

Serological evidence of canine arthropod-borne infections in an ecotone area of a natural reserve at the Pantanal, Brazil

Evidência sorológica de infecções transmitidas por artrópodes caninos em uma área de ecótono de uma reserva natural no Pantanal, Brasil

Bruno Ricardo Soares Alberigi da Silva¹ , Norma Labarthe² , Flávia de Oliveira Cardoso³ , Caroline Magalhães Cunha³ , Caroline Almeida de Carvalho⁴ , Flavia Mendes-de-Almeida⁵  & Celeste da Silva Freitas de Souza⁶ 

¹Veterinary, Doctorate, MSc. Programa de Pós-graduação em Medicina Veterinária – PPGMV, Universidade Federal Rural do Rio de Janeiro – UFRJ, Seropédica, RJ, Brasil

²Professor. Programa de Pós-graduação em Bioética, Ética Aplicada e Saúde Coletiva, Escola Nacional de Saúde Pública Sérgio Arouca – ENSP, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brasil

³MSc. Laboratório de Imunomodulação e Protozoologia, Instituto Oswaldo Cruz – IOC, Fundação Oswaldo Cruz – Fiocruz, Rio de Janeiro, RJ, Brasil

⁴Doctor. Laboratório de Imunomodulação e Protozoologia, Instituto Oswaldo Cruz – IOC, Fundação Oswaldo Cruz – Fiocruz, Rio de Janeiro, RJ, Brasil

⁵Professor. Departamento de Patologia e Clínica Veterinária, Universidade Federal Fluminense – UFF, Niterói, RJ, Brasil

⁶Doctor. Laboratório de Imunomodulação e Protozoologia, Instituto Oswaldo Cruz – IOC, Fundação Oswaldo Cruz – Fiocruz, Rio de Janeiro, RJ, Brasil

Abstract

Arthropod-borne infections are dependent on environmental conditions; therefore, anthropomorphic meddling may disrupt the natural balance that maintains wildlife. It is common to find dogs roaming in Brazilian natural reserves, what favors the spillover of pathogens among species. The aim of this study was to determine the canine seroprevalence of *Ehrlichia canis*, *E. ewingii*, *Anaplasma platys*, *A. phagocytophilum*, *Borrelia burgdorferi*, *Leishmania infantum* and *Dirofilaria immitis* using 84 serum samples from dogs from the border area of the SESC-Pantanal reserve (RPPN SESC-Pantanal 16°40'51''S; 56°17'45''W) stored at the Laboratório de Protozoologia e Imunomodulação, Instituto Oswaldo Cruz. Samples were tested with SNAP® Canine *Leishmania* Antibody Test (IDEXX Laboratories) or DPP® canine visceral leishmaniasis test (Bio-Manguinhos) for the presence of *L. infantum* antibodies and with SNAP® 4Dx Plus Test (IDEXX Laboratories) for *D. immitis*, *Ehrlichia* spp., *Anaplasma* spp. and *B. burgdorferi* seroprevalences. The seroprevalence for tick-borne parasites was 79.8%, 13.1% for *L. infantum* and 7.1% for *D. immitis*. Since tick-borne parasites were the most frequent among the examined dogs it may be suggested that these parasites, ticks and hosts display special resilience skills to overcome the hostile local conditions. The low *L. infantum* prevalence suggests that the local biodiversity, especially the bird abundance, depurates *Leishmania* circulation. The mosquito-borne *D. immitis* prevalence was higher than expected, suggesting that the local flooding regime provides suitable mosquito-breeding spots. On the other hand, the only known vector in the area feeds on birds, therefore reducing mosquitoes worm burden and impairing *D. immitis* transmission.

Keywords: vector-borne diseases, biodiversity, environment, natural reserve.

