FULL ARTICLE

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Dogs naturally infected by *Rangelia vitalii, Babesia canis vogeli,* and *Ehrlichia canis* in São Paulo, Brazil

Cães naturalmente infectados por Rangelia vitalii, Babesia canis vogeli, *e* Ehrlichia canis *em São Paulo, Brasil*

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ABSTRACT

Several agents can cause hemoparasitic diseases in dogs, and blood-sucking arthropods transmit these diseases. These agents can cause several clinical manifestations and, in some cases, can kill the host. Because these agents are essential in animal health, this study aims to detect the frequency of *Ehrlichia canis*, *Rickettsia rickettsii*, *Anaplasma platys*, and *Rangelia vitalii* by real-time PCR and *Babesia vogeli* in dogs in the southern region of the city of São Paulo, São Paulo. Of the 98 dog samples, 18 (18.4%) tested positive with real-time polymerase chain reaction for at least one studied agent. Of these 18 samples, 17 tested positive for a single agent (11.2% for *B. canis vogeli*, 1.02% for *R. vitalii*, and 5.1% for *E. canis*), and one showed co-infection with *B. canis vogeli* and *R. vitalii.* The results demonstrate the presence of hemoparasites in the studied animals, which can influence the quality and life expectancy of these animals. The *Rangelia* detection warns small animal clinicians to include it as a differential diagnosis for hemoparasitosis.

Keywords: Hemoparasitic diseases. Molecular diagnosis. qPCR. Ticks. Vector-borne diseases.

RESUMO

As hemoparasitoses em cães podem ser causadas por diversos agentes, sendo essas doenças transmitidas por artrópodes hematófagos. Esses agentes podem causar diversas manifestações clínicas e, em alguns casos, podem matar o hospedeiro. Este estudo teve como objetivo detectar por PCR em tempo real a frequência *de Ehrlichia canis, Rickettsia rickettsii, Anaplasma platys, Rangelia vitalii* e *Babesia canis vogeli* em amostras de cães da zona sul da cidade de São Paulo, Brasil. Das 98 amostras de cães, 18 (18,4%) testaram positivo com reação em cadeia da polimerase em tempo real para pelo menos um agente estudado. Destas 18 amostras, 17 testaram positivo para um único agente (11,2% para *B. canis vogeli*, 1,02% para *R. vitalii* e 5,1% para *E. canis*), e uma apresentou coinfecção com *B. canis vogeli* e *R. vitalii*. Os resultados demonstram a presença de hemoparasitas nos animais estudados, o que pode influenciar a qualidade e a expectativa de vida desses animais. Além disso, é o primeiro relato da detecção de *R. vitalli* na zona sul de São Paulo e serve de alerta para os clínicos de pequenos animais incluírem esse agente como diagnóstico diferencial para as hemoparasitoses.

Palavras-chave: Doenças hemoparasitárias. Diagnóstico molecular. qPCR. Carrapatos. Doenças transmitidas por vetores.

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Introduction

Several agents can cause hemoparasitic diseases in dogs and are usually transmitted by hematophagous arthropods (Mongruel et al., 2018; Otranto et al., 2009). They are relevant in small animal practice and can have clinical manifestations of varying intensities, often with poor prognoses. The definitive diagnosis is usually not achieved in the clinical routine, and the presumptive diagnosis is based on the anamnesis, clinical manifestations, and abnormalities in the blood investigations. A presumptive diagnosis can impair the animal's well-being, lead to the discontent of the guardian, and cause bacterial resistance, as well as put the affected dogs and concerned individuals at risk due to the potentially zoonotic character of some agents (Mongruel et al., 2018; Otranto et al., 2009).

Symptoms in sick dogs include increased rectal temperature, weight loss, lethargy/apathy, pale mucous membranes, changes in lymph nodes, diffuse hemorrhages, hematuria, jaundice, and petechiae; and may cause death in some animals (Irwin & Hutchinson, 1991; Harrus et al., 1997; Sato et al., 2020). The most frequent alterations in the blood count are thrombocytopenia, hemolytic anemia, leukocytosis or leukopenia, hyperglobulinemia, hypoalbuminemia, hemoglobinuria, and proteinuria (Harrus et al., 1997; Irwin & Hutchinson, 1991; Sato et al., 2020).

