Original Article

Survey of canine tick-borne diseases in Lábrea, Brazilian Amazon: 'accidental' findings of *Dirofilaria immitis* infection

Pesquisa de agentes transmitidos por carrapatos em cães de Lábrea, Amazonas: achados "acidentais" de *Dirofilaria immitis*

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Abstract

Blood samples were collected from 99 domestic dogs from the urban and rural areas of the Lábrea municipality, state of Amazonas, Brazil. Canine serum samples were tested by immunofluorescence assay against *Rickettsia* spp., which revealed that only 3.0% (1/33) and 7.6% (5/66) of the dogs from urban and rural areas, respectively, reacted positively to at least one *Rickettsia* species. DNA was extracted from canine blood and tested by a battery of PCR assays targeting protozoa of the genera *Babesia* and *Hepatozoon,* and bacteria of the genera *Rickettsia* and *Ehrlichia* and family *Anaplasmataceae.* All samples were negative in the PCR assays targeting the genera *Babesia, Hepatozoon, Ehrlichia* and *Rickettsia.* For *Anaplasmataceae,* 3% (1/33) and 39.4% (26/66) of the urban and rural dogs, respectively, yielded amplicons that generated DNA sequences 100% identical to the corresponding sequence of *Wolbachia* endosymbiont of *Dirofilaria immitis.* Because of these results, all canine DNA samples were further tested in a PCR assay targeting filarial nematodes, which was positive for 18.2% (6/33) and 57.6% (38/66) urban and rural dogs, respectively. Filarial-PCR products generated DNA sequences 100% identical to *D. immitis.* While tick-borne infections were rare in Lábrea, *D. immitis* infection rates were among the highest reported in South America.

Keywords: Dogs, ticks, *Rickettsia, Wolbachia, Dirofilaria immitis,* Amazon.

Resumo

Amostras de sangue foram coletadas de 99 cães domésticos de áreas urbana e rural do município de Lábrea, estado do Amazonas. Soros caninos foram testados pela técnica de imunofluorescência indireta contra *Rickettsia* spp., resultando em apenas 3,0% (1/33) e 7,6% (5/66) de cães soropositivos nas áreas urbana e rural, respectivamente. DNA foi extraído do sangue canino e testado por diferentes protocolos da PCR para detecção de protozoários dos gêneros *Babesia* e *Hepatozoon,* e bactérias dos gêneros *Rickettsia* e *Ehrlichia* e da família *Anaplasmataceae.* Todas as amostras foram negativas nos protocolos de PCR para os gêneros *Babesia, Hepatozoon, Ehrlichia* e *Rickettsia.* Para *Anaplasmataceae,* 3% (1/33) e 39,4% (26/66) dos cães de áreas urbana e rural, respectivamente, geraram sequências de DNA 100% idênticas ao endosimbionte *Wolbachia* de *Dirofilaria immitis.* Posteriormente, as amostras foram testadas pela PCR para nematódeos filarídeos, resultando em 18,2% (6/33) e 57,6% (38/66) de amostras positivas nas áreas urbana e rural, respectivamente. Os produtos geraram sequências de DNA 100% idênticas a *D. immitis.* Em contraste com várias outras regiões do Brasil, infecções transmitidas por carrapatos foram raras em Lábrea. Por outro lado, as frequências de infecção por *D. immitis* estiveram entre as mais altas relatadas na América do Sul.

Palavras-chave: Cães, carrapatos, *Rickettsia, Wolbachia, Dirofilaria immitis,* Amazônia.

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Introduction

In recent years, there has been a substantial improvement in our knowledge of tick-borne diseases among domestic dogs in Brazil. Many recent studies have shown that canine babesiosis (caused by *Babesia vogeli*), hepatozoonosis (caused by *Hepatozoon canis*), monocytic ehrlichiosis (caused by *Ehrlichia canis*), and anaplasmosis (caused by *Anaplasma platys*) are endemic in many parts of the country (RAMOS et al., 2010; SPOLIDORIO et al., 2011; VIEIRA et al., 2011; COSTA-JÚNIOR et al., 2012; SILVA et al., 2012; DEMONER et al., 2013). While *H. canis* seems to be primarily associated with ticks of the genus *Amblyomma* in Brazil (FORLANO et al., 2007; DEMONER et al., 2013), the other agents mentioned above have been associated with *Rhipicephalus sanguineus* sensu lato ticks (DANTAS-TORRES, 2008; VIEIRA et al., 2011).

