Short Communication

jfms OPEN REPORTS

Journal of Feline Medicine and Surgery Open Reports
1–8

DOI: 10.1177/20551169221148672
journals.sagepub.com/home/jfmsopenreports

This paper was handled and processed by the American Editorial Office (AAFP) for publication in *JFMS Open Reports*

SAGE

Molecular detection of feline hemoplasmas and retroviruses in free-roaming and shelter cats within a university campus

Ana Carolina Yamakawa[1], Amanda Haisi[1], Louise Bach Kmetiuk[2], Maysa Pellizzaro[3], Juliana Cristina Rebonato Mendes[2], Aurea Maria Oliveira Canavessi[4], Leila Sabrina Ullmann[1], Wagner Antônio Chiba de Castro[5], João Pessoa Araújo Júnior[1], Andrea Pires dos Santos[6] and Alexander Welker Biondo[2,6]

Abstract

Objectives The aim of the present study was to assess the frequency of hemoplasma, feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) infections in cats living in an on-campus shelter and free-roaming cats within a university campus in Brazil.

Methods Blood samples were tested using quantitative PCR for hemoplasma, FIV and FeLV. Positive hemoplasma samples were sequenced. Associations between hemoplasma detection and living situation, sex, flea and/or tick parasitism, and coinfection with FIV and FeLV, were assessed using Fisher's exact test and the respective odds ratios were calculated.

Results Overall, 6/45 (13.3%) cats tested positive: four (8.9%) were infected with '*Candidatus* Mycoplasma haemominutum' and two (4.4%) with *Mycoplasma haemofelis*. All positive samples were from free-roaming cats (6/15; 40.0%) and had statistically significantly lower packed cell volumes ($P=0.037$). Although 5/23 (21.7%) males and 1/22 (4.6%) females were positive, no statistically significant association between sex and hemoplasma infection was found ($P=0.19$). Viral quantitative PCR (qPCR) was performed on 43/45 samples, among which 2/43 (4.7%) were positive for FIV and none for FeLV. Only one cat (2.3%) was coinfected with hemoplasma and FIV ($P=0.26$). In addition, 4/6 (66.7%) cats that tested positive for hemoplasmas were infested by fleas ($P=0.0014$) and/or ticks ($P=0.25$).

Conclusions and relevance These results show that even if the free-roaming cat population is clinically healthy and has adequate access to food, it may present flea infestation and hemoplasma infection with lower packed cell volume values.

Keywords: Animal health; coinfection; feline immunodeficiency virus; hemotropic mycoplasma; molecular diagnosis

Accepted: 13 December 2022

[1]Biotechnology Institute, São Paulo State University (UNESP), Botucatu, SP, Brazil
[2]Graduate College of Cell and Molecular Biology, Federal University of Paraná (UFPR), R dos Funcionários, Curitiba, PR, Brazil
[3]Institute of Collective Health, Federal University of Bahia, Salvador, BA, Brazil
[4]Department of Animal Science, São Paulo University (USP), Piracicaba, SP, Brazil
[5]Latin-American Institute of Life and Nature Sciences, Federal University for Latin American Integration, Foz do Iguaçu, PR, Brazil
[6]Department of Comparative Pathobiology, Purdue University, West Lafayette, IN, USA

Corresponding author:
Alexander Welker Biondo PhD, Zoonoses & Shelter Medicine at the Federal University of Paraná, Funcionários Street, 1540, Curitiba, PR 80060-000, Brazil;
Email: abiondo@ufpr.br

Correction (March 2023): Article updated to include additional co-author and affiliation.

Introduction

Hemotropic mycoplasmas (hemoplasmas) are small pleomorphic bacteria that infect the erythrocytes of several mammalian species, including cats.[1,2] The transmission cycle has not been completely elucidated. Although fleas[3–5] and ticks[6] have primarily been implicated in hemoplasma transmission, blood transfusion and aggressive contact may also be transmission routes.[7] Although most hemoplasma infections are asymptomatic, clinical signs – depending on the hemoplasma species – have been described, including fever and hemolytic anemia,[8] which can be severe in susceptible and immunosuppressed animals.[1]

Mycoplasma haemofelis (Mhf),[9] '*Candidatus* Mycoplasma haemominutum' (CMhm),[10] '*Candidatus* Mycoplasma turicensis' (CMtc)[7] and '*Candidatus* Mycoplasma haematoparvum-like'[11] have been identified as infecting cats worldwide, with variable pathogenicity. Mhf is the most pathogenic species to infect cats.[1] The zoonotic potential of hemoplasmas has also been reported in association with arthropod vectors[12] and immunosuppressed individuals.[13] Coinfections with multiple hemoplasma species may also occur.[14–16]

