Is the free-ranging jaguar (*Panthera onca*) a reservoir for *Cytauxzoon felis* in Brazil?

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ABSTRACT

This study investigated the occurrence of *Cytauxzoon felis* and *Babesia* spp. in free-ranging jaguars (*Panthera onca*), domestic dogs (*Canis lupus familiaris*) and domestic cats (*Felis catus*) from the Cerrado, Amazon and Pantanal biomes of Brazil. Blood samples were collected from 30 jaguars, 129 dogs and 22 cats for detection of the 18S rRNA genes of piroplasmids. All of the jaguars from the Pantanal (n = 22) and Cerrado (n = 4) and three of four jaguars from the Amazon were positive for *C. felis*, but no dogs or cats were positive for the agent. All of the jaguars and domestic cats were negative for *Babesia* spp., while dogs from the Cerrado (7.9%; 5/63) and Amazon (10.6%; 5/47) biomes tested positive for the hemoparasite. *Cytauxzoon* nucleotide sequences detected were closely related to *C. felis*; and *Babesia* nucleotide sequences showed 100% of identity with *Babesia vogeli*. Although the pathogenicity of *Cytauxzoon* spp. genotypes that circulate in Brazil is still unknown, free-ranging jaguars probably play an important role in the maintenance of *C. felis* in nature. In addition, even though there is no evidence of the circulation of *Babesia* spp. between jaguars and dogs, the presence of this hemoparasite should be monitored in jaguar populations.

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1. Introduction

Cytauxzoon felis and *Babesia* spp. are protozoan hemoparasites belonging to the Piroplasmida Order that are transmitted by tick bites and infect a wide range of mammals worldwide (Meinkoth and Kocan, 2005; Penzhorn, 2006). *C. felis* has historically been viewed as highly fatal for domestic cats (*Felis catus*) while wild felids remain asymptomatic; however, recent studies have reported cats surviving infections as well as wild cats with fatal infections (Brown et al., 2010; Meinkoth et al., 2000; Nietfeld and Pollock, 2002; Rizzi

et al., 2015). *C. felis* appears to be observed primarily in the south-central regions of the United States of America (USA), where the disease has been extensively studied and the bob cat (*Lynx rufus*) has been identified as the natural reservoir of the parasite (Brown et al., 2010; Shock et al., 2013). Among the other felids, *C. felis* has also been diagnosed in lions (*Panthera leo*), tigers (*Panthera tigris*), pumas (*Puma concolor*), cheetahs (*Acinonyx jubatus*), ocelots (*Leopardus pardalis*), tigrinas (*Leopardus tigrinus*) and jaguars (*Panthera onca*) (André et al., 2009; Filoni et al., 2012; Lewis et al., 2012; Peixoto et al., 2007; Rotstein et al., 1999; Soares, 2001; Yabsley et al., 2006; Zinkl et al., 1981).

Babesia spp. are responsible for causing major tick-borne diseases in the world, namely, babesiosis. Feline babesiosis is most frequently reported in wild and domestic cats from Africa (Penzhorn et al., 2004; Penzhorn, 2006). High parasite loads of *Babesia* were associated with the death of lions during the canine distemper epidemic in the Serengeti in 1994, where the climatic conditions probably favored coinfections with *Babesia* spp. and

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Fig. 1. Location of study areas (dots) in Brazilian Biomes.

altered the host-parasite relationship (Munson et al., 2008). *Babesia* spp. have been reported in leopards (*Panthera pardus*), pampas cat (*Leopardus colocolo*), pumas, cheetahs, and lions (André et al., 2011; Averbeck et al., 1990; Ayoob et al., 2010; Bosman et al., 2007; Githaka et al., 2012; Kelly et al., 2014; Lopez-Rebollar et al., 1999; Penzhorn et al., 2001; Williams et al., 2014; Yabsley et al., 2006).

