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Molecular detection of *Rickettsia rickettsii, Ehrlichia canis* and *Rangelia vitalli* in *Rhipicephalus sanguineus* senso latu collected from dogs in Brazil

[*Detecção molecular de* Rickettsia rickettsii, Ehrlichia canis *e* Rangelia vitalli *em* Rhipicephalus sanguineus *sensu lato coletados de cães no Brasil*]

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ABSTRACT

This study evaluated by molecular methods the presence of major canine tick-borne agents in ticks infesting domestic dogs of a hospital population in a neglected area of the southern zone of the São Paulo Metropolitan region, which is characterized by an extensive urban area surrounded and interspersed by forest remnants of the original Atlantic rainforest. During 2017, 106 tick specimens – 71 adults and 33 nymphs of *Rhipicephalus sanguineus* sensu lato (s.l.), and two adults of *Amblyomma aureolatum* – were collected from 41 dogs that were attended in a Veterinary Teaching Hospital in São Paulo City, Brazil. By molecular analyses, 4.2% (3/71) of the *R. sanguineus* s.l. adult ticks contained the bacterium *Rickettsia rickettsii*, 2.8% (2/71) contained the bacterium *Ehrlichia canis*, and 4.2% (3/71) contained the protozoan *Rangelia vitalii*. These results indicate that domestic dogs of the southern zone of the São Paulo metropolitan region might be exposed to three of the major tick-borne agents affecting dogs in Brazil, *R. rickettsii*, *E. canis* and *R. vitalii*. In addition, the findings reinforce the circulation of the human pathogen *R. rickettsii* in the study area in a likely enzootic cycle involving dogs and *R. sanguineus* ticks.

Keywords: *Rhipicephalus sanguineus* senso latu, *Amblyomma aureolatum*, dogs, tick-borne diseases

RESUMO

Este estudo avaliou, por métodos moleculares, a presença dos principais agentes etiológicos de doenças caninas transmitidas por carrapatos que infestam cães domésticos de uma população hospitalar, em uma área negligenciada da zona sul da região metropolitana de São Paulo, caracterizada por uma extensa área urbana circundada e intercalada por remanescentes florestais de Mata Atlântica. Durante o ano de 2017, 106 espécimes de carrapatos – 71 adultos e 33 ninfas de Rhipicephalus sanguineus *sensu lato (s.l.), e dois adultos de* Amblyomma aureolatum *– foram coletados de 41 cães atendidos em um Hospital Veterinário Universitário da cidade de São Paulo, Brasil. Por análises moleculares, 4,2% (3/71) de* R. sanguineus *s.l. adultos continham a bactéria* Rickettsia rickettsii, *2,8% (2/71) continham a bactéria* Ehrlichia canis *e 4,2% (3/71) continham o protozoário* Rangelia vitalii. *Esses resultados indicam que cães domésticos da zona sul da região metropolitana de São Paulo podem estar expostos a três dos principais agentes carrapatos que afetam cães no Brasil,* R. rickettsii, E. canis *e* R. vitalii. *Além disso, os achados reforçam a circulação do patógeno humano* R. rickettsii *na área de estudo, em um provável ciclo enzoótico envolvendo cães e carrapatos* R. sanguineus.

Palavras-chaves: Rhipicephalus sanguineus *sensu lato*, Amblyomma aureolatum, *cães; doenças transmitidas por carrapatos*

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INTRODUCTION

Ticks are hematophagous arthropods that parasitize terrestrial vertebrates worldwide (Guglielmone *et al.,* 2014*).* In Brazil, the domestic dog is exposed to several tick species, and consequently, to tick-borne agents that might cause important diseases (Labruna and Pereira, 2001). In this regard, the most important tick-borne pathogens of dogs in Brazil are the bacterium *Ehrlichia canis*, the agent of canine monocytic ehrlichiosis (Aguiar *et al.*, 2013*),* the protozoa *Rangelia vitalii* and *Babesia vogeli*, agent of canine rangeliosis and canine babesiosis, respectively (Gottlieb *et al.*, 2016) and the bacterium *Rickettsia rickettsii*, the agent of Brazilian spotted fever (Labruna *et al.,* 2009*).* While the three agents are highly pathogenic to dogs, *R. rickettsii* also affects humans, in which Brazilian spotted fever has manifested with a 55% fatality rate (Faccini-Martínez *et al.,* 2021*).*

The São Paulo Metropolitan region is the largest populated area of Brazil, where nearly 21 million people occupy an area of nearly 2,200km^2. It is located on a plateau on the Serra do Mar Range, about 800 meters above sea level. The urban area is surrounded and interspersed by forest remnants of the original Atlantic rainforest, where several tick species have been reported on dogs (Ogrzewalska *et al.*, 2012). The aim of the present study was to evaluate by molecular methods the presence of major canine tick-borne agents in ticks infesting domestic dogs of a hospital population in a neglected area of the São Paulo Metropolitan region.