Despite worldwide distribution, the prevalence of feline hemoplasmas has been found to be geographically variable. The prevalence of hemoplasmas infecting cats ranges from 8.5% to 13.2% among animals in European countries,[14,15,17,18] from 25.4% to 38.05% in Asia,[19–21] from 3.1% to 23.3% in North America[22,23] and from 0.24% to 7.8% – depending on the mycoplasma species – in Chile.[24] Hemoplasma prevalence has been extensively studied in Brazil, with prevalence ranging from 6.5% to 36.4% in different regions.[8,16,25–28] Previous studies in Brazil evaluated the prevalence of hemoplasma infection among free-roaming cats, which ranged from 32% to 40%,[25,28] and among domiciled cats, which ranged from 6.5% to 35.5%.[26,27]

Given that feline retroviruses have been mostly associated with immunosuppression, hemoplasma may be aggravated by coinfection with feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV), thereby impairing effective treatment and prognosis.[29] While FeLV is associated with a greater severity of illness, FIV infection may increase the risk of coinfections.[29] Unsurprisingly, retrovirus infection has been associated with higher hemoplasma infection worldwide.[14,15,17,21–23]

Accordingly, the aim of the present study was to assess hemotropic mycoplasma infections, potential associated risk factors and coinfections among free-roaming and shelter cats living on a major university campus in southeastern Brazil.

Materials and methods

Study area

Blood samples were acquired from cats living on the campus of the 'Luiz de Queiroz' School of Agriculture, University of São Paulo, located in Piracicaba, Sao Paulo, Brazil (22°42′37.4″ S; 47°37′58.3″ W). According to the campus administration, the free-roaming cat population was estimated to be 250 animals at the time of the study.[30] The present study was approved by the Ethics Committee for Animal Use of the Federal University of Paraná (protocol no. 103/2017).

Blood sample and tick/flea collection

The two cat populations comprised 51 cats living in an on-campus shelter and the estimated 250 free-roaming cats within the campus limits.[30] Cats were caught, and sampling was carried out between July and October 2017. These samples were also screened for other pathogens, as previously reported.[30,31] Free-roaming cats were caught by means of bait-trapping, in cages distributed throughout the university area, followed by physical restraint and blood sampling through jugular vein puncture. A total of 30/51 shelter cats (58.8%) and 15/250 free-roaming cats (6%) were caught and sampled. Sample sizes ranging from 2% to 80% of the cat population have been described in previous studies; thus, the present study focused on 27–124 cats (simple random sampling with 95% confidence and 5% precision).[31] The relatively low number of free-roaming cats sampled reflected difficulties in catching them, as previously documented in studies conducted on feral cats.[32]

The sampled cats were examined for ticks and fleas; when found, these were collected and preserved in isopropyl alcohol, followed by taxonomic identification using morphological keys.[33]

Molecular analyses

DNA extraction was performed using a commercial kit (Illustra Blood Genomic Prep Mini Spin Kit; GE Healthcare), following the manufacturer's recommendations. A quantitative PCR (qPCR) was performed to identify the presence of feline hemoplasma, FIV and FeLV infection using SYBR Green detection. The qPCR for detecting feline hemoplasmas included 0.6 µl (10 pM) of forward 5′-ATACGGCCCATATTCCTACG-3′ and reverse HBT-R 5′-TGCTCCACCACTTGTTCA-3′ primers,[34] 10 µl 2 × SYBR Green PCR master mix (qPCR Master Mix GoTaq; Promega), 4.8 µl nuclease-free water and 4 µl of the DNA sample. DNA from a cat blood sample positive for Mhf and nuclease-free water were used as positive and negative controls, respectively.

For the detection of FIV and FeLV, each qPCR assay included 0.4 µl (10 pM) of each specific primer: forward qFIV-Sdeg 5′-RTGGTTYACAGCCTTYTCDGC-3′ and reverse qFIV-ASdeg 5′-GGTACGATCATAYTCWGC TGTCA-3′; or forward FFeLV-U3 5′-AACAGCAGA AGTTTCAAGGCC-3′ and reverse RFeLV-U3 5′-TTA TAGCAGAAAGCGCGCG-3′, respectively.[35] The qPCR for FIV and FeLV included 10 µl 2 × SYBR Green PCR master mix (Promega GoTaq qPCR Master Mix), 4.2 µl

nuclease-free water and 5 µl of the sample. A cat blood sample positive for FIV and FeLV and nuclease-free water were used as positive and negative controls, respectively.

All qPCR runs used the same cycling conditions, including an initial denaturation at 95°C for 5 mins, followed by 40 cycles at 95°C for 15 s and also at 60°C for 1 min, followed by the melting curve using the AriaMX real-time PCR system (Agilent). Samples were considered positive for feline mycoplasma if Ct ⩽34 with a melting temperature of 83°C. Regarding FIV and FeLV, samples were considered positive if Ct⩽34 with melting temperatures of 80°C and 85.5°C, respectively.

