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Original Article

Molecular detection of vector-borne agents in cats in Southern Brazil

Detecção molecular de agentes transmitidos por vetores em felinos domésticos no Sul do Brasil

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Abstract

This study used serological and molecular methods to investigate the occurrence of vector-borne pathogens (VBP) with zoonotic potential in cats neutered at the University Veterinary Hospital in Canoinhas, Santa Catarina. The combined PCR and serological results revealed that 17 (56.6%) cats were positive for one or more pathogens. The sampled cats had antibodies to *Ehrlichia* spp. (7/30), *Anaplasma phagocytophilum* (3/30) and *Leishmania infantum* (2/30). The PCR assay detected DNA closely related to *Ehrlichia canis* in 6/30 cats, *Mycoplasma haemofelis* in 2/30 cats, *A. phagocytophilum* and *Cytauxzoon* sp. in one cat each. While *Bartonella clarridgeiae* and *B. henselae* were detected in two cats each, and *B. koehlerae* was detected in one cat.

Keywords: *Anaplasma*, *Bartonella, Ehrlichia*, *Leishmania*, *Mycoplasma*, cat.

Resumo

Como os felinos podem ser parasitados por diversos patógenos transmitidos por vetores (PTV), alguns com caráter zoonótico, este estudo objetivou detectar por métodos sorológicos e moleculares, patógenos transmitidos por vetores hematófagos, em gatos atendidos em um Hospital Veterinário Universitário em Santa Catarina. Os resultados da PCR e da sorologia combinados, revelaram que 17 (56,6%) gatos foram positivos para um ou mais patógenos. Na sorologia, foram positivos 7/30 gatos para *Ehrlichia*, 3/30 para *Anaplasma phagocytophilum* e 2/30 para *Leishmania infantum*. Na PCR foi detectado DNA filogeneticamente associado a: *Ehrlichia canis* em 6/30 gatos; *Mycoplasma haemofelis*, em 2/30 gatos; *A. phagocytophilum* e *Cytauxzoon* sp. em 1/30 gatos cada. Enquanto *Bartonella clarridgeiae* e *B. henselae* foram detectadas, cada uma, em dois gatos, *B. koehlerae* foi detectada em um gato.

Palavras-chave: *Anaplasma*, *Bartonella*, *Ehrlichia*, *Leishmania*, *Mycoplasma*, gato.

Introduction

Feline vector-borne diseases (FVBDs) are caused by a wide range of pathogens, including viruses, bacteria, protozoa, and helminths that are transmitted by blood-sucking arthropods, such as fleas, ticks, and flies (HARTMANN & BERGMANN, 2017). The vector-borne diseases (VBDs) are becoming more common not only in humans, but also in dogs and cats (BANETH et al., 2012; MAIA et al., 2014; ANDRÉ et al., 2017) in Brazil (ANDRÉ et al., 2017) and worldwide (MAIA et al., 2014) as a result of changing climate. Some fvbds can be life-threatening for cats while others represent a major zoonotic concern (MAIA et al., 2014; ANDRÉ et al., 2015). Although the occurrence of *Bartonella* spp., *Leishmania* spp. and hemoplasmas has been extensively investigated among cats, further studies are necessary regarding the dispersion of Anaplasmataceae agents, piroplasmids and *Hepatozoon* spp. in domestic and stray cats (OTRANTO & DANTAS-TORRES, 2010; ANDRÉ et al., 2017; GUIMARÃES et al., 2019).

Nowadays, the extensive contact between domestic cats and humans has favored the transmission of zoonotic agents. Therefore, it is crucial to increase the FVBP surveillance aiming at determining the real impact of these agents on Public Health (UDELL & SHREVE, 2017). Currently, no data on vector-borne agents in cats is available in the municipality of Canoinhas, SC, southern Brazil, so molecular and serological methods were used to investigate the DNA presence and/or serological exposure to FVBP, namely *Anaplasma*, *Ehrlichia*, *Babesia/Theileria, Bartonella*, *Mycoplasma*, *Cytauxzoon* and *Hepatozoon* in domestic cats in this region.

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Material and Methods

Population and studied area

Blood and ectoparasite samples were collected from 30 apparently healthy domestic cats neutered at the Veterinary Hospital of the University of Contestado (UnC) in Canoinhas (latitude 26° 10' 38" S and longitude 50º 23' 24" W), SC, Brazil, between November and December 2017. The cats aged from 8 months to 7 years: 15 males (46.7% were less than 2 years old, 40% between 2 and 5 years old and 13.3% with more than 5 years old) and 15 females (80% were less than 2 years old and 20% between 2 and 5 years old). All performed procedures followed the ethical guidelines of the Ethics Committee of the UnC (number 11/17). The owners answered a questionnaire regarding animal gender, breed, living conditions, age and use of acaricides/insecticides.

Blood and ectoparasites sampling

Blood samples were collected from the cephalic or jugular vein. A fraction of the sample was stored in DNAse and RNAse free sterile microtubes containing anticoagulant (EDTA) while another fraction was centrifuged to separate the serum. Both whole blood and serum samples were stored at -20°.

Ectoparasite specimens were collected and preserved in 70% ethanol (Êxodo Científica®). Ticks and fleas were identified using previously described taxonomic keys (BICHO & RIBEIRO, 1998; BARROS-BATTESTI et al., 2006).

Hemogram and Giemsa-stained blood smears

The hemogram was performed according to the routine technique. The leukocyte differential count and the search for the FVBP were performed manually in May-Grünwald-Giemsa - stained blood smears.

FVBP molecular screening and characterization

For the FVBP molecular screening and characterization, a 200 µL whole blood aliquot from each cat was used to extract the DNA using the QIAamp DNA Blood Mini kit (QIAGEN®, Valencia, CA, USA), following the manufacturer's instructions. The DNA concentration and quality were measured using the 260/280nm absorbance ratio (Nanodrop®, Thermo Fisher Scientific, Waltham, MA, USA). The amplifiable DNA was detected by a PCR assay targeting a fragment of mammalian glyceraldehyde-3-phosphate dehydrogenase (*gapdh*), according to Birkenheuer et al. (2003).