MATERIALS AND METHODS

During 2017, ticks were collected by convenience from dogs that were attended for any purpose at the Veterinary Teaching Hospital of the University of Santo Amaro (23^{o}43’43’’S, 46^{o}42’46’’W), São Paulo City, Brazil. The hospital is in the southern zone of the São Paulo Metropolitan region, in an area characterized by households surrounded by fragments of the Atlantic rainforest. All dogs attended at the hospital during this study were residents of the southern zone. This study was approved by the Ethics Committee on the Use of Animals of Santo Amaro University, with approval number 01/2016.

Ticks collected from dogs were stored in vials containing 70% ethanol and submitted to taxonomic identification according to Barros-Battesti *et al.* (2006). Thereafter, ticks were processed individually by DNA extraction through the guanidine isothiocyanate/phenol-chloroform protocol (Sangioni *et al.,* 2005*)* and tested by three independent protocols of Taqman real-time PCR assays: a genus-specific protocol targeting the genus *Rickettsia* (Labruna *et al.,* 2004), a species-specific protocol targeting *E. canis (*Doyle *et al.,* 2005), and a species-specific protocol targeting *R. vitalii (*Soares *et al.,* 2018*).*

Samples positive by the *Rickettsia* genus-Taqman assay were further tested by two conventional PCR protocols, one targeting a 401-bp fragment of the rickettsial citrate synthase gene (*gltA*) (Labruna *et al.,* 2004), and the other targeting a 632-bp of the rickettsial 190-kDa outer membrane protein gene (*ompA*), as previously described (Eremeeva *et al.,* 1994). PCR products were treated with ExoSAP-IT (USB Corp., Cleveland, OH, USA) and underwent DNA sequencing in an ABI automated sequencer (Applied Biosystems/Perkin Elmer, model ABI Prism 3500 Genetic, Foster City, CA, USA). The resultant *gltA* and *ompA* sequences were compared with GenBank data by BLAST analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and were deposited in GenBank under the accession numbers MT304455 to MT304459).

RESULTS

We collected 106 tick specimens from 41 dogs. The ticks were identified as 71 adults (39 males and 32 females) and 33 nymphs of *Rhipicephalus sanguineus* sensu lato (s.l.), and two (1 male and 1 female) adults of *Amblyomma aureolatum.* Four (4 males) *R. sanguineus* s.l. adults yielded rickettsial DNA by the *Rickettsia* genus-Taqman assay. These four adults yielded *gltA* and *ompA* amplicons through conventional PCR. Reliable *gltA* partial sequences were generated from two ticks, whereas *ompA* partial sequences were generated from three ticks. No *gltA* or *ompA* sequence could be generated from one *R. sanguineus* s.l. specimen that was positive by the Taqman assay. All *gltA* and *ompA* partial sequences were 100% identical to *R. rickettsii* sequences available in GenBank (CP003305). Based on these DNA sequences, we considered

that 4.2% (3/71) of the *R. sanguineus* s.l. adults contained *R. rickettsii.* For the other two Taqman assays, two *R. sanguineus* s.l. adults yielded *E. canis* DNA, and three *R. sanguineus* s.l. adults yielded *R. vitalii* DNA (Table 1). There was no coinfection.