PCR product sequencing

For the hemoplasma-positive samples, a conventional PCR was performed using the following primers: 16S_HAEMOforw 5′-GGCCCATATTCCT(AG)CGGGAA-G3′ and 16S_HAEMOrev 5′-AC(AG)GGATTACTAGT GATTCCA-3′.[36] These targeted the 16S rDNA gene of *Mycoplasma* species (~1000 base pairs). The reaction contained 1× commercial mix (GoTaq Green Master Mix; Promega), 0.6 µl of each primer (10 pM), 8.8 µl nuclease-free water and 5 µl of DNA sample. The cycling conditions were 94°C for 3 mins, followed by 35 amplification cycles at 94°C for 45 s, 59°C for 45 s and 72°C for 90 s, with a final extension of 10 mins at 72°C carried out in the Verti thermal cycler (Applied Biosystems). The amplified products were separated by means of electrophoresis on 1.5% agarose gel for 40 mins at 100 V, and were analyzed using an ultraviolet light transilluminator (GE-ImageQuant LAS 500; GE Healthcare Bio-Sciences).

The PCR products were purified using commercial magnetic beads (Speed Bead magnetic carboxylate-modified particles, with 0.05% sodium azide; GE Healthcare). Concentration and purity were evaluated using a commercial spectrophotometer (NanoDrop; Thermo Scientific), and amplicons were sequenced in both directions through the Sanger method. Sense and antisense sequences were trimmed and assembled using Geneious Prime (2021). Consensus sequences were then aligned with *Mycoplasma* species sequences obtained from cat samples worldwide, which are available in the GenBank nucleotide database, using MUSCLE alignment. The phylogenetic tree was constructed with Geneious Tree Builder using the neighbor-joining method, including 1000 bootstrap replicates and an outlier sequence of *Mycoplasma pneumoniae* (GenBank ID: NR041751.1).

Statistical analyses

Statistical analyses were performed with the aim of detecting associations between hemoplasma infection and risk variables, including living situation (in a shelter or free-roaming), sex, flea and/or tick parasitism, and coinfection with FIV and FeLV. These analyses to find associated risk factors were performed using Fisher's exact test (confidence level = 0.05) and, in cases of statistically significant results ($P < 0.05$), the odds ratio (OR) and 95% confidence interval (CI). The previous serological status with regard to *Rickettsia* species[30] and *Toxoplasma gondii*[31] was also analyzed as an associated risk factor for hemoplasma positivity. Packed cell volume (PCV) was evaluated by means of short blood centrifugation in microhematocrit tubes and measured directly. The normality of the distribution of PCV values was evaluated with the Shapiro–Wilk test (alpha = 0.05), and comparisons were then made using the Student's *t*-test (confidence level = 0.95). Statistical analyses were performed using SAS Studio, release 3.8 (SAS Institute).

Results

Overall, 6/45 cats (13.3%; 95% CI 5.18–6.82) were positive for hemoplasma species, among which 6/15 (40.0%; 95% CI 7.53–4.47) were free-roaming and 0/30 (0%) were shelter cats (Table 1). A total of four *Amblyomma* species larvae, one *Rhipicephalus sanguineus* adult and five *Amblyomma sculptum* sensu lato nymphs were collected from the free-roaming cats. No molecular investigation was performed on the tick samples.

Sequencing analyses showed that 4/45 samples (8.9%) were infected with CMhm and 2/45 (4.4%) with Mhf species. Each positive amplified PCR product was separated on agarose gel, such that a single band was shown for each sample, which suggested that there was no hemoplasma coinfection. The sequences available in the GenBank database that showed the highest similarity to the sequences of the present study were from Italy (GenBank ID: KR905447.1 [100% identical]; KR905457.1 [97.5% and 99.1%]) and Eurasia (GenBank ID: DQ825456 [99.2%]) for CMhm; and from Thailand (GenBank ID: KU645929.1 [two samples that were 100% identical]) for Mhf. These results are illustrated in the phylogenetic tree (Figure 1).

The situation of being free-roaming was associated with higher hemoplasma positivity (OR infinite; $P < 0.001$) and with flea parasitism (OR 37, 95% CI 4.04–338.92; Fisher's exact test, $P = 0.001$) (Table 2). No significant statistical association was found between hemoplasma qPCR positivity and sex (Fisher's exact test, $P = 0.19$), FIV (Fisher's exact test, $P = 0.26$), or the presence of ticks (Fisher's exact test, $P = 0.25$), *T gondii* (Fisher exact test, $P = 0.06$) or *Rickettsia parkeri* (Fisher's exact test, $P = 0.38$) (Table 1). Since none of the shelter cats were found to be positive for hemoplasmas, no calculations or tests of associated risk factors were performed.

