Molecular detection of *Leishmania infantum*, Babesia vogeli, and Rangelia vitalii in dogs from the Embu-Guaçú municipality around the Guarapiranga Reservoir, São Paulo

Detecção molecular de *Leishmania infantum*, Babesia vogeli e Rangelia vitalii em cães do município de Embú-Guaçú no entorno da represa Guarapiranga, São Paulo

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ABSTRACT
Vector-borne diseases are of great importance worldwide in human and veterinary medicine, as several parasites can cause disease in multiple different hosts. In this work, we investigated the prevalence of some vector-borne parasitic diseases in dogs in Embu-Guaçu, São Paulo, Brazil. Molecular diagnosis was performed by PCR using oligonucleotide markers specific for protozoans with significance in both animal and human health, such as *Leishmania infantum*, *Rangelia vitalii*, and *Babesia vogeli*. Molecular diagnostic results showed a high occurrence (16.25%, 13/80) of *Babesia vogeli*, but negative results for *Leishmania infantum* and *Rangelia vitalii*. Further studies should be conducted with a larger number of dogs, and vector control and educational measures should be implemented within the population to minimize the impact of vector-borne diseases on animal health.

Keywords: Sandfly; Tick; Zoonosis; Atlantic Rainforest, Vectors

RESUMO
As doenças transmitidas por vetores são de grande importância em todo o mundo na medicina humana e veterinária, pois vários parasitas podem causar doenças em vários hospedeiros diferentes. Neste trabalho, investigou-se a prevalência de algumas doenças parasitárias vetoriais em cães em Embu-Guaçu, São Paulo, Brasil. O diagnóstico molecular foi realizado por PCR utilizando marcadores oligonucleotídeos específicos para protozoários com significância tanto na saúde animal quanto na humana, como *Leishmania infantum*, *Rangelia vitalii* e *Babesia vogeli*. Os resultados diagnósticos moleculares mostraram alta ocorrência (16,25%, 13/80) de *Babesia vogeli*, mas resultados negativos para *Leishmania infantum* e *Rangelia vitalii*. Outros estudos devem ser realizados com um maior número de cães, e o controle de vetores e medidas educativas devem ser implementadas dentro da população para minimizar o impacto das doenças transmitidas por vetores na saúde animal.

Palavras-chave: Flebotomíneos; Carrapatos; Zoonose; Mata Atlântica; Vetores

1 INTRODUCTION
Vector-borne diseases are of great importance worldwide in human and veterinary medicine, as several parasites can cause disease in multiple different hosts (Massard and Fonseca, 2004). Due to the close contact between animals and humans, some of these diseases are considered public health problems with zoonotic potential because the animal reservoir of the disease may be kept in the home environment, which in many cases compromises animal welfare (Braga and Silva, 2013).

Included among the diseases of veterinary interest transmitted by arthropods are hemoparasitic diseases such as babesiosis and canine rangeliosis, transmitted by the ticks *Rhipicephalus sanguineus* and *Amblyomma aureolatum*, respectively (Costa-Junior et al., 2012; Araujo et al., 2015; Soares et al., 2015; Mongruel et al., 2018). Visceral leishmaniasis (VL), an important zoonosis, is transmitted by the sandfly *Lutzomyia longipalpis* and is considered a neglected
tropical disease, showing a clear and non-random distribution in Brazil (Lainson and Rangel, 2005; Lindoso and Lindoso, 2009; Azevedo et al., 2019).

The species *Leishmania infantum* is the causative agent of VL in the Americas (Silva, 2007; Marcili et al., 2014; Ribeiro et al., 2019). Dogs (*Canis familiaris*) are among the main vertebrate hosts in the urban environment, which in the wild include Canidae, rodents, and marsupials (Gontijo and Melo, 2004). This disease is a major public health concern due to its expansion, urbanization, and the severity of its different clinical forms (Figueiredo et al., 2012; Cardim et al., 2015).

Canine babesiosis is a disease caused by members of the *Babesia* genus (Baneth, 2018), which are erythrocyte parasites (Carret et al., 1999; Hunfeld et al., 2008). *Babesia vogeli* is the subspecies most commonly diagnosed in dogs in Brazil (Gottlieb et al., 2016). The causative agent of rangeliosis is *Rangelia vitalii*, a protozoan belonging to the order Piroplasmida that is an erythrocyte, leukocyte, and endothelial cell parasite (Lemos et al., 2012). Both diseases have similar symptoms such as fever, apathy, anemia, and jaundice (da Silva et al., 2011; Spolidorio et al., 2011; Quadros et al., 2015), but cutaneous hemorrhages are evident only in rangeliosis (Loretti and Barros, 2004; Lemos et al., 2017).

