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Identification of vector-borne pathogens in dogs and cats from Southern Brazil

J. Malheiros[a,*], M.M. Costa[b], R.B. do Amaral[c], K.C.M. de Sousa[c], M.R. André[c], R.Z. Machado[c], M.I.B. Vieira[a]

[a] Programa de Pós-graduação em Bioexperimentação, Faculdade de Agronomia e Medicina Veterinária, Universidade de Passo Fundo (UPF), Passo Fundo, RS, Brazil
[b] Laboratório de Análises Clínicas Veterinárias, Faculdade de Agronomia e Medicina Veterinária, Universidade de Passo Fundo (UPF), Passo Fundo, RS, Brazil
[c] Departamento de Patologia Veterinária, Faculdade de Ciências Agrárias e Veterinárias—FCAV, Universidade Estadual Paulista (UNESP), Jaboticabal, SP, Brazil

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ABSTRACT

Dogs and cats are often infected with vector-borne pathogens and play a crucial role as reservoirs and hosts in their life cycles. The aim of the present study was to investigate the occurrence of vector-borne pathogens among dogs and cats in the northwestern region of Rio Grande do Sul (RS) State, Brazil. One hundred and ten blood samples were collected from dogs (n = 80) and cats (n = 30). Laboratory analysis were carried out through stained blood smears, indirect enzyme-linked immunosorbent assay (ELISA) for *Babesia vogeli* and *Ehrlichia canis* (only for dogs) and polymerase chain reaction (PCR) aiming the detection of pathogens. The following pathogens were screened by PCR among dogs and cats: *Babesia* spp. and *Hepatozoon* spp. (*18S rRNA* gene), *Anaplasma* spp. (*16S rRNA* gene), and *Ehrlichia* spp. (*dsb* gene for dogs and *16S rRNA* gene for cats) and *Bartonella* spp. (*nuoG* gene only for cats). Using blood smears structures morphologically compatible with piroplasms were found in 5.45% (6/110) of the samples. Anti-*B. vogeli* and anti-*E. canis* antibodies were detected in 91% (73/80) and 9% (7/80) of the dogs, respectively. All the seropositive dogs to *E. canis* were also to *B. vogeli*. Nineteen (17.3%) animals were positive to hemoparasites by PCR. After sequencing *Rangelia vitalii* 6/80 (7.5%), *B. vogeli* 3/80 (4%), *Hepatozoon* spp. 1/80 (1%), and *Anaplasma* spp. 1/80 (1%) were found in the dogs, and *B. vogeli* 2/30 (7%) and *Bartonella* spp. 6/30 (20%) were detected in the screened cats. No sample was positive for genes *dsb* and *16S rRNA* of *Ehrlichia* spp. Only those animals which were positive for *R. vitalii* showed findings compatible with rangeliosis, such as anemia (100%), thrombocytopenia (67%), jaundice (50%), external bleeding (50%), and anorexia (50%). This is the first time that *B. vogeli* detected among cats in Southern Brazil.
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1. Introduction

Humans and animals that live in tropical and subtropical regions have been brought to desolation by injury of parasitic diseases around the world (Dantas Torres and Otranto, 2014). In this context, vector-borne diseases (VBD) are known as important emerging and re-emerging illnesses (Vilhena et al., 2013), which are ubiquitous and easily disseminated (Maia et al., 2015). *Babesia* spp. (Schnittger et al., 2012), *Rangelia vitalii* (Fighera et al., 2010), *Hepatozoon* spp. (Aydin et al., 2015), *Ehrlichia* spp. (Corales et al., 2014), *Anaplasma* spp. (Aktas et al., 2015), and *Bartonella* spp. (Mogollon-Pasapera et al., 2009; Chomel et al., 2014) have been pointed out as important VBD pathogens that infect the animals such as domestic animals.

In Brazil, *Babesia vogeli* and *Ehrlichia canis* are transmitted by the tick *Rhipicephalus sanguineus* sensu lato (s.l.), whereas *R. vitalii* is transmitted by *Amblyomma aureolatum* (Dantas-Torres and Otranto, 2014). *Hepatozoon canis* is admittedly transmitted by *R. sanguineus* s.l.; however, in this country, studies have demonstrated that transmission also occurs by *Amblyomma ovale* (Forlano et al., 2005; Demoner et al., 2013). Although *A. phagocytophilum* is transmitted by ticks of the genus *Ixodes* in several regions of the world, this agent has been molecularly detected in *Amblyomma cajennense* and *R. sanguineus* s.l. ticks in Brazil (Santos et al., 2013). *A. platys* seems to be transmitted by *R. sanguineus* s.l. (Cardoso et al., 2015; Maia et al., 2015), but Brazilian epidemiological data also suggest its transmission by *A. cajennense* (Dahmani et al., 2015). On

* Corresponding author.
E-mail addresses: jenifermalheiros@hotmail.com, jenifer@unicruz.edu.br (J. Malheiros).