Resumo

Infecções transmitidas por artrópodes são dependentes das condições ambientais, portanto, alterações antropomórficas podem romper o equilíbrio natural que mantém a vida selvagem. É comum encontrar cães vagando nas reservas naturais brasileiras, o que favorece a adaptação interespécifica de patógenos. O objetivo deste estudo foi determinar a soroprevalência de *Ehrlichia canis*, *E. ewingii*, *Anaplasma platys*, *A. phagocytophilum*, *Borrelia burgdorferi*, *Leishmania infantum* e *Dirofilaria immitis* utilizando 84 amostras de soro obtidas de cães do ecótono da Reserva SESC-Pantanal (RPPN SESC-Pantanal 16°40'51''S; 56°17'45''W). As amostras foram testadas por SNAP® Canine *Leishmania* Antibody Test (IDEXX Laboratories) ou pelo teste DPP® leishmaniose visceral canina (Bio-Manguinhos) para detecção de anticorpos contra *L. infantum* e por SNAP® 4Dx Plus Test (IDEXX Laboratories) para detecção de



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*Correspondence

Bruno Ricardo Soares Alberigi da Silva
Programa de Pós-graduação em Medicina Veterinária – PPGMV, Universidade Federal Rural do Rio de Janeiro – UFRJ
Rodovia BR 465, Km 07, s/n Zona Rural,
Campus Seropédica,
CEP 23890-000 - Seropédica (RJ), Brasil
E-mail: bruno.alberigi@gmail.com

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D. immitis, *Ehrlichia* spp., *Anaplasma* spp. e *B. burgdorferi*. A soroprevalencia para doenças transmitidas por carrapatos foi 79.8%, 13.1% para *L. infantum* e 7.1% para *D. immitis*. Como os parasitos transmitidos por carrapatos foram os mais frequentes, é possível sugerir que estes parasitos, carrapatos e hospedeiros sejam resilientes para superar a hostilidade do Pantanal. A soroprevalência de *L. infantum* foi baixa, sugerindo que a biodiversidade, especialmente a abundância avícola, depure a circulação de *Leishmania*, reduzindo o risco da ocorrência de leishmaniose visceral. A prevalência de *D. immitis* foi relativamente alta, sugerindo que as enchentes do Pantanal possam prover criadouros para os mosquitos. Além disso, o único vetor competente da área é atraído por aves, o que pode diminuir a transmissão do parasito para a população canina.

Palavras-chave: doenças transmitidas por vetores, biodiversidade, meio ambiente, reserva natural.

Introduction

Besides the close living of domestic animals and wildlife, global climate changes favors arthropods dispersion and colonization in new areas worldwide. Consequently there is an expected arthropod-borne diseases spread, which imposes the need for continuous surveillance (Beugnet & Chalvet-Monfray, 2013; Dantas-Torres, 2015). Mosquitoes and ticks are the two leading arthropod groups in transmitting different pathogens to domestic animals, wildlife and humans (Brites-Neto et al., 2015; Adhikari, 2018). Among these vectors, sandflies must be mentioned once they vector *Leishmania infantum*, a parasite that infects and threatens the lives of more than a million humans/year (Lainson & Rangel, 2005; World Health Organization, 2015).

Among the most frequently detected canine arthropod-borne parasites worldwide are *Ehrlichia canis*, *Anaplasma phagocytophilum*, *Anaplasma platys*, *Borrelia burgdorferi*, *Leishmania infantum* and *Dirofilaria immitis*, (Labarthe et al., 2003; Bowman et al., 2009; Villeneuve et al., 2011; Cardoso et al., 2012; Day, 2011). *D. immitis* dispersion is favored by its capacity of being vectored by many different mosquito species (Ludlam et al., 1970) while the bacteria and the protozoan demand specific vectors (Vieira et al., 2011). Even in face of this demand *E. canis*, *E. ewingii*, *A. platys*, *A. phagocytophilum*, *L. infantum*, and *B. burgdorferi* infect diverse mammalian hosts and circulate in different areas of the world (Sykes, 2014; Lainson & Rangel, 2005; Akhouni et al., 2016).