Differences in the mean PCV values were statistically significant (Student's *t*-test, $P = 0.037$), such that the mean PCV was 28.2% (95% CI 20–35) for positive cats and 34.6% (95% CI 20–35) for negative cats (Table 1). Although hemoplasmas have been shown to be opportunistic

Table 1 Feline samples with sex and location information, and packed cell volume (PCV) and quantitative PCR (qPCR) results

Sample	Sex	Location	PCV (%)	qPCR		
				Feline *Mycoplasma* species	FIV	FeLV
1	Female	Shelter	NE	–	–	–
2	Male	Shelter	NE	–	–	–
3	Female	Shelter	NE	–	–	–
4	Male	Free-roaming	NE	+	–	–
5	Male	Free-roaming	NE	–	–	–
6	Female	Free-roaming	40	–	–	–
7	Male	Free-roaming	30	+	–	–
8	Female	Free-roaming	30	–	–	–
9	Female	Free-roaming	30	–	–	–
10	Male	Free-roaming	20	+	+	–
11	Male	Shelter	50	–	–	–
12	Female	Shelter	45	–	NE	NE
13	Female	Shelter	39	–	–	–
14	Male	Shelter	37	–	–	–
15	Female	Shelter	32	–	–	–
16	Female	Shelter	39	–	–	–
17	Female	Shelter	40	–	–	–
18	Female	Shelter	32	–	–	–
19	Male	Shelter	35	–	–	–
20	Male	Shelter	33	–	–	–
21	Male	Shelter	35	–	–	–
22	Female	Shelter	29	–	–	–
23	Male	Shelter	35	–	–	–
24	Male	Shelter	35	–	–	–
25	Male	Shelter	39	–	–	–
26	Male	Shelter	36	–	–	–
27	Female	Shelter	40	–	–	–
28	Female	Shelter	21	–	–	–
29	Female	Shelter	40	–	–	–
30	Female	Shelter	27	–	+	–
31	Male	Shelter	26	–	–	–
32	Female	Shelter	31	–	–	–
33	Female	Shelter	37	–	–	–
34	Female	Shelter	42	–	NE	NE
35	Male	Shelter	40	–	–	–
36	Male	Shelter	31	–	–	–
37	Male	Shelter	NE	–	–	–
38	Male	Free-roaming	30	–	–	–
39	Female	Free-roaming	28	–	–	–
40	Female	Free-roaming	37	–	–	–
41	Male	Free-roaming	31	+	–	–
42	Male	Free-roaming	25	+	–	–
43	Male	Free-roaming	36	–	–	–
44	Female	Free-roaming	35	+	–	–
45	Male	Free-roaming	21	–	–	–

+ = tested positive; – = tested negative; NE = not enough sample; FIV = feline immunodeficiency virus; FeLV = feline leukemia virus

pathogens associated with arthropod-borne diseases,[37] previous studies on the same cat population found anti-*T gondii* (Fisher's exact test, $P=0.06$), anti-*Rickettsia rickettsii* (Fisher's exact test, $P=0.07$) and anti-*R parkeri* ($P=0.38$) antibodies that did not present any associated risk factor for coinfection.[30,31]