The performed conventional PCR (cPCR) assays targeted the 16S rRNA gene of *Ehrlichia* (MURPHY et al., 1998), *Anaplasma* (MASSUNG et al., 1998), and *Mycoplasma* (MAGGI et al., 2013), and the 18S rRNA of *Cytauxzoon* sp. (BIRKENHEUER et al., 2006), *Hepatozoon* spp. (INOKUMA et al., 2002), *Babesia* spp. (JEFFERIES et al., 2007) and kinetoplast DNA *Leishmania* spp. (RODGERS et al., 1990; LOPES et al., 2016). Additionally, the cPCR also targeted *gltA* (BILLETER et al., 2011), *rpoB* (DINIZ et al., 2007), *ftsZ* (PAZIEWSKA et al., 2011), and *nuoG* gene fragments (ANDRÉ et al., 2015) of *Bartonella* spp. and the *msp2* gene fragment (DRAZENOVICH et al., 2006) of *A. phagocytophilum.*

The DNA positive controls for *Leishmania infantum* (JUSI et al., 2015), *Bartonella henselae* (André et al., 2015), *Mycoplasma* sp. (GONÇALVES et al., 2015), *Hepatozoon caimani* (BOUER et al., 2017), *Babesia vogeli* (Jaboticabal strain), *Cytauxzoon* sp. (ANDRÉ et al., 2009), *Ehrlichia canis* (Jaboticabal strain), and *Anaplasma* sp. (BENEVENUTE et al., 2017) were obtained from naturally infected animals. Ultra-pure water (Nuclease-Free Water Promega®, Wisconsin, USA) was used as a negative control in all PCR assays. The gels were imaged under ultraviolet light using the Image Lab Software, version 4.1 (Bio-Rad®).

The reaction products of PCR-positive samples were purified using the Silica Bead DNA gel extraction kit (Thermo Fisher Scientific®, USA). Purified amplified DNA fragments were submitted to phylogenetic analysis and sequence confirmation using an automatic sequencer (ABI PRISM 3700 DNA Analyser/ Applied Biosystem®) in the Center for Biological Resources and Genomic Biology (CREBIO, Jaboticabal, São Paulo, Brazil). The sequences were aligned with GenBank data using the MAFFT software, version 7 (KATOH & STANDLEY, 2013). Phylogenetic inference was based on Bayesian Inference (BI) analysis, using the MrBayes X-SEDE (3.2.6) (RONQUIST & HUELSENBECK, 2003). All phylogenetic analyses used the CIPRES Science Gateway (MILLER, 2010). The trees were examined in Treegraph 2.0.56-381 beta (STOVER & MULLER, 2010).

Serological screening for selected FVBP

The serological screening for FVBP was performed by the indirect fluorescence antibody test (IFAT) using slides sensitized with *E. canis* (ANDRÉ et al., 2010), *A. phagocytophilum* (SOUSA et al., 2017) and *L. infantum* (OLIVEIRA et al., 2008) antigens as previously described. Briefly, antigen slides were removed from storage and allowed to thaw at room temperature for 30 min. Ten microliters of twofold serum dilutions (starting at 1:64, the cut-off for *E. canis* and *A. phagocytophilum,* and 1:80 for *Leishmania infantum*) were placed in the wells of antigen-sensitized slides. On each slide, serum samples from dogs naturally infected with *E. canis* (NAKAGHI et al., 2008) and *L. infantum* (OLIVEIRA et al., 2008) as well as from horses experimentally infected with *A. phagocytophilum* (SOUSA et al., 2017) were used as positive controls. Dog and horse serum samples negative to the studied VBP were kindly supplied by IMUNODOT Diagnostics Ltda. (Jaboticabal, SP, Brazil). Slides were incubated at 37 °C in a moist chamber for 30 min, washed three times in PBS (pH 7.2) for 5 min, and air dried at room temperature. Then, the slides were incubated with fluorescein isothiocyanate, labeled anti-cat IgG (Sigma®, St. Louis, USA) in tested serum samples and anti-dog IgG and anti-horse IgG (Sigma®, St. Louis, USA) in positive and negative control serum samples, which were diluted following the manufacturer instructions. These slides were incubated again at 37 °C, washed three times in PBS, once more in distilled water, and air dried

at room temperature. Finally, slides were overlaid with buffered glycerin (pH 8.7), covered with glass coverslips, and examined in a fluorescence microscope (Olympus®, Tokyo, Japan).

Statistical analyses

Data were not normally distributed according to the Shapiro–Wilk test. Therefore, the quantitative hematological variables of positive and negative FVBP groups were compared by the nonparametric Mann Whitney U-test, at P <0.05.

Results

The May-Grünwald-Giemsa–stained blood smears (n=30) showed no inclusions suggestive of FVBP. However, the PCR and IFAT techniques detected FVBP and the results are shown in Table 1. Of the 30 domestic cats, 17 (56.6%) were positive to at least one FVBP by either serological or molecular techniques. Among cats positive for only one agent, *Ehrlichia* spp. was the most common pathogen (36.6%), followed by *Anaplasma* spp. and *Bartonella* spp. (13.3% each), and *Mycoplasma* spp. and *Leishmania* spp. (6.6% each) (Table 1). Five cats were positive to at least two pathogens from different genera (Table 2). Despite this, all cats were considered healthy based on history, physical examination and body condition scores.

Table 1. FVBP positive cats according to the results of the serological and molecular techniques used in this study.

Pathogen	Number of positive cats (%)		
	PCR	IFAT	Total
Ehrlichia spp.	6 (20.0)	7 (23.3)	11 (36.6)
Anaplasma spp.	1 (3.3)	3 (10.0)	4 (13.3)
Bartonella spp.	4 (13.3)	-	4 (13.3)
Mycoplasma spp.	2 (6.6)	-	2 (6.6)
Leishmania spp.	0 (0.0)	2 (6.6)	2 (6.6)
Cytauxzoon spp.	1 (3.3)	-	1 (3.3)
Hepatozoon spp.	0 (0.0)	-	0 (0.0)
Babesia spp.	0 (0.0)	-	0 (0.0)

According to the owners, 76.6% of the cats were not controlled for ectoparasites. A total of 80% (24/30) of the cats had parasites, of which 95.8% (23/24) were parasitized by fleas of the species *Ctenocephalides felis* Bouché, 1835, and 4.2% (1/24) was parasitized by an adult female tick, *Amblyomma aureolatum* Pallas, 1772.

The owners reported that most cats did not have a history of recent fights (83.3%), were not aggressive with people (76.7%), had access to the street (80%), and lived with other cats (83.3%).

Furthermore, the hematological results indicated that the monocyte count was significantly lower in FVBP-free cats (Table 3), whereas monocytosis was observed in two cats seropositive for FVBP.

Out of 30 samples, two (6.6%) were positive for *M. haemofelis.* The cluster was composed of *M. haemofelis* 16S rRNA sequences previously detected in domestic cats in Switzerland, Australia, and the United Kingdom (Figure 1).