In Brazil, as in other South American countries, *D. immitis* and *E. canis* are the most prevalent arthropod-borne parasites (Labarthe et al., 2018; Bendes et al., 2017; Willi, et al., 2017; Labarthe et al., 2014; Vezzani et al., 2011; Guilarte et al., 2011; Melo et al., 2011; Aguiar et al., 2007; Vieira et al., 2011). This wide distribution of *E. canis* is probably due to the fact that its vector is the wide-spread *Rhipicephalus sanguineus* (Miranda & Måttar, 2015; Vieira et al., 2011; Dantas-Torres, 2008), a prevalent species all over Brazil (Labruna & Pereira 2001). On the other hand *A. phagocytophilum* and *B. burgdorferi* are vectored by the *Ixodes ricinus* complex (Nieto & Foley, 2009) and *E. ewingii* by *Amblyomma americanum* ticks either rare or erroneously reported in Brazil (Dantas-Torres et al., 2009), therefore ehrlichiosis and anaplasmosis of Brazilian dogs are most probably due to *E. canis* and *A. platys* infections. Although rare, *E. ewingii*, *A. phagocytophilum* or *B. burgdorferi* canine infections have been documented (Santos et al., 2011; Silveira et al., 2015; Cordeiro et al., 2012; Montandon et al., 2014; Vieira et al., 2011). *L. infantum* is endemic in Latin America, Asia, Middle East and in the Mediterranean basin (Leblois et al., 2011). In the Americas it is mainly transmitted by *Lutzomyia longipalpis* (Lainson & Rangel, 2005), a different vector species from the Old World (Senghor et al., 2011).

E. canis infections are widely distributed among domestic and wild canids (Sykes, 2014). It is known to be widely spread in the Brazilian territory (Vieira, et al., 2011). A national survey showed dot-ELISA seroprevalence of 19.8%, confirming that the bacterium is present in all Brazilian geographic regions (Labarthe et al., 2003) and local surveys using various diagnostic tests and different canine populations have been published confirming its wide distribution (Macieira et al., 2005; Oliveira et al., 2000; Aguiar et al., 2007; Souza et al., 2010; Silva et al., 2010). A survey conducted in the Pantanal biome showed that in both urban and rural areas the majority of dogs were seropositive for *E. canis* (70.9%), with similar infection rates in both environments (Melo et al., 2011). *E. ewingii* is seldom reported in Brazil although molecular analysis detected five infected dogs (Oliveira et al., 2009).

A. platys is also wide spread worldwide (Sykes & Foley, 2014). In Brazil its known prevalence ranges from 10.3% to 15.8% (Sales et al., 2007; Ferreira et al., 2007). It must be emphasized that 160 of the 2,861 dogs' blood samples from an urban area of the Pantanal area were shown to be infected by *A. platys* (Sales et al., 2007).

The Brazilian canine *L. infantum* prevalence varies from 19% to 40.3% in the Northeastern region (Dantas-Torres et al., 2006; Figueiredo et al., 2017) and from 12 to 30% in the Southeastern region (Figueiredo et al., 2017). In an urban area of the Pantanal (Mid-Western region) the known prevalence of leishmaniasis is 61.7% (Moura et al., 1999).

The definitive hosts of *D. immitis* are canids, although other mammals can be infected. Brazilian *D. immitis* prevalence varied over time from 7.9% (Guerrero et al., 1989) to 2% (Labarthe et al., 2003) and afterwards rebounded to 23.1% showing hyperenzootic areas with infection rate of 62% (Labarthe et al., 2014). In the urban area of Cuiabá, at the Pantanal, the known prevalence is only 1% (Ramos et al., 2015).

Therefore, since there are different arthropod-borne parasites infecting dogs in various Brazilian areas and because the close living of dogs and wildlife favors the spillover of bioagents that can affect wildlife, domestic animals and humans, the aim of this survey was to investigate if the canine population living by an ecotone presented serologic evidence of arthropod-borne pathogens infections..

Material and methods

The Pantanal is the largest wetland area of the world, covering approximately 170,000km². During the flooding season animals are trapped in smaller dry areas where they share resources, vectors and parasites intimately. When drought arrives they roam around the entire environment, therefore dispersing parasites and vectors (Bechara et al., 2000; Martins et al., 2004; Labruna et al., 2005). The "reserve", Reserva Particular do Patrimônio Natural (RPPN SESC- Pantanal) is a 87,871 hectares area in the Pantanal (North - 16°28'31"S and 56°01'49"W; East - 16°42'30"S and 56°00'06"W; South - 16°51'50"S and 56°23'19"W; West - 16°47'18"S and 56°30'56"W) (Figure 1) harboring approximately 95 mammalian species and 340 bird species among other animal groups (Alho et al., 1987, 2011; Marchini, 2003; Brandão et al., 2011). There are two villages



Figure 1. Geographical delimitation of the SESC Pantanal reserve (RPPN SESC Pantanal, 16° 40'51"S; 56° 17'45" W). Source: Fiocruz (2012).

on the edge of the reserve and, given that human groups keep dogs as pets or for hunting, it is not uncommon to find dogs roaming in the reserve.