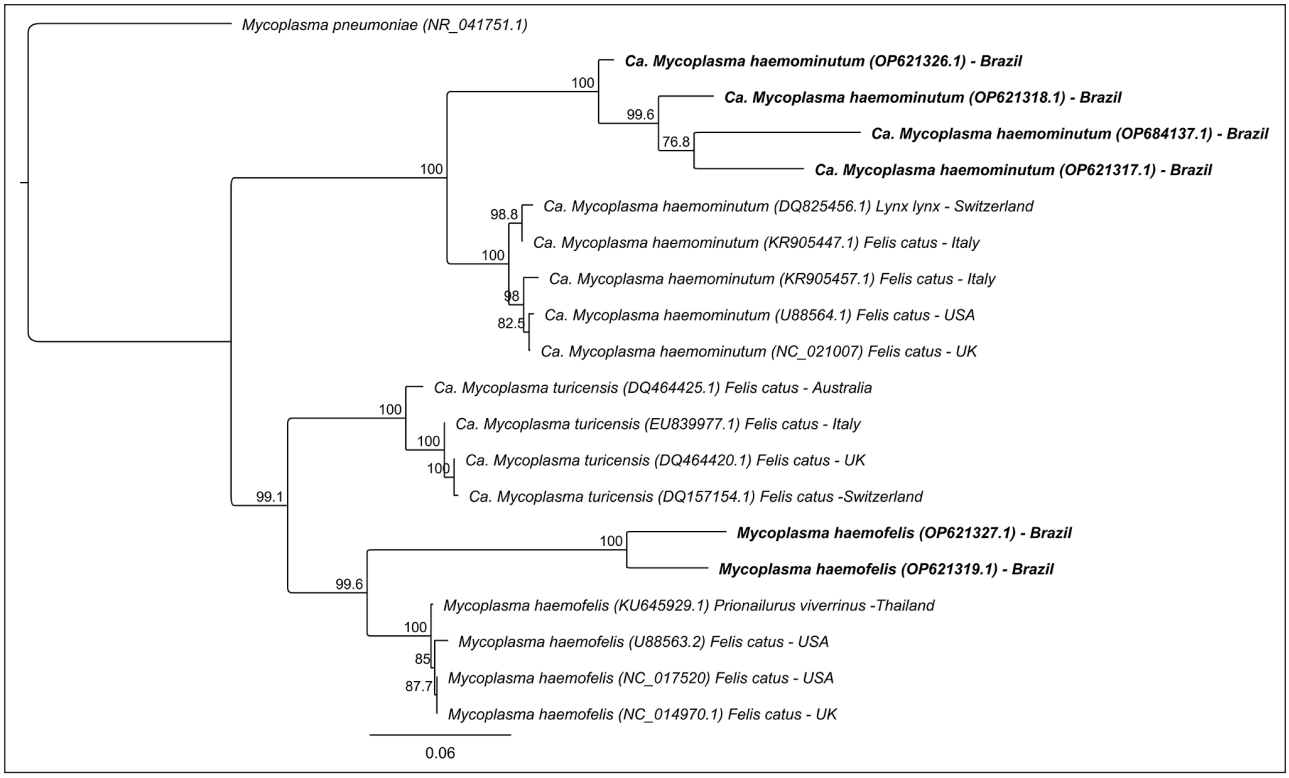

Figure 1 Phylogenetic relationships among 20 hemoplasmas inferred from 16S rDNA gene sequences. The data set was resampled 1000 times. Bootstrap support values ⩾50% are indicated, and the feline hemoplasmas from the present study are highlighted in bold. Species names are followed by GenBank accession number, host and country of origin. The tree was rooted to *Mycoplasma pneumoniae* (GenBank ID: NR_041751.1)

Discussion

The frequency of hemoplasma-positive free-roaming cats (n = 6/45; 13.3%) found in the present study was around twice as high as reported in another study – also conducted in the state of São Paulo – among free-roaming cats within the area of a zoo, in which the frequency was 3/46 (6.5%) among clinically healthy cats.[26] Nevertheless, the frequency found in the present study can still be considered low.

Here, the most frequent hemoplasma species was CMhm (8.9%), followed by Mhf (4.4%). This prevalence corroborates the findings from previous studies, which showed a higher prevalence of CMhm than Mhf among cats, both in Brazil[8,16,26] and in other countries.[18,21,23] In a comparative study, CMhm infection (n = 63/594; 10.6%) was higher than Mhf infection (n = 48/594; 8.1%) among both client-owned and stray cats within a university campus in Madrid, Spain.[14] In addition, a higher hemoplasma prevalence was observed among stray cats.[14] CMhm may cause persistent infection and be less virulent than other species.[38]

Furthermore, the similarity among CMhm and Mhf 16S rDNA sequences reported by the present study, compared with sequences from different continents, suggests poor genetic diversity. However, in this study, and according to most of the published data,[8,16,18,21,23,26] the only sequence analyzed was from the conservative gene 16S rDNA. Although sequence similarities with more than 97% similarity between bacterial isolates based on the 16S rDNA gene suggest that the species are highly correlated,[39] a combination of analyses of multiple genetic markers, such as 23S rRNA and rpoB, is a more suitable option for achieving better phylogenetic characterization.[40,41]

The FIV positivity observed in the present study (4.6%) was lower than that seen in a study on domestic cats in northeastern Brazil (n = 12/200; 6%)[42] and higher than among healthy cats treated in a university animal hospital in southern Brazil (n = 2/91; 2.2%).[43] However, the lack of FeLV detection indicates that there was no circulation of the virus, that the infection was at an abortive stage or that there was low qPCR sensitivity.[29] Lower FIV positivity and no FeLV detection could be explained by the sensitivity of the assay used in the present study since qPCR detection correlates with circulating viral loads, and, in some cats, the viral load may have fallen below the detection limit.[44] In the present study, no statistically significant correlation between coinfection with feline hemoplasma and FIV was detected ($P = 0.26$). Nonetheless, FIV has already been considered to be a

Table 2 Feline hemoplasma infection and associated risk factors among cats on a university campus

Variable	n	Positive for feline hemoplasma (%)	OR	95% CI	*P* value	Reference
Situation	45	6 (13.3)				
In shelter	30	0	–	–	–	This study
Free-roaming	15	6 (40.0)				
Sex	45	6 (13.3)				
Male	23	5 (21.7)	5.83	0.62–54.65	0.19	This study
Female	22	1 (4.6)				
FIV	43	6 (14.0)				
Negative	41	5 (12.2)	7.2	0.39–134.22	0.26	This study
Positive	2	1 (50.0)				
FeLV	43	6 (14.0)				
Negative	43	0	–	–	–	This study
Positive	0	6 (14.0)				
Presence of fleas	45	6 (13.3)				
Yes	6	4 (66.7)	37	4.04–338.92	0.0014	This study
No	39	2 (33.3)				
Presence of ticks	45	6 (13.3)				
Yes	2	1 (16.7)	7.6	0.41–141.54	0.25	This study
No	43	5 (83.3)				
*Toxoplasma gondii**	45	6 (13.3)				
Seropositive	8	3 (37.5)	6.8	1.06–43.48	0.06	Kmetiuk et al[31]
Seronegative	37	3 (8.1)				
*Rickettsia rickettsii**	45	6 (13.3)				
Seropositive	17	0	1.27	1.05–1.54	0.07	Mendes et al[30]
Seronegative	28	6 (13.3)				
*Rickettsia parkeri**	45	6 (13.3)				
Seropositive	18	1 (5.6)	3.86	0.41–36.23	0.38	Mendes et al[30]
Seronegative	27	5 (18.5)				

