



## Survey of protozoan vector-borne diseases in dogs from Atlantic Rainforest fragment around Billings Dam, São Paulo, Brazil

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**ABSTRACT:** *Vector-borne diseases are currently one of the biggest public health concerns worldwide. Dogs, being the closest companion animals to humans, are considered the main reservoir of some of these diseases in the urban environment. Therefore, the study of the disease behavior in dogs can help to understand the disease affecting human health. Serological and molecular diagnoses of *Babesia vogeli*, *Rangelia vitalli*, *Leishmania infantum*, and other trypanosomatids, were performed by immunochromatographic and PCR assays, respectively, on dogs in a dog shelter located in an Atlantic Forest fragment near the Billings Dam, São Bernardo do Campo, São Paulo-Brazil. Our molecular diagnostic results showed a high prevalence of *Babesia vogeli*, at 20.9% (17/81). No other protozoan was detected in any of the tests. Determining the prevalence of major vector-borne diseases is essential to establish preventive and control measures for zoonotic diseases in animals kept in shelters, in order to minimize the impact of vector-borne diseases on animal health.*

**Key words:** *Rhhipicephalus, serology, molecular diagnosis, Phlebotomine, ticks.*

### Levantamento de protozoários transmitidos por vetores em cães de um fragmento de Mata Atlântica nos arredores da Barragem Billings, São Paulo, Brasil

**RESUMO:** *As doenças transmitidas por vetores são atualmente um dos maiores problemas de saúde pública. Os cães, sendo os animais de companhia mais próximos dos seres humanos, são considerados os principais reservatórios de algumas dessas doenças no ambiente urbano, e o estudo de seu comportamento em cães ajuda a entender a doença como um todo na saúde humana. Diagnósticos sorológicos e moleculares de *Babesia vogeli*, *Rangelia vitalli*, *Leishmania infantum* e outros tripanossomatídeos, em um abrigo para cães localizado em um fragmento da Mata Atlântica próximo à Barragem Billings, São Bernardo do Campo, São Paulo, Brasil. Foram realizadas sorologias e diagnósticos moleculares, no ensaio de PCR foram utilizados marcadores moleculares de oligonucleotídeos específicos para alguns protozoários de importância na saúde animal, como *Babesia vogeli*, *Rangelia vitalli*, *Leishmania infantum* e outros tripanossomatídeos. Nossos resultados de diagnóstico molecular mostraram uma alta prevalência de 20,9% (17/81) de *Babesia vogeli*. Nenhum outro protozoário foi detectado em nenhum dos testes. A determinação da prevalência das principais doenças transmitidas por vetores é essencial para estabelecer medidas preventivas e de controle de doenças zoonóticas em animais mantidos em abrigos. Essas medidas devem ser propostas para minimizar o impacto de doenças transmitidas por vetores na saúde animal.*

**Palavras-chave:** *Rhhipicephalus, sorologia, diagnóstico molecular, flebotomíneos, carrapatos.*

## INTRODUCTION

Vector-borne diseases are one of the biggest public health problems (DANTAS-TORRES, 2008; MAGGI et al., 2019). Dogs, being the closest companion animals to humans, are considered the main reservoir of some of these diseases in the urban environment, and studying the behavior of the disease in dogs can help in our understanding of the disease as a whole and of how it integrates within human health (DANTAS-TORRES, 2008; DANTAS-TORRES et al., 2014).

Visceral leishmaniosis, caused by *Leishmania infantum*, is now considered one of the most serious vector-borne diseases. In Brazil, it is responsible for 90% of all human and canine (PAHO, 2019). *Lutzomyia longipalpis* is the main vector, which is widely distributed (KRAUSPENHAR et al., 2003; MONGRUEL et al., 2018), but other species of this genus are also considered vectors (WALKER et al., 2000; MORAES-FILHO et al., 2011; DANTAS-TORRES et al., 2019).

