Molecular prevalence and factors associated with *Babesia vogeli* infection in dogs in the Cerrado Mato-Grossense region of Brazil

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ABSTRACT: Canine babesiosis is a common haemoparasitosis in Brazil. Caused by parasites of the genus Babesia, it is transmitted by ixodid ticks and affects domestic and wild canids. The objective of this study was to verify the prevalence of Babesia species (spp.) using molecular methods in dogs living in urban and rural areas of Cuiabá, Mato Grosso State, Brazil, and to identify the main factors associated with infection. A total of 407 samples from 407 dogs were evaluated using a polymerase chain reaction (PCR) technique, among which Babesia species (spp.) was amplified in 10 (2.5%). Although, no statistical association was found among the variables studied (p>0,05), greater positivity was observed in dogs<1 year of age, male sex, those with free access to the street, and the presence of ticks. PCR samples positive for Babesia spp. were submitted to sequencing and compared in GenBank and exhibited a high degree of similarity with Babesia vogeli sequences.

Key words: babesiosis, molecular identification, Cuiaba.

Prevalência molecular e fatores associados à infecção por *Babesia vogeli* em cães do cerrado Mato-Grossense

RESUMO: Babesiose canina é uma hemoparasitose comum no Brasil. Causada por parasitos do gênero Babesia, é transmitida por carrapatos ixodídeos e acomete canídeos domésticos e silvestres. O objetivo deste trabalho foi verificar a prevalência molecular da infecção por Babesia spp. em cães residentes em áreas urbanas e rurais do município de Cuiabá, estado de Mato Grosso, Brasil, e relacionar os principais fatores associados à infecção. Para a pesquisa foram avaliados 407 cães usando a PCR. Das 407 amostras analisadas, 10 (2,5%) amplificaram DNA de Babesia spp. Não foi observada associação estatística entre as variáveis estudadas (p>0,05), porém observou-se maior positividade em cães com idade inferior a um ano, machos, com livre acesso à rua e com a presença de carrapatos. Amostras positivas nas PCRs para Babesia spp. foram submetidas a sequenciamento e comparadas no GenBank, mostrando alto grau de similaridade com as sequências de *B. vogeli*.

Palavras-chave: babesiose, identificação molecular, Cuiaba.

First detected by PASSOS et al. (2005) in Minas Gerais, Brazil, using a polymerase chain reaction (PCR) technique, *Babesia* is known to cause babesiosis in dogs in Brazil (SILVA et al., 2016). It has also been reported in France, Australia, Japan, South Africa, and the United States. *Rhipicephalus sanguineus sensu lato* is the biological vector of this protozoan species in Brazil (PASSOS et al., 2005; SCHOEMAN, 2009).

Among *Babesia* species (spp.), *Babesia vogeli* is the least pathogenic, and usually causes mild disease in adult dogs, but more severe illness in puppies, immunosuppressed dogs, or animals co-infected with other pathogens. Weakness, anorexia, paleness of the mucosae, fever, lymphadenomegaly, and splenomegaly are common clinical signs of the disease, similar to infections caused by other *Babesia* spp. (AZMI et al., 2017; WANG et al., 2018).

For many years, microscopic detection of the parasite using the blood smear stain technique has been considered the gold standard for the diagnosis of acute babesiosis. However, the low sensitivity of the technique makes detection difficult in cases of low parasitemia. Molecular techniques, such as PCR, have been applied and have shown to be more efficient in detecting and identifying *Babesia* spp. infections due to their high specificity and sensitivity (MTSHALI & MTSHALI, 2013; DAVITKOV et al., 2015). However, this sensitivity is decreased in samples obtained from asymptomatic dogs naturally.
infected with *Babesia* in the chronic disease phase when low fluctuations in parasitaemia are observed (IRWIN, 2009; SOUSA et al., 2013).

Based on these data and the few studies investigating babesiosis in the region, the present study was designed to verify the prevalence of *Babesia* spp. in the city of Cuiabá-MT, Brazil, and to identify the main factors associated with the infection, in addition to identifying the circulating species.

A total of 407 domiciled dogs evaluated in a cross-sectional study for visceral leishmaniasis conducted in endemic, urban, and rural areas in the municipality of Cuiabá, Mato Grosso State, Brazil (ALMEIDA et al. 2013) were used in the research. Sampling was calculated using an 8% prevalence rate for canine babesiosis (O’DWYER et al., 2009) based on the canine population of 64,290 from data provided by the Cuiabá Municipal Health Department, and an acceptable error rate of 5%, estimating a minimum of 400 dogs.

Owners were required to provide informed consent allowing enrollment of their dogs in the study. After the dogs were evaluated for body condition score, lymphadenopathy, and eye and dermatological changes, a questionnaire including this information was completed. Factors associated with infection, such as sex, race, age, street access, presence of ticks, origin, displacement to other regions, rural or urban residence area, permanence indoors, environment fumigation, and the presence of vegetation were also addressed in the questionnaire.