*Indirect immunofluorescence assay was used to determine serological status[30,31]
OR = odds ratio; CI = confidence interval; FIV = feline immunodeficiency virus; FeLV = feline leukemia virus

significant risk factor for hemoplasma infection among stray and client-owned cats.[14]

In the present study, cats infected with hemoplasma showed significantly lower hematocrit, which was unexpected since CMhm has been described as less pathogenic than Mhf, despite its higher prevalence.[1] In another study, also conducted in southern Brazil, similar results were reported,[16] which suggests that a more pathogenic strain may be circulating in this region of Brazil.

Hematophagous arthropods such as fleas and ticks have been described as natural vectors of hemoplasmas.[2–6] In the present study, flea parasitism was associated with hemoplasma positivity ($P = 0.0014$). The results here are concordant with those from previous research in which fleas positive for hemoplasma were described. Such results highlight the role of fleas as possible vectors.[2,4] Although no molecular tests were performed on the ticks and fleas found in the present study, a previous study showed that hemoplasma DNA was absent or had low prevalence in flea and tick samples.[40] Similar to previous reports,[14] the presence of ticks did not show any significant association with hemoplasma infection ($P = 0.25$) in the present study.

Studies conducted among free-roaming, shelter and owned cats have shown that males are more likely to be infected with hemoplasmas, possibly due their aggressive and exploratory behavior.[14,15,17,21–23] Although no significant association with sex ($P = 0.19$) was found in the present study, this result may have reflected the limited sampled size. Nonetheless, all the hemoplasma-positive cats in the present study were free-roaming, which is in line with previous studies, where it has been shown that stray cat behavior, such as aggressive interactions and potential vector exposure, may be a risk factor for hemoplasma infection.[14,17,25]

The limitation of this study was that only 15 (6.0%) of the estimated population of 250 free-roaming cats and 30/51 (58.8%) of the shelter cats were tested. This may impair extrapolation of the findings to the whole population, particularly with regard to free-roaming cats.[30] This small, free-roaming cat sample was a result of difficulties in catching the cats,[32] together with the potential

risk to life posed by trapping, the temporary duration of permission for sampling from the campus and an excess of cat food offered by visitors to the campus at several (more than 40) campus locations, which strongly competed with cat bait traps. A more representative free-roaming cat sample is needed in future studies, and this should extend to other university campuses.

Conclusions

The results suggest that free-roaming cat populations may present a higher risk of flea infestation and hemoplasma infection. Although hemoplasma infection was not highly prevalent or associated with FIV and FeLV in this population, such coinfections may pose a threat to animal health. Thus, free-roaming cats should be considered to be subject to health and welfare risk, even if provided with food and relatively safe conditions within major university campuses.

Author Note Alexander Welker Biondo is also affiliated to Purdue University, USA.

Acknowledgements The authors thank the Luiz de Queiroz Campus Administration Center (PUSP-LQ) for supporting the study and sample processing, and Dr Taylor W Bailey of Purdue University for reviewing the English language.

Conflict of interest The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding The authors received no financial support for the research, authorship, and/or publication of this article.

Ethical approval The work described in this manuscript involved the use of non-experimental (owned or unowned) animals. Established internationally recognised high standards ('best practice') of veterinary clinical care for the individual patient were always followed and/or this work involved the use of cadavers. Ethical approval from a committee was therefore not specifically required for publication in *JFMS Open Reports*. Although not required, where ethical approval was still obtained, it is stated in the manuscript.

Informed consent Informed consent (verbal or written) was obtained from the owner or legal custodian of all animal(s) described in this work (experimental or non-experimental animals, including cadavers) for all procedure(s) undertaken (prospective or retrospective studies). No animals or people were identifiable within this publication, and therefore additional informed consent for publication was not required.