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the other hand, *Bartonella* is transmitted by fleas, lice, and phlebotomines. Nonetheless, the DNA of this bacterium was detected in flies (Chomel and Kasten, 2010; Telford and Wormser, 2010; Yao et al., 2011) and the vector competence of *Ixodes ricinus* tick for *B. birtlesii* and *B. henselae* has been proven (Cotté et al., 2008; Reis et al., 2011).

Dogs and cats are often infected with vector-borne pathogens and play a crucial role as reservoirs and hosts in their life cycles (Day, 2011; Maia et al., 2015). Given the impact of VBD, studies have been conducted to determine their geographical prevalence (Yuasa et al., 2012). In Brazil, dogs and cats may harbor a wide variety of parasitic agents; however, information on their distribution and epidemiology is still fragmented (Dantas-Torres and Otranto, 2014). Therefore, the aim of the present study was to investigate the occurrence of vector-borne pathogens in dogs and cats in the northwestern region of Rio Grande do Sul (RS) State, Brazil.

2. Materials and methods

2.1. Ethical statement

This study was approved by the Committee on Animal Research and Ethics of Universidade de Passo Fundo, RS, Brazil, protocol no. 015/2014.

2.2. Blood specimens from dogs and cats

Blood samples were collected in EDTA tubes from dogs (n = 80) and cats (n = 30) treated at the Veterinary Hospital of Universidade de Passo Fundo (HV-UPF), in Passo Fundo, in the northwestern region of RS, Brazil, between June and December 2014. Animals with clinical signs (jaundice, pale mucous, epistaxis, splenomegaly) and/or blood disorders (anemia, leukopenia or leukocytosis and thrombocytopenia), presence of ticks (routine procedure), or which had been referred for surgical sterilization and no clinical signs and hematological alterations compatible with VBD (elective procedure) were included in the present study. Blood was collected by venipuncture and used in serological and molecular tests. A blood sample was collected from the pinna in order to obtain blood smears and later staining by Giemsa and Panotic methods.

2.3. Serology

The presence of anti-*B. vogeli* and anti-*E. canis* IgG antibodies in the serum of dogs was identified by indirect enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (Imunodot Diagnósticos Ltda®, Brazil) for each pathogen researched. Optical density (OD) was calculated from the average of negative controls x 2.5, according to the manufacturer's instructions. The serological analysis of cats was not be performed.

2.4. PCR amplification and sequencing

The DNA was extracted from the blood samples using a commercially available kit (DNeasy Blood & Tissue Kit®, Qiagen, Hilden, Germany), following the manufacturer's extraction protocol. To verify the existence of amplifiable DNA in the samples, a PCR assay for the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was performed as previously described (Birkenheuer et al., 2003).

The following agents were screened by PCR: *Babesia* spp., *Hepatozoon* spp., *Anaplasma* spp., *Ehrlichia* spp. and *Bartonella* spp. The PCR was carried out according to the protocols previously described for genes *18S rRNA* of *Babesia* spp. (Jefferies et al., 2007) and *Hepatozoon* spp. (O'Dwyer et al., 2013), *16S rRNA* of *Anaplasma* spp. (Massung et al., 1998), and *Ehrlichia* spp. (Murphy et al., 1998), *dsb* of *Ehrlichia* spp. (Doyle et al., 2005), and *nuoG* of *Bartonella* spp. (André et al., 2015a). Table 1 provides information on primers and reaction conditions. *Hepatozoon* sp., *Ehrlichia canis,* and *Bartonella* spp. DNA-positive controls were obtained from naturally infected cats from Campo Grande, MS, Brazil (André et al., 2015a; André et al., 2015b). *Anaplasma* sp. DNA positive control was obtained from a naturally infected dog from Campo Grande, MS, Brazil (Sousa et al., 2013).

After amplification by conventional PCR assays, the amplicons were subjected to 1% agarose gel electrophoresis and stained with ethidium bromide. The positive PCR products were purified using Silica Bead DNA Gel Extraction Kit (Fermentas, Lithuania). The sequencing of *18S rRNA* gene for *Babesia* spp. and *Hepatozoon* spp., and *16S rRNA* gene for *Anaplasma* spp. was carried out using the BigDye® Terminator v3.1Cycle Sequencing Kit and ABI PRISM 310 DNA Analyzer (Applied Biosystems®).

