Parasitology Research (2022) 121:2561–2570
https://doi.org/10.1007/s00436-022-07602-8

PROTOZOOLOGY - ORIGINAL PAPER

Piroplasmid infection is not associated with clinicopathological and laboratory abnormalities in cats from Midwestern Brazil

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Received: 11 February 2022 / Accepted: 14 July 2022 / Published online: 25 July 2022

Abstract

Feline piroplasmids include the genera *Babesia* spp., *Cytauxzoon* spp., and *Theileria* spp. In Brazil, there are few reports regarding these hemoprotozoans; however, clinicopathological and molecular data are scarce. This study aimed to characterize the clinical relevance of these parasites through hematological, biochemical, and molecular approaches. For this purpose, 166 cats from Brasilia, Federal District, Midwestern Brazil, were screened using a quantitative polymerase chain reaction (qPCR) for piroplasmids based on the LSU4 mitochondrial gene, which resulted in an overall prevalence of 36/166 (21.7%). Twelve of 166 samples (7.2%) were positive for *C. felis*, while 19/166 (11.4%) were positive for *Babesia vogeli*. No samples tested positive for *Theileria* spp. *Babesia vogeli* and *Cytauxzoon* spp. LSU4 sequences showed identities of 97–100% and 99.3%, respectively, to US isolates. The hematological and biochemical findings did not differ significantly between the cats that tested positive and negative for piroplasmids. Although the lack of abnormalities in clinical and laboratory parameters does not eliminate the possibility that these cats were sick and recovered, it may suggest that the Brazilian strain of *Cytauxzoon* spp. is not as pathogenic as that from the USA, despite the high molecular identity with North American isolates.

Keywords Feline · Piroplasmida · *Babesia vogeli* · *Cytauxzoon* spp. · Hematology · Biochemistry

Introduction

Piroplasmids are among the most important tick-borne agents of domestic and wild animals worldwide (Mehlhorn and Schein 1993; Uilenberg, 2006; Jalovecka et al. 2018). The classification of piroplasmids is no longer based only on their morphological features and involved vector, since advances in molecular techniques and phylogenetic analyses have helped to elucidate the relationship among these agents (Lack et al. 2012; Schreeg et al. 2016; Schnittger et al. 2022). Feline piroplasmids (phylum Apicomplexa, order Piroplasmida) include the genera *Babesia* spp., *Theileria* spp., and *Cytauxzoon* spp. (Alvarado-Rybak et al. 2016).

Even though *Babesia* spp. have been reported in domestic cats in almost all continents, except the Antarctic and Australia (Penzhorn and Oosthuizen 2020), to date, clinical disease has only been observed in cats from South Africa (Bosman et al. 2007, 2013, 2019). The reported molecular prevalence rates range from 0.8% in Italy (Spada et al. 2014) to 39.5% in Thailand (Do et al. 2021). Clinical disease has been observed in cats infected by different *Babesia* species,

Section Editor: Domenico Otranto

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namely *B. felis*, *B. leo*, *B. microti*, *B. lengau*, and Western Cape strain of *Babesia* sp., and is characterized by lethargy, anemia, fever, icterus, and neurological signs (Bosman et al. 2013). *Babesia vogeli* has been described in apparently healthy cats from Qatar (Alho et al. 2017), the Caribbean (Kelly et al. 2017), Thailand (Simking et al. 2010), Trinidad and Tobago (Georges et al. 2008), Portugal (Maia et al 2014), and Brazil (André et al., 2014, 2015, 2022; Malheiros et al 2016).

Cytauxzoon spp. has been reported in cats from North and South America, Africa, Asia, and Europe (Wang et al., 2017). Among the *Cytauxzoon* spp. species that have been identified thus far, *C. felis* harbors the most concerns as it causes fatal diseases in cats, primarily in the USA (Meier and Moore., 2000; Birkenheuer et al., 2006; Sherrill and Cohn, 2015; Qurollo 2019; Wikander et al. 2020a). Although there is an increasing number of cytauxzoonosis cases reported from other continents over the last decade, it is not always clear what role *Cytauxzoon* spp. play in the clinical findings (Varshney et al. 2009; Maia et al. 2013; Carli et al. 2014; Legroux et al. 2017; Nentwig et al. 2018; Zou et al. 2019). In the USA, bobcats (*Lynx rufus*) are considered the primary wildlife reservoir host for *C. felis* with a molecular prevalence as high as 60–79% in some states (Shock et al. 2011; Zieman, 2017). Similarly in Brazil, *Cytauxzoon* spp. appear to be significantly more prevalent in wild felids (e.g., jaguars and ocelots) than in domestic cats (André et al. 2009, 2014, 2015; Furtado et al. 2017).

