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Research of hemoparasites in ticks collected from dogs resident in the municipality of Itu, São Paulo, SP

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ABSTRACT

OBJECTIVE

The aim of this study was to report the occurrence of *Ehrlichia canis*, *Babesia canis vogeli* and *Rangelia vitalli* in ticks collected from dogs living in the city of Itu, São Paulo/SP, Brazil.

METHODS

DNA was extracted from 200 tick samples using the extraction kit PureLink Genomic DNA Kit (Invitrogen®) and real-time PCR was performed for the detection of *Ehrlichia canis*, *Rangelia vitalli* and *Babesia canis vogeli*.

RESULTS

We tested 200 ticks, 1/200 (0.5%) of the genus *Amblyomma aureolatum* and 199/200 (99.5%) of *Rhipicephalus sanguineus*. The results show an occurrence rate of positivity only in *R. sanguineus*, being 0.5% (1/200) for *E. canis*; 41% (82/100) for *B. c. vogeli*. No ticks were positive for *R. vitalli*.

CONCLUSIONS

The detection of *B. canis vogeli* and *E. canis* in ticks collected from dogs living in the municipality of Itu, São Paulo State, Brazil; show the dispersion of these pathogens in the country and the role of *R. sanguineus* s.l. as a vector of these pathogens cannot be neglected.

DESCRIPTORS

Ehrlichia canis, *Babesia canis vogeli*, *Rangelia vitalli*, Ticks.

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INTRODUCTION

Of the approximately 1825 tick species described in the world, only about 10% are of direct public health importance, due to the possibility of parasitizing humans[1,2]. Several other tick species that have never been described parasitizing humans assume indirect importance in public health, as they contribute to the enzootic maintenance of infectious agents in nature[3].

Among the species with great importance for public and animal health, we can mention *Amblyomma sculptum* and *Amblyomma aureolatum*, vectors of the bacterium *Rickettsia rickettsii*, the causative agent of Brazilian Spotted Fever[4]; the last one also transmits the protozoan *Rangelia vitalli*, the pathogenic agent that causes the disease Canine Rangeliosis[5]. The tick *Rhipicephalus sanguineus* sensu lato (s.l.) is cosmopolitan and capable of transmitting microorganisms such as *Ehrlichia canis* and *Babesia canis vogeli* in domestic dogs[6].

Canine Monocytic Ehrlichiosis (CME) is caused by *Ehrlichia canis*, an obligate intracellular bacterium found inside monocytes and macrophages of domestic dogs. It is a multisystem disease, with acute, subclinical or chronic clinical presentations. Transmission occurs in the blood meal of the infected *R. sanguineus tick*[7].

Canine Babesiosis is an emerging disease of great importance in veterinary medicine due to its distribution, infectivity and pathogenesis[8], whose etiological agent is intraerythrocyte protozoa of the genus *Babesia*[9], species *Babesia canis*, in which it was subdivided into three subspecies: *Babesia canis canis*, *Babesia canis rossi* and *Babesia canis vogeli*[9]. In Brazil, the prevalent subspecies in dogs is B. canis vogeli and is distributed in tropical, subtropical and Mediterranean regions[9,10], present in both urban and rural environments[11], but mainly in urban and peripheral areas[12].

The protozoan *Rangelia vitalli* belongs to the Order *Piroplasmorida* and infects erythrocytes and endothelial cells of canids[13]. In a recent work carried out by Soares and collaborators[5]; it was reported that the species *A. aureolatum* is the only one that demonstrated vector competence for *R. vitalli*, as it was able to acquire and transmit the agent between domestic dogs.

Considering the risk for the occurrence of pathogens transmitted by these Ixodidae[1] in domestic dogs, it is essential to carry out research regarding the occurrence of *E. canis*, *B. canis vogeli* and *R. vitalli* in ticks in Brazil.

METHODS

Ticks were collected from 289 dogs that were screened at the Centro de Controle de Zoonoses de Itu, São Paulo, for pre-surgical evaluation of sterilization from March to November 2016. The ticks were preserved in flasks containing 70° alcohol. and duly identified according to Barros-Battesti[14].

The study was approved by the Ethics Committee for the Use of Animals at Universidade Santo Amaro (CEUA 15/2018).

Ticks were processed individually. DNA extraction was performed with the Purelink Genomic DNA Extraction Kit (Invitrogen®), according to the manufacturer's instructions. The eluates obtained from DNA were properly identified and stored at -20º C for further molecular analysis.