The expansion of hemoparasites is directly proportional to the increase in the population of arthropods. This fact is observed globally, due to climate and environmental changes, favoring the access of these parasites to new habitats (Shaw et al., 2001).

The southern region of São Paulo is characterized by urbanized regions in areas of fragmentation of the Atlantic Forest, with one-fourth of the population living in informal settlements (slum communities) (Boletim CEInfo..., 2012). These conditions are ideal for *Amblyomma aureolatum* and *Rhipicephalu ssanguineus* to thrive, which are the vectors responsible for transmitting some agents associated with hemoparasitic diseases (Scinachi et al., 2017).

Molecular studies of blood parasites in the studied area provide essential epidemiological information for the prophylaxis, control, and treatment of diseases (Aguiar et al., 2013; Shaw et al., 2001) and are also paramount for maintaining the health and well-being of the population.

Using real-time PCR, this study aims to detect the frequency of *Ehrlichia canis*, *Rickettsia rickettsii*, *Anaplasma platys*, *Rangelia vitalii,* and *Babesia vogeli* in dogs in the southern region of the city of São Paulo, São Paulo.

Materials and methods

We used whole blood samples collected from dogs residing in different locations in the southern region of the municipality of São Paulo, including environments located close to areas of the Atlantic Forest (São Paulo, 2002), by a private laboratory in the South Zone of São Paulo between January and July 2018. The laboratory donated these samples for this study. The study was approved by the Animal Use Ethics Committee of Santo Amaro University (CEUA 17/2018).

Whole blood from dogs was processed individually. DNA was extracted using the PureLink™ Genomic DNA Kit (Thermo Fisher Scientific) following the manufacturer's instructions. The obtained DNA samples were identified and stored at -20°C until use for molecular diagnosis.

Dog samples were tested using real-time polymerase chain reaction (RT-PCR) to detect the genetic material of *A. platys*, *B. canis vogeli, E. canis, R. vitalii,* and *Rickettsia* spp. The primers used were targeted to *A. platys* 18s rRNA genes (Khatat et al., 2017), *B. canis vogeli* hsp70 (Paulino et al., 2018), *E. canis* dsb gene (Doyle et al., 2005), *R. vitalii* hsp70 (Soares et al., 2011), and citrate synthase from *Rickettsia* spp. (Labruna et al., 2004).

Samples were individually tested for each hemoparasite in 96-well plates subjected to thermal variations through an initial cycle of 95°C for 5 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min (Labruna et al., 2004). Data were amplified, acquired, and analyzed using the multicolor detection system for real-time PCR (7500 Real-Time PCR Systems - Applied BioSystems, Foster City, CA, USA).

Results

Of the 98 dogs tested, 18 (18.4%) tested positive in the molecular test for at least one hemoparasite studied. Of these 18 samples, 17 tested positive for a single agent: 11.2% for *B. canis vogeli*, 1.02% for *R. vitalii*, and 5.1% for *E. canis*. Co-infection with *B. canis vogeli* and *R. vitalii* was

detected in one sample. *R. rickettsii* and *A. platys* were not detected in the evaluated samples.

Discussion

In Brazil, hemoparasitic diseases were reported throughout the territory, as described in the states of Recife (Ramos et al., 2010), Maranhão (Costa et al., 2015), Paraíba (Rotondano et al., 2017), Rio Grande do Sul (Lasta et al., 2013), and São Paulo (Azevedo et al., 2020; Castelli et al., 2020; O'Dwyer et al., 2009). The incidence of these diseases in the country varied according to the agent found, vectors involved, sample characteristics, and sampled regions.