ORCID iD Alexander Welker Biondo https://orcid.org/0000-0002-4182-5821

References

1 Barker EN. **Update on feline hemoplasmosis.** *Vet Clin North Am Small Anim Pract* 2019; 49: 733–743.
2 Tasker S. **Haemotropic mycoplasma: what's their real significance in cats?** *J Feline Med Surg* 2010; 12: 369–381.
3 Lappin MR. **Update on flea and tick associated diseases of cats.** *Vet Parasitol* 2018; 254: 26–29.
4 Shaw SE, Kenny MJ, Tasker S, et al. **Pathogen carriage by the cat flea *Ctenocephalides felis* (Bouché) in the United Kingdom.** *Vet Microbiol* 2004; 102: 183–188.
5 Hornok S, Meli ML, Perreten A, et al. **Molecular investigation of hard ticks (*Acari: Ixodidae*) and fleas (*Siphonaptera: Pulicidae*) as potential vectors of rickettsial and mycoplasmal agents.** *Vet Microbiol* 2010; 140: 98–104.
6 Taroura S, Shimada Y, Sakata Y, et al. **Detection of DNA of '*Candidatus Mycoplasma haemominutum*' and *Spiroplasma* sp. in unfed ticks collected from vegetation in Japan.** *J Vet Med Sci* 2005; 67: 1277–1279.
7 Willi B, Boretti FS, Cattori V, et al. **Identification, molecular characterization, and experimental transmission of a new hemoplasma isolate from a cat with hemolytic anemia in Switzerland.** *J Clin Microbiol* 2005; 43: 2581–2585.
8 Raimundo JM, Guimarães A, Rodrigues RB, et al. **Hematological changes associated with hemoplasma infection in cats in Rio de Janeiro, Brazil.** *Rev Bras Parasitol Vet* 2016; 25: 441–449.
9 Neimark H, Johansson KE, Rikihisa Y, et al. **Proposal to transfer some members of the genera *Haemobartonella* and *Eperythrozoon* to the genus *Mycoplasma* with descriptions of '*Candidatus* Mycoplasma haemofelis', '*Candidatus* Mycoplasma haemomuris', '*Candidatus* Mycoplasma haemosuis' and '*Candidatus* Mycoplasma wenyonii'.** *Int J Syst Evol Microbiol* 2001; 51: 891–899.
10 Foley JE and Pedersen NC. **'*Candidatus* Mycoplasma haemominutum', a low virulence epierythrocytic parasite of cats.** *Int J Syst Evol Microbiol* 2001; 51: 815–817.
11 Sykes JE, Drazenovich NL, Ball LM, et al. **Use of conventional and real-time polymerase chain reaction to determine the epidemiology of hemoplasma infections in anemic and nonanemic cats.** *J Vet Intern Med* 2007; 21: 685–693.
12 da Costa Vieira RF, Vidotto O, Vieira TSWJ, et al. **Molecular investigation of hemotropic mycoplasmas in human beings, dogs and horses in a rural settlement in southern Brazil.** *Rev Inst Med Trop Sao Paulo* 2015; 57: 353–357.
13 dos Santos AP, Dos Santos RP, Biondo AW, et al. **Hemoplasma infection in HIV-positive patient, Brazil.** *Emerg Infect Dis* 2008; 14: 1922–1924.
14 Díaz-Regañón D, Villaescusa A, Ayllón T, et al. **Epidemiological study of hemotropic mycoplasmas (hemoplasmas) in cats from central Spain.** *Parasit Vectors* 2018; 11: 140. DOI: 10.1186/s13071-018-2740-9.
15 Ravagnan S, Carli E, Piseddu E, et al. **Prevalence and molecular characterization of canine and feline hemotropic mycoplasmas (hemoplasmas) in northern Italy.** *Parasit Vectors* 2017; 10: 132. DOI: 10.1186/s13071-017-2069-9.
16 dos Santos AP, de Oliveira Conrado F, Messick JB, et al. **Hemoplasma prevalence and hematological abnormalities associated with infection in three different cat populations from southern Brazil.** *Rev Bras Parasitol Vet* 2014; 23: 428–434.
17 Bergmann M, Englert T, Stuetzer B, et al. **Risk factors of different hemoplasma species infections in cats.** *BMC Vet Res* 2017; 13. DOI: 10.1186/s12917-017-0953-3.

18 Willi B, Boretti FS, Baumgartner C, et al. **Prevalence, risk factor analysis, and follow-up of infections caused by three feline hemoplasma species in cats in Switzerland.** *J Clin Microbiol* 2006; 44: 961–969.

19 Do T, Kamyingkird K, Bui LK, et al. **Genetic characterization and risk factors for feline hemoplasma infection in semi-domesticated cats in Bangkok, Thailand.** *Vet World* 2020; 13: 975–980.

20 Ghazisaeedi F, Atyabi N, Zahraei Salehi T, et al. **Molecular characterization and phylogenetic analysis of feline hemoplasmas in domestic cats in Iran.** *Vet Res Forum* 2017; 8: 67–73.

21 Tanahara M, Miyamoto S, Nishio T, et al. **An epidemiological survey of feline hemoplasma infection in Japan.** *J Vet Med Sci* 2010; 72: 1575–1581.

22 Stojanovic V and Foley P. **Infectious disease prevalence in a feral cat population on Prince Edward Island, Canada.** *Can Vet J* 2011; 52: 979–982.

23 Sykes JE, Terry JC, Lindsay LL, et al. **Prevalences of various hemoplasma species among cats in the United States with possible hemoplasmosis.** *J Am Vet Med Assoc* 2008; 232: 372–379.

24 Vergara RW, Galleguillos FM, Jaramillo MG, et al. **Prevalence, risk factor analysis, and hematological findings of hemoplasma infection in domestic cats from Valdivia, Southern Chile.** *Comp Immunol Microbiol Infect Dis* 2016; 46: 20–26.