A recent study described clinical illness in domestic dogs due to *Rickettsia rickettsii,* the agent of Rocky Mountain spotted fever (RMSF), for the first time in Brazil (LABRUNA et al., 2009). RMSF affecting humans in Brazil has been reported since the beginning of the previous century. The agent is transmitted by *Amblyomma* spp. ticks (LABRUNA, 2009). In fact, domestic dogs have been considered excellent sentinels for infection by *R. rickettsii* and other spotted fever group (SFG) *Rickettsia* species in southeastern Brazil (SANGIONI et al., 2005; HORTA et al., 2007; PINTER et al., 2008). In addition, canine serosurvey in different parts of Brazil have indicated that dogs are infected with different SFG-*Rickettsia* species, such as *R. rickettsii, Rickettsia parkeri,* or *Rickettsia amblyommii,* all associated with ticks (PINTER et al., 2008; SAITO et al., 2008; MELO et al., 2011; SPOLIDORIO et al., 2013; SZABÓ et al., 2013).

Comparative studies comprising dogs from urban and rural areas of Brazil have shown that urban dogs are infested almost solely by *R. sanguineus* s.l. ticks, while rural dogs are infested mainly by different *Amblyomma* species, and in some cases, also by *R. sanguineus* s.l. (LABRUNA et al., 2000; SZABÓ et al., 2001; COSTA et al., 2013). This scenario has resulted in *B. vogeli* and *E. canis* infection prevalence to be generally higher in urban than in rural dogs (AGUIAR et al., 2007; SPOLIDORIO et al., 2013; VIEIRA et al., 2013) because the ecologic niche of *R. sanguineus* s.l. in Brazil is typically in human dwellings (LABRUNA & PEREIRA, 2001). On the other hand, because *H. canis* and SFG-*Rickettsia* spp. are primarily associated with *Amblyomma* spp. ticks in Brazil, the prevalence of these agents has been generally higher among rural than urban dogs (LABRUNA et al., 2007; MELO et al., 2011; O'DWYER, 2011; SPOLIDORIO et al., 2013).

Although multiple studies on canine tick-borne diseases have been conducted in Brazil, no study was conducted in the state of Amazonas, northern Brazil. Because Amazonas preserves 98% of its Amazon forest, it bears the second lowest population density of Brazil (2.23 inhabitants/Km²); its population in the year 2012 (3,807,923 inhabitants, with 60% living in the capital Manaus) represented less than 2% of the Brazilian population (official data available at www.ibge.gov.br). These conditions have motivated the present study, which aimed to evaluate tick-borne agents infecting domestic dogs in Lábrea, a Municipality in the southern part of the state of Amazonas.

Materials and Methods

Study site

During February 2009, domestic dogs were sampled in the urban and rural areas of Lábrea, a municipality in the southern part of the state of Amazonas. Lábrea is a large municipality, encompassing an area of 68,229 Km², with elevation varying from 61 to 75 m. It has a population of 41,600 inhabitants (population density: 0.61 inhabitant/km²), living mostly in the urban area, whereas the rural area is comprised mostly of riverine families that usually do not raise livestock, because of the scarcity of high-water level lands. The weather is typically humid equatorial, with >2,000 mm annual precipitation, and mean temperature above 25°C through the year (SILVA et al., 2008a). Lábrea has a typical landscape of lowland Amazonian rainforest, composed mostly by Igapós (permanently flooded land, roots of vegetation always submerged) and Várzeas (higher than Igapós, land is only submerged when rivers are at their highest during the wet season); some Low Plateau (high-water level lands, never submerged) also exists, such as the urban area and the Indian villages.