Canine babesiosis is a hemolytic disease that, in Brazil, is caused by *Babesia vogeli*, in

which all clinical signs of the disease are caused by erythrocyte lysis (SOARES et al., 2018; DANTAS-TORRES et al., 2019). It is transmitted by the tick *Rhipicephalus sanguineus* sensu lato, which has a cosmopolitan distribution, nesting behavior, and easily adapts to changes in temperature and humidity (WALKER et al., 2000; MORAES-FILHO et al., 2011; DANTAS-TORRES et al., 2019).

*Rangelia vitalli*, the causative agent of canine rangelirosis, is a protozoan piroplasma that infects erythrocytes, leukocytes, and endothelial cells in dogs (CARINE et al., 1914; FIGHERA et al., 2010; MONGRUEL, 2018). It is more common in wild canids because its unique vector, the tick *Amblyomma aureolatum*, is exclusively found in areas of the Atlantic rainforest where the temperature and humidity conditions are favorable for its development (SOARES et al., 2018; DANTAS-TORRES et al., 2019).

In this study, we performed serological and molecular diagnoses of some protozoans with importance to health that are transmitted by different arthropods, such as *Babesia vogeli*, *Rangelia vitalli*, and *Leishmania infantum*, in a dog shelter located in an Atlantic Forest fragment in the influential area

of Billings Dam in the Riacho Grande district, São Bernardo do Campo, São Paulo-Brazil.

## MATERIALS AND METHODS

### *Geographical area, study population, and sample collection*

This study was conducted in a private shelter located in the Riacho Grande district of the city of São Bernardo do Campo in the metropolitan region of São Paulo, located at 23°48' 11.2" S and 46°32' 07.6" W (Figure 1). The study area is an Atlantic Forest fragmentation close to the Billings Dam. The kennels are rustic, made of wood, and the area for dogs is surrounded by the native Atlantic Rainforest of the region. Dogs in the shelter were mixed-breed animals that had been abandoned nearby, dogs that were born in the shelter from pregnant bitches, dogs that had been adopted more than once and returned due to lack of adaptation to the adopted home, and even purebred species that were part of clandestine kennels or were abandoned because they were born with some deformity or disability. It was not possible to obtain parameters to measure the age of the dogs.

