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

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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*, *Anaplasmata 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;56º17´45´´W). Samples were tested with SNAP⁵ Canine *Leishmania* Antibody Test (IDEXX Laboratories) or DPP⁶ canine visceral leishmaniasis test (BIO-Manguinhos) for the presence of *L. infantum* antibodies and with SNAP⁵ 4Dx Plus Test (IDEXX Laboratories) for *D. immitis*, *Ehrlichia spp.*, *Anaplasmata spp.* and *B. burgdorferi* seroprevalences. The seroprevalence for tick-borne parasites was 79.8%, 13.1% for *L. infantum* and 71% 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 interspecífica de patógenos. O objetivo deste estudo foi determinar a soroprevalência de *Ehrlichia canis*, *E. ewingii*, *Anaplasmata platys*, *A. phagocytophilum*, *Borrelia burgdorferi*, *Leishmania infantum* e *Dirofilaria immitis* utilizando 84 amostras de soro obtidas de cães do ecotone da Reserva SESC-Pantanal (RPPN SESC-Pantanal 16°40´51´´S;56º17´45´´W). As amostras foram testadas por SNAP⁵ Canine *Leishmania* Antibody Test (IDEXX Laboratories) ou pelo teste DPP⁶ leishmâniose visceral canina (Bio-Manguinhos) para detecção de anticorpos contra *L. infantum* e por SNAP⁵ 4Dx Plus Test (IDEXX Laboratories) para detecção de...
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 threaten 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; Akhoundi 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; Bendas et al., 2017; Willi et al., 2017; Labarthe et al., 2014; Vezzani et al., 2011; Guilarte et al., 2011; Melo et al., 2011; Aguilar 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 & Mätäar, 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; Aguilar 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 widespread 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 dog’s 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; Figueredo et al., 2017) and from 12% to 30% in the Southeastern region (Figueredo 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 170,000 km². 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 an 87,871 hectares area in the Pantanal (North - 16°28´31"S and 56°17´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
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 ºC 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., Anaplasma spp. and L. infantum (1/30-3.3%); and for Ehrlichia spp., Anaplasma spp. and L. infantum (1/30-3.3%) (Table 1).

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 | Ehr | Anap | Leish | Di | Ehr+ Anap | Ehr+ Anap+B | Di+ Ehr | Di+ Ehr+ Anap+B | 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 ºC) with droughts when temperature can change from 30 ºC to 10 ºC 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
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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 C 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 it’s 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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