Sampling dogs

A total of 99 dogs were sampled in the present study: 33 in the urban area and 66 in the rural area. Among rural dogs, 40 were owned by riverines (living on the banks of Purus River), while 26 were owned by Indians. The geographic localities of the dogs sampled are detailed in Table 1. The 33 urban dogs were randomly sampled according to their availability in the homes that were visited in the urban area. This sample size (n=33) was calculated using Epi Info 7, considering a 5,000 population size, 15% expected frequency (based on the study of Labruna et al. (2007)), 10% confidence limits, and a 90% confidence level. The 66 rural dogs encompassed all canine populations that were present at each of the 6 riverine communities and the 2 Indian villages during our visits. During sampling, the following dog-related information was obtained through a questionnaire that was applied to the owner: gender, age, breed, rearing mode (restrained or free-roaming), access to forests, living place history, and tick infestation history. From each dog, blood was collected by venipuncture in two vials: one with EDTA anticoagulant and frozen at –20°C until DNA extraction; and the other without EDTA, from which sera were separated by centrifugation and kept frozen until tested by serological methods. At the time of blood collection, every dog had its whole body examined for the presence of ticks. If ticks were found, they were collected and brought to the laboratory for taxonomic identification according to Onofrio et al. (2006) and Martins et al. (2010). Procedures of this work have been approved by the Animal Ethical Commission of the Faculty of Veterinary Medicine of the University of São Paulo (protocol no. 1667/2009).

Serologic analyses

Canine serum samples were tested by immunofluorescence assay (IFA) against six *Rickettsia* isolates from Brazil: *R. rickettsii* strain Taiaçu, *R. parkeri* strain At24, *R. bellii* strain Mogi, *R. amblyommii* strain Ac37, *R. rhipicephali* strain HJ#5, and *R. felis* strain Pedreira. Slides were prepared as previously described (LABRUNA et al., 2007). Each canine serum was diluted in 2-fold increments with PBS starting from the 1:64 cut-off dilution. Ten microliters of diluted serum were added to each well of the antigen slides. Slides were incubated at 37°C for 30 min in a humid chamber. The slides were rinsed once and then washed twice for 10 min per wash in PBS. Incubation of the slides was performed with fluorescein isothiocyanate-labeled goat anti-dog IgG (Sigma Diagnostics, USA) and washed as described previously. The slides were mounted with buffered glycerin under coverslips. In each slide, a serum previously shown to be nonreactive (negative control) and a known reactive serum (positive control) were tested. For each sample, the endpoint titer reaction with each of the six rickettsial antigens was determined. Slides were read in an ultraviolet microscope (Olympus BX60, Japan) at 400 magnification.

Molecular analyses

DNA was extracted from each blood sample using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. Blood DNA samples were individually tested by a battery of PCR assays targeting protozoa of the genera *Babesia*, and *Hepatozoon,* and bacteria of the genera *Rickettsia* and *Ehrlichia*, and family *Anaplasmataceae.* Genus or family-specific primers are shown in Table 2. In each PCR assay, blank controls (water) and an appropriate positive control sample (DNA extracted from canine blood infected by *B. vogeli, H. canis* or *E. canis,* and *R. parkeri*-infected Vero cells) were run together with the canine DNA clinical samples. PCR products of the expected size for each assay were purified with ExoSAP-IT (Amersham Biosciences, USA) and sequenced in an automatic

Table 1. Geographic localities where 99 dogs were sampled in the urban and rural areas of Lábrea municipality, state of Amazonas, northern Brazil, during January 2009.

Locality name	Coordinates		Living habits	Main landscape	Elevation (m)	Number sampled dogs
	South	West				
Urban area	07o15'55"	64o48'08"	Urban	Low Plateau	62	33
Rural areas*						
Carmelitas	07o16'51"	64o50'10"	Riverine	Várzea	56	3
Boca do Ituchi	07°18'36"	64°50'51"	Riverine	Várzea	56	6
Maciari	07°16'57"	64°51'05"	Riverine	Várzea	58	5
Samaúma	07°18'50"	65°08'39"	Riverine	Várzea	60	6
Santa Rosa	07°20'28"	64°59'50"	Riverine	Várzea	65	12
Bacural	07°15'02"	64°53'03"	Riverine	Igapó	41	8
Jarauara	07°16'42"	65°10'31"	Indian village	Low Plateau	81	15
Araça	07°18'54"	64°55'56"	Indian Village	Low Plateau	84	11

*banks of Purus River. m = meters.

Table 2. Primers used in the present study.