Seven cats 23.1% (7/30) were positive for *E. canis* with the following titers: 64 (six animals) and 128 (one animal). Further, six animals (19.8%) were positive for *Ehrlichia* spp. in the 16S rRNA PCR assay, of which two cats (6.6%) were also positive in the IFAT. The only tick found was collected from an *E. canis* seropositive cat. Although apparently healthy, 54.5% of the positive animals for *Ehrlichia* (PCR and/or IFAT) had hematological disorders such as lymphopenia (27.27%), neutropenia (9.09%) and hyperproteinemia (18.18%). The phylogenetic analysis indicated that the *Ehrlichia* 16S rRNA sequences (seq. 1, 2 and 3) detected in three different cats were closely related to the *E. canis* sequences identified in a domestic cat (MH234591) in Brazil and in ticks in

Table 2. FVBP co-positivity assessed by molecular and serological techniques in blood samples of cats in Canoinhas, SC, Brazil.

Pathogens detected (Diagnostic technique)	Total of positive cats (%)
Ehrlichia spp. (IFAT) + *Bartonella* spp. (PCR)	2 (6.6)
Ehrlichia spp. (PCR) + *Bartonella* spp. (PCR)	
Ehrlichia spp. (PCR) + *Cytauxzoon* spp. (PCR)	1 (3.3)
Ehrlichia spp. (PCR/ IFAT) + *Bartonella* spp. (PCR) + *Leishmania* spp. (IFAT)	1 (3.3)
Ehrlichia spp. (IFAT) + *Mycoplasma* spp. (PCR) + *Anaplasma* spp. (IFAT)	1 (3.3)

Table 3. Mean ± Std. Error of Mean (SE) for the hematological parameters of domestic felines -positive and negative for FVBP.

Parameter	Ranges for healthy cats (Weiss, Wardrop, 2010)	Positive (n=17) (mean ± SE)	Negative (n=13) (mean ± SE)	P-value (test)
Hematocrit (%)	24-45	42.71 ± 1.53	39.62 ± 1.59	0.171
RBC count (x 10^6/mL)	5.0-10.0	7.8 ± 0.30	7.3 ± 0.28	0.291
WBC count (x 10^3/mL)	5,500-19,500	10,065 ± 821.9	11,862 ± 1,326	0.298
Band neutrophils	0-300	110.6 ± 32.3	66.81 ± 34.4	0.232
Segmented neutrophils	2,500-12,500	5,486 ± 688.4	7,154 ± 1211	0.298
Lymphocytes	1,500-7,000	3,757 ± 523.6	4,294 ± 690.4	0.532
Monocytes	0-850	293.3 ± 75.0	148.6 ± 63.8	0.046
Eosinophils	0-1,500	311 ± 42.6	198.3 ± 46.0	0.085
PPT	5.4-7.8	7.08 ± 0.22	6.71 ± 0.14	0.205

Note: No significant difference was observed between groups for the Mann-Whitney (U) at p≥0.05.

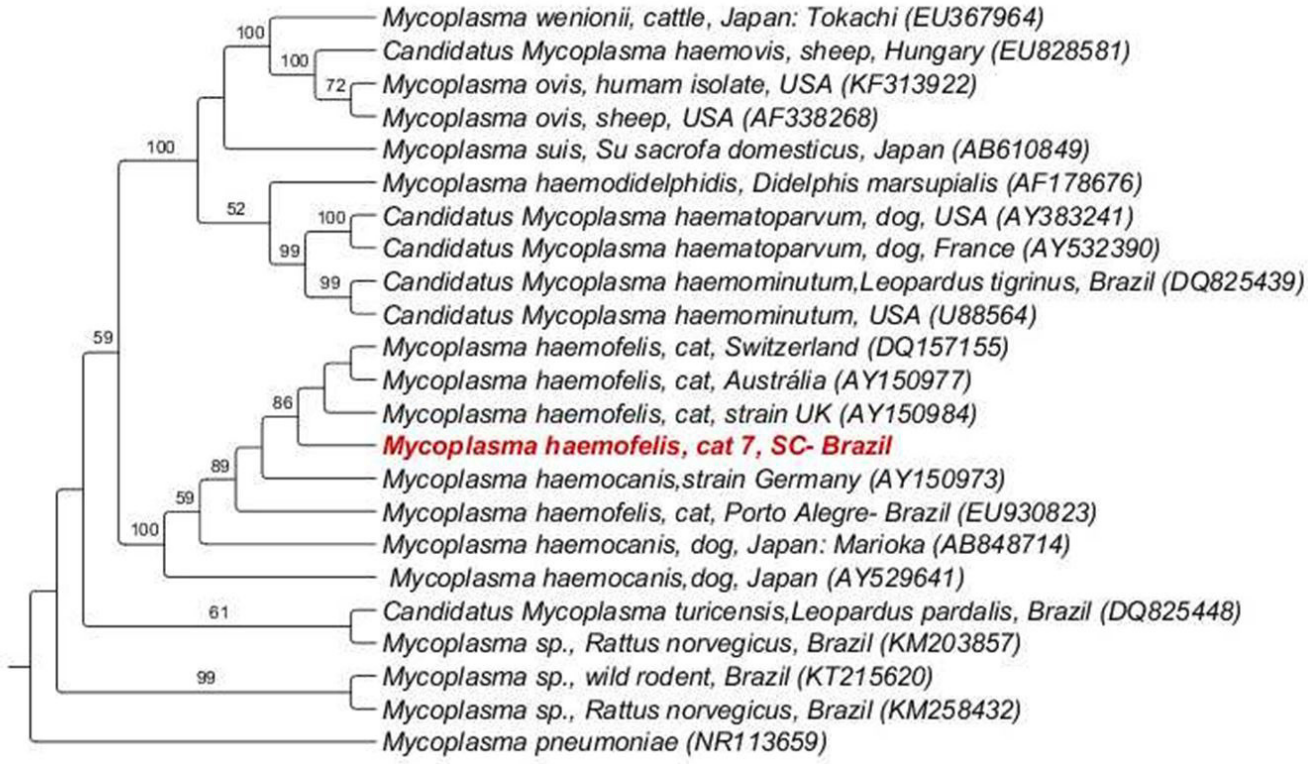

Figure 1. Phylogenetic relationships based on the sequence of 16S rRNA fragment amplified from *Mycoplasma* species isolated from domestic cats, compared with known species of the genus *Mycoplasma*. The phylogenetic tree was constructed from 1.300 bp sequences, inferred using the Bayesian method and GTRGAMMA+I evolutionary model. Numbers at nodes correspond to Bayesian posterior probabilities over 50, using *Mycoplasma pneumoniae* (NR113659) as outgroup.

the USA (MF059355) and Mexico (MG029082), supported by a high Bayesian posterior probability (100%) (Figure 2).