The jaguar is the largest feline in the Americas and the only representative of the genus *Panthera* on the American continent (Seymour, 1989). The jaguar is a top predator and as such plays an important role in balancing ecosystems (Soulé and Terborgh, 1999). This study investigated the presence of *C. felis* and *Babesia* spp. and verified possible transmission between jaguars and domestic animals in three Brazilian biomes—the Cerrado, Pantanal and Amazon.

2. Materials and methods

2.1. Study area

The current study was conducted in Brazil. Specifically, jaguars and domestic animals were sampled in the Cerrado biome, in the region of Emas National Park (ENP) (−18,061146S; −52,941067W); in the Pantanal biome, on the Caiman Ecological Refuge (−19,80319S; −56,27373W) and Barranco Alto Ranch (−19,57643S; −56,16144W); and in the transitional area between the Cerrado and Amazon biomes, in Cantão State Park (CSP) (−9,64503S; −50,13065W) (Fig. 1).

The Cerrado is the second largest Brazilian biome and an ecological hotspot, with approximately 80% of its area being degraded by human activities (Cavalcanti and Joly, 2002). The ENP is one of the largest protected areas of the Cerrado grasslands and one of the last refuges for the native fauna, including the jaguar (Klink and Moreira, 2002). The native vegetation surrounding the park

has been converted to large-scale crop plantations, mainly soybean, corn and, more recently, sugar cane, as well as, to a lesser extent, extensive livestock ranching.

The Pantanal is one of the largest contiguous habitats for jaguars outside the Amazon forest (Sanderson et al., 2002) and the largest wetland in the world. Rural properties in this area mainly engage in extensive cattle ranching and, in some cases, ecotourism (Harris et al., 2005).

The CSP is located in the transitional area between the Amazon forest and the Cerrado grasslands. The main economic activity of the surrounding rural properties is extensive livestock ranching, and indigenous lands are found nearby (Morton et al., 2006).

2.2. Animals and biological samples collection

Between February 2000 and May 2009, 29 free-ranging jaguars were captured as part of the Jaguar Long Term Monitoring Program conducted by the Brazilian Non-Governmental Organization Jaguar Conservation Fund. Two techniques to capture the animals were used: trained hounds and metal cage traps (Furtado et al., 2008). In addition, one juvenile jaguar, raised on an indigenous land near CSP, was also part of this sampling effort.

The jaguars were anesthetized intramuscularly with a combination of tiletamine-zolazepam (Zoletil® or Telazol®) at an average dose of 9.7 mg/kg. Immediately after the animal was completely immobilized, physiologic parameters (heart rate, respiratory rate and rectal temperature) were evaluated and monitored at 10 min intervals. Blood samples were collected by internal femoral vein puncture in vacuum tubes with an anticoagulant (EDTA) and physical examinations were conducted.

All adult jaguars were fitted with a radiocollar. The animals were placed in a shaded and quiet area to recover from anesthesia. Recaptures of five individuals were performed at ≥60 day-intervals. After

Table 1

Piroplasm species and GenBank accession numbers used for design of the primers that amplify the 18S rRNA gene region.

Species	GenBank	Amplicon (bp)
<i>B. canis</i>	AY272047; AY072926; AY649326	341
<i>B. rossi</i> ^a	AB935165; DQ111760; JQ613105	340
<i>B. vogeli</i>	AY072925; AY371197; AY371198; DQ439545; EF636702	340
<i>B. gibsoni</i>	AF175300; AF271082; AF396748	339
<i>B. felis</i>	AF244912; AY452707	372
<i>B. leo</i>	KC790444; AF244911; KC790441	369
<i>B. rodhaini</i>	M87565	364
<i>B. capreoli</i>	KX839234	341
<i>B. microti</i>	AB071177	374
<i>Babesia</i> spp.	AF244914; KC790442; JQ861965; JQ861971; AF244913	340–374
<i>C. felis</i> ^b	AF399930; AY531524; AY679105; L19080; GU903911	366
<i>Cytauxzoon</i> spp.	EF094470; EU622908; AY309956; KT361081; KT361080	365
<i>T. annulata</i>	DQ287944	354
<i>T. ovis</i>	FJ603460	358
<i>T. equi</i>	KJ573374	353
<i>Theileria</i> spp.	DQ866842; AF036336; KP410271; AF078816; JQ923446	349–361

^a 2 mismatches in forward primer.