Recently, *Theileria* sp. has emerged as the third piroplasmid species occurring in cats, even though the clinical significance is still unknown. Until now, *Theileria* spp. has only been molecularly detected in cats from Brazil (André et al. 2014; 2015) and Chile (Sacristán et al., 2019).

Despite the detection of piroplasmids in cats from Brazil (Maia et al. 2013; André et al. 2014, 2015, 2017, 2022; Malheiros et al. 2016; Pedrassani et al. 2019; Raimundo et al. 2021), the hematological and biochemical abnormalities associated with these infections have not been assessed to date. In addition, molecular data are still incipient and based only on short fragments of the 18S rRNA gene. Therefore, in order to shed some light on the clinical significance of piroplasmid infection in cats from Brazil, the present work aimed to investigate the occurrence and clinicopathological disorders as well as molecular features associated with piroplasmid infection in cats from Midwestern Brazil.

Material and methods

Animals and sampling sites

This study was approved by the Ethics Committee of the University of Brasilia, under the protocol number UnB Doc 40/2017. Between June 2016 and September 2017, 166 domestic cats (*Felis catus*) were selected by convenience from animals attended in the urban area of Brasília (15° 47′ 38″ S, 47° 52′ 58″ O) from private clinics of the Federal District (FD) or the Veterinary Hospital of the University of Brasilia, regardless of age, sex, breed, and health status. All owners were required to fill out a form regarding the reason for the appointment, the habits of each animal, and the presence of ectoparasites, as well as the housing type (house or apartment), outdoor access, and contact with other dogs or cats. Unfortunately, we were not able to obtain epidemiological data from all cats.

Hematological and biochemical analysis

Blood samples were collected from all cats, either from cephalic or femoral veins, into ethylenediaminetetraacetic acid (EDTA)–coated tubes for complete blood count (CBC) and DNA extraction, and tubes containing a clotting activator (serum samples) for biochemical analysis. All the hematological and biochemical analyses were performed at the Veterinary Clinical Pathology Laboratory, from the College of Agronomy and Veterinary Medicine, University of Brasilia, Brasilia, DF.

The CBC and the concentration of hemoglobin were obtained using an automatic cell counter (ABC Vet Horiba® ABX Diagnostics, Brazil). The packed cell volume (PCV) was determined by microhematocrit centrifugation. Mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were calculated. Plasma protein concentration was determined by refractometry (models SZJ-D and RTP-20 ATC). Differential leukocyte counts were obtained by direct observation of 100 leukocytes in Diff-Quick (Newprov®) stained blood smears using a light microscope (CX40RF200, Olympus, Japan). All blood smears were checked for the presence of platelet aggregates and hemoparasite inclusions. For checking parasite inclusions, a screening at low magnification (40 × objective lens) was done initially. Then, the blood smear was examined at least in 300 fields using the 100 × oil immersion objective, selecting an area that was well stained, free of stain precipitate, and well populated with red blood cells.

Serum samples were analyzed for the activity of alanine aminotransferase (ALT), alkaline phosphatase (ALP), total serum protein, albumin, gamma-glutamyl transferase (GGT), urea, and creatinine in an automatic biochemistry analyzer (Cobas c111 Roche®). FIV and FeLV tests were processed using Idexx® manufactured kits.

The reference interval of CBC and biochemistry analysis used in our lab is in accordance with Weiss and Wardrop (2011), and Kaneko et al. (2008), respectively. Hematological abnormalities were considered anemia (PCV < 24%, and/or red blood cells < 5.0×10^6/μL and/or hemoglobin < 8.0 g/

dL), leukopenia (white blood cells < 5.500×10^3/μL), or thrombocytopenia (platelets < $300{,}000 \times 10^3$/μL).

