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Molecular detection of *Hepatozoon canis* and *Babesia canis vogeli* in domestic dogs from Cuiabá, Brazil

Detecção molecular de *Hepatozoon canis* e *Babesia canis vogeli* em cães domésticos de Cuiabá, Brasil

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Abstract

The objective of this study was to report for the first time infection by *Hepatozoon* spp. and *Babesia* spp. in 10 dogs from the city of Cuiabá, State of Mato Grosso, central-western Brazil. A pair of primers that amplifies a 574 bp fragment of the 18S rRNA of *Hepatozoon* spp., and a pair of primers that amplifies a 551 bp fragment of the gene 18S rRNA for *Babesia* spp. were used. Six dogs were positive for *Babesia* spp., and 9 were positive for *Hepatozoon* spp. Co-infection of *Babesia* spp. and *Hepatozoon* spp. was seen in 5 dogs. Sequenced samples revealed 100% identity with *B. canis vogeli*, and *H. canis*. This is the first molecular detection of *H. canis* in domestic dogs from Cuiabá. Additionally, it is described for the first time the presence of *B. canis vogeli* circulating among dogs in Cuiabá.

Keywords: *Hepatozoon canis*, *Babesia canis vogeli*, dogs, Mato Grosso, Brazil.

Resumo

O objetivo deste estudo foi relatar pela primeira vez a infecção por *Hepatozoon* spp. e *Babesia* spp. em cães domésticos provenientes da cidade de Cuiabá, estado de Mato Grosso. Foram utilizados pares de *primers* que amplificam um fragmento de 574 pb do gene 18S rRNA de *Hepatozoon* spp., e 551 pb do gene 18S rRNA para *Babesia* spp. Dos 10 cães amostrados, 6 apresentaram-se positivos para *Babesia* spp., e 9 foram positivos para *Hepatozoon* spp. pela PCR. Co-infecção entre *Babesia* spp. e *Hepatozoon* spp. ocorreu em 5 cães. As amostras revelaram 100% de identidade com *B. canis vogeli*, e as amostras que foram positivas para *Hepatozoon* spp. foram 100% idênticas a *H. canis*. Esta é a primeira identificação molecular de *H. canis* em cães domésticos em Cuiabá. Adicionalmente, descrevemos pela primeira vez a presença de *B. canis vogeli* circulando entre cães em Cuiabá.

Palavras-chave: *Hepatozoon canis*, *Babesia canis vogeli*, cães, Mato Grosso, Brasil.

Hepatozoon species known to infect dogs are tick-borne protozoans transmitted through the ingestion of infected ticks that cause disease in dogs throughout the world. This infection is easily misdiagnosed by veterinarians as general symptoms are similar to those seen in other tick-borne diseases such as ehrlichiosis and babesiosis (McCULLY et al., 1975; MURATA et al., 1991). Similar clinical and hematological findings for canine hepatozoonosis

and babesiosis include fever, lethargy, anorexia, weight loss, anemia, neutrophilia and thrombocytopenia (GREENE et al., 2006). Two *Hepatozoon* species have been described in dogs, *H. canis* (RUBINI et al., 2005; PALUDO et al., 2005) and *H. americanum* (BANETH et al., 2000); the former is responsible for all Brazilian cases reported in domestic dogs, and the latter is responsible for North American cases. Recently, new genotypes of *Hepatozoon* spp. including one closely related to *H. americanum* were identified in wild carnivores from Brazil (ANDRÉ et al., 2010). While *Rhipicephalus sanguineus* has been incriminated as a vehicle in the transmission of *H. canis* (SPOLIDORIO et al., 2009), other studies have found evidence that *Amblyomma ovale* is

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a natural vector as this tick has been found to be naturally infected by *H. canis*, and able to acquire and transmit *H. canis* to dogs (FORLANO et al., 2005; RUBINI et al., 2009). *Rhipicephalus sanguineus* is the most common ixodid tick in urban areas in Brazil, and is also responsible for the transmission of *Babesia* sp. in dogs (LABRUNA; PEREIRA, 2001). In Brazil two *Babesia* species infecting dogs have been reported, *B. canis vogeli* (PASSOS et al., 2005) and *B. gibsoni* (TRAPP et al., 2006). However, in the city of Cuiabá, State of Mato Grosso, central-western Brazil, species of *Hepatozoon* sp. and *Babesia* sp. have not yet been reported in domestic dogs.

The objective of this study was to report the first molecular characterization of *Hepatozoon* spp. and *Babesia* spp. in 10 domestic dogs from the city of Cuiabá, Mato Grosso, seen at two university veterinary hospitals (Universidade Federal de Mato Grosso and Universidade de Cuiabá), between July and December 2009. They presented different symptoms and were diagnosed with *Babesia* sp. and/or *Hepatozoon* sp. infection by direct visualization of parasites in Giemsa-stained blood smears.