2.5. Phylogenetic analysis

Consensus sequences were obtained through the analysis of the sense and antisense sequences using the CAP3 program (http://mobyle.pasteur.fr/cgi-bin/MobylePortal/portal.py). Comparisons with sequences deposited in GenBank were done using the basic local alignment search tool (BLAST) (Altschul et al., 1990). The sequences were aligned with sequences published in GenBank using Clustal/W (Thompson et al., 1994) in Bioedit v. 7.0.5.3 (Hall, 1999). Phylogenetic inference was based on maximum likelihood (ML) inference. The ML phylogenies were inferred with RAxML-HPC BlackBox 7.6.3 software (Stamatakis et al., 2008) (which includes an estimation of bootstrap node support) through the CIPRES Science Gateway (Miller et al., 2010), using a GTR + GAMMA model of evolution and 1000 bootstrapping replicates. The best model of evolution was selected by the program jModelTest2 on XSEDE (Darriba et al., 2012) under the Akaike Information Criterion (AIC) (Posada et al., 2004), through the CIPRES Science Gateway (Miller et al., 2010). The trees were visualized in Treegraph 2.0.56-381 beta (Stover and Muller, 2010).

3. Results

The results of blood smears, serology and PCR-positive animals submitted to elective procedures and routine ones are shown in Table 2.

3.1. Blood smears

Structures morphologically compatible with piroplasms were found in 6 (5.45%) dogs (Fig. 1).

3.2. Serology

The serum samples of dogs with OD value higher than 0.3325 and 0.4175 were considered to be positive for the presence of anti-*B. vogeli* and anti-*E. canis* antibodies, respectively. Among the sampled dogs, 91% (73/80) were seropositive for *B. vogeli* and 9% (7/80) for *E. canis*. Interestingly, all dogs seropositive to *E. canis* also showed seropositivy for *B. vogeli*.

3.3. PCR and sequencing

The PCR-positive samples are shown in Table 3. All the PCRs for the *16S rRNA* and *dsb* of *Ehrlichia* spp. were negatives. At the time of collection, 50% (3/6) of dogs positive for *R. vitalii*, and both dogs positive for *Hepatozoon* spp. and *Anaplasma* spp. were infested by ticks, which unfortunately were not identified.

Table 1
Primer sets for PCR amplification of VBD pathogens.

Pathogen	Gene	Primers and Probes (5′-3′)	Product size (bp)	PCR conditions	Reference
Babesia spp. (n-PCR)	18S rRNA	BTF1 GGCTCATTACAACAGTTATAG BTR1CCCAAAGACTTTGATTTCTCTC BTF2CCGTGCTAATTGTAGGGCTAATAC BTR2 GGACTACGACGGTATCTGATCG	790	1°) 94 °C 3 min, 58 °C 1 min, 72 °C 2 min, 45 cycles (94 °C 30 s, 58 °C 20 s, 72 °C 30 s), 72 °C 7 min, 2°) Just the annealing temperature was increased to 62 °C.	Jefferies et al., 2007
Hepatozoon spp.	18S rRNA	HepF300 GTTTCTGACCTATCAGCTTTCGACG Hep900CAAATCTAAGAATTTC ACCTCTGAC	581	94 °C 3 min, 35 cycles (94 °C 45 sec, 56 °C 1 min, 72 °C 1 min), 72 °C 7 min	O'Dwyer et al., 2013
Anaplasma spp. (n-PCR)	16S rRNA	ge3a CACATGCAAGTCGAACGGATTATTC ge10r TTCCGTTAAGAAGGATCTAATCTCC ge9f AACGGATTATTCTTTATAGCTTGCT) ge2 GGCAGTATTAAAAGCAGCTCCAGG	546	1°) 95 °C 2 min, 40 cycles (94 °C 30 s, 55 °C 30 s, 72° 1 min), 72 °C 5 min, 2°) The same conditions were as described, except that 30 cycles were used.	Massung et al., 1998
Ehrlichia spp. (n-PCR)	16S rRNA	ECC AGAACGAACGCTGGCGGCAAGC ECB CGTATTACCGCGGCTGCTGGCA HE3 TATAGGTACCGTCATTATCTTCCCTAT ECAN5CAATTATTTATAGCCTCTGGCTATAGGA	396	1°) 94 °C 3 min, 30 cycles 94 °C 1 min, 65 °C 2 min, 72 °C 2 min. 2°) 3 cycles 94 °C 1 min, 55 °C 2 min, 72 °C 1.5 min.	Murphy et al., 1998
Ehrlichia spp. (qPCR)	dsb	321 TTGCAAAATGATGTCTGAAGATATGAAACA 671 GCTGCTCCACCAATAAATGTATCYCCTA PROBE FAM AGCTAGTGCTGCTTGGGCAACTTTGAGTGAA BHQ-1	409	95 °C 5 min, 40 cycles 95 °C 15 s, 60 °C 1 min.	Doyle et al., 2005
Bartonella spp. (qPCR)	nuoG	F-Bart CAATCTTCTTTTGCTTCACC R-Bart TCAGGGCTTTATGTGAATAC PROBE TEXAS RED TTYGTCATTTGAACACG BHQ2a-Q	93	95 °C 3 min, 40 cycles 95 °C 10 min, 52,8 °C 30 sec.	André et al., 2015a,b

Table 2
Results of the blood smear, ELISA and PCR according to the type of procedure.