^b 1 mismatch in forward primer.

being fitted with radiocollar, jaguars from the Cerrado and Pantanal regions were monitored through radiotelemetry and camera traps. The monitoring period comprises the interval between an individual's capture day and the last location obtained until November 2008, except one animal that was monitored until February 2010.

Between May 2008 and January 2010, blood samples were also collected from 129 domestic dogs and 22 domestic cats from rural properties surrounding the protected areas. Domestic dogs were sampled in 28 rural properties from the Cerrado, six properties from the Pantanal and 13 from the Amazon. Domestic cats were sampled in six rural properties from the Cerrado, seven from the Pantanal and three from the Amazon. Blood samples were collected by jugular or cephalic vein puncture in vacuum tubes with anticoagulant (EDTA).

All blood samples were transported to the research base, separated into aliquots with or without the addition of an equal volume of saturated saline (100 mM Tris, 100 mM EDTA, 2% SDS), and frozen at -20°C .

Handling procedures were consistent with the Ethical Principles in Animal Research adopted by the Bioethics Commission of the School of Veterinary Medicine and Animal Science of University of São Paulo (FMVZ-USP) and were approved by permit number 1471/2008. Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) granted field permits to work in ENP, Pantanal and CSP (Permits number 14637, 11214 and 11628, respectively).

2.3. DNA extraction

Biological samples were transported, on average, for 12 h, in coolers with ice packs to São Paulo-SP and stored at -20°C .

DNA extraction was performed by pre-incubating the blood with proteinase K for 4 h at 56°C and then for 15 min at 70°C (Rubini et al., 2005) under constant agitation. From 200 μl aliquots of the blood, DNA was isolated using a GFX[®] Genomic Blood DNA Purification Kit (GE Healthcare) according to the manufacturer's instructions. Each DNA sample was eluted in 100 μl of TE buffer.

2.4. Standardization of PCR

Primers that amplify fragments of 339–374 bp of the 18S rRNA gene region were designed based on the conserved DNA sequences of piroplasm species (Table 1): Piro F (5'-TGACACAGGGAGGTAGT-3') and Piro R (5'-CAACAAAATAGAACCAAG-3'). The amplification reaction was performed in a total of 25 μl containing 1 $\times$ Go Taq Colorless Mastermix (Promega[®]), 10 pmol of each primer and 5 μl

of extracted DNA. Ultrapure water was used as negative control and DNA from domestic dogs that tested positive for *Babesia vogeli* and DNA from a puma that tested positive for *C. felis* were used as positive controls. PCR reactions were performed using the following thermal cycles: 95°C for 5 min, 52°C for 1 min, 72°C for 2 min and 35 repetitive cycles of 94°C for 30 s, 52°C for 20 s and 72°C for 20 s, followed by a final extension at 72°C for 7 min. The purified amplicon of *B. vogeli* and *C. felis*, previously sequenced, were quantified using the Nanodrop 2000 (Thermo Fisher Scientific, Wilmington, DE) and the copy number was calculated. The sensitivity of PCR was performed with ten-fold dilutions (10^5 – 10^0) of amplicons using a canine negative DNA pool as a matrix (Helps et al., 2001). The specificity of the assay was tested with positive samples of *Babesia bovis*, 'Candidatus Mycoplasma haemominutum', 'Candidatus Mycoplasma turicensis', *Ehrlichia canis*, *Hepatozoon* spp., *Mycoplasma haemofelis*, *Rickettsia* sp. strain NOD and *Rangelia vitalli*. Moreover, the primers were aligned with 72 sequences available in GenBank, including species of the genera *Babesia*, *Cytauxzoon*, *Rangelia* and *Theileria*, to analyze the nucleotide identity (Supplementary material).