Molecular analysis

DNA extraction

DNA extraction was performed at the Veterinary Molecular Biology Laboratory from the College of Agronomy and Veterinary Medicine, University of Brasilia, Brasilia, Federal District (FD). EDTA-whole blood was stored at 4–8 °C for no more than 7 days before the extraction step. DNA was extracted from blood samples using a commercial kit (Blood Genomic Prep Mini Spin Kit, Promega Corporation®, WI, EUA), according to the manufacturer's recommendations. The DNA sample concentration and quality were evaluated by optical spectrophotometry (Nanodrop, Thermo Scientific®). DNA was stored at − 20 °C until PCR analysis.

PCR assays

The quantitative PCR assays (qPCR) were performed at the Vector-Borne Disease Diagnostic Laboratory (College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA). All samples were then screened for the presence of piroplasmid DNA using a broad-range qPCR assay targeting a 108–173 bp fragment of the mitochondrial large subunit (mtLSU) DNA (Qurollo et al., 2017). The qPCR was performed using the primers BAB-LSU4 F (ACCTGT CAARTTCCTTCACTAAMTT), BMIC-LSU4 F (TTGCGA TAGTAATAGATTTACTGC), and BAB-LSU R (TCTTAA CCCAACTCACGTACCA). Briefly, the amplification reaction was performed using the Thermocycler Biorad CFX96 Real-Time System C1000 Touch. The qPCR assays contained 12.5 μL of SSO Advanced SYBR Universal Supermix 2×(BioRad, Hercules, USA), 5 μL DNA template, 0.3 μL of BAB primers (0.6 μM), 0.2 μL of BMIC primer (0.4 μM), and molecular grade water to a final volume of 25 μL. The amplification protocol used was as follows: 3 min at 98 °C, followed by 40 cycles of 15 s at 98 °C, 15 s at 60 °C, and 15 s at 72 °C. The melting curve was acquired using 0.5 °C steps, with holds of 2 s, from 65 to 95 °C. The results were assessed through observation of amplification and melting curves. In all qPCR assays, plasmids encoding mt LSU fragments of *B. microti*-like (GenBank access number KC207827) and *B. rossi* (KC207823.1) were used as positive controls. DNA from a dog negative for vector-borne agents and ultrapure sterilized water (Sigma-Aldrich Inc., Germany) was used as negative and no template control (NTC), respectively. All amplicons were subjected to electrophoresis in a 2% agarose gel stained with *GelRed® Nucleic Acid* Gel Stain (Biotium, Inc., US), regardless of the presence or absence of amplification curves on qPCR. Only samples that showed valid sequencing results, previously positive on qPCR or at least on gel electrophoresis, were considered positive. Thus, the samples were classified into the following groups: positive group (all samples that were positive for *Cytauxzoon* spp. or *Babesia* spp.); negative group (any negative results); *Babesia* group (only positive for *Babesia* spp.); or *Cytauxzoon* group (only positive for *Cytauxzoon* spp.). A housekeeping PCR targeting the glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) gene was performed to confirm the presence of mammal genomic DNA and rule out the presence of PCR inhibitors (Birkenheuer et al., 2003) for all samples of this study.

Amplicon sequencing and BLAST analysis

Amplicons from all qPCR-positive samples were directly submitted (without purification) for bidirectional Sanger sequencing to confirm the results (GENEWIZ, Inc., Raleigh, NC). Geneious Prime (v.2020.0.3) was used to align and analyze DNA results with reference sequences from GenBank. The primer regions were manually trimmed. Identity, query coverage, and *e*-values were assessed by the BLASTn tool (using default parameters), available in the NCBI GenBank database (Altschul et al., 1990).

Statistical analysis

The effects of age, test result, sex, type of residence, previous life on the street, time in shelters, contact with dogs, results of FIV and FeLV tests, and ectoparasites on the test result (positive or negative) were tested using a general linear model (PROC GLM) and means compared using Duncan's multiple range test, with $P < 0.05$ used as a significant difference. Transformations by logarithm were carried out if the coefficient of variance was greater than 25%. The effect of age, test result, sex, type of residence, previous life on the street, time in shelters, contact with dogs, results of FIV and FeLV tests, and ectoparasites of the animal on the test outcome (0 = negative and 1 = positive) were evaluated using logistic regression (PROC LOGISTIC). A chi-square test of frequencies was used to see the effect of the test result on age, test result, sex, type of residence, previous life on the street, time in shelters, contact with dogs, results of FIV and FeLV tests, and ectoparasites, anemia, leukopenia, and thrombocytopenia (PROC FREQ). All data were analyzed in SAS (Statistical Analysis System Institute, Cary, NC).