Animals	Procedure		Diagnosis		
	Elective	Routine	Blood Smear	ELISA *B. canis* and *E. canis*	PCR
Dog 52		X	N	1	*Anaplasma* spp.
Dog 69		X	N	N	*Hepatozoon* spp.
Dog 86	X		N	1	*Babesia vogeli*
Dog 94	X		N	1	*Babesia vogeli*
Dog 98		X	Piroplasms	1	*Rangelia vitalii*
Dog 102	X		N	N	*Babesia vogeli*
Dog 106		X	Piroplasms	2	*Rangeia vitalii*
Dog 107		X	Piroplasms	2	*Rangelia vitalii*
Dog 108		X	Piroplasms	1	*Rangelia vitalii*
Dog 109		X	Piroplasms	1	*Rangelia vitalii*
Dog 110		X	Piroplasms	1	*Rangelia vitalii*
Cat 31		X	N	–	*Bartonella* spp.
Cat 71	X		N	–	*Bartonella* spp.
Cat 72	X		N	–	*Bartonella* spp.
Cat 73	X		N	–	*Babesia vogeli*
Cat 74	X		N	–	*Bartonella* spp.
Cat 82	X		N	–	*Bartonella* spp.
Cat 84	X		N	–	*Bartonella* spp.
Cat 100	X		N	–	*Babesia vogeli*

N: negative. 1: *B. canis*. 2: *E. canis*.

Table 3
The genetic sequencing results.

Animals	PCR	GenBank access number[a]	GenBank (BLAST) similarity (%)
Dog 98	18S rRNA *Babesia* spp.	KT323926	*Rangelia vitalii* KF218606 – 99%
Dog 106	18S rRNA *Babesia* spp.	KT323927	*Rangelia vitalii* KF218606–99%
Dog 107	18S rRNA *Babesia* spp.	KT323928	*Rangelia vitalii* KF218606 – 99%
Dog 108	18S rRNA *Babesia* spp.	KT323931	*Rangelia vitalii* KF218606 – 99%
Dog 109	18S rRNA *Babesia* spp.	KT323929	*Rangelia vitalii* KF218606 – 99%
Dog 110	18S rRNA *Babesia* spp.	KT323920	*Rangelia vitalii* KF218606 – 99%
Dog 86	18S rRNA *Babesia* spp.	KT323933	*Babesia vogeli* HM590440 – 100%
Dog 94	18S rRNA *Babesia* spp.	KT323934	*Babesia vogeli* HM590440 – 100%
Dog102	18S rRNA *Babesia* spp.	KT323936	*Babesia vogeli* HM590440 – 100%
Cat 73	18S rRNA *Babesia* spp.	KT323932	*Babesia vogeli* HM590440 – 100%
Cat 100	18S rRNA *Babesia* spp.	KT323935	*Babesia vogeli* HM590440 – 100%
Dog 69	18S rRNA *Hepatozoon* spp.	KT323037	*Hepatozoon canis* AY150067 – 100% (Spanish Fox)
Dog 52	16S rRNA *Anaplasma* spp.	KT323925	*Anaplasma phagocytophilum* CP006618 – 100% (European sample)

[a] Access number of positive samples in this study deposited in GenBank.