To confirm the agent and identify possible co-infections, two specific PCR protocols that separately amplified *Babesia* spp. and *C. felis* were performed. For *C. felis* detection, a 284 bp fragment of the 18S rRNA gene was amplified using the primers and amplification conditions described by Birkenheuer et al. (2006). For *Babesia* spp. detection, a 340 bp fragment of the 18S rRNA gene was amplified by PCR using the primers 455–479 F and 793–772 R as described by Birkenheuer et al. (2003). The amplification reactions were performed in a total of 25 μl containing 1 $\times$ GoTaq Colorless Mastermix (Promega[®]), 10 pmol of each primer and 5 μl of extracted DNA. For *C. felis*, DNA from a puma that tested positive for the agent was used as the positive control; and for *Babesia* spp., DNA of an isolate of *B. vogeli* from a domestic dog was used as the positive control. Ultrapure water was used as the negative control.

Aliquots of 5 μl of each of the amplified products were analyzed in 1.5 or 2% agarose gel with gel Red (0.05 $\mu\text{l}/\text{ml}$; Uniscience[®]) by electrophoresis at 80 V for 30 min in TAE buffer and visualized under a UV transilluminator. PCR fragments were estimated by comparison with known amounts of electrophoretic standards, using a 100 bp ladder (Invitrogen[®]).

Diagnoses were presented according to the species and study area sampled. An animal that had at least one positive result during capture or recapture was considered to be positive (Patterson and Lello, 2003).

2.5. DNA integrity

All samples that were PCR negative were tested for the glyceraldehydes-3-phosphate (GAPDH) gene to confirm the integrity of nucleic acids and to discard the presence of PCR inhibitors. PCR was carried out with 1× GoTaq Green MAsTernix (Promega®), 10 pmol of each primer GAPDH fel S (5'-GCCATCAATGACCCCTTCAT-3') and GAPDH fel AS (5'-GCCGTGGAATTTGCCGT-3') (Leutenegger et al., 1999), 2.5 µl of the DNA sample and ultrapure water to a final volume of 25 µl. Reaction conditions were 94 °C for 3 min, 35 cycles of 94 °C for 20 s, 55 °C for 30 s and 72 °C for 30 s, and final extension at 72 °C for 5 min. The reaction was considered positive when an amplicon of 81 (dog) or 164 bp (cats and jaguar) was present.

Aliquots of 5 µl of each of the amplified products were analyzed in 2% agarose gel with gel Red (0.05 µl/ml; Uniscience®) by electrophoresis at 80 V for 30 min in TAE buffer and visualized under a UV transilluminator. PCR fragments were estimated by comparison with known amounts of electrophoretic standards, using a 100 bp ladder (Invitrogen®).

2.6. Sequencing and phylogenetic analysis

Sequencing and phylogenetic analysis were performed with six positive samples that were randomly selected and include the following: two domestic dogs, *C. lupus familiaris* 31 and 117, and one jaguar, *P. onca* 156, from the Cerrado, two jaguars, *P. onca* 136 and 143, from the Pantanal and one jaguar, *P. onca* 149, from the Amazon. The PCR products were purified with the GFX™ PCR DNA and Gel Band Purification Kit® (GE Healthcare) according to the manufacturer's recommendations and quantified in comparison to the Low Mass DNA Ladder® (Invitrogen). Bidirectional sequencing was performed using the Sanger's method (Sanger et al., 1977) with BigDye Terminator 3.1 Cycle Sequencing Kit (Applied Biosystems) and ABI-3500 sequencer (Applied Biosystems™). The quality of the sequences was determined using Phred electropherogram quality analysis software (Togawa and Brigido, 2003), available at <http://asparagin.cenargen.embrapa.br/phph/>, last updated 2012. The final sequence was obtained with the Cap-Conting application of the Bioedit Sequence Alignment Editor version 7.2.5 program (Hall, 1999), and subject to comparison with the GenBank database (<http://www.ncbi.nlm.nih.gov/BLAST>). The nucleotide sequences generated for this study were aligned with homologous sequences of *C. felis* and *Babesia* spp. retrieved from the GenBank database using CLUSTAL/W method implemented in MEGA 6.06 program (Tamura, 2013). The values of the nucleotide identity were calculated using the Bioedit Sequence Alignment Editor program version 7.2.5 (Hall, 1999).