25 de Bortoli CP, André MR, Seki MC, et al. **Detection of hemoplasma and *Bartonella* species and co-infection with retroviruses in cats subjected to a spaying/neutering program in Jaboticabal, SP, Brazil.** *Rev Bras Parasitol Vet* 2012; 21: 219–223.

26 André MR, Baccarim Denardi NC, Marques de Sousa KC, et al. **Arthropod-borne pathogens circulating in free-roaming domestic cats in a zoo environment in Brazil.** *Ticks Tick Borne Dis* 2014; 5: 545–551.

27 Munhoz AD, Simões IGPC, Calazans APF, et al. **Hemotropic mycoplasmas in naturally infected cats in northeastern Brazil.** *Rev Bras Parasitol Vet* 2018; 27: 446–454.

28 de Santis ACGA, Herrera HM, de Sousa KCM, et al. **Molecular detection of hemotrophic mycoplasmas among domiciled and free-roaming cats in Campo Grande, state of Mato Grosso do Sul, Brazil.** *Brazilian J Vet Parasitol* 2014; 23: 231–236.

29 Hartmann K. **Clinical aspects of feline retroviruses: a review.** *Viruses* 2012; 4: 2684–2710.

30 Mendes JCR, Kmetiuk LB, Martins CM, et al. **Serosurvey of *Rickettsia* spp. in cats from a Brazilian spotted fever-endemic area.** *Rev Bras Parasitol Vet* 2019; 28: 713–721.

31 Kmetiuk LB, Pellizzaro M, Yamakawa AC, et al. **Occurrence of antibodies anti-*Toxoplasma gondii* among sheltered and free-roaming cats within a university campus.** *Semin Ciências Agrárias* 2021; 42: 1685–1694.

32 Cowen S, Clausen L, Algar D, et al. **Using genetics to evaluate the success of a feral cat (*Felis catus*) control program in north-western Australia.** *Animals* 2019; 9. DOI: 10.3390/ani9121050.

33 Martins TF, Onofrio VC, Barros-Battesti DM, et al. **Nymphs of the genus *Amblyomma* (*Acari: Ixodidae*) of Brazil: descriptions, redescriptions, and identification key.** *Ticks Tick Borne Dis* 2010; 1: 75–99.

34 Criado-Fornelio A, Martinez-Marcos A, Buling-Saraña A, et al. **Presence of *Mycoplasma haemofelis, Mycoplasma haemominutum* and piroplasmids in cats from southern Europe: a molecular study.** *Vet Microbiol* 2003; 93: 307–317.

35 Tandon R, Cattori V, Gomes-Keller MA, et al. **Quantitation of feline leukaemia virus viral and proviral loads by TaqMan® real-time polymerase chain reaction.** *J Virol Methods* 2005; 130: 124–132.

36 Hoelzle K, Winkler M, Kramer MM, et al. **Detection of *Candidatus* Mycoplasma haemobos in cattle with anaemia.** *Vet J* 2011; 187: 408–410.

37 André MR, Filgueira KD, Calchi AC, et al. **Co-infection with arthropod-borne pathogens in domestic cats.** *Rev Bras Parasitol Vet* 2017; 26: 525–531.

38 Sacristán I, Acuña F, Aguilar E, et al. **Assessing cross-species transmission of hemoplasmas at the wild-domestic felid interface in Chile using genetic and landscape variables analysis.** *Sci Rep* 2019; 9: 1–14. DOI: 10.1038/s41598-019-53184-4.

39 Drancourt M and Raoult D. **Sequence-based identification of new bacteria: a proposition for creation of an orphan bacterium repository.** *J Clin Microbiol* 2005; 43: 4311–4315.

40 Pontarolo GH, Kühl LF, Pedrassani D, et al. **'*Candidatus* Mycoplasma haemoalbiventris', a novel hemoplasma species in white-eared opossums (*Didelphis albiventris*) from Brazil.** *Transbound Emerg Dis* 2021; 68: 565–572.

41 Kim KS, Ko KS, Chang MW, et al. **Use of rpoB sequences for phylogenetic study of *Mycoplasma* species.** *FEMS Microbiol Lett* 2003; 226: 299–305.

42 Lacerda LC, Silva AN, Freitas JS, et al. **Feline immunodeficiency virus and feline leukemia virus: frequency and associated factors in cats in northeastern Brazil.** *Genet Mol Res* 2017. DOI: 10.4238/gmr16029633.

43 Biezus G, Machado G, Ferian PE, et al. **Prevalence of and factors associated with feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) in cats of the state of Santa Catarina, Brazil.** *Comp Immunol Microbiol Infect Dis* 2019; 63: 17–21.

44 Crawford PC and Levy JK. **New challenges for the diagnosis of feline immunodeficiency virus infection.** *Vet Clin North Am Small Anim Pract* 2007; 37: 335–350.