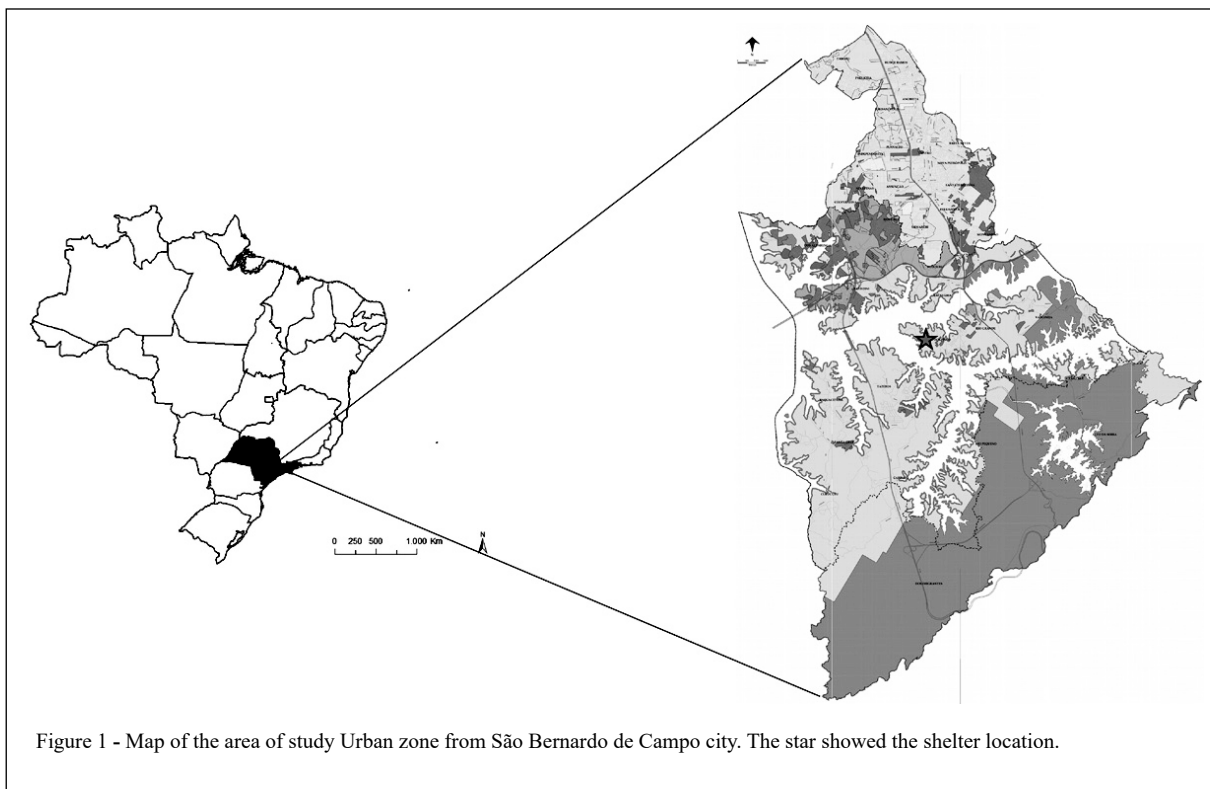


Figure 1 - Map of the area of study Urban zone from São Bernardo de Campo city. The star showed the shelter location.

Peripheral whole blood was collected from all 81 dogs that lived in the shelter on January 15, 2019. All dogs were inspected for the presence of ectoparasites and samples were collected.

In dogs that tested positive for serological and molecular markers of disease, fine needle aspiration of the popliteal lymph nodes was performed to isolate the parasite.

#### Serological screening

Serological screening was performed on peripheral whole blood immediately after collection by immunochromatographic assay, using the DPP® Rapid Diagnostic Kit for Canine Leishmaniosis (Biomanguinhos/Fiocruz) for the detection of K9/K26/K39-reactive antibodies, according to manufacturer's instructions.

#### DNA extraction

For DNA extraction, the commercial PureLink Genomic DNA Mini Kit (Invitrogen) was used according to the manufacturer's instructions.

#### Molecular markers for diagnosis

For the specific molecular diagnosis of *Leishmania infantum*, PCR was performed using L-like Cathepsin genes (SILVA et al., 2019) and ribosomal internal transcribed spacer (ITS) (SCHONIAN et al., 2003) as targets.

For detection of other trypanosomatid species, the V7V8 region of SSU rDNA was used, as this is considered the trypanosomatid barcode (MAIA DA SILVA et al., 2004). The characterization of isolates was performed by sequencing SSU rDNA and gGAPDH (MARCILI et al., 2014).

For *Babesia vogeli* and *Rangelia vitalli*, real-time PCR was performed using the sense hsp70-F and antisense hsp70-R primers (PAULINO et al., 2018), and sense Rv751-770 and antisense Rv930-911 primers (SOARES et al., 2014), respectively.

#### Isolation and culture of *Leishmania* parasites

For dogs positive by serological and/or molecular examination, parasite isolation was performed by fine needle aspiration of popliteal lymph nodes (MARCILI et al., 2014). Samples were inoculated into tubes of biphasic medium consisting of BAB (blood agar base, 15% rabbit blood) as the solid phase and LIT or RPMI medium, containing fetal bovine serum and antibiotics, as the liquid phase.

## RESULTS

The 81 samples were submitted for *Leishmania infantum* serology by the DPP® Rapid

Test distributed by the Brazilian Ministry of Health, and 2.46% (2/81) were positive.

Molecular tests for the trypanosomatid barcode region (V7V8 SSU rDNA) and the gGAPDH gene were negative for all samples. Specific molecular markers for the diagnosis of the *Leishmania* genus (ITS1 SSU rDNA) and *L. infantum* species (cathepsin L-like) were also negative for all samples tested.

Additionally, fine needle aspiration of the popliteal lymph nodes in dogs that had a positive reaction to the DPP® test was performed to attempt to isolate parasites. All cultures were negative for trypanosomatid parasites. The molecular diagnosis of *Rangelia vitalli* was also negative for all samples tested. A total of 20.9% (17/81) of the samples were positive for *Babesia vogeli*. No ectoparasites were found in the examined dogs.