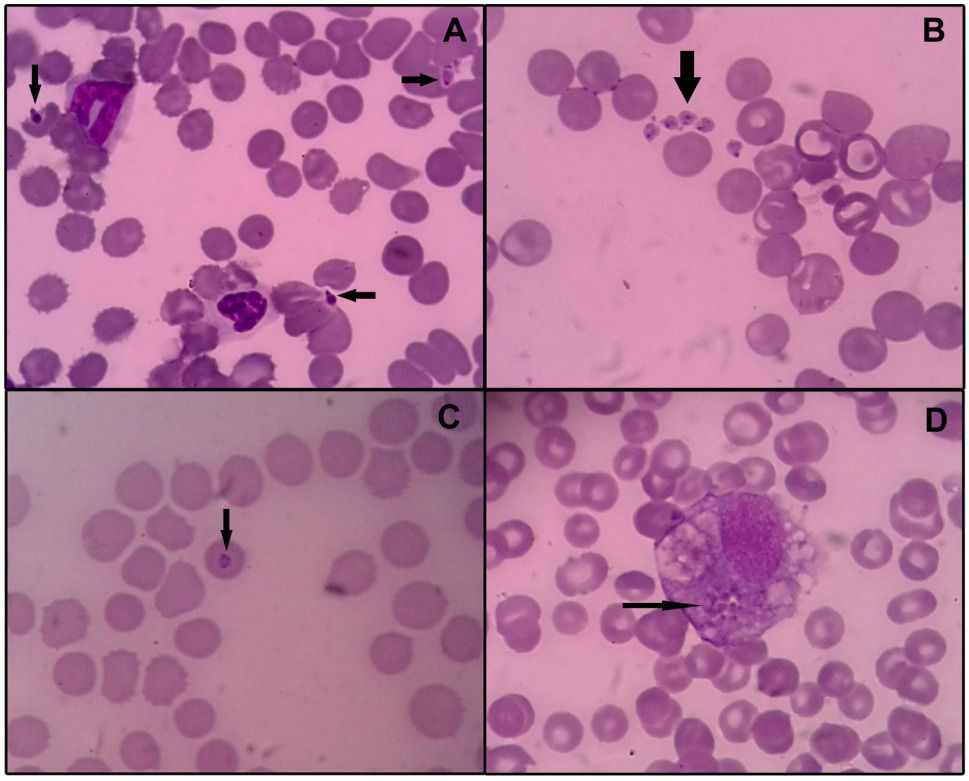

Fig. 1. Intra- and extracellular piroplasms found in naturally infected dogs in this study. (A and C) piroplasms intraerythrocytic (arrows) (A–Panotic and C–Giemsa). (B) Extracellular piroplasms (arrow) (Panotic). (D) Piroplasms within a macrophage (arrow) (Panotic) (1000×).

3.4. Phylogenetic analysis

The phylogenetic tree of the sample positive for the 18S rRNA of *Babesia* spp. (Fig. 2) showed that the six isolated positive for *R. vitalii* by PCR were located in the same clade of the group of *R. vitalii* detected in domestic dogs in RS and wild carnivores of the species *Cerdocyoun thous* (GenBank accession numbers HQ150006 and KF964151, respectively). By the isolated sequences from dog and cats positive for *Babesia* spp. by PCR, they presented in the same clade of *Babesia vogeli* (value bootstrap 99/100).

Phylogenetically, the isolated PCR positive for *Hepatozoon* was found within the same clade of *H. canis* (value bootstrap 92/100) detected in a red fox from Porto Alegre, RS, Brazil by Criado-Fornelio et al. (2006) (Fig. 3).

The positive fragments of the *16S rRNA* gene of *Anaplasma* spp. presented in the same clade of *A. phagocytophilum* (value bootstrap 80/100) isolated by European dog (GU236670) (Fig. 4).

4. Discussion

Dogs and cats are the most popular animals worldwide (Dantas-Torres and Otranto, 2014). In Brazil, the *Instituto Brasileiro de Geografia e Estatística (IBGE)* data indicate that 44.3% of households have at least one dog and 11.7% at least one cat. Unfortunately, some arthropods such as fleas, lice and ticks can transmit pathogens to these animals, like bacteria and protozoa (Dantas-Torres and Otranto, 2014).

The pathogen *E. canis* has been reported around the world, mainly in tropical and subtropical regions (Dantas-Torres and Otranto, 2011). Frequently, *E. canis* is detected in dogs in several regions of Brazil (Vieira et al., 2013), but in Southern region only Aguiar et al. (2013) detected *E. canis* by PCR in dogs the State of Paraná. By investigating the context in which *R. sanguineus* s.l. is a vector for *E. canis*, Moraes-Filho et al. (2011) observed the presence of two lineages of this tick. The temperate lineage (from Spain) was detected in RS, Brazil (where the present study was undertaken); Chile; Argentina and Uruguay; and tropical lineage (from South Africa) detected in other Brazilian regions. In Mexico, an study found no molecular evidence of transmission of *E. canis* by lineage temperate of *R. sanguineus* s.l. (Almazán et al., 2016). Furthermore, recently, Moraes-Filho et al. (2015) showed that temperate lineage has no vectorial competence to transmit *E. canis*.

On the other hand, serological tests for the detection of anti-*E. canis* antibodies revealed 9% (7/80) of positive dogs. Similarly, the study of Krawczak et al. (2012), conducted in the central region of RS, showed 4.43% of seropositive animals for *E. canis* in the immunofluorescence assay. However, the research of Lasta et al. (2013) did not detect any seropositive animal, in the city of Porto Alegre, state capital of RS. According to the Krawczak et al. (2012), cross-reactions with other species of *Ehrlichia*, or even with other agents of the family Anaplasmataceae might occur but, in this study, the only dog that was positive for *Anaplasma* spp. was not positive for *E. canis*.