One cat was positive for *Anaplasma* spp. in the 16S rRNA nPCR but negative to *A. phagocytophilum* in the *msp-2* qPCR assay. Three cats seropositive for *A. phagocytophilum* had titers ranging from 64 to 512 but none was positive for *Anaplasma* spp. in both PCR and IFAT.

The phylogenetic analysis indicated that the *Anaplasma* 16S rRNA sequence is phylogenetically related to *A. phagocytophilum.* The phylogenetic relationships inferred by Bayesian analysis indicated five main clades, supported by posterior probabilities ranging from 54.2 to 99.8% (Figure 3). The sequence detected in a domestic cat in Santa Catarina, in the present study, was positioned in the main clade comprising the *A. phagocytophilum/Anaplasma* spp. sequences previously detected in animals and ticks in Brazil and worldwide, corroborating the BLASTn analysis, which indicated 100% identity with a sequence of *Anaplasma* sp. previously detected in a domestic cat in Brazil.

Two of the four positive cats for *Anaplasma* spp. (IFAT or PCR) had anemia, neutropenia, monocytosis, hypoproteinemia, and lymphopenia.

In this study, 13.3% (4/30) of the sampled cats had *Bartonella* spp. DNA. The four qPCR (*nuoG*) positive samples for *Bartonella* spp were also positive for at least one targeted gene in cPCR assays. All four were positive for the *gltA* gene, three for the *ftsZ* gene and three for the *rpoB* gene. The sampled animals, sequencing confirmed *B. clarridgeiae* (6.6%, 2/30), *B. henselae* (6.6%, 2/30) and *B. koehlerae* (3.3%, 1/30) infections. The analysis on the sequenced products showed that they presented 99.8-100% identicalness with *B. henselae* (access numbers MF314832, KY913627 and KX499336); 100% with *B. clarridgeiae* (access number KY913636) and 100% with *B. koehlerae* (access numbers FJ832089 and AF176091) (Table 4). Cases in which *gltA* and *rpoB* sequences from a single cat corresponded to different feline *Bartonella* species were considered coinfections. Therefore, one cat (3.3%, 1/30) was concurrently infected with *B. clarridgeiae* and *B. henselae.*

The *B. clarridgeiae gltA* sequences amplified in this study were closely positioned to sequences previously detected in fleas in Chile (KY913636) and in a cat in Thailand (KX001761), supported by a 100% posterior probability. Additionally, the amplified *B. koehlerae gltA* sequence detected in cat #29 was closely related to a *B. koehlerae* sequence (AF176091) detected in a cat in the USA, supported by a high Bayesian posterior probability of 100%. The detected *B. henselae* sequence was closely related to a *B. henselae* sequence (AF176091) previously detected in fleas in Chile (KY913627), supported by a high Bayesian posterior probability of 100% (Figure 4).

The *Bartonella koehlerae rpoB* sequence was positioned near to *B. koehlerae* sequences detected in a dog in Israel (FJ832089),

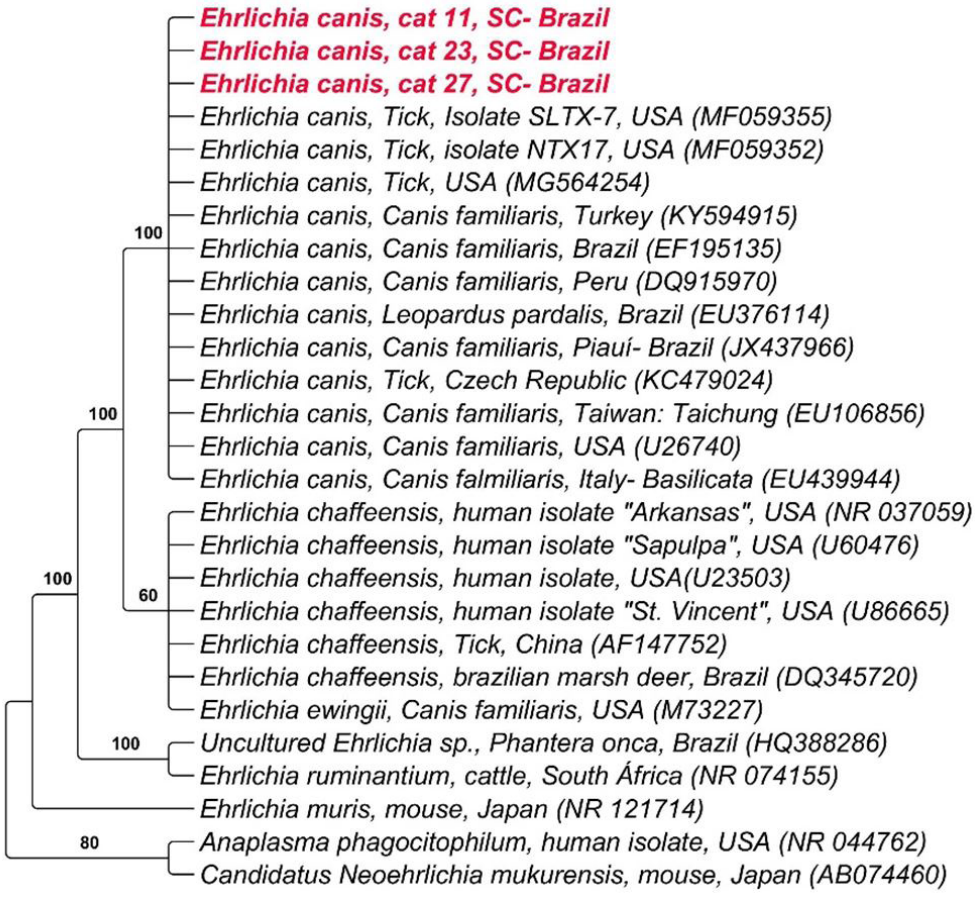

Figure 2. Phylogenetic relationships based on the sequence of 16S rRNA fragment amplified from *Ehrlichia* species isolated from domestic cats, compared with known species of the genus *Ehrlichia*. The phylogenetic tree was constructed from 350 bp sequences, inferred using the Bayesian method and GTRGAMMA+I evolutionary model. Numbers at nodes correspond to Bayesian posterior probabilities over 50, using *Anaplasma phagocytophilum* (NR044762) and '*Candidatus* Neoehrlichia mikurensis' (AB074460) as outgroups.