Results

Epidemiological and clinical findings

The overall molecular prevalence for piroplasmids assessed by LSU-based qPCR was 36/166 (21.7%). Out of the 36

positive cats, *Cytauxzoon* spp. were detected in 12/166 (7.2%) animals and 19/166 (11.4%) were positive for *Babesia* sp. None of the cats was positive for *Theileria* spp. by qPCR analysis. No co-infections were detected. Some hematological and biochemical data are lacking, mainly due to the small volume or blood obtained during collection, preventing complete analysis, or due to the presence of clots in these samples. Figure 1 compares our data with all other piroplasmid reports that have been described so far in Brazil.

No clinical parameters were significantly different between positive versus negative animals, even when compared with the groups positive for *Babesia* spp. or *Cytauxzoon* spp. Interestingly, we did not find a high frequency of ectoparasites in this study, even for the positive groups (Table 1). A summary of the clinicopathologic features of the piroplasmid-positive cats is shown in Supplementary material 1.

Hematological and biochemistry profile

Similar to the clinical findings, there was no significant difference between the positive and negative groups in the blood test profile (Table 2), and also regarding the biochemistry panel. Concerning anemia, leukopenia, and thrombocytopenia frequencies, no significant differences were found between the positive and negative groups (Table 3), or even for the comparison between the *Babesia* and *Cytauxzoon* groups (Table 4). Intra-erythrocytic inclusions suggestive of piroplasmids were not found in any sampled blood smears.

Sequencing analysis

Thirty-one LSU sequences were obtained (GenBank access numbers — *Babesia vogeli*: OM502556, OM502557, OM502558, OM502559, OM502560, OM502561, OM502562, OM502563; *Cytauxzoon felis*: OM502564, OM502565). All sequences shared 97–100% identity to either *B. vogeli* (GenBank accession number KC207825.1) or 12 sequences of *C. felis* (KC207821.1). The identity among the *Babesia* sequences obtained in this study ranged from 99.3 to 100%, while *Cytauxzoon* spp. sequences identified in this study shared 99.3% nucleotide identity with each other. Cycle quantification (Cq) values found relatively low parasitemia and the Cq ranged from 33.32 to 39.62, with 50% of samples with Cq values higher than 36. The melting temperature (T*m*) for *Babesia vogeli* sequences was 76.4 (standard deviation: 0.464), and the Tm for the *Cytauxzoon* spp. sequences was 77.0 (standard deviation: 0.300) (Table 5). The *Tm* values obtained in this study were consistent with previously published data (Qurollo et al. 2017).