The detection of *A. platys* by PCR may range from 7.8% to 88% in Brazil (Santos et al., 2013). In RS, the detection of this pathogen is described in the study of Lasta et al. (2013), who found 14.07% of positive dogs in PCR. In addition, *A. platys* has also been reported in cats in Brazil (Lima et al., 2010; Correa et al., 2011), but no cat was positive in our study. On the other hand, few are the reports on molecular detection of *A. phagocytophilum* in Brazil (Santos et al., 2011; Santos et al., 2013; Silveira et al., 2015). Our study indicates the presence of *Anaplasma* spp. closely related to *A. phagocytophilum* (GU236670, bootstrap value 80/100). Additionally, *Anaplasma* spp. closely related to *A. phagocytophilum* has been detected in wild carnivores (André et al., 2012), wild birds

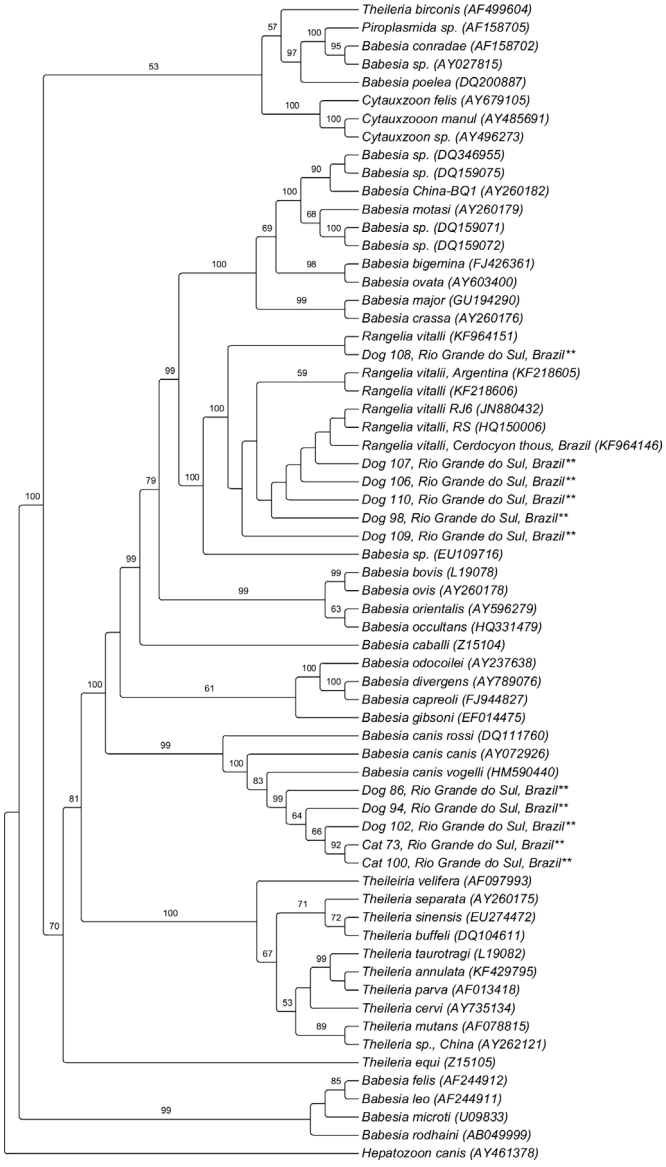

Fig. 2. Phylogenetic tree of piroplasmids (*Babesia* spp., *Cytauxzoon* sp., *Theileria* spp. and *Rangelia vitalii*) (*18SrRNA)* based on and maximum likelihood, using a GTRGAMMA model of evolution. Numbers correspond to bootstrap values over 50. **Samples from the present study.

(Machado et al., 2012), wild deer (Silveira et al., 2014), and free-roaming cats (André et al., 2014) in Brazil.

Regarding rangeliosis, after been overlooked for over 50 years, is currently regarded as a re-emerging disease in Brazil (Soares et al., 2014). Reports of infected canids are more frequent in the southern and southeastern regions, including the states of Rio de Janeiro (RJ) (Lemos et al., 2012), São Paulo (SP) (Pestana, 1910; Carini and Maciel, 1914), and RS (Loretti and Barros, 2005; Fighera et al., 2010). Aside from Brazil, rangeliosis has already been described in Argentina (Eiras et al., 2014) and in Uruguay (Sarasúa and Donati, 1976; Soares et al., 2015), countries where *A. aureolatum* is also found (Guglielmone et al., 2002; Venzal et al., 2005). In RS, where the disease appears to be more common, a retrospective study of Fighera et al. (2010) identified 35 cases of *R. vitalii* infection in necropsy and histopathological samples examined from 1985 to 2009. Besides these studies, other ones conducted in RS in recent years demonstrate the presence of *R. vitalii* in dogs (Krauspenhar et al., 2003; Soares et al., 2011), including a study of ours about domestic dogs in which four positive animals were detected (Gottlieb, 2014), in addition to positive results for one wild carnivore (Soares et al., 2014). Based on these results, we suggest that further studies be carried out in other regions of RS and across Brazil in order to get to know the current epidemiological profile of the disease.