Figure 3. Phylogenetic relationships based on the sequence of 16S rRNA fragment amplified from *Anaplasma* species isolated from domestic cats, compared with known species of the genus *Anaplasma*. The phylogenetic tree was constructed from 932 bp sequences, inferred using the Bayesian method and GTR+G+I evolutionary model. Numbers at nodes correspond to Bayesian posterior probabilities over 50, using *Neorickettsia sennetsu* (M73219) as outgroup.

supported by 100% posterior probability. In addition, the amplified *B. henselae rpoB* sequences are closely related to *B. henselae* sequences previously detected in *Rattus rattus* Linnaeus, 1758 in New Zealand (MF 314832) and a cat in New Caledonia (JN646670), supported by a high Bayesian posterior probability of 100% (Figure 5).

Finally, the amplified *B. henselae ftsZ* sequences were closely related to *B. henselae* sequences previously detected in *Acinonyx jubatus* Schreber, 1775 in Zimbabwe (KX499336) and in a cat in Guatemala (KP822812), supported by high Bayesian posterior probability of 100% (Figure 6). The amplified *ftsZ B. koehlerae* sequence detected in the cat #29 presented 100% identity with a *Bartonella* sp. previously detected in *Leopardus wiedii* Schinz, 1821 from Brazil (GU903912), and was closely related to *B. koehlerae* from *Panthera leo* Linnaeus, 1758 from South Africa (KX499334) and to *Bartonella* sp. from a margay from Brazil (GU903912), supported by a Bayesian posterior probability of 75% (Figure 6, Table 4).

A concatenated phylogenetic tree of the genes was constructed to achieve more robust support to identify *Bartonella* species in this study. The amplified *B. henselae* sequences detected in cats #6 and #15 were closely related to *B. henselae* strain Houston-1 (BX897699), supported by a high Bayesian posterior probability of 99-100%. The amplified *B. koehlerae* sequence detected in cat #29 was closely related to *B. koehlerae* C-29 (KL407334) supported by a high Bayesian posterior probability of 100% (Figure 7).

Two cats were seropositive for *L. infantum,* with 80 and 160 titers. However, all 30 cats (including the two positives) were negative for *L. infantum* based on the PCR assay targeting the kinetoplast DNA. Even though one cat (3.3%) was positive to *Cytauxzoon* sp. in the 18S rRNA cPCR, the low amount of amplified DNA resulted in weak band intensity in agarose gel electrophoresis, precluding a high-quality sequencing.

None of the sampled cats was positive for either *Babesia/Theileria* spp. or *Hepatozoon* spp. in the PCR assays.

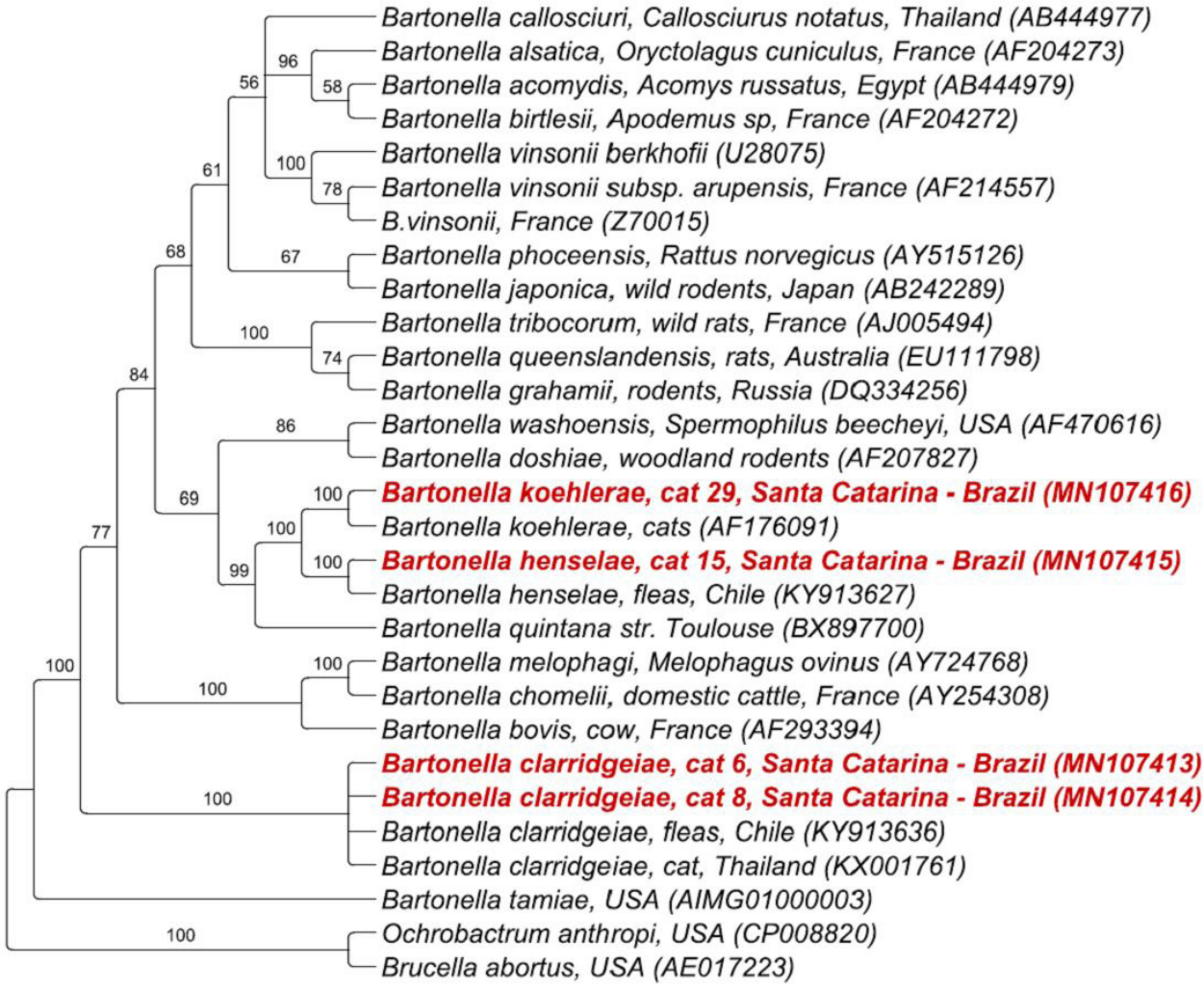

Figure 4. Phylogenetic relationships based on the sequence of a citrate synthase (*gltA*) gene fragment amplified from *Bartonella* species isolated from domestic cats, compared with known species of the genus *Bartonella*. The phylogenetic tree was constructed from 767 bp sequences, inferred using the Bayesian method and GTR+G+I evolutionary model. Numbers at nodes correspond to Bayesian posterior probability over 50, using *Brucella abortus* and *Ochrobactrum anthropi* as outgroups.