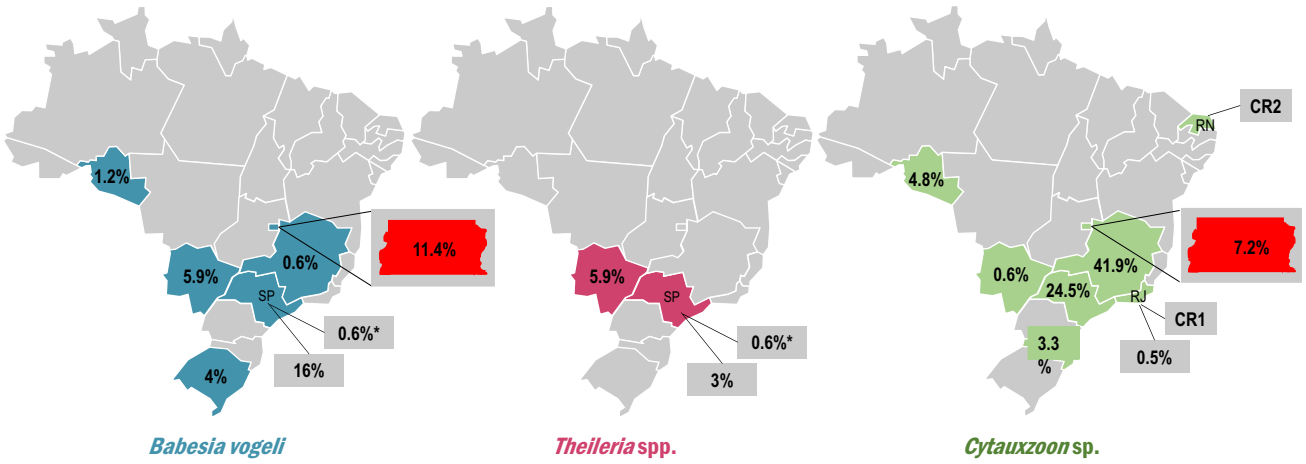

Fig. 1 Comparison of the occurrence of piroplasmids in domestic cats reported so far in Brazil. Legend: In blue, pink, and green, the occurrence from previous studies in Brazil of *Babesia vogeli* (André et al., 2014, 2015, 2022; Malheiros et al., 2016), *Theileria* spp. (André et al., 2014; 2015; 2022), and *Cytauxzoon* spp. (Maia et al., 2013; André et al., 2015, 2017, 2022; Pedrassani et al., 2019; Raimundo et al., 2021), respectively. In red is the occurrence found in the present study. Notes: (1) The state of São Paulo (SP) has two different reports of *Babesia vogeli* or *Theileria* spp. in domestic cats: the first report was described by André et al. (2014), with an occurrence of 16% for *Babesia vogeli* and 3% for *Theileria* spp. Furthermore, a recent article by André et al. (2022) found 0.6% positivity for *Babesia vogeli* or *Theileria* spp., as the study failed to differentiate the two agents (*). (2) The state of Rio de Janeiro (RJ) has two different reports of *Cytauxzoon* sp.: the first one, it was found occurrence of 0.5%. Another study described a case report (CR), with only 1 case detected (CR1), precluding data on the occurrence of *Cytauxzoon* sp. (3) The state of Rio Grande do Norte (RN) registered only two cases of *Cytauxzoon* sp. (CR2)

Table 1 Frequency data on piroplasmid positive versus negative cats epidemiological features

Variable/total	Negative cats, *n* (%)	Positive cats, *n* (%)		
		Overall	*Babesia vogeli*	*Cytauxzoon* spp.
Sex	131 (81.4)	30 (18.6)	18 (60.0)	12 (40.0)
Female	26 (19.8)	5 (16.7)	3 (16.6)	2 (16.6)
Spayed female	36 (27.5)	6 (20.0)	5 (27.7)	1 (8.3)
Male	25 (19.1)	6 (20.0)	4 (22.2)	2 (6.6)
Spayed male	44 (33.6)	13 (43.3)	6 (33.3)	7 (58.3)
Age (years)	126 (80.2)	27 (17.3)	16 (59.2)	11 (40.7)
0–1	42 (32.5)	3 (11.1)	1 (6.2)	2 (18.1)
> 1–2	14 (10.8)	5 (18.5)	3 (18.7)	2 (18.1)
> 2–7	31 (24.0)	9 (3.3)	5 (55.5)	4 (36.3)
> 7	42 (32.5)	10 (7.0)	7 (43.7)	3 (27.2)
Type of residence	126 (81.3)	29 (18.7)	18 (62.0)	11 (37.9)
House	49 (38.9)	12 (41.4)	8 (44.4)	4 (36.3)
Apartment	75 (59.5)	17 (58.6)	10 (55.5)	7 (63.6)
Hotel	1 (0.8)	0	0	0
Farm	1 (0.8)	0	0	0
Lifestyle	127 (80.8)	30 (19.1)	18 (60.0)	12 (40.0)
Outdoor	21 (16.5)	6 (20.0)	3 (16.6)	3 (25.0)
Indoor	106 (83.4)	24 (80.0)	15 (83.3)	9 (75.0)
Origin from a shelter?	112 (80.0)	28 (20.0)	16 (57.1)	12 (42.8)
Yes	55 (49.1)	10 (35.71)	8 (50.0)	2 (16.6)
No	57 (50.9)	18 (64.9)	8 (50.0)	10 (83.3)
Contact with other cats?	127 (80.8)	30 (19.1)	18 (60.0)	12 (40.0)
Yes	94 (74.0)	21 (70.0)	13 (72.2)	8 (66.6)
No	33 (25.9)	9 (30.0)	5 (27.7)	4 (33.3)
Contact with dogs?	126 (81.2)	29 (18.7)	17 (58.6)	12 (41.3)
Yes	30 (23.8)	4 (13.7)	4 (23.5)	0
No	96 (76.1)	25 (86.2)	13 (76.4)	12 (100.0)
Ectoparasites?	127 (80.9)	30 (19.1)	18 (60.0)	12 (40.0)
Yes	15 (11.8)	3 (10.0)	1 (5.5)	2 (16.6)
No	91 (71.7)	22 (73.3)	13 (72.2)	9 (75.0)
Not observed	21 (16.5)	5 (16.7)	4 (22.2)	1 (8.3)
FIV test	127 (80.9)	30 (19.1)	18 (60.0)	12 (40.0)
Positive	0	1 (3.3)	1 (5.5)	0
Negative	79 (62.2)	17 (56.7)	10 (55.5)	7 (58.3)
Not tested	48 (37.8)	12 (40.0)	7 (38.8)	5 (41.6)
FeLV test	127 (80.9)	30 (19.1)	18 (60.0)	12 (40.0)
Positive	10 (7.9)	2 (6.7)	1 (5.5)	1 (8.3)
Negative	69 (54.3)	16 (53.3)	10 (55.5)	6 (50.0)
Not tested	48 (37.8)	12 (40.0)	7 (38.8)	5 (41.6)