A single infection with *B. canis vogeli* was found in the DNA of 11.2% of the processed samples. This occurrence was lower than that reported by O'Dwyer et al. (2009) (8% [12/150]), Castelli et al. (2020) (16.25% [13/80]), and Azevedo et al. (2020) (20.9% [17/81]) from the interiors of the state of São Paulo. O'Dwyer et al. (2009) conducted a study on dogs from rural areas in three cities, Castelli et al. (2020) processed whole blood samples obtained from a condominium with a biome similar to that of the present study, and Azevedo et al. (2020) analyzed samples from shelter dogs. The characteristics of the sampled animals may explain these differences because the population of *R sanguineus* is directly proportional to the number of dogs present in the environment (Castelli et al., 2020; Azevedo et al., 2020).

In this study, real-time PCR showed a single *E. canis* infection in 5/98 (5.1%) of the samples analyzed. In Maranhão, only 9 out of 322 dogs (2.8%) were positive for this hemoparasite (Costa et al., 2015). Rotondano and collaborators found in a random sampling of 719 healthy dogs in the state of Paraíba, 8.9% positivity (Rotondano et al., 2017), in disagreement with a study carried out years earlier by the same researchers, but with dogs from a veterinary hospital, where the percentage of positive samples for *E. canis* it was equivalent to 25% (Rotondano et al., 2015). Perez et al. (2006) demonstrated an association between *E. canis* in humans and human monocytic ehrlichiosis. In Brazil, sequences of *E. canis* similar to those described by Perez et al. (2006) and human parasitism by R. sanguineus (Dantas-Torres et al., 2006) have already been described. However, there is still no description of this association in the country.

Although *A. platys* was not detected in this study, it has been identified by nested PCR in Rio Grande do Sul (Lasta et al., 2013) with a prevalence of 14.05% (28/199). Of these animals, 53 were stray dogs, and 146 were semi-domestic dogs. One of the hypotheses for this difference may be the diversity of tick populations found in the states of São Paulo and Rio Grande do Sul, which may result in vector competence with greater or lesser efficiency for this agent, as observed for *E. canis* (Moraes-Filho et al., 2015).

Furthermore, *Rickettsia* spp. was not found in the evaluated dogs. *R. rickettsii* was only reported once in dogs in Brazil as they have a short period of bacteremia (Piranda et al., 2011). Although the ticks commonly involved in the transmission of *R. rickettsii* are *A. aureolatum* and *A. sculptum*, *R. sanguineus* s.l. can also transmit the bacteria to dogs (Piranda et al., 2011) and are recognized as the primary vector of this pathogen in humans in some areas of northern Mexico (Eremeeva et al., 2011). Regarding *Rickettsia* spp., only serological studies have found positivity in dogs (Binder et al., 2021; Carvalho et al., 2021; Piranda et al., 2011). *R. vitalli* was detected in 2/98 processed whole blood samples in this study. This was the first report of *R. vitalli* infection in dogs at the south zone of São Paulo.

In 2019, Silva et al. (2019) reported the case of a dog with rangeliosis in the North Zone of the municipality. The South and North zones have environmental characteristics supporting the parasitism hypothesis by *A. aureolatum* ticks (Scinachi et al., 2017). This tick has vector competence in transmitting *R. vitalli* (Soares et al., 2018).

These results indicate that the pathogens transmitted by ticks should be monitored. Small animal clinicians working in the region should consider rangeliosis as a differential diagnosis in cases of hemoparasitic diseases.

Conclusion

The results demonstrate the presence of DNA from B. canis vogeli, *E. canis*, and *R. vitalli* in dogs from the southern region of the city of São Paulo, which can influence the quality and life expectancy of these animals. The *Rangelia* detection warns small animal clinicians to include it as a differential diagnosis for hemoparasitoses diseases.

Conflict of Interest

This is an original manuscript that has not been published before or submitted for publication elsewhere and will not be submitted until a decision has been taken for acceptability by Brazilian Journal of Veterinary Research and Animal Science. All the authors meet the criteria for authorship and assume the responsibility for the manuscript contents. We declare that there are no conflicts of interest.

Ethics Statement

The study was reviewed and approved by the Research Ethics Committee. University of Santo Amaro, Brazil. Protocol number is 17/2018.

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