## DISCUSSION

In the present study, 20.9% of dogs were positive for *Babesia vogeli*, which is much higher than previous studies conducted in São Paulo and other locations in the Southeast region of Brazil (SILVA et al., 2014; O'DWYER et al., 2009; MONGRUEL et al., 2018). It is known that high canine population density favors both an increase in the number of *R. sanguineus* ticks and their permanence in the environment, as well as pathogen circulation in the population. A study conducted in a dog shelter on *Rickettsia rickettsii* (PACHECO et al., 2011) demonstrated an increase in both the vectors and agent in the studied population, corroborating the results obtained in this study. However, during sample collection the dogs did not have a tick infestation.

Vector-borne diseases are important in human and animal health worldwide, with cycles involving vertebrates, invertebrates, and their interaction with the environment concerning different levels of anthropic action (ALBERIGI et al., 2019). The São Bernardo do Campo municipality is a predominant region of the Atlantic Forest fragments (SÃO BERNARDO DO CAMPO, 2019).

The Atlantic Rainforest has climatic conditions favorable for the development of *Amblyomma* spp. and phlebotomine (PINTER et al., 2004; SCINACHI et al., 2017); however, positive results were observed only for parasites of the genus *Babesia*, which is transmitted by ticks of the species *R. sanguineus* that are nidicolous and closely associated with canines in urban areas (GALAY et al., 2018).

In forested areas, adult *A. aureolatum* ticks feed on wild carnivores (GUGLIEMONE et al., 2003),

whereas in rural areas near forests, these ticks feed on domestic canines, enabling the transfer of these ectoparasites from dogs to humans (GUGLIEMONE et al., 2003; PINTER et al., 2004). *A. aureolatum* is the only species capable of transmitting *R. vitalli*, thus all clinical cases are related to an infestation of this tick (SOARES et al., 2018; SILVA et al., 2019). All dogs sampled in this study were kept in enclosures without access to the woods around the shelter, which corroborates the negative results for *R. vitalli*.

Positive results of serological tests for *Leishmania* may result from cross-reactions with other pathogens, most commonly with other trypanosomes, such as *Trypanosoma caninum* (ALVES et al., 2012), and leishmania vaccines (SOLANO-GALEGO et al., 2017; MARCONDES et al., 2013). This may account for the positive serological tests in this study, as the parasitological diagnosis was negative for serologically positive dogs. In addition, the molecular diagnosis was negative for all samples based on cathepsin L-like and ITS SSU rDNA markers. Despite the low parasitemia presented in *Leishmania infantum* infections, the use of the whole blood instead of the popliteal lymph node samples for molecular diagnosis is based to the high sensitivity and specificity of the marker based on Cathepsin L-like.

The canine population in the city of São Paulo was estimated at 2,507,401 in 2012, with a human:dog ratio of 4.34, and a higher concentration of dogs in urban areas than rural areas of the municipality (CANATTO et al., 2012). The proportion of dogs adopted (73.9%) was significantly higher than that of dogs purchased (26.1%), and the frequency of dog adoption is correlated with the frequency of households with a dog and greater levels of social exclusion (CANATTO et al., 2012). In this context, dog shelters play an important role in dog care and the adoption process.

Sanitation guidelines for animals kept in shelters should be proposed to minimize the impact of vector-borne diseases on animal health, as well as for the prevention and control of zoonotic diseases.

## CONCLUSION

This study showed that *Babesia vogeli* is highly prevalent in dogs from the Atlantic Rainforest fragment around Billings Dam, São Paulo-Brazil. These results also emphasized the need to test for multiple vector-borne diseases in dogs suspected of infection, to facilitate appropriate treatment and to promote the adoption of animals abandoned and kept in shelters.

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## BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

This work was analyzed and approved by the research ethics committee on the use of animals at the Universidade Santo Amaro (UNISA) (authorization no. 19/2018).

## DECLARATION OF CONFLICTS OF INTERESTS

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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