The *C. felis* and *Babesia* nucleotide sequences of these data set have been deposited in the GenBank database under the accession numbers: KY346853 to KY346855. A phylogenetic tree was constructed with the program MEGA 6.06 (Tamura et al., 2013) using the Maximum Likelihood and Neighbor-Joining methods based on the Kimura 2-parameter model with a discrete Gamma distribution and 1000 bootstrap replicates.

3. Results

Almost all captured jaguars (96.7%, n = 29) were in good physical condition and showed no clinical signs of any apparent disease. Only one of the female jaguars captured in the Amazon presented with low body weight and dehydration; it also had no incisors, no upper left canine and the lower canines were worn. Jaguars from the Cerrado and Pantanal regions were monitored, on average, for periods of 21.7 months (range of 1–91 months).

Table 2

Results of molecular tests by study areas and species sampled between February 2000 and January 2010.

Biome	Species	Examined	Positive for	
			<i>C. felis</i> (%)	<i>Babesia</i> spp. (%)
Cerrado	Jaguar	4	4 (100)	0
	Domestic dog	63	0	5 (7.9)
	Domestic cat	7	0	0
Pantanal	Jaguar	22	22 (100)	0
	Domestic dog	19	0	0
	Domestic cat	10	0	0
Amazon	Jaguar	4	3 (75)	0
	Domestic dog	47	0	5 (10.6)
	Domestic cat	5	0	0

All of the samples analyzed were positive for the endogenous control GAPDH in the DNA extractions. The sensitivity analysis of the designed primers (Piro F and Piro R) showed a limit of detection of 2 copies per reaction for both *B. vogeli* (KY346855) and *C. felis* (KY346854), which was confirmed in quadruplicate performed in 2 distinct runs. The specificity assay demonstrated no amplification of 'Ca. Mycoplasma haemominutum', 'Ca. Mycoplasma turicensis', *M. haemofelis*, *Hepatozoon* spp., *Rickettsia* sp. strain NOD, *R. vitalli*, *E. canis* and *B. bovis*. In silico analysis, with alignment of 72 sequences available in GenBank, showed 100% to 94% (1 mismatch) of identity with the genera *Babesia*, *Cytauxzoon* and *Theileria*, except the species *B. rossi*, *B. lengau* and *B. bovis* (Supplementary material).

Results of molecular tests are shown in Table 2. Five recaptured jaguars (one from the Cerrado and four from the Pantanal) presented with the same diagnosis as their initial captures. Four rural properties from the Cerrado (14.3%) and two from the Amazon (15.4%) had dogs only infected with *B. vogeli* but no dogs or cats were infected with *C. felis*.

The four *Cytauxzoon* nucleotide sequences (330 bp) had 99.6% of identity between them. As *P. onca* 136 and 143 had one degenerate base in the same position, a comparison with other sequences from the GenBank database was not included. *P. onca* 153 and 156 showed 100% and 99.6% of identity, respectively, with *C. felis* from a domestic cat with a fatal infection from the USA (AY679105); 98.7% and 99% of identity from a captive ocelot (GU903911) and a tigrina (DQ382277) from Brazil; 91.2% and 90.9% of identity with *Cytauxzoon* spp. from wildcat (*Felis silvestris*) (KT361081) and 90.9% and 90.6% of identity from lynx (*Lynx lynx*) (KT361080) from Europe.

Babesia spp. nucleotide sequences (304 bp) from the domestic dogs were identical and showed 100% of identity with *B. vogeli* from dogs (KT333456 KU710803) and cat (EF636702) from Brazil.