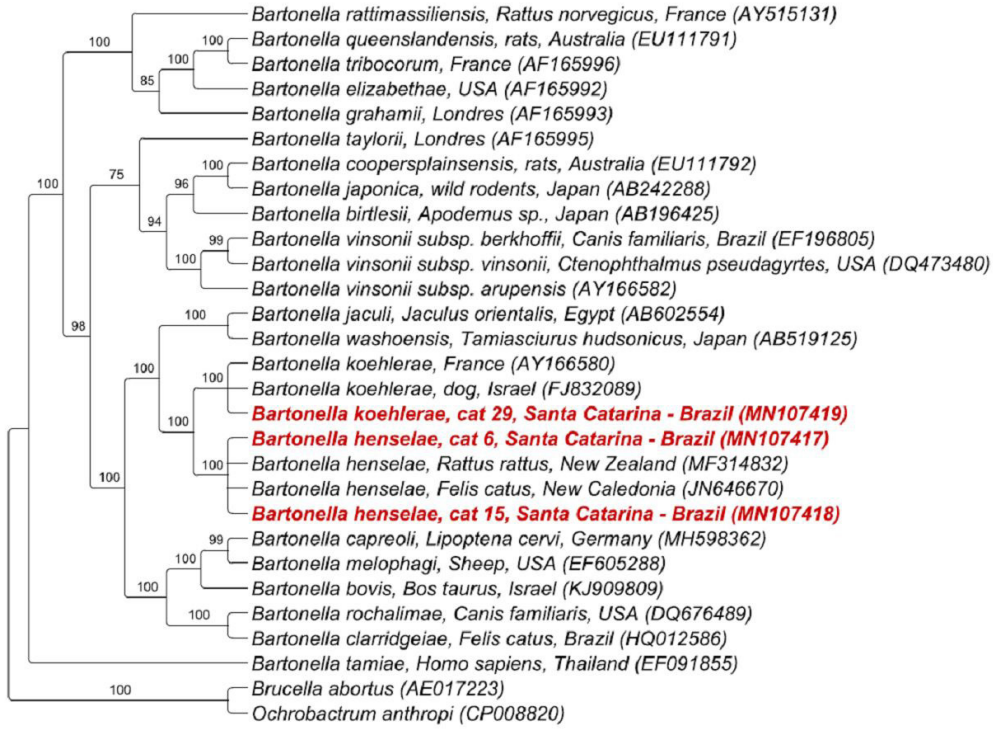

Figure 5. Phylogenetic relationships based on the sequence of a *rpoB* gene fragment amplified from *Bartonella* species isolated from domestic cats, compared with known species of the genus *Bartonella*. The phylogenetic tree was constructed from 720 bp sequences, inferred using the Bayesian method and GTR+G+I evolutionary model. Numbers at the nodes correspond to Bayesian posterior probability over 80, using *Brucella abortus* and *Ochrobactrum anthropi* as outgroups.

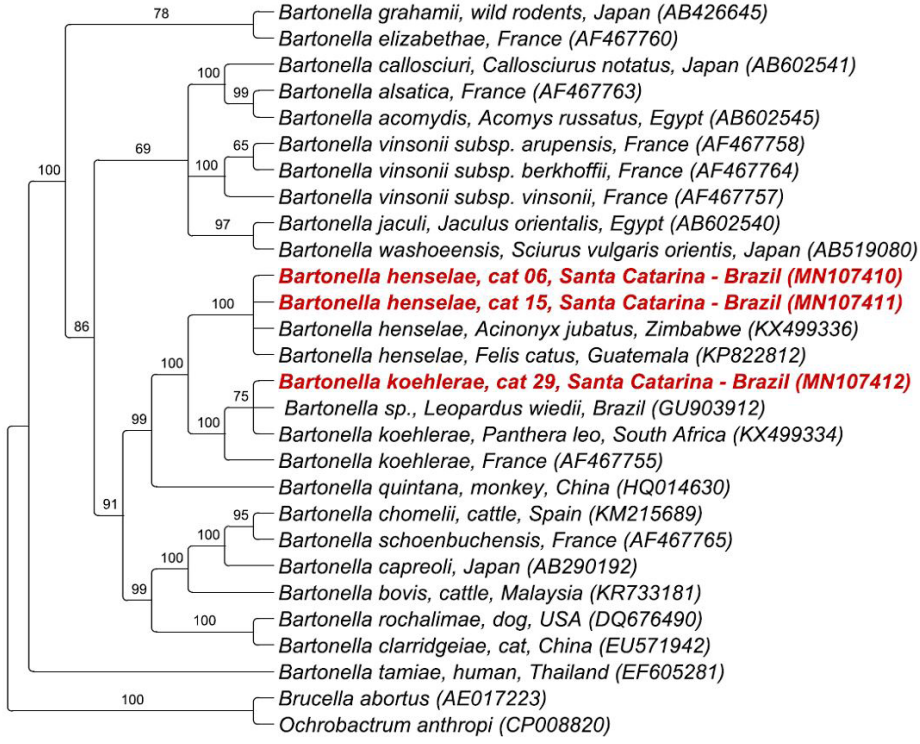

Figure 6. Phylogenetic relationships based on the sequence of a *ftsZ* gene fragment amplified from *Bartonella* species isolated from domestic cats, compared with known species of the genus *Bartonella*. The phylogenetic tree was constructed from 600 bp sequences, inferred using the Bayesian method and GTR+G+I evolutionary model. Numbers at the nodes correspond to Bayesian posterior probability over 80, using *Brucella abortus* and *Ochrobactrum anthropi* as outgroups.

Table 4. *Bartonella* species identified in cats sampled in Canoinhas, State of Santa Catarina, southern Brazil, according to conventional PCR assays based on three different genes and BLAST analysis.