Real-time PCR for: a) *Babesia canis vogeli* was performed using sense hsp70-F and antisense hsp70-R primers associated with a specific internal fluorogenic probe (5'-Hex/AGCGCCAGGCCACCAAGGACGCT-3'-IABlkFQ), obtaining the amplification of a fragment of the hsp70 gene[15]; b) for *E. canis*, real-time PCR was performed using primers Dsb-321 and Dsb-671, in addition to the specific probe TaqMan (5`-AGCTAGTGCTGCTTGGGCAACTTTGAGTGAA - 3´) 5' FAM/BHQ – 1 3', obtaining an amplified nucleotide sequence of the dsb gene[16] is shown; c) for *R. vitalli* he used the oligonucleotide primers called sense Rv751-770 and antisense Rv930-91, in addition to a TaqMan probe [5'-6-FAM (CCT TAT CAA ATC ATT CTT C) MGB NFQ -3']. This pair of primers corresponds to the amplification of a fragment of the hsp70 gene[13].

The reactions were performed in 96-well plates subjected to thermal variations corresponding to an initial cycle of 95°C for 5 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for one-minute[28]. Amplification, acquisition, and data analysis were performed using the multicolor detection system for Real-Time PCR (7500 Real-Time PCR Systems – Applied BioSystems, Foster City, CA, USA).

RESULTS

A total of 200 ticks were tested, 1/200 (0.5%) of the species of the genus *Amblyomma aureolatum* and 199/200 (99.5%) of Rhipicephalus sanguineus. The results show an occurrence rate of positivity only in R. sanguineus, being 0.5% (1/200) for E. canis; 41% (82/100) for *B. canis vogeli*. No ticks were positive for *R. vitalli*.

DISCUSSION

The largest number of ticks collected in the present work was of the species *R. sanguineus* (97%), a fact that was already expected, because they were collected from dogs that are their main hosts (64/65) and such arthropods have a nidicola habit with great proximity to dogs and humans[12].

Ticks of the species *A. aureolatum* can be found parasitizing dogs, with wild carnivores as their main natural hosts in a native area of the Atlantic Forest, the lowest percentage of the finding of such tick species in dogs in the present study.

There is a complex interaction between hosts, vectors, arthropods and pathogens and the low prevalence of *E. canis* in the present study may be related to the decrease in infected animals for the maintenance of the pathogen in a tick population, as there is no vertical transmission in the tick population *R. sanguineus*[19].

Since *B. canis vogeli* causes a chronic and mild disease, it provides infected dogs for a longer time and consequently "food" for the ticks to maintain their infected populations for a longer time in the environment, also highlighting that in the cycle of this hemoparasite, transovarian transmission in *R. sanguineus* it is of great importance in the maintenance of this protozoan, causing ticks to remain infected for several generations[20]. This information was corroborated by the results found in the present study, in which 41% (82/100) of the ticks were positive for *B. canis vogeli*.

No sample was positive for *R. vitalli*, even though a specimen of *Amblyomma aureolatum*, vector of the protozoan[5] was collected in the present study, which suggests the need to monitor this pathogen in the city, as this infection can cause severe disease in domestic dogs[21].

In the present study, positivity of 0.5% (1/200) of the samples for *E. canis* was detected through real-time PCR; 41% (82/100) for *B. canis vogeli*. The low percentage found for the bacterium stands out, as studies show the high prevalence of the disease in dogs in the country[22]. Probably due to the high infection found in these ticks for *B. canis vogeli*, could somehow inhibit the infection for *E. canis*, which would explain the difference in the results found; but further studies are needed to verify if the vector competence of the arthropod for these pathogens occurs in the same way and if co-infections in the vector may inhibit the proliferation of one of the pathogens.

It is important to emphasize that due to the nidicola behavior of *R. sanguineus* s.l., the environmental differences have little impact on the environmental infestation of this vector[23], sug-

gesting that sanitary management and responsible custody are important behaviors in the control of these hemoparasitoses.

CONCLUSION

Detection of *B. canis vogeli* and *E. canis* in ticks collected from dogs residing in the municipality of Itu, in the State of São Paulo, Brazil; show the dispersion of these pathogens in the country and; the role of *R. sanguineus* s.l. as a vector of these pathogens cannot be neglected.

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