The phylogenetic analysis shows that *P. onca* 153 (KY346853) and 156 (KY346854) sequences segregated with *C. felis*, and *C. lupus familiaris* 31 (KY346855) segregated with *B. vogeli* sequences retrieved from GenBank (Fig. 2).

4. Discussion

The *C. felis* infection in all but one jaguar suggests the high prevalence of this pathogen in the study sites and the important role of jaguars in the maintenance of the parasite in the wild. This is the first report of *C. felis* in free-ranging jaguars from the Cerrado, Pantanal and Amazon biomes.

In Brazil, *C. felis* had been detected in jaguars, ocelots, pumas and lions kept in captivity (André et al., 2009; Filoni et al., 2012; Peixoto et al., 2007). A higher incidence of *C. felis* in free-ranging felids is probably related to a higher exposure to vectors in the natural environment. The jaguar raised in the indigenous land was the only negative result for *C. felis* in our study, probably because this animal did not move through natural habitats and had less contact with ticks.

Fig. 2. Phylogenetic tree of the 18S rRNA gene nucleotide sequences of *C. felis* from jaguars and *B. vogeli* from domestic dogs using Maximum Likelihood and Neighbor-Joining methods based on the Kimura 2-parameter model with a discrete Gamma distribution and 1000 bootstrap replicates. The circles indicate the sequences from this study. Bootstrap values superior of 70 are shown at the nodes. Bar represents substitutions number per site.

In the USA, *Dermacentor variabilis* and *Amblyomma americanum* are responsible for transmitting *C. felis* (Blouin et al., 1984; Reichard et al., 2009; Reichard et al., 2010). However, these two tick species do not occur in South America, where other ixodid species are probably involved in the hemoparasite transmission (Peixoto et al., 2007). In the present study, jaguars were mainly parasitized by different species of the *Amblyomma* genus (data not shown), that could have acted as vector for *C. felis*.

Nearly 100% of infected jaguars did not show clinical signs or alterations in their movement patterns during the monitoring period suggesting that they did not develop clinical disease and strengthening the hypothesis of the involvement of jaguars as potential carriers of *C. felis* in the study areas. The poor physical condition observed in a single jaguar from the Amazon was likely due to the animal's advanced age and not related to the presence of *C. felis*. André et al. (2009) suggested that Brazilian wild felids could act as potential reservoirs of *C. felis*, similar to bobcats and pumas in the USA (Blouin et al., 1984; Glenn et al., 1983; Rotstein et al., 1999; Shock et al., 2011). Although not common, fatal infections caused by *C. felis* were reported in bobcats (Nietfeld and Pollock, 2002), tigers (Garner et al., 1996) and lions (Peixoto et al., 2007)—highlighting the importance of monitoring the agent in jaguars, and not discarding the potential threat to immunosuppressed animals or animals with low genetic variability.

The PCR developed in the present study revealed high sensitivity and expected specificity when tested with the main hemoparasites of the studied species. It demonstrates the utility of this assay in screening studies since the primers match with different species of piroplasms. Comparing 298 bp from the *Cytauxzoon* nucleotide sequence from our jaguars with sequences from the other Brazilian jaguar described in the literature (EU376527), 100% of identity occurred with *P. onca* 153, and 99.6% of identity with *P. onca* 156 was found. Additionally, our sequences were closely related either to the domestic cat with fatal infection (AY679105) and the domestic cat with survival infection (AF399930). The identical sequences appear to support the hypothesis of a common source of *C. felis* infection to wild and domestic cats, but larger fragment sequencing and different target genes are still needed to confirm this hypothesis.

The four jaguars that tested positive in the recapture events confirmed the presence of the agent after the time intervals of 7–38 months, suggesting reinfection during that time or persistent infections, as reported for bobcats (Brown et al., 2010; Meinkoth and Kocan, 2005).