Blood samples from these dogs were collected in EDTA anticoagulant tubes, and kept frozen at -20°C until DNA extraction. DNA was extracted from each blood sample using the DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions, and eluted in 100 μL of TE buffer, provided by the same DNA extraction kit. Five microliters of extracted DNA were used for polymerase chain reaction (PCR) amplification. DNase-free water was used as a negative control for DNA extractions and PCR assays. Primers HEP144-169 (5'-GGTAATTCTAGAGCTAATA-3') and HEP743-718 (5'-ACAATAAAGTAAAAACA-3'), which amplify a 574-bp fragment of the 18S rRNA of *Hepatozoon* spp., and primers BAB143-167 (5'-CCGTGCTAATTGTAGGGCTAATACA-3') and

BAB694-667 (5'-GCTTGAAACACTCTARTTTCTCAAAG-3'), which amplify a 551-bp fragment of the gene 18S rRNA of *Babesia* spp., were used according to Almeida (2011). PCRs were carried out in a total of 50 μL water-solution containing 1x PCR buffer, 1.5 mM MgCl_2 , 0.2 mM dNTPs, 1 U of Platinum Taq DNA Polymerase (Invitrogen, Carlsbad, CA), and 0.2 mM of each primer. PCR cycle conditions for *Hepatozoon* spp. consisted of a initial denaturation for 5 minutes at 95°C , and 35 repetitive cycles of 30 seconds at 95°C , 30 seconds at 50°C , and 60 seconds at 72°C , followed by a 5 minutes final extension at 72°C . PCR cycle conditions for *Babesia* spp. primers consisted of a initial denaturation for 5 minutes at 95°C , and 35 repetitive cycles of 30 seconds at 95°C , 30 seconds at 58°C , and 30 seconds at 72°C , followed by a 7 minutes final extension at 72°C . PCR products were electrophoresed through a 1.5% agarose gel (Invitrogen, Carlsbad, CA), stained with ethidium bromide (AMRESCO, Solon, OH), and examined by UV transillumination. Amplicons of the expected size were purified with ExoSap (GE Healthcare Pittsburgh, PA) and sequenced in an automatic sequencer (Applied Biosystems/PerkinElmer, model ABI Prism 310 Genetic, Foster City, CA) according to the manufacturer's protocol. Partial sequences obtained were submitted to BLAST analysis (ALTSCHUL et al., 1990) to determine the closest similarities to corresponding sequences.

From the 10 canine samples examined, 6 yielded amplicons through the PCR for *Babesia* spp., and 9 through the PCR for *Hepatozoon* spp. Five dogs presented amplicons by both PCR protocols, indicating co-infection by *Babesia* sp. and *Hepatozoon* sp., which was confirmed by DNA sequencing in at least three of these dogs (Table 1). With the exception of 3 positive samples (that showed a weak positive signal), all PCR products were DNA-sequenced. By BLAST analysis, the sequences obtained from the *Babesia*-PCR showed to be 100% identical to available

Table 1. Results of PCR and sequencing of *Hepatozoon canis* and *Babesia canis vogeli* in 10 dogs seen at two university veterinary hospitals in the city of Cuiabá, State of Mato Grosso, Brazil.

Dogs	Age	Clinical signs	Blood smear cytology	PCR results (Closest GenBank similarity)	
				<i>Babesia</i> primers	<i>Hepatozoon</i> primers
1	24 months old, female	Lymphadenopathy, myoclonus, pale mucous	<i>Hepatozoon</i> sp.	-	+ (100% <i>H. canis</i>)
2	8 months old, female	Lymphadenopathy, osteosarcoma, pale mucous	<i>Hepatozoon</i> sp.	-	+ (100% <i>H. canis</i>)
3	2 months old, male	Diarrhea, vomiting, anorexia and pale mucous	<i>Babesia</i> sp.	+ (100% <i>B. canis vogeli</i>)	+ (100% <i>H. canis</i>)
4	36 months old, male	Anorexia, vomiting and nasal secretion	<i>Babesia</i> sp.	+ (100% <i>B. canis vogeli</i>)	-
5	144 months old, female	Polyuria and polydipsia	<i>Babesia</i> sp.	-	+ *
6	20 months old, female	Lymphadenopathy and fever	<i>Babesia</i> sp.	+ (100% <i>B. canis vogeli</i>)	+ (100% <i>H. canis</i>)
7	1 month old, male	Diarrhea, vomiting, anorexia and pale mucous	<i>Babesia</i> sp.	+ (100% <i>B. canis vogeli</i>)	+*
8	3 months old, male	Anorexia, lymphadenopathy, pale mucous, diarrhea and fever	<i>Babesia</i> sp.	-	+ (100% <i>H. canis</i>)
9	1 month old, male	Diarrhea, vomiting and anorexia	<i>Babesia</i> sp.	+ (100% <i>B. canis vogeli</i>)	+ (100% <i>H. canis</i>)
10	3 months old, female	Lymphadenopathy, diarrhea, myoclonus and pale mucous	<i>Babesia</i> sp.	+ (100% <i>B. canis vogeli</i>)	+ *

(-) = negative; (+) = positive; * DNA sequencing not performed due to low amount of amplified DNA.

sequences of *B. canis vogeli* (AY371196; EF052632; DQ297390; AY371195; AY371194). The partial sequence (18S rRNA) generated from dog number 10 was deposited in the GenBank and assigned the nucleotide accession number JF295087. The sequences obtained from the *Hepatozoon*-PCR showed to be 100% identical to *H. canis* (AY461375; HM212626; GU371448; GU371447; GQ176245; FJ743476; FJ497022; FJ497021; FJ497020; EU571737; AY150067; AY471615; AF418558). The partial sequence (18S rRNA) of *H. canis* generated from dog number 2 in this study was deposited into the GenBank and assigned the nucleotide accession number JF295088. This isolate of *H. canis* was designated strain Cuiabá of *H. canis* and was stored frozen at -86 °C where it is available upon request. Regarding clinical signs in these infected dogs, no pathognomonic symptoms could be associated to canine infection by *H. canis* or *B. canis vogeli*.

Recently, André et al. (2010) reported *H. canis* in Brazilian wild carnivores, including animals from Cuiabá, but to our best knowledge there has been no previous report of *H. canis* in domestic dogs in the city of Cuiabá, State of Mato Grosso, and this is the first report confirmed by molecular biology tools. Additionally, it is described for the first time the molecular detection of *B. canis vogeli* among domestic dogs in Cuiabá.

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