Target		Primer sequence (5′-3′)		Annealing T (°C)	Amplicon size (nt)	Reference
Organisms	Gene	Forward	Reverse			
Anaplasmataceae	16S rRNA	GGTACCYA-CAGAAGAAGTCC	TGCACTCATCGTTTA-CAG	55	344	Inokuma et al. (2000)
Ehrlichia spp.	*dsb*	GATGATGTTTGAA-GATATSAAACAAAT	CTATTTTACTTCTTA-AAGTTGATAWATC	52	401	Doyle et al. (2005)
Ehrlichia spp.	*dsb*	ATTTTTAGRGATTTTC-CAATACTTGG*	CTATTTTACTTCTTA-AAGTTGATAWATC*	52	349	Almeida et al. (2013)
Babesia spp.	18S rRNA	CCGTGCTAATTG-TAGGGCTAATACA	GCTTGAAACACTC-TARTTTTCTCAAAG	58	551	Almeida et al. (2012)
Hepatozoon spp.	18S rRNA	GGTAATTCTAGAGCTA-ATACATGAGC	ACAATAAAGTAAAAAA-CAYTTCAAAG	50	574	Almeida et al. (2012)
Rickettsia spp.	*gltA*	GCAAGTATCGGTGAG-GATGTAAT	GCTTCCTTA-AAATTCAATAAATCAG-GAT	50	401	Labruna et al. (2004)
Filarioidea	*cox1*	GCTTTRTCTTTTTGG-KTTACTTTT	TAGTRTCATA-AAAAGAAGTATTAAA	50	370	To et al. (2012)

*Used in a heminested reaction. T = temperature. nt = nucleotides.

sequencer (Applied Biosystems/PerkinElmer, model ABI Prism 310 Genetic, USA) according to the manufacturer's protocol, using the same primers (forward and reverse) used for the PCR. Partial sequences obtained were submitted to Blast analysis (ALTSCHUL et al., 1990) to determine the closest similarities in GenBank.

Because the *Anaplasmataceae* PCR assay generated products that were 100% identical to the *Wolbachia* symbiont of the canine heartworm *Dirofilaria immitis* (see Results section), we decided to test the canine DNA samples with primers targeting the cytochrome oxidase subunit 1 gene (*cox1*) of filarial nematodes (Table 2), as previously described (TO et al., 2012). PCR products were purified and sequenced as described above.

Results

Of the 99 sampled dogs (33 urban, 66 rural), 56 were males and 43 females. One-third of the dogs were ≤1 year old, whereas two-thirds were >1 year old (precise age was not possible to be determined for many adult dogs, especially those living in the Indian villages). Among the 33 urban dogs, 25 were free-roaming, with free access to the streets; rarely some of them were taken to forest areas. All 66 rural dogs had free access to forest; however, only 45 had access to low plateau forest areas. All sampled dogs had lived in the site where they were sampled for at least one year or since they were born. Owners reported previous tick infestation in 23 dogs, 1 urban and 22 rural; however, ticks crawling on walls in human dwellings were never seen. Riverines were unanimous in reporting that their dogs became tick-infested especially when they visited low plateau forest areas. Our canine examinations revealed that almost all dogs were free of ticks at the time of blood collection; only 5 dogs were found infested by ticks, which included the following species: *Amblyomma naponense, Amblyomma oblongoguttatum, Amblyomma ovale,* and *Amblyomma*

scalpturatum (Table 3). All these five dogs had visited low plateau forest areas in the preceding days.

Overall, sera from only 3.0% (1/33) and 7.6% (5/66) of the dogs from urban and rural areas, respectively, reacted positively to at least one *Rickettsia* species. The single reactive urban dog reacted solely to *R. rhipicephali* (endpoint titer 128). Among the reactive rural dogs, 1 reacted to the 6 *Rickettsia* species (endpoint titers varying from 128 to 512), 1 reacted to *R. rickettsii, R. parkeri, R. amblyommii,* and *R. rhipicephali* (endpoint titers 128 to 256), 2 reacted to *R. rickettsii* and *R. parkeri* (endpoint titers 64 to 128), and 1 reacted solely to *R. bellii* (endpoint titer 64).

