year) or older (≥10 years) animals (2.6%, *P*=0.006); while significantly higher positive rates of *Bartonella* species were observed in both ≤1 year and 1 to 10-year old groups (5.6% to 7.2%) than in older animals (≥10 years) (1.3% to 1.7%, *P*=0.019 to 0.025).

Between the two breed types, mixed breeds had significantly higher positive rates (6.7% to 7.6%) than purebreds (2.1% to 2.6%) for both hemoplasmas and *Bartonella* spp. (*P*=0.002 to 0.008). Cats with stray history had >2 times higher positive rates (8.0% to 9.1%) than those without stray history (2.7% to 3.5%, *P*=0.001 to 0.015). Further analysis by multiple logistic regression indicated that age (*P*=0.018) and breed (*P*=0.025) were significantly associated with hemoplasma infections after the adjustment for stray history (Table 3). Age (*P*=0.014 and 0.044), stray history (*P*=0.028) and ectoparasiticide use (*P*=0.022) were significantly associated with *B. henselae* infection, while only age (*P*=0.012 and 0.032) was significantly associated with *B. clarridgeiae* infection (Table 3).

The fact that both hemoplasma and *Bartonella* infections are more frequently observed in mixed breed cats than purebreds may relate to their living environments, since mixed breed cats are more likely to be allowed for outdoor activities in China. Outdoor cats are more prone to the exposure to arthropod vectors that potentially carry pathogens. Another possibility is that most mixed breed cats are adopted from places where they were housed in groups with higher chances to socialize with infected-cats. The behavior changes in cats over the age might also be a contributor to the variation of infection. For instance, higher hemoplasma-positive rates were observed in 1 to 10-year old cats that are generally more aggressive in interacting with each other, thus increasing the risk of infection. On the other hand, older cats (≥10 years) might spend less time roaming outside [17], thus reducing the risk of vector exposure, resulting in lower infections for both hemoplasmas and *Bartonella*. Among other risk factors, the significantly higher prevalence of hemoplasmas and *Bartonella* in cats with stray history, and that of *B. henselae* in cats without ectoparasiticide use, were apparently related to the higher chances of animal exposure to vectors (e.g., fleas).

We were able to retrieve medical records of 224 sampled cats from veterinarians in Beijing for analyzing the relationship between infection and anemia, but observed that the overall hemoplasma-positive rate was not statistically different between cats with and without anemia (9.2% vs. 12.9%; *P*=0.372) (Table 2). This observation suggested that hemoplasma infection contributed no more than other possible factors to the overall rate of feline anemia in Beijing. However, there is a lack of more direct evidence

Table 3. Multivariate analysis of risk factors for infection of feline hemoplasmas and *Bartonella* spp. in cats

	OR (CI_{95})	*P*
1. Hemoplasmas		
Age (n=668)		
≤1 year	1.030 (0.283–3.745)	0.964
1–10 years	3.099 (1.209–7.943)	**0.018**
≥10 years	Ref.	
Breed (n=668)		
Mixed breed	2.888 (1.139–7.321)	**0.025**
Purebreds	Ref.	
Former strays (n=668)		
Yes	1.436 (0.629–3.278)	0.390
No	Ref.	
2. *Bartonella henselae*		
Age (n=668)		
≤1 year	4.325 (1.342–13.940)	**0.014**
1–10 years	3.147 (1.029–9.627)	**0.044**
≥10 years	Ref.	
Former strays (n=668)		
Yes	Ref.	
No	2.283 (1.093–4.772)	**0.028**
Ectoparasiticide use (n=668)		
Yes	2.610 (1.145–5.949)	**0.022**
No	Ref.	
3. *Bartonella clarridgeiae*		
Age (n=668)		
≤1 year	5.424 (1.452–20.258)	**0.012**
1–10 years	3.988 (1.124–14.144)	**0.032**
≥10 years	Ref.	
Breed (n=668)		
Mixed breed	2.631 (0.973–7.116)	0.057
Purebreds	Ref.	
Former strays (n=668)		
Yes	Ref.	
No	1.878 (0.778–4.532)	0.161

OR=odds ratio, CI_{95}=95% confidence interval. Bold values denote statistical significance at the $P<0.05$ level.

on the relationship between hemoplasma-infection and anemia because the medical records were not individually paired between anemia status and hemoplasma infection. We could only retrieve four paired records, showing three (75%) of the four *Mhf*-positive cats had anemia, weakly supporting *Mhf* as a potential cause of feline anemia.

In summary, our year-round survey between 2017 and 2018 indicated the presence of three hemoplasmas and two *Bartonella* species in cats in two Chinese metropolitan cities. Age of animals and their stray history are the two major factors positively associated with the infection rates. The presence of zoonotic *Bartonella* species indicates a significant risk for CSD in humans.

CONFLICT OF INTEREST. The authors declare no conflict of interest with respect to the publication of this manuscript.

ACKNOWLEDGMENTS. We would like to thank participating veterinarians from China Agricultural University Veterinary Teaching Hospital, Meilian Zhonghe Animal Hospital, Puppy Town Animal Hospital and Kang Cheng Pet Hospital for their assistance in sample collection. The positive control DNA samples of hemoplasmas and *Bartonella* species were kindly provided by LPet Veterinary Diagnostic Center (Beijing, China) and Dr. Qiyong Liu at the National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing.

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