Table 1. Results of molecular analyses for detection of DNA of *Rickettsia rickettsii*, *Ehrlichia canis* and *Rangelia vitalii* in 106 ticks that were collected from 41 infested dogs at the Veterinary Teaching Hospital of the University of Santo Amaro, located in the southern zone of São Paulo Metropolitan Area, Brazil

Collected ticks			No. ticks with detected DNA (%)		
Species	Stage	No. tested	*R. rickettsii*	*E. canis*	*R. vitalii*
Rhipicephalus sanguineus	Adults	71	3 (4.2)	2 (2.8)	3 (4.2)
	Nymphs	33	0	0	0
Amblyomma aureolatum	Adults	2	0	0	0

DISCUSSION

Except for two *A. aureolatum* specimens, all ticks collected in this study were identified as *R. sanguineus* s.l., which is an exotic species that has adapted to live inside households of the Brazilian cities, and consequently, it is the most frequent tick infesting urban dogs in Brazil (Labruna and Pereira, 2001, Ribeiro *et al.,* 1997, Szabó *et al.,* 2001). In contrast, *A. aureolatum* is native to the Atlantic rainforest of the São Paulo metropolitan region (Ogrzewalska *et al.,* 2012, Barbieri *et al.* 2015); therefore, the two specimens collected in this study were likely linked to dogs that had access to forest fragments of the study area.

Brazilian spotted fever has been endemic for decades in the southern zone of the São Paulo Metropolitan region, where *R. rickettsii* is transmitted to humans and dogs primarily by *A. aureolatum* (Ogrzewalska *et al.,* 2012, Scinachi *et al.,* 2017). In fact, a recent study reported a human fatal case in association with *R. rickettsii*-infected *A. aureolatum* ticks in a locality just 8 km from the veterinary hospital of the present study (Savani *et al.,* 2019) (Fig. 1). Previous studies from that metropolitan area reported that up to 11% of the *A. aureolatum* ticks from dogs were infected by *R. rickettsia* (Ogrzewalska *et al.,* 2012, Pinter and Labruna, 2006). Indeed, the low sample size of *A. aureolatum* in the present study (only two specimens) justify our negative results for rickettsiae in this *Amblyomma* species. On the other hand, our finding of *R. rickettsii* in 4.2% of the *R. sanguineus* s.l. adult ticks from dogs agrees with two previous studies in two different sites of the southern zone of the São Paulo Metropolitan region, in which *R. rickettsii* was detected in 1.3% (Moraes-Filho *et al.,* 2009) and 11.3% (Ogrzewalska *et al.,* 2012*)* of the dog-associated *R. sanguineus* s.l. ticks. The presence of *R. rickettsii* in *R. sanguineus* s.l. ticks were possibly related to horizontal transmission via rickettsemic dogs that were primarily infected via *R. rickettsii*-infected *A. aureolatum* ticks, a condition that has been demonstrated experimentally (Piranda *et al.,* 2011). Indeed, this condition highlights the role of *R. sanguineus* s.l. as an important vector of *R. rickettsii* for dogs in the study area, but so far not to humans, since to our knowledge, human infestation by *R. sanguineus* s.l. has never been reported in the São Paulo metropolitan region.

The tick-borne protozoan *R. vitalii* – originally described infecting dogs in the São Paulo metropolitan region more than a hundred years ago (Carini and Maciel, 1914), was recognized as a valid species only during this century (Gottlieb *et al.,* 2016). In addition, a recent study demonstrated that *A. aureolatum* is the main vector of *R. vitalii*, for which *R. sanguineus* s.l. was not a competent vector (Soares *et al.,* 2018). Therefore, because *R. vitalii* causes persistent blood infection in dogs for several months (Soares *et al.,* 2018), we infer that our finding of *R. vitalii* in three *R. sanguineus* s.l. adult ticks is likely to be related to ingested blood meal containing *R. vitalii.* Similarly, our finding of *E. canis* in two *R. sanguineus* adult ticks (2.8% infection rate) could also be related to ingested blood meal, since *E. canis* causes persistent infection in dogs (Vieira *et al*., 2011). Alternatively, it could be related to active infection in these ticks, since *R. sanguineus* s.l. is the natural vector of *E. canis* in the study area (Moraes-Filho *et al*., 2015) and previous reports of *E. canis*-infection rates in *R. sanguineus* s.l. in Brazil varied from 2.3 to 6.2% (Moraes-Filho *et al.,* 2015).