The absence of *Cytauxzoon* spp. in domestic dogs was expected since *C. felis* is a specific parasite of felines (Kier et al., 1982; Meinkoth and Kocan, 2005), but the agent was also absent in the domestic cats sampled. As the pathogenic potential of *Cytauxzoon* isolates in Brazil has not been utterly assessed, this absence cannot be related to the high fatality rate observed in the USA (Meinkoth and Kocan, 2005). Cats from our study were in good physical condition and apparently had no clinical signs of the infectious disease. In domestic cats in Brazil, *C. felis* has already been reported in the state of Rio de Janeiro in a cat co-infected with 'Ca. Mycoplasma haemominutum', which died four days after the onset of symptoms (Maia et al., 2013) and in the state of Minas Gerais (André et al., 2015). Conversely, cats from catteries in central-west Brazil (Miceli et al., 2013) and from a zoo environment in southeast Brazil (André et al., 2014) were PCR negative for the agent. The pathogenicity of *Cytauxzoon* spp. genotypes that circulate in domestic and wild cats in Brazil remains unknown. André et al. (2014) suggested that *C. felis* circulates more often in wild felids compared to cats in Brazil, a statement corroborated by our findings.

Babesia spp. was not detected in jaguars or domestic cats from the three study areas, and only dogs from the Cerrado and the Amazon surroundings tested positive for *B. vogeli*. As in the present

study, no free-ranging jaguars in Venezuela, French Guiana and the Pantanal were found to be positive for *Babesia* spp. (Criado-Fornelio et al., 2009; Thoisy et al., 2000). André et al. (2011) reported captive jaguars seropositive for *B. vogeli*, however, the same individuals were negative when performing molecular testing.

The low exposure of dogs to *Babesia* spp. in the present study agree with the frequencies of 5.2% and 18.8% found in dogs from Rio de Janeiro (O'Dwyer et al., 2001) and from Minas Gerais (Maia et al., 2007) in Brazil. The identification of *B. vogeli* in two positive dogs agrees with the findings of Passos et al. (2005) and Maia et al. (2007) that this is the species responsible for most cases of canine babesiosis in Brazil.

The absence of *Babesia* spp. in domestic cats is consistent with the literature (Dantas-Torres and Figueredo, 2006), stating that cats have a lower predisposition to contract the infection than dogs (Ayoob et al., 2010). Feline babesiosis was diagnosed and has been reported regularly in South Africa (Ayoob et al., 2010; Penzhorn et al., 2004). In Brazil, *B. vogeli* was recently detected in domestic cats from São Paulo (André et al., 2014), Mato Grosso (André et al., 2015) and Rio Grande do Sul (Malheiros et al., 2016). Regarding the wild felids, *Babesia* closely related to *B. leo* was detected in a pampas cat kept in captivity (André et al., 2011).

The vector of *B. vogeli*, the tick *Rhipicephalus sanguineus* s.l., was identified parasitizing domestic dogs in the three study areas but was not found in jaguars (data not shown). This reinforces the absence of *Babesia* in jaguars and the specificity of *B. vogeli* to this vector.

Munson et al. (2008) highlighted the importance of *Babesia* spp. showing that coinfection with the canine distemper virus resulted in the death of lions in the outbreak of 1994 in Serengeti. Thus, the presence of *Babesia* spp. in domestic dogs from the Cerrado and the Amazon surroundings should be monitored, as well as its occurrence in jaguars. *Babesia* spp. possibly have specificity for host species and the infection by species usually diagnosed in domestic animals could be fatal to wildlife, especially in stressful situations and in animals that are immunosuppressed or in endangered populations (Penzhorn, 2006).

5. Conclusions

The results of this study suggest the participation of free-ranging jaguars in the maintenance of *C. felis* in nature and the existence of a sylvatic cycle of the agent in the study areas. Otherwise, jaguars probably are not the normal host for *Babesia* spp., but the occurrence of this hemoparasite in dogs from surrounding properties should be carefully monitored due to the history related to the agent.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.tbd.2017.02.005>.

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