B. vogeli has already been identified in Africa, Asia, Europe, Americas, and Australia. In Brazil, *B. vogeli* and *B. gibsoni* are the species identified in dogs so far (Passos et al., 2005; Trapp et al., 2006; Dantas-Torres and Otranto, 2014); nevertheless, the vector for *B. gibsoni* has not been detected in Brazil yet (Trapp et al., 2006). On the other hand, *R. sanguineus* s.l.is widely distributed in Brazil and adapts well to urban areas, which may contribute towards the endemic status of babesiosis in some Brazilian regions (Costa et al., 2015). Furthermore, dog-specific *Babesia* species have been sporadically detected by molecular methods in cats, also in Brazil, where new *Babesia* spp. and *Theileria* spp. genotypes related to ruminant and equine piroplasmosis have been recently reported in cats in southeastern and midwestern regions of Brazil (André et al., 2014, 2015a), thus highlighting the need for molecular characterization of circulating piroplasmids in pets.

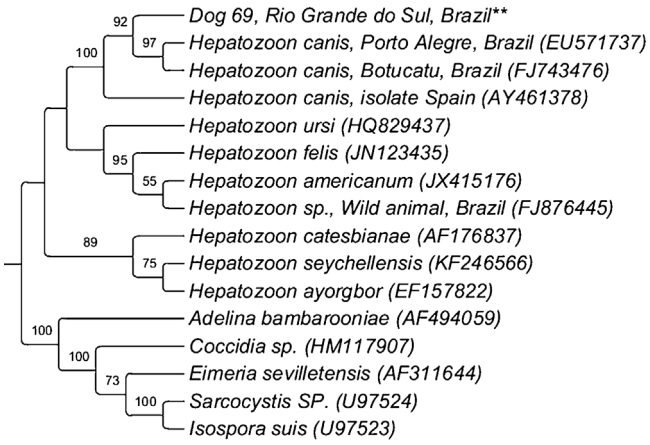

Fig. 3. Phylogenetic tree of *Hepatozoon* spp. (*18SrRNA*) based on and maximum likelihood, using a GTRGAMMA model of evolution. Numbers correspond to bootstrap values over 50. **Samples from the present study.

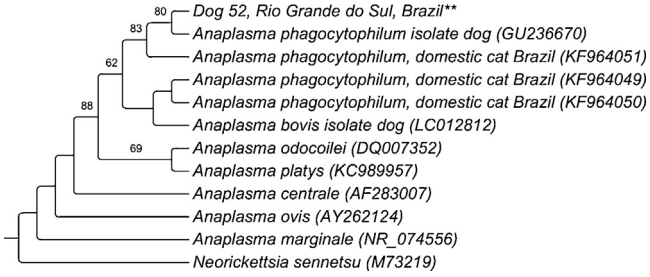

Fig. 4. Phylogenetic tree of *Anaplasma* spp. (*16SrRNA*) based on and maximum likelihood, using a GTRGAMMA model of evolution. Numbers correspond to bootstrap values over 50. **Samples from the present study.

We detected seropositive results for *B. vogeli* in 91% of the tested dogs, but Vieira et al. (2013) found 60.3% of positive dogs in indirect immunofluorescence (IIF), also in southern Brazil. In this study, out of 91%, 2 of 3 PCR-positive dogs for *B. vogeli* were also seropositive. Therefore, the animals tested herein were assumed to be in the chronic stage of the disease, which is often asymptomatic (Schnittger et al., 2012). Consequently, the levels of circulating parasites were below the PCR detection threshold, which, according to some authors (Birkenheuer et al., 2004; Jefferies et al., 2007; Irwin, 2009), may lead to false-negative results at this stage. Moreover, cross-reactions with antigens of other piroplasmids, such as *R. vitalii*, might have occurred when all the animals with positive PCR results for *R. vitalii* were considered to be also positive in the serum tests for anti-*B. vogeli* antibodies. So, what is known that *A. aureolatum*, tick involved in the transmission of *R. vitalii*, is found in this Brazilian state (Evans et al., 2000; Martins et al., 2010; Gauger et al., 2013; Soares et al., 2014) and also, unlike other Brazilian regions, the RS is not regarded as an endemic area for babesiosis. Additionally, seven animals were seropositive for anti-*B. vogeli* and anti-*E. canis* antibodies, including two positive dogs for *R. vitalii* in PCR, demonstrating possible exposure to these agents, as reported in other Brazilian studies (Trapp et al., 2006; Sousa et al., 2013). However, although *R. sanguineus* s.l. is potentially involved in the transmission of at least nine pathogens that infect dogs in Brazil (Dantas-Torres, 2008), the vectorial competence of temperate lineage of *R. sanguineus* s.l. found in RS for *B. vogeli* is still unknown.