Blood samples (5 ml) were collected from the cephalic or jugular vein and stored in tubes containing ethylenediamine tetraacetic acid (EDTA) for molecular analysis. Extraction of DNA from these samples was performed using the phenol/chloroform/isoamyl alcohol method, according to a protocol described by ALMEIDA et al. (2013). A pair of *Babesia* oligonucleotides was used for PCR (forward, 5′–CCG TGC TAA TTG TAG GCC TAA TAC A–3′ and reverse, 5′–GCT TGA AAC ACT CTA RTT TTC TCA AAG–3’) to amplify a region of approximately 550 base pairs of the 18S ribosomal RNA (rRNA) gene, according to ALMEIDA et al. (2012a). The amplified products were separated using 1.5% agarose gel electrophoresis, stained with Gel Red (Biotium) and visualized on an ultraviolet transilluminator (UV-gel electrophoresis, stained with Gel Red (Biotium). Amplified fragments were subjected to sequencing using the Sanger method on an automated sequencer (ABI-3500, Applied Biosystems, Foster City, CA, USA).

The data obtained were transferred to a database and statistically analyzed using EpilInfo version 3.3.2 software (Centers for Disease Control & Prevention, Atlanta, GA, USA), and evaluated using chi-squared or Fisher’s exact test to test the association between independent variables and the presence of *Babesia* spp. DNA, considering a significance level of 5%. All variables were tested in multivariate analysis.

The specific fragment of the 18S rRNA gene from *Babesia* spp. was detected in 2.5% (10 of 407 [95% confidence interval [CI] 1.0–4.2%]) of the samples analysed. All positive amplicons for *Babesia* spp. were purified, sequenced, and compared in GenBank, which revealed 99% identity with the following deposits: KT323936.1, KT323935.1, KU710803.1, EF052627.1, all of which were identified as *B. vogeli*.

The prevalence of infection determined in the present study was relatively low, and is in agreement with other studies from different regions of Brazil, such as MELO et al. (2016) in Mato Grosso (3.1%), SOUZA et al. (2013) in Mato Grosso do Sul (3.3%), SILVA et al. (2012) in Maranhão (3.3%), RAMOS et al. (2010) in Pernambuco (7.3%), O’DWYER et al. (2009) in São Paulo (8%), COSTA-JÚNIOR et al. (2012) in Minas Gerais (10.8%), RIBEIRO et al. (2017) in Paraná (10.9%), PAULINO et al. (2018) in Rio de Janeiro (14%), MORAES et al. (2015) in Pará (15.7%).

To our knowledge, the present study was the first to estimate the prevalence of infection by *Babesia* spp in the city of Cuiabá - Mato Grosso, with evidence of autochthonous cases, because all positive amplicons were from animals born in the city and without previous displacement to other regions (Table 1). However, this is not the first molecular description of *B. vogeli* reported in the city. SPOLIDORIO et al. (2011) detected the presence of this piroplasma in six sick dogs treated at veterinary hospitals. In a study involving blood donor dogs admitted to a veterinary hospital in the same capital, SEABRA DA CRUZ et al. (2017) detected infection by *B. vogeli* and stressed the importance of more sensitive and specific diagnostic methods, such as PCR, and screening animals for this purpose. In a prevalence study in Poconé, a municipality located 100 km from Cuiabá, Pantanal Matogrossense region, MELO et al. (2016) reported dogs and ticks parasitized with the same species.

The detection of *B. vogeli* in urban and rural dogs in the present study was probably due to the predominance of *Rhipicephalus sanguineus*, the main tick species observed in the region (ALMEIDA et al., 2012b; MELO et al., 2016), and the unique known natural vector.
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Considered to be less pathogenic, B. vogeli manifests subclinically or with only mild disease, exhibiting greater severity in splenectomized animals and puppies (SCHOEMAN, 2009; WANG et al., 2018). Of the infected dogs, seven (58.3%) exhibited some clinical sign at the time of data and sample collection including lymphadenomegaly, progressive weight loss, petechiae, alopecia, and/or conjunctivitis. However, the stage of infection was unknown and other tick-borne diseases could not be excluded (WANG et al., 2018). The other three positive dogs

Table 1 - Univariate and multivariate analysis of variables considered associated with Babesia vogeli infection in dogs from Cuiabá, Mato Grosso.