The aim of the present study was to detect infection by the hemoprotozoan parasites *Babesia vogeli*, *Rangelia vitalii*, and *Leishmania infantum* in dogs from a residential condominium in the Embu-Guaçú municipality, part of the Guarapiranga Reservoir, which has had a confirmed case of canine VL.

2 MATERIALS AND METHODS

This study was conducted in a residential condominium in the city of Embu-Guaçú-SP. This municipality has an area of 463 ha, bordering over 400 ha of native Atlantic Forest vegetation (23° 49' 04.9" S 46° 47' 13.0" W) (Figure 1). This site was chosen based on the notification of a case of canine VL through serological diagnosis.

A total of 80 animals were restrained for physical examination and collection of biological material. Approximately 0.5 mL of blood from the cephalic vein or, if this was not possible, jugular vein was collected from each animal and stored in microtubes containing absolute ethanol (da Costa et al., 2015). All dogs were inspected for the presence of ticks. All procedures were performed in accordance with the Ethics Committee on Animal Use (nº 22/2018 CEUA UNISA).

For DNA extraction, the PureLink Genomic DNA Mini Kit (Invitrogen) was used according to the manufacturer's instructions. For the specific molecular diagnosis of *Leishmania infantum*, PCR was performed using the cathepsin L-like gene (Silva et al., 2019) and ribosomal internal transcribed spacer (ITS SSUrDNA) (Schonian et al., 2003) as targets. For *Babesia vogeli* and *Rangelia vitalli*,
real-time PCR was performed using the sense Hsp70 gene (Peleg et al., 2010), and sense Rv751-770 and antisense Rv930-911 primers (Soares et al., 2018), respectively.

3 RESULTS

Molecular tests for the genus *Leishmania* (ITS1 SSUrDNA) and species *L. infantum* (cathepsin L-like gene) were negative for all samples. The molecular diagnosis of *Rangelia vitalli* was also negative for all samples tested.

A total of 16.25% (13/80) of the samples were positive for the molecular diagnosis of *Babesia vogeli*. No ticks were found on the examined dogs.

4 DISCUSSION

Vector-borne diseases are significant in both veterinary medicine and public health. Veterinarians, in collaboration with medical doctors, play a fundamental role in the prevention and treatment of vector-borne diseases (Savic et al., 2014).

Rangeliosis is generally a rural or peri-urban hemoparasitosis due to the vector habitats being associated with forest and rural environments (Labruna et al., 2005). There is also a positive relationship between altitude and the presence of *A. aureolatum*, which is confirmed in Southeast Brazil, as most reports of rangeliosis are related to the mountainous Atlantic Forest region (Pinter et al., 2016).

The municipality of Embu-Guaçú chosen for this study contains Atlantic Forest fragments. Despite favorable conditions for the *A. aureolatum* vector, *Rangelia vitalli* infection was not found in any of the sampled dogs; however, 13 animals were found to be infected with *Babesia vogeli*.

*B. vogeli* is the most commonly diagnosed subspecies of *Babesia* in dogs in Brazil (Lemos et al., 2012; Oliveira et al., 2009). It is transmitted by the tick *Rhipicephalus sanguineus*, the primary host of which is dogs, that is considered one of the main ectoparasites of dogs in Brazil (Labruna, 2004). *R. sanguineus* is the most widespread tick in the world, is well adapted to its environment, and can be found parasitizing dogs in both rural and urban areas (Moraes-Filho et al., 2011), leading to the transmission of pathogens such as *B. vogeli* in many different environments. A total of 16.25% of the dogs in this study were positive for *B. vogeli*, which is much higher than that found in other studies conducted in the Southeast region of Brazil, including São Paulo (O’Dwyer et al., 2009).

Despite the implementation of the Ministry of Health’s Surveillance and Control Program, VL is booming in Brazil’s large urban centers (Cardim et al., 2013; Von Zuben and Donalisio, 2016). Data from 1999 to 2017 showed that 7,328 cases of VL were reported in the state of São Paulo, and 2,858 were confirmed as autochthonous (Hiramoto et al., 2019).
The metropolitan region of São Paulo was investigated in 2007 after notification of a human case of VL, but the transmission mechanism is poorly understood (Camargo-Neves, 2008). The unregulated transportation of small animals is a risk factor, as it allows the migration of parasitized animals to non-endemic areas.

Epidemiological and entomological surveillance are of great importance to prevent the spread of parasitic diseases; much of the control of VL is concentrated on the vector *Lu. longipalpis*; however, the possibility of other sandflies acting as vectors cannot be disregarded (Galvis-Ovallos et al., 2017).

The metropolitan region of São Paulo is extremely heterogeneous in environmental, social, and economic aspects, and several significant diseases in veterinary and human medicine are neglected. Thus, further studies should be conducted with a larger number of dogs sampled from distinct areas, and vector control and educational measures put in place within the population.

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CONFLICT OF INTEREST

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

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