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### DOMESTIC DOGS AS SENTINELS OF *Borrelia burgdorferi* INFECTIONS TO HUMAN IN TWO REGIONS OF PANTANAL BIOME

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#### Abstract

At rural areas of Brazil, people keep dogs to guard their properties or for companionship in the field. Aware of these facts, this study aimed to evaluate the role of domestic dogs as sentinel of *Borrelia burgdorferi* in the Pantanal wetland (sub-region of Nhecolândia) and adjacent plateau region (Urucum rural settlement). We carried out serology (Enzyme Linked Immunosorbent Assay) and molecular test (PCR) in 139 dogs. Our results indicate for the first time the occurrence of *B. burgdorferi* infecting dogs in the western of Brazil. Overall, 28% (39/139) of sampled dogs were seropositive to *B. burgdorferi*. The use of a serological test combined with the molecular test allowed the identification of animals with acute (n=20) and chronic infection (n=15). Furthermore, distinct patterns of infections were recorded between the studied areas: in the Nhecolândia we found only four dogs (5%) seropositive contrasting with 35 dogs (56%) parasitized by *B. burgdorferi* in the Urucum rural settlement.

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#### Introduction

Ticks are known to transmit *Borrelia burgdorferi*, the etiological agent of Lyme disease, which is an important zoonotic disease in the United States and Europe (Krupka and Straubinger, 2010). In Brazil at the 90's, the first human cases of the disease were observed, accompanied with tick bites history (Yoshinari et al., 1993; Joppert et al., 2001). Due to some differences in the clinical manifestations and the difficulty of isolating the etiologic agent *Borrelia* spp., the disease was named Baggio-Yoshinari Syndrome (Yoshinari et al., 2010). This syndrome is caused by spirochetes with atypical morphology and the transmission of the parasites occurs by ticks, probably from the genera *Amblyomma* and *Rhipicephalus* (Yoshinari et al., 2010). Therefore, the emergence of this parasite in Brazil and the similar zoonotic disease (Yoshinari et al., 1993; Joppert et al., 2001) demonstrates the importance in fulfill gaps in the knowledge about occurrence, risks to human infection, epidemiology and transmission cycles (Joppert et al., 2001; Montadon et al., 2014).

At rural areas of Brazil, most of people keep dogs to guard their properties, as pets, for companionship in the field, and in some cases, dogs are also used in subsistence hunting. Direct contact with wild animals may be a source of spillover and spill back infection (Daszak et al., 2000; Thompson et al., 2009), and since dogs, people and wild animals share the same environment, dogs have been suggested as sentinels to estimate the risks for vector-borne infection to humans (Castañera et al., 1998; Roque et al., 2008; Salb et al., 2008; Rabinowitz et al., 2009; Wang et al., 2012). A number of characteristics contribute to the usefulness of dogs as sentinels, since they could be subject to a greater exposure to parasites due to behavior and habitat use (Rabinowitz et al., 2009).

Since dogs have been reported to be exposed to *B. burgdorferi* in some areas of Brazil, such as Cotia County (São Paulo state), Minas Gerais, and rural settlements of Paraná state (Joppert et al., 2001; Montadon et al., 2014; Nascimento et al., 2016), this study aimed to evaluate the role of domestic dogs in the enzooty of *Borrelia burgdorferi* in the Pantanal wetland (sub-region of Nhecolândia) and adjacent plateau (the Urucum rural settlement).

#### Methodology

The study was carried out in two distinct areas of the Pantanal, Mato Grosso do Sul state: (a) Nhecolândia sub-region and (b) Urucum rural settlement. The first area has a phytophysognomy characterized by deciduous or semi deciduous forests known as "cordilheiras" (long narrow strands of vegetation bordering

water bodies) or "capões" (forest patches surrounded by field floods). The topography is characterized by elevated portions that do not undergo regular flooding, floodplains and permanent or temporary ponds. The second area, Urucum rural settlement, located near to the Brazil-Bolivian border, approximately 18 km from the urban area of Corumbá, Mato Grosso do Sul state. The total area is 1,979 hectares, which is divided in 84 small properties. Approximately 87 families live in Urucum (Cardoso, 2013). The main economic activities are cassava and vegetables growing, dairy farming and breeding of chickens, pigs and cattle. A census survey carried out by the Zoonoses Control Center (ZCC) estimated domestic dogs' population in approximately 300 animals (ZCC, non-published data).

Sampling were conducted in August 2013 and July of 2014 at Nhecolândia and July and August of 2015 at Urucum rural settlement. Approximately 4 ml of venous blood was collected from each animal using sterile techniques: 2 ml were stored in a tube with Ethylenediamine Tetra acetic Acid (EDTA) for molecular testing, and the other 2 ml were stored in tubes without EDTA for serological testing.