Cat ID	% identity according to Blast analysis (GenBank ID)		
	rpoB gene	***gltA gene***	***ftsZ gene***
#6	100% *B. henselae* (MF314832)	100% *B. clarridgeiae* (KY913636)	99,81% *B. henselae* (KX499336)
#8	negative	100% *B. clarridgeiae* (KY913636)	negative
#15	100% *B. henselae* (MF314832)	100% *B. henselae* (KY913627)	100% *B. henselae* (KX499336)
#29	100% *B. koehlerae* (FJ832089)	100% *B. koehlerae* (AF176091)	100% *Bartonella* sp. (GU903912)

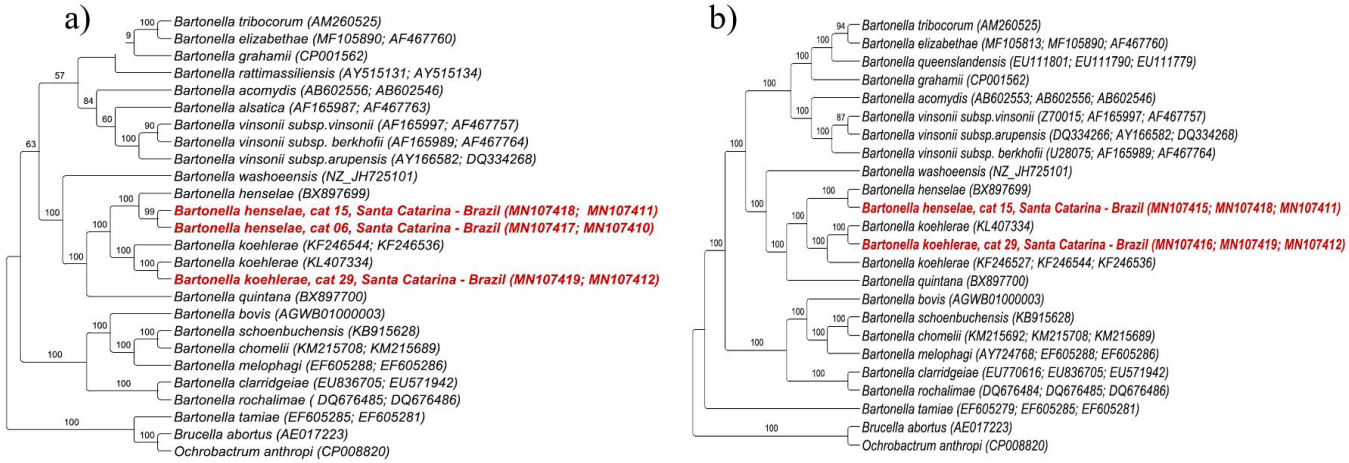

Figure 7. Concatenated phylogenetic analysis of *Bartonella* sequences: a) *rpoB* and *ftsZ* sequences (4902 bp after alignment), b) *rpoB*, *gltA* and *ftsZ* sequences (6294 bp after alignment), based on the topology generate on the Bayesian method. The values of support of posterior probability than 50% are shown in each branch, using *Brucella abortus* and *Ochrobactrum anthropi* as outgroups.

Discussion

The FVBP geographical distribution and prevalence are on the rise worldwide (BANETH et al., 2012; ANDRÉ et al., 2017) while an increasing number of FVBP has been detected in humans (SANTOS et al., 2008; EYER-SILVA et al., 2017). No molecular studies investigating FVBP in cats had been previously conducted in the Canoinhas municipality, SC, southern Brazil. The results of the IFAT and PCR assays revealed a wide variety of FVBP species such as *Mycoplasma*, *Ehrlichia*, *Anaplasma*, *Bartonella,* and *Cytauxzoon* in household cats of the region. The low number of cats sampled in the present study, an acknowledged limitation of this work, precludes any statistical inference on the real prevalence of FVBP in the studied area.

Ehrlichia canis was the most common FVBP detected in this study and, interestingly, the only cat parasitized by an *Amblyomma* tick was positive for this pathogen as well. The seropositivity found in the present study suggests the exposure to *Ehrlichia* spp. in a feline population studied. However, the occurrence of cross-reactivity with other Anaplasmataceae family agents cannot be ruled out (WEN et al., 1997; ORTUÑO et al., 2005). *Ehrlichia canis*-like DNA was also detected in domestic cats in Mato Grosso (BRAGA et al., 2014b), Mato Grosso do Sul (ANDRÉ et al., 2015), Rio Grande do Norte (ANDRÉ et al., 2017), and Rio de Janeiro (GUIMARÃES et al., 2019), and in wild felids maintained in captivity in the state of São Paulo and in the Federal District (ANDRÉ et al., 2010; 2012).

Of the 11 cats positive for *Ehrlichia*, two (one positive in PCR and another positive in IFAT) had hyperproteinemia, which has also been found in *E. canis* seropositive cats in Rio de Janeiro (GUIMARÃES et al., 2019). In fact, this finding may be attributed to the increase of gamma globulins concentration during the febrile stage and persistence during subclinical and chronic stages of monocytic canine ehrlichiosis (RISTIC & HOLLAND, 1993). Lymphopenia was also observed in seropositive cats to *Ehrlichia* spp. in Mato Grosso (BRAGA et al., 2013) whereas the authors in the present study observed that positive cats to *Ehrlichia* sp. in PCR tended to be lymphopenic.

The *Bartonella* species detected in the cats sampled in Canoinhas suggests that these animals may act as a source of infection to humans via scratching contaminated with flea feces or bites contaminated with infected blood (PENNISI et al., 2013).

Herein, *B. henselae, B. clarridgeiae* and *B. koehlerae* were detected in cats in the present study. *Bartonella* spp., an increasingly important Gram-negative intracellular bacteria in veterinary and human medicine (HARTMANN & BERGMANN, 2017), was detected in four *C. felis*-infested cats. In fact, fleas are the main vector for *B. henselae* among cats (CHOMEL & KASTEN, 2010). *Bartonella henselae*, the agent of the cat scratch disease (CSD), has been frequently reported in cats, which are considered the major reservoir for human infection (PENNISI et al., 2013). On the other hand, *Bartonella clarridgeiae* has been incriminated as a minor causative agent of CSD (CHOMEL & KASTEN, 2010). Indeed, *B. henselae* and *B. clarridgeiae* have been already detected in domestic cats in other Brazilian states (BRAGA et al.,

2012a, 2015; MICELI et al., 2013; ANDRÉ et al., 2014, 2015). Additionally, *B. henselae* (PITASSI et al., 2015) and *B. clarridgeiae* (VIEIRA-DAMIANI et al., 2015) have been detected in blood donors in Brazil, raising suspicions regarding *Bartonella* transmission among humans via blood transfusion (SILVA et al., 2016).

Previously, a small *Bartonella-gltA* sequence fragment (96-bp) sharing 99% identity with *B. koehlerae* was detected in a margay (*L. wiedii*) maintained in captivity in a Brazilian zoo (FILONI et al., 2012). Recently, *B. koehlerae* was detected in cats in Rio de Janeiro (RAIMUNDO et al., 2019). Although *B. koehlerae* has been isolated from cat blood, the routes of transmission among cats have not been fully established yet (PENNISI et al., 2013).