| Variables                  | N     | Positive (%) | Univariate Analysis | Multivariate Analysis |
|----------------------------|-------|--------------|---------------------|-----------------------|
|                            |       |              | p / OR (CI)         | p                     |
| Sex                        |       |              |                     |                       |
| Male                       | 221   | 7 (3.2)      | 0.249               | 0.31                  |
| Female                     | 186   | 3 (1.6)      | 0.50 (0.11-1.7)     |                       |
| Breed                      |       |              |                     |                       |
| CRD                        | 77    | 2 (2.6)      | 0.59                | 0.93                  |
| SRD                        | 330   | 8 (2.4)      | 1.07 (0.33-4.74)    |                       |
| Age                        |       |              |                     |                       |
| Indefinite age             | 23    | 0 (0.0)      | 0.56                | 0.58                  |
| <1 year                    | 57    | 3 (5.3)      |                     |                       |
| 1-3 years                  | 152   | 3 (2.0)      |                     |                       |
| 3-6 years                  | 112   | 3 (2.7)      |                     |                       |
| >6 years                   | 63    | 1 (1.6)      |                     |                       |
| Access to the street       |       |              |                     |                       |
| No                         | 131   | 2 (1.5)      | 0.32                | 0.40                  |
| Yes                        | 276   | 8 (2.9)      | 1.92 (0.49-5.59)    |                       |
| Tick                       |       |              |                     |                       |
| No                         | 74    | 2 (2.7)      | 0.56                | 0.88                  |
| Yes                        | 333   | 8 (2.4)      | 0.88 (0.22-2.66)    |                       |
| City Origin                |       |              |                     |                       |
| Cuiabá                     | 378   | 10 (2.6)     | 0.47                | 0.37                  |
| Others                     | 29    | 0 (0.0)      | 0.92 (0.90-0.95)    |                       |
| Dog habitat                |       |              |                     |                       |
| Indoor                     | 6     | 0 (0.0)      | 0.86                | 0.69                  |
| Outdoor                    | 401   | 10 (2.5)     | 0.98 (0.97-0.99)    |                       |
| Vegetation at home         |       |              |                     |                       |
| No                         | 70    | 1 (1.4)      | 0.46                | 0.54                  |
| Yes                        | 337   | 9 (2.7)      | 1.89 (0.31-3.10)    |                       |
| Residence Area             |       |              |                     |                       |
| Rural Area                 | 112   | 2 (1.8)      | 0.45                | 0.59                  |
| Urban Area                 | 295   | 8 (2.7)      | 0.65 (0.21-2.61)    |                       |
| Insecticide use            |       |              |                     |                       |
| No                         | 342   | 9 (2.6)      | 0.50                | 0.60                  |
| Yes                        | 65    | 1 (1.5)      | 0.57 (0.34-3.19)    |                       |

N, number of animals; OR, odds ratio; CI, confidence interval; CRD - With defined Breed; SRD – Without defined Breed;
were subclinical. In a survey conducted in Croatia, a higher prevalence of *B. vogeli* was observed in dogs with subclinical infection compared to those with clinical signs (BECK et al., 2009). The presence of *B. vogeli* in clinically healthy dogs in our study corroborates the available literature, which considers *B. vogeli* to be the least pathogenic strain that causes relatively mild disease, usually subclinical infection (BECK et al., 2009; IRWIN 2009; SCHOEMAN, 2009; IONITA et al., 2011; WANG et al., 2018).

Regarding factors associated with infection (Table 1); although, male and defined-breed dogs exhibited higher positivity, variables, such as sex and breed, revealed no statistical association with *Babesia* spp. infection as COSTA-JÚNIOR et al. (2009), SILVA et al. (2012), ARAÚJO et al. (2015), and PAULINO et al. (2018) reported in their studies.

Although, no statistical difference was observed in relation to age and infection with *Babesia* spp., the frequency of positive dogs was higher in the group of animals<1 year of age (5.3%). Accounting for factors such as immature immune system associated with the stress of adapting to a new environment and a new diet, young and weaned dogs are actually more susceptible to disease (PAULINO et al., 2018). However, some reports in the literature described a higher prevalence in adult dogs (GUIMARÃES et al., 2016). Although, environmental control of ticks is performed by some owners, this important prophylactic measure was not statistically significant in this study, corroborating the results of SILVA et al. (2012). ADASZEK et al. (2011) highlighted that properly performed ectoparasite prophylaxis considerably limits *Babesia* spp. infection.

In conclusion, the present study demonstrated that the prevalence of babesiosis in naturally infected dogs, based on molecular data, is low in Cuiabá city, with *B. vogeli* being the circulating agent in both urban and rural regions, demonstrating a similar prevalence. The detection of this piroplasma in the Brazilian cerrado should be brought to the attention of veterinarians and tutors so that effective measures can be implemented to control canine babesiosis.

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**BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL**

This study was approved by the Ethics Committee for the Use of Animals of the Universidade Federal de Mato Grosso (UFMT), Brazil, under protocol number 23108.019868/099.

**DECLARATION OF CONFLICT OF INTERESTS**

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

**AUTHORS’ CONTRIBUTIONS**

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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