The totals of each column and line encompass missing data; therefore, some features do not add up to 100% or 166 samples

Discussion

In this study, we characterized the infection by piroplasmids in cats from Midwestern Brazil, combining, for the first time, epidemiological, clinicopathological, and molecular approaches. The lack of abnormalities in clinicopathological parameters allows us to infer important points. Firstly, we did not find a high frequency of ectoparasites on the cats in this study, including the group of positive cats. This could indicate that the infection was transmitted by ectoparasites at a prior date and was no longer present on the cats or perhaps these cats were infected by an alternate means of transmission such as direct cat to cat (e.g., vertical or horizontal transmission) (Jefferies et al. 2007; Yeagley et al.

Table 2 Mean values of the hematologic and biochemistry data on piroplasmid negative and positive cats

Variable	Negative cats	Positive cats	R2	CV
Hematologic features				
Red blood cells ($\times 10^6$/μL)	8.2	7.9	0.105361	18.73
Hemoglobin (g/dL)	12.2	12.1	0.097027	17.05
Hematocrit (%)	36.1	35.9	0.103486	18.26
MCV (fL)	44.1	46.7	0.142121	6.60
MCHC (%)	33.9	33.8	0.067015	5.42
White blood cells ($\times 10^3$/μL)	12.905	11.893	0.127745	37.05
Band neutrophils (/μL)	104	45	0.169910	658.55
Segmented neutrophils (/μL)	8.880	8.993	0.356266	79.36
Lymphocytes (/μL)	3.063	2.162	0.192080	86.21
Monocytes (/μL)	201	109	0.092897	240.18
Eosinophils (/μL)	613	520	0.174782	113.79
Basophils (/μL)	123	64	0.113875	335.43
Platelets ($\times 10^3$/μL)	299.443	337.600	0.115101	44.43
Total plasma protein (g/dL)	7.5	7.7	0.184556	9.34
Biochemistry				
ALT (UI/L)	85.3	83.4	0.175915	135.67
Alkaline phosphatase ALP (UI/L)	46.6	65.2	0.318110	165.69
Creatinine (mg/dL)	1.5	1.6	0.241948	25.42
Urea (mg/dL)	73	74	0.336914	77.28
Total protein (g/dL)	7.5	8.0	0.280317	14.47
Albumin (g/dL)	3.1	2.7	0.477411	24.75
Gamma glutamyltransferase (UI/L)	3.7	3.5	0.377943	88.17

R2, coefficient of determination; *CV*, coefficient of variation

Table 3 Frequency data on piroplasmid negative and positive cats versus hematological abnormalities

qPCR or gel result/ variable, *n* (%)	Anemia		Leukopenia		Thrombocytopenia	
	No	Yes	No	Yes	No	Yes
Negative cats	118 (95.1)	6 (4.8)	115 (92.7)	9 (7.2)	57 (46.7)	65 (53.2)
Positive cats	28 (93.3)	2 (6.6)	29 (96.6)	1 (3.3)	19 (63.3)	11 (36.6)
Total	146 (94.8)	8 (5.1)	144 (93.5)	10 (6.4)	76 (50.0)	76 (50.0)