The 16S rRNA-*Anaplasma* genotypes closely related to *A. phagocytophilum* have been detected in little-spotted-cats, *Leopardus pardalis* Linnaeus, 1758 (ANDRÉ et al., 2012), stray cats (ANDRÉ et al., 2014; 2017), coatis (SOUSA et al., 2017), and wild birds (MACHADO et al., 2012; WERTHER et al., 2017) in Brazil. However, in the present study, the sampled animals were negative for *A. phagocytophilum* in *msp-2*-qPCR assays, indicating that an *Anaplasma* genotype closely related to the human granulocytic anaplasmosis agent circulates in South America (ANDRÉ, 2018). Similarly, the cat #18 was negative for *A. phagocytophilum* in *msp-2* qPCR, despite presenting a 16S rRNA *Anaplasma* genotype closely related to *A. phagocytophilum*. The *Anaplasma*-positive cat in this study had no hematological disorders, corroborating the report by Pennisi et al. (2017) that cats seem to remain chronic carriers after infected with *A. phagocytophilum*.

There are few reports on the occurrence of piroplasmids among cats in Brazil (ANDRÉ et al., 2014, 2015, 2017; MALHEIROS et al., 2016). Regarding the molecular detection of piroplasmids, one cat was positive for *Cytauxzoon* sp. whereas none was positive for *Babesia/Theileria* spp. Even though 18S rRNA *Cytauxzoon* sp. closely related to *Cytauxzoon felis* has already been detected in cats in Mato Grosso do Sul (ANDRÉ et al., 2015), Rio de Janeiro (MAIA et al., 2013), and Rio Grande do Norte (ANDRÉ et al., 2017), clinical cytauxzoonosis has never been described in domestic cats in Brazil so far. Nonetheless, fatal cytauxzoonosis was reported among lions maintained in captivity in a zoo in Volta Redonda, Rio de Janeiro (PEIXOTO et al., 2007). While neotropical wild felids have been incriminated as potential reservoirs for *Cytauxzoon* spp. in Brazil (ANDRÉ et al., 2009; FURTADO et al., 2017), the vectors remain unknown in South America (ANDRÉ et al., 2017). Similarly, though there are reports on cats being infected with both *Babesia vogeli* (ANDRÉ et al., 2014, 2015; MALHEIROS et al., 2016) and *Theileria* spp. associated with ruminants and equids (ANDRÉ et al., 2014, 2015) in Brazil, the clinical relevance of these findings is unknown.

Likewise, *Hepatozoon* spp. has been scarcely reported in cats in Brazil. For instance, *H. felis* (BORTOLI et al., 2011; BRAGA et al., 2016) and *Hepatozoon* sp. closely related to *H. americanum* (ANDRÉ et al., 2015) have been reported in cats in Brazil. The real clinical significance of these infections deserves further investigation.

Albeit the absence of *Leishmania* DNA in whole blood samples from seropositive cats has been previously reported (BRAGA et al., 2014a; COURA et al., 2018), feline leishmaniasis is an emergent feline disease (PENNISI & PERSICHETTI, 2018). And, even though the role of cats in the epidemiological cycles of leishmaniasis remains unclear, they may act as a reservoir for this parasite since sandflies feed on them (COURA et al., 2018). In Santa Catarina state, two autochthonous cases of canine visceral leishmaniasis were first detected in 2010 (FIGUEIREDO et al., 2012) while the first autochthonous case of human visceral leishmaniasis was diagnosed in 2017 (SMS, 2017), both in Florianópolis city. Because Canoinhas is not an endemic area for visceral leishmaniasis, we did not expect to find animals seropositive for *Leishmania* spp. The positive results in serology may be explained by serological cross-reactions between *Leishmania* and *Trypanosoma* species, as previously reported in dogs (LONGONI et al., 2012).

Hemoplasmas are one of the most prevalent flea-borne bacterial pathogens in cats (SHAW et al., 2004). In this study, despite two cats being infected with *M. haemofelis*, the most pathogenic hemoplasma, the erythrocyte count was within the reference values in both cats, albeit one had lymphopenia. According to Tasker et al. (2018), *M. haemofelis* chronic infection is usually not associated with significant anemia and carrier cats may show no evidence of anemia. Although '*Candidatus* Mycoplasma haemominutum' is the most frequent hemoplasma species found in cats (BRAGA et al., 2012b; ANDRÉ et al., 2014; TASKER et al., 2018), often associated with chronic or subclinical infections (HARTMANN & BERGMANN, 2017; TASKER et al., 2018), this agent was not detected in the cats of this study. Since blood-sucking arthropods (ticks and fleas) are suspected to be the vectors of feline hemoplasmas among domestic cats (SHAW et al., 2004; TASKER et al., 2018), and flea infestation was quite common in the sampled cats, including those positive for hemoplasmas, it is most likely that fleas acted as hemoplasma-vectors to these animals.

Furthermore, five cats were simultaneously positive for *Ehrlichia* and other FVB pathogens, such as *Bartonella*, *Cytauxzoon*, and *Mycoplasma*. Previously, co-positivity to *Ehrlichia/ Anaplasma/ Cytauxzoon* and *Mycoplasma* was reported in two cats in Rio Grande do Norte (ANDRÉ et al., 2017); to *Cytauxzoon* and '*Candidatus* Mycoplasma haemominutum*'* in one cat in Rio de Janeiro (MAIA et al., 2013), and to '*Candidatus* Mycoplasma haemominutum' and *Babesia vogeli*, *Bartonella* spp., and to '*Candidatus* Mycoplasma turicensis*'* or *Mycoplasma haemofelis* in stray cats in São Paulo (ANDRÉ et al., 2014). André et al. (2017) concluded that most likely that co-positivity is frequent in cats living in geographic areas where the competent vectors are present. According to these authors, the effect of multiple arthropod-borne hemoparasites in the pathogenesis of feline vector-borne diseases warrants further investigation. Although FBVP-positive cats were apparently healthy in the present study, veterinarians should keep in mind that cats co-positive to multiple FVBP may present a non-specific disease, which may further complicate the diagnosis, treatment and prognosis (BANETH et al., 2012; MAIA et al., 2014; ANDRÉ et al., 2017).

Conclusion

The results allow concluding that apparently healthy cats, with no clinical signs of infection, are exposed to multiple FVBP in the Canoinhas region, southern Brazil. The adoption of effective

prophylaxis and control measures against arthropod vectors is much needed to prevent infection of cats, as well as the potential transmission to other animals and humans.

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