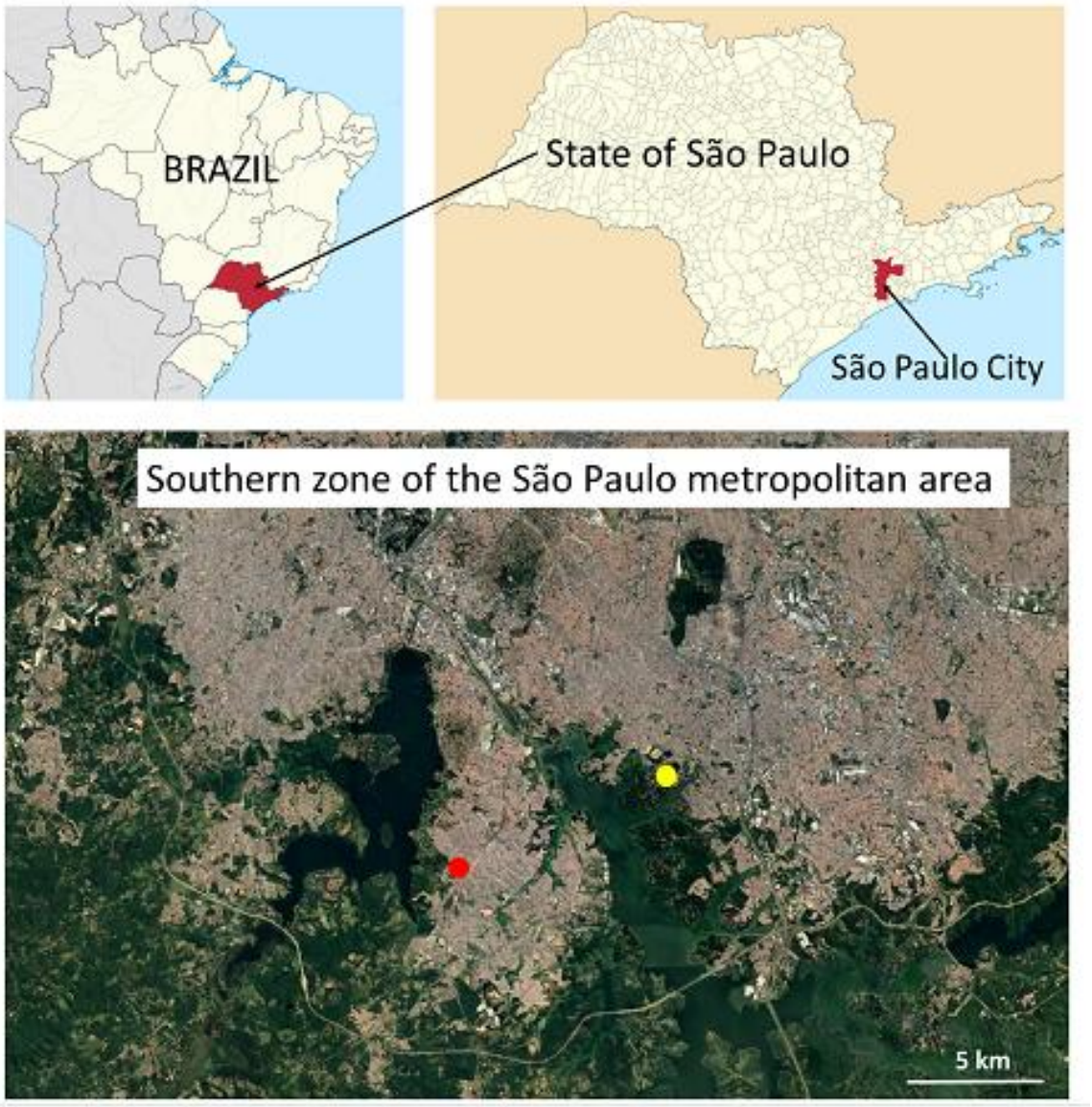


Figure 1. Location of the Veterinary Teaching Hospital of the University of Santo Amaro (red circle) in the southern zone of the São Paulo Metropolitan area in the state of São Paulo, Brazil. Yellow circle indicates the neighborhood where a human fatal case of Brazilian spotted fever associated to *Rickettsia rickettsii*-infected *Amblyomma aureolatum* ticks was recently reported by Savani *et al.* (2019).

In conclusion, our results indicate that domestic dogs of the southern zone of the São Paulo metropolitan region might be exposed to three of the major tick-borne agents affecting dogs in Brazil, *R. rickettsii, E. canis* and *R. vitalii.* In addition, our findings reinforce the circulation of the human pathogen *R. rickettsii* in the study area in a likely enzootic cycle involving dogs and *R. sanguineus* ticks.

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