For molecular tests we used genomic DNA extracted from 200 µl of blood with EDTA using the QIAamp Blood DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. To detect *B. burgdorferi* the primers flgE 262 FW (5 –TCCTCCGGGATTACATAACAAG–3 ) and flgE 262 Rev (5 –TGGGTGCAAATGTAGGTGAA–3) were used for amplification of a 262-bp fragment from the coding region of the conserved gene that synthesizes the flagellar hook (flgE) of *B. burgdorferi* (Lopes et al., 2017). Each reaction included negative and positive control samples from *B. burgdorferi*. PCR products were visualized in 2% agarose gel after ethidium bromide staining under ultraviolet light.

For the detection of anti-*Borrelia* IgG antibodies in serum samples we used the Enzyme-Linked Immunoabsorbent Assay (ELISA) test (Soares et al., 1999). The cut-off value for the IFAT adopted was 1:40. The cut-off value for the ELISA was defined as the mean optical absorbance of the negative controls +20%, and to each reaction plate, we added 2 positive and 2 negative control sera. In the present study we considered an animal infected when a positive result was found on any of the diagnostic tests used: serology and molecular. The animal was considered in an acute (initial) phase of infection when negative serology and positive molecular test were observed. The infection was considered in the chronic phase when the animal presented positivity at serology and negativity to the molecular test.

## Results and Discussion

In this study a total of 139 dogs (77 in the Nhecolândia and 62 at the Urucum rural settlement) were sampled. Overall, 28% (39/139) of dogs were positive for *B. burgdorferi*. Considering only results of molecular test, we showed positivity for twenty dogs (14%). Additionally, the serological test showed fifteen dogs (11%) exposed to the parasite (Table 1). However, distinct patterns of infections were noted between the sampled areas (Table 1). In the Nhecolândia we found only four dogs (5%) seropositive, and all examined dogs had negative molecular results in this area, contrasting with 35 dogs (56%) parasitized by *B. burgdorferi* in the Urucum rural settlement.

**Table 1.** Patterns of infection evidenced by serological and molecular tests carried out to detect *Borrelia burgdorferi* in blood samples of dogs surveyed in the two distinct areas of Pantanal region, Brazil. In parentheses results expressed in percentages.

	Serology- PCR-	Serology- PCR+	Serology+ PCR-	Serology+ PCR+
Urucum rural settlement (N=62)	27 (44%)	20 (32%)	11 (18%)	4 (6%)
Nhecolândia(n=77)	73 (95%)	0 (0%)	4 (5%)	0 (0%)
Total samples (n=139)	100 (72%)	20 (14%)	15 (11%)	4 (3%)

Our results indicate for the first time the occurrence of *B. burgdorferi* infecting dogs in the western of Brazil. In addition, the use of a serological test combined with the molecular test allowed to identify animals with acute (n=20) and chronic infection (n=15). In fact, for vector-borne bacterias such as *Anaplasma* spp., *B. burgdorferi*, and *Ehrlichia* spp., the number of intravascular organisms fluctuates over time (Beall et al., 2008). Therefore, PCR testing at a single time, especially during chronic phase of infection that is characterized by cryptic bacteremias, may produce a false negative result. In this context, serological and PCR assays should be used together to maximize the diagnosis (Maggi et al., 2014).

The first evidences of *B. burgdorferi* parasitizing human in Brazil dates from the 90's (Yoshinari et al., 1993), since the Borreliose of Lyme was considered an emergent zoonosis in the country. According to Joppert et al. (2001), Lyme disease in Brazil (Baggio-Yoshinary Syndrome) seems to be endemic of areas where ticks and wild mammals are present and in close contact to human residents, a context found at the Urucum Settlement.

As the infection by *B. burgdorferi* is characterized by a chronic disease without bacteremias (Constantino et al., 2017), the detection of *B. burgdorferi* infecting dogs of the Urucum settlement highlights that this domestic animal has important role as a source of infection to humans, since 38.7% (24/62) of sampled dogs presented bacteremias, expressed by positive PCR, allowing the infection of other ticks. Additionally, the occurrence of 32% of seronegative dogs with positivity in the molecular test indicate acute infections characterized by patent bacteremias, suggesting high prevalence of *B. burgdorferi* infection in ticks at the Urucum settlement.

Our results provide strong evidences that domestic dogs have an important role as sentinels of *B. burgdorferi* infections to humans in the Urucum settlement, a fact that should be carefully observed. Surprisingly, our results in the Nhecolândia region suggests that dogs do not have importance in the epidemiology of borreliosis, probably because in this region, the expressive diversity of mammals acts as a diluent for ticks. Moreover, the occurrence of Lyme diseases seems to be related to areas with higher density of ixodids ticks, wild mammals, local people and domestic dogs (Joppert et al. 2001).

## Conclusions

Our results has shown the importance and efficiency of dogs as sentinels for *B. burgdorferi*, since we found significant rates of infection on the dogs sampled, notably at Urucum settlement. The combination between serological and molecular tests allowed to identify distinct patterns of infections (acute and chronic patterns), which show the efficiency in the association of two different analysis methods. The results at the Urucum settlement demonstrate the needs of more observations at this border region, due to the possibility of an active transmission cycle of the parasite in this area.

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