Studies conducted in Brazil for research of *Hepatozoon* in dogs report levels of prevalence around 58% (Spolidorio et al., 2009) and 67% (Rubini et al., 2005), or as high as 84.3% in dogs from a rural area in southeastern (Miranda et al., 2014). On the other hand, we found only one dog positive for *Hepatozoon* spp. in PCR, as well as in study of Lasta et al. (2009), which had already detected this pathogen in a dog in the city of Porto Alegre. Apart from these reports, there are no more records of the presence of *Hepatozoon* spp. in dogs in the RS and the face of such prevalence differences, Miranda et al. (2014) report that the prevalence rates for infection may vary depending on the study design, sampling method, characteristics of the canine population, vector distribution, and diagnostic methods.

The phylogenetic analysis showed *Hepatozon* in the same clade of *H. canis* detected in Spanish red foxes. However, another study performed in RS identified foxes infected with *Hepatozoon* phylogenetically related to *H. americanum* detected in canids from the USA (Criado-Fornelio et al., 2006). In addition to the detection of *Hepatozoon* in dogs, the present study did not find any positive cat despite the fact that *H. canis* (Rubini et al., 2008), *H. felis* (Bortoli et al., 2012) and a new genotype of *Hepatozoon* sp. (André et al., 2015b) have been occasionally reported in cats in Brazil.

According to previous studies performed in Brazil, the molecular prevalence of *Bartonella* in cats ranges from 4.3% to 97.3% (Souza et al., 2010; Staggemeier et al., 2010; Bortoli et al., 2012; Braga et al., 2012; Miceli et al., 2013; André et al., 2014, 2015b). In these studies, it is important to take into account the different molecular methods used (conventional PCR or qPCR), in addition to the type of sampled population (stray cats, owned cats, or catteries). In our study, the frequency of positive cats for *Bartonella* spp. was equal to 20%, in which all animals were owned, but four of them lived in the same house, which could have facilitated transmission. Similar results were found by Miceli et al. (2013) and Staggemeier et al. (2014), who identified 17% and 25.53% of positive cats for *Bartonella* spp. in catteries in the state of Mato Grasso and RS, respectively.

None of the positive cats for *Bartonella* spp. in qPCR had clinical signs of infection. It is known that cats are the major reservoirs for *B. henselae*, *B. clarridgeiae*, and *B. koehlerae*, and that the infection is usually subclinical or shows nonspecific clinical signs (Maia et al., 2014). While *Ctenocephalides* fleas had been incriminated as vectors for *B. henselae* and *B. clarridgeiae, Ixodes* ticks have been pointed out as vectors for *B. henselae* and *B. birtlesii* (Cotté et al., 2008; Reis et al., 2011).

In this study, a higher frequency of positive animals was observed among animals submitted to elective procedures (14/19), probably because these animals could be chronic and asymptomatic carriers of these pathogens. Therefore, the only animals with positive blood smears with later molecular confirmation were those positive for *R. vitalli* (6/6), given that these animals had been submitted to routine procedures and were the only ones with acute disease compatible with rangeliosis at the time of collection. The isolates, consistent with those described in the literature (França et al., 2014), were anemia (100%), thrombocytopenia (67%), jaundice (50%), external bleeding (50%), and anorexia (50%).

All of the other animals with positive PCR results had negative blood smears and showed no clinical and/or laboratory findings compatible with infection by their respective agents (data not shown). In this case, infected dogs and cats may sometimes present with nonspecific clinical signs, low parasitemia due to subclinical or chronic disease, hindering the diagnosis of VBD (Spolidorio et al., 2009; Solano-Gallego and Baneth, 2011; Maia et al., 2014). Furthermore, serological assays may have cross-reactions, and direct detection by blood smears has poor sensitivity and specificity. Thus, molecular methods play an important role in the detection and differentiation of these agents (Little, 2010).

5. Conclusion

This study shows the presence of *R. vitalii, B. vogeli*, *Hepatozoon* spp., *Anaplasma* spp. and *Bartonella* spp. in pets. In cats, *B. vogeli* is detected for the first time in this region, showing the need for

more studies that aim to determine the role of these animals in the epidemiology of VBD.

Furthermore, we highlight the importance of pathogens research transmitted by arthropods in RS, Brazil, because, unlike other Brazilian regions, very little is known about the agents circulating in pets and wild animals and even more so, what are the vectors involved, which may hinder correct diagnosis, treatments and tick control measures in this region.

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