Serum samples obtained non probabilistically by convenience from 84 dogs living on the edge of the Reserva Particular do Patrimônio Natural (reserve), Pantanal, Brazil and maintained in the Laboratório de Imunomodulação e Protozoologia do Instituto Oswaldo Cruz (Authorization LW-16/10- CEUA/Oswaldo Cruz Foundation) at -20 °C were used.

Samples were tested with the DPP® canine visceral leishmaniasis test (Bio-Manguinhos, Brazil) or with the SNAP® *Leishmania* antibody test (IDEXX Laboratories, Inc., Maine, USA) for detection of antibodies against *L. infantum* and with SNAP® 4Dx Plus (IDEXX Laboratories, Inc., USA) for detection of *D. immitis* antigens; *E. canis* or *E. ewingii* antibodies; *A. platys* or *A. phagocytophilum* antibodies; and *B. burgdorferi* antibodies. The serum samples were taken from the freezer in batches of approximately 10 units and used after achieving room temperature. Each test was carried out according to the manufacturer's recommendations.

The number of positive or negative samples for each parasite was recorded on Microsoft excel spread sheet.

Results

The overall prevalence of the pathogens was 79.8% (67/84) for tick-borne parasites antibodies, 13.1% (11/84) for *L. infantum* antibodies and 7.1% (6/84) for *D. immitis* infection. No dog was found to be seroprevalent to all surveyed parasites and 14 were seronegative to all of them (16.7%). There were 40 dogs with evidence of single infections: for *Ehrlichia* spp. (34/40-85%); for *Anaplasma* spp. (3/40-7.5%); for *L. infantum* (2/40-5%) and for *D. immitis* (1/40-2.5%). The 30 dogs with evidence of co-infections presented antibodies against *Ehrlichia* spp.: for *Ehrlichia* spp. and *Anaplasma* spp. (16/30-53.3%); for *Ehrlichia* spp. and *L. infantum* (6/30-20%); for *Ehrlichia* spp. and *D. immitis* (4/30-13.3%); for *Ehrlichia* spp., *Anaplasma* spp. and *L. infantum* (2/30-6.7%); for *Ehrlichia* spp.,

Table 1. Number of dogs living on an ecotone area of the Brazilian Pantanal according to serology results for *Ehrlichia* spp. (Ehr); *Anaplasma* spp. (Anap); *Borrelia burgdorferi* (B); *Leishmania infantum* (Leish) and *Dirofilaria immitis* (Di).

SNeg	Serological results									
	Ehr	Anap	Leish	Di	Ehr+ Anap	Ehr+ Anap+B	Di+ Ehr	Di+ Ehr+ Anap+Leish	Ehr+Anap + Leish	Ehr+ Leish
14	34	3	2	1	16	1	4	1	2	6

SNeg: seronegative.

Anaplasma spp. and *B. burgdorferi* (1/30-3.4%); and for *Ehrlichia* spp., *Anaplasma*, spp., *L. infantum* and *D. immitis* (1/30-3.4%) (Table 1).

Discussion

It is well known that landscape influences population density, diversity and movement patterns of parasites, vectors and hosts. In the Pantanal, a biome that alternates flooding with very hot temperature (40 °C) with droughts when temperature can change from 30 °C to 10 °C in 24 hours (Brandão et al., 2011), seasons act as a strong dynamics changing force.