Table 4 Frequency data on piroplasmid results versus hematological abnormalities

qPCR or gel result/ variable, *n* (%)	Anemia		Leukopenia		Thrombocytopenia	
	No	Yes	No	Yes	No	Yes
Babesia vogeli	17 (94.4)	1 (5.5)	17 (94.4)	1 (5.5)	13 (72.2)	5 (27.7)
Cytauxzoon spp.	11 (91.6)	1 (8.3)	12 (100.0)	0	6 (50.0)	6 (50.0)
Total	28 (93.3)	2 (6.6)	29 (96.6)	1 (3.3)	19 (63.3)	11 (36.6)

2009; Saleh et al. 2021) or even ingestion of ticks or infected tissues (Hornok et al. 2015, 2016; de Sousa et al. 2018; Corduneanu et al. 2019, 2020). Interestingly, the vector(s) of *Cytauxzoon* spp. (André et al. 2015; de Sousa et al. 2018) and *B. vogeli* (Hartmann et al. 2013) in cats from Brazil remains unknown.

We found a moderate molecular prevalence of *Cytauxzoon* spp. (7.2%; 12/166) in domestic cats from Brazil. Previously, only 3.3% (1/30) in cats from Southern Brazil (Pedrassani et al. 2019) and 0.6% in Mato Grosso do Sul, also in Midwestern Brazil (André et al. 2015), tested positive for *Cytauxzoon* spp. Recently, a high prevalence (41.9%) for *Cytauxzoon* spp. was reported among cats in the state of Minas Gerais, Brazil (André et al. 2022). Regarding *B. vogeli*, 11.4% (19/166) of the cats in our study tested positive, a similar rate to a previous study from Southeast Brazil 16% (6/37), and higher than other places in the country (André et al. 2015, 2022; Malheiros

Table 5 Cycle of quantification (Cq) values, melting temperature (Tm), and sequencing results of piroplasmid sequences detected in cats from Brasilia, Federal District, Brazil, using LSU4-qPCR and electrophoresis gel

Sample's number	Sequencing results LSU	LSU4 Cq	Tm (°C)
1	*Babesia vogeli*	33.32	76.0
2	*Babesia vogeli*	33.89	76.5
3	*Babesia vogeli*	34.44	76.5
4	*Cytauxzoon felis*	34.73	77.0
5	*Babesia vogeli*	35.07	76.0
6	*Babesia vogeli*	35.44	76.0
7	*Babesia vogeli*	35.57	76.5
8	*Cytauxzoon felis*	35.58	77.0
9	*Babesia vogeli*	35.64	76.5
10	*Cytauxzoon felis*	35.86	77.0
11	*Cytauxzoon felis*	36.42	77.0
12	*Cytauxzoon felis*	36.55	77.0
13	*Cytauxzoon felis*	36.88	77.0
14	*Babesia vogeli*	37.31	77.0
15	*Babesia vogeli*	37.33	75.5
16	*Babesia vogeli*	38.01	76.5
17	*Babesia vogeli*	38.13	76.5
18	*Cytauxzoon felis*	38.55	77.0
19	*Babesia vogeli*	38.57	76.5
20	*Cytauxzoon felis*	38.84	77.0
21	*Babesia vogeli*	39.11	76.5
22	*Babesia vogeli*	39.30	76.5
23	*Cytauxzoon felis*	39.32	77.5
24	*Babesia vogeli*	39.46	77.5
25	*Babesia vogeli*	39.57	77.0
26	*Cytauxzoon felis*	39.62	76.5
27	*Babesia vogeli*	*	-
28	*Cytauxzoon felis*	*	-
29	*Babesia vogeli*	*	-
30	*Babesia vogeli*	*	-
31	*Cytauxzoon felis*	*	77.5

Tm, melting temperature

et al. 2016). These worldwide differences may be related to epidemiological features associated with vectors (species, prevalence, rate of infection, and attractivity to cat infestation in each studied region) (Hamel et al. 2012), environmental conditions (geographic variation) (Díaz-Regañón et al. 2017), the lifestyle of sampled cats (indoor versus outdoor cats), diagnostic methods (quantitative PCR (qPCR) or conventional PCR (cPCR)) (Gadkar and Filion 2014; Persichetti et al. 2016; Qurollo et al. 2017; Do et al. 2021), phase of infection (molecular tests provide evidence of likely active infection, and fluctuating parasitemia in carrier cats could affect the identification of reservoir hosts (Kidd, 2019; Wikander et al. 2020a), as well as the sample size (surveys with a high number versus a low number of susceptible animals) used in each study (Do et al. 2021).