Tick-borne parasites were by far the most frequent among the examined dogs (79.8%), suggesting that these parasites, ticks and hosts display special resilience skills to overcome the variable and hostile conditions of the Pantanal. Since *R. sanguineus*, the main vector of the most prevalent arthropod-borne parasite detected (*Ehrlichia* spp.), is a tick species known to present catholic feeding habits and to be able to adopt different strategies to survive in rough conditions (Labruna et al., 2005; Dantas-Torres et al., 2010), it is possible to infer that they can overcome the flooding season finding alternative refuges and uncommon hosts. Although not especially attractive to *R. sanguineus*, capybaras (*Hydrochaeris hydrochaeris*) can host those ticks (Fernanda Passos Nunes, personal communication) therefore; *R. sanguineus* survival strategy could include infesting the Brazilian *Rickettsia rickettsii* efficient amplifier capybara (Krawczak et al., 2014). Since

R. sanguineus can get infected with *R. rickettsii* (Piranda et al., 2011) among other parasites, it may be suggested that the domestic dogs of the villages are exposed to the risk of being infected by this tick-borne pathogen. In face of the global climatic changes this may become a public health concern because tick's attraction towards humans is enhanced in high temperatures (Parola et al., 2008).

The unexpected finding of a *B. burgdorferi* seropositive dog raises the question on which species could be the local competent vector as well as the local competent reservoir of the bacterium. The detected antibody is produce towards C₆ peptide a marker only expressed when the bacterium is transmitted to the dog and known to be specific (Marques et al., 2002; Littman et al., 2006). Therefore and mainly because local biodiversity is still to be completely unveiled, further surveys must be conducted to elucidate *B. burgdorferi* ecology in the Pantanal.

The observed *L. infantum* seroprevalence was lower than expected for the Pantanal even though no culling of seropositive dogs nor insecticide spraying were carried out at the surveyed villages. Since there was no direct human interference on local *L. infantum* infection-rate in the villages, it can be speculated that the complexity of the biodiversity of the area must have diluted the risk of infection either by diminishing competent vectors abundance or by offering an enormous richness of *L. infantum* refractory hosts in which *Lu. longipalpis* feeds on. It is well known that these sandflies are strongly attracted to birds (Lainson & Rangel, 2005), that *L. infantum* infects only a narrow range of mammal species (Lainson & Rangel, 2005) and that the abundance and richness of birds in the Pantanal is astonishing (Signor & Pinho, 2011), suggesting that birds might play a key role for the natural control of the number of canine *L. infantum* infections in the area.

The only mosquito vectored parasite surveyed, *D. immitis*, was the least seroprevalent, what can, at first sight, suggest low competent vector population or low microfilaremic canids in the area. As a matter of fact, the competent vectors *Ochlerotatus scapularis* and *O. taeniorhynchus* were not captured in the reserve or on its edge and the only known competent vector genus captured in the area was *Culex* (unpublished data). Many *Culex* species are known to blood-feed on birds (Forattini, 2002) therefore, reducing the mosquitos' worm burden. Furthermore, it must be considered that the test kit used detects *D. immitis* active infection and not antibodies as the other surveyed pathogens, what certainly makes seroprevalence lower. Since *D. immitis* was the only medically preventable infection studied and even though 7.1% of the dogs were infected, it shows that dogs were neglected and received no veterinary care. The trifling way dogs are cared for at the villages enhances the need for health programs in the area to guarantee both wild and domestic animals welfare and human health.

In conclusion it may be said that the abundance and richness of biodiversity of the Pantanal tends to enhance health of all kinds of life, although when a high-density of one host species is observed it may point out to disturbance that are difficult to diagnose or control. That could be the reason why tick-borne diseases were so prevalent while sandfly-borne and mosquito-borne seemed to be under natural control. Furthermore, it is reasonable to suggest that if no action is taken towards environmental care aiming to control capybaras population in the area allied to better peridomestic tick control, a Brazilian Spotted Fever outbreak may occur.

Conclusions

All the surveyed vector-borne parasites were found to infect the canine population living by the edge of the RPPN SESC- Pantanal. *E. canis* was the most prevalent pathogen, pointing out to the fact that ticks and tick-borne diseases must receive specific attention in order to promote the one health approach in that important biodiverse environment. These results suggest that the biological richness and abundance of the reserve contribute to human, domestic animals and wildlife welfare in the area.

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