To the best of our knowledge, this is the first study performed in Brazil that associated molecular detection of piroplasmids with clinical and laboratory findings in cats. The positive group for *Babesia vogeli* presented as asymptomatic animals and without laboratory abnormalities. Previous studies have also reported apparently healthy cats infected with *B. vogeli* (Georges et al. 2008; Maia et al. 2014; Alho et al. 2017; André et al. 2022), which suggests that this piroplasmid may be non-pathogenic in cats. Ultimately, that could mean *Babesia vogeli* infection in domestic cats is probably a consequence of the presence of the cosmopolitan vector *Rhipicephalus sanguineus* sharing places with both dogs and cats (André et al. 2022).

Concerning *Cytauxzoon* sp., our clinicopathologic findings were more like those describing cats infected with *Cytauxzoon* spp. in Europe (Carli et al., 2012; Carli et al., 2014; Díaz-Regañón et al., 2017) than they were to cats with acute cytauxzoonosis in the USA (Birkenheuer et al., 2006; Hartmann et al., 2013; Sherrill and Cohn, 2015; Lloret et al., 2015). This could support a different strain or species of *Cytauxzoon* in South America that is less pathogenic than *C. felis* from North America. That hypothesis corroborates a recent study that described a high occurrence of *Cytauxzoon* in Brazil in apparently asymptomatic domestic cats (André et al., 2022), and Moghaddam et al. (2020), which suggested that these cats might have been infected by a less pathogenic strain of *Cytauxzoon* as well. However, it is noteworthy that our study did not target acutely ill cats. Indeed, while specific clinical and clinicopathologic abnormalities are not explicitly described, it is known that *C. felis* infections can be detected in asymptomatic apparently healthy cats with a high frequency in regions of the USA where *C. felis* is endemic (Haber et al., 2007; Rizzi et al., 2015; Wikander et al., 2020b; Reichard et al., 2021). Comparative whole genome, or at least complete mitochondrial genome sequencing, between Brazilian and American isolates should be performed (Uilenberg et al., 2018; André et al., 2022).

The lack of piroplasmids in blood smears in this study as well as the high Cq values obtained in most samples are likely associated with low parasitemia, which can be found in the chronic phase of hemoparasitosis (Hartmann et al., 2013). In that regard, the majority of positive cats for *Cytauxzoon* spp. in Brazil might act as chronic carriers (André et al., 2017; Grillini et al., 2021), and they probably only experienced a limited schizogonic phase (Legroux et al., 2017; Nentwig et al., 2018), which in turn would not be associated with clinical signs as observed in North American cats. It is worthy to note that these cats from Brazil could also have recovered from severe illness, instead of being asymptomatic all along.

Conclusion

We found a moderate occurrence of piroplasmids in cats from Midwestern Brazil, a new geographic locality for *Cytauxzoon* spp. in domestic cats. Considering the absence of clinical, hematological, and biochemical abnormalities observed in piroplasmid-positive cats, the tick-borne infections detected herein were of unknown clinical significance. We have not verified the possibility of previous acute disease, chronic manifestations after prolonged infection, or the development of more severe diseases in immunosuppressed cats or the presence of co-infections. Even so, this information set strengthens the hypothesis that the Brazilian strain of *Cytauxzoon* spp. is different from the North American ones.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00436-022-07602-8.

Funding The authors would like to thank the Federal Agency for the Support and Improvement of Higher Education (CAPES — Finance Code 001) for the graduate scholarships granted to Camila Manoel de Oliveira and Daniela Maciel Figueiredo. We also would like to thank CNPq (National Council for Scientific and Technological Development) for providing a fellowship to Liliane Maria do Rosario Batista and the Federal District Research Support Foundation (FAPDF) for providing financial support (0193.001495/2016). MRA received a fellowship from CNPq (National Council for Scientific and Technological Development — Productivity Grant Process #302420/2017–7).

Declarations

Conflict of interest The authors declare no competing interests.

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