Blood samples from all 99 dogs were negative in the PCR assays targeting protozoa of the genera *Babesia* and *Hepatozoon,* and bacteria of the genera *Rickettsia* and *Ehrlichia.* In the PCR targeting members of the family *Anaplasmataceae,* 3% (1/33) and 39.4% (26/66) of the urban and rural dogs, respectively, yielded amplicons of the expected size (Table 3), which were all demonstrated by DNA-sequencing to be 100% identical (291/291-bp) to the corresponding sequence of *Wolbachia* endosymbiont of *D. immitis* (GenBank accession numbers AF088187, Z49261). Because of these results, all canine DNA samples were further tested in a PCR assay targeting filarial nematodes. A total of 18.2% (6/33) and 57.6% (38/66) of the urban and rural dogs, respectively, yielded amplicons of the expected size, which include all 27 dogs that were PCR-positive for *Anaplasmataceae.* PCR products of 4 urban and 8 rural dogs were submitted to DNA-sequencing, resulting in sequences 100% identical (321/321-bp) to a number of corresponding sequences of *D. immitis* in GenBank (accession numbers AM749229, EU159111, FN391553, KC107805).

GenBank nucleotide sequence accession numbers for partial sequences generated in this study are KF977877 for the 16S rRNA partial sequence of *Wolbachia* endosymbiont of *D. immitis,* and KF977878 for the *cox1* partial sequence of *D. immitis*

Table 3. Number of dogs that yielded positive results by PCR for *Wolbachia* endosymbiont and the filarial nematode *Dirofilaria immitis,* and by serology for *Rickettsia* spp. organisms.

Locality name	Living habits	Number sampled dogs	Number of tick-infested dogs (%)	Number infected dogs (%)		
				Wolbachia sp.	*Dirofilaria immitis*	*Rickettsia* spp.
Urban area	Urban	33	0	1 (3.0)	6 (18.2)	1 (3.0)
Rural areas						
Carmelitas	Riverine	3	0	1 (33.3)	2 (66.7)	0
Boca do Ituchi	Riverine	6	0	3 (50.0)	3 (50.0)	1 (16.7)
Maciari	Riverine	5	0	1 (20.0)	2 (40.0)	0
Samaúma	Riverine	6	1(16.7)[a]	3 (50.0)	5 (83.3)	0
Santa Rosa	Riverine	12	1 (8.3)[b]	4 (33.3)	6 (50.0)	2 (16.7)
Bacural	Riverine	8	0	4 (50.0)	4 (50.0)	0
Jarauara	Indian village	15	3 (20.0)[c]	7 (46.7)	9 (60.0)	1 (6.7)
Araça	Indian Village	11	0	3 (27.3)	7 (63.6)	1 (9.1)
Total		99	5 (5.1)	27 (27.3)	44 (44.4)	6 (6.1)

[a] dog infested by *A. naponense* (3 nymphs), *A. oblongoguttatum* (4 nymphs), and *A. scalpturatum* (6 nymphs); [b] dog infested by *A. naponense* (1 nymph), *A. oblongoguttatum* (3 males, 1 female, 1 nymph), and *A. scalpturatum* (3 nymphs); [c] dogs infested by *A. scalpturatum* (4 nymphs), *A. ovale* (1 female), and *Amblyomma* sp. (13 larvae).

Discussion

In the present study, none of the dogs sampled were found to be infected by tick-borne agents, through molecular methods. These results suggest that the Lábrea urban area and the surrounding banks of the Purus River are not endemic or have low endemicity for canine tick-borne diseases. These results largely contrast to all other regions of Brazil that have been investigated, where recent studies have indicated, by molecular methods, that a considerable proportion of the dogs was infected by *E. canis, A. platys, B. vogeli,* and/or *H. canis* (RAMOS et al., 2010; SPOLIDORIO et al., 2011; VIEIRA et al., 2011; COSTA-JÚNIOR et al., 2012; SILVA et al., 2012; DEMONER et al., 2013). Indeed, these results are directly related to the absence of infestations by *R. sanguineus* s.l. on the dogs of the present study because this tick species is the only known vector of *E. canis* and *B. vogeli* and the possible vector of *A. platys* (DANTAS-TORRES, 2008; VIEIRA et al., 2011). In Brazil, *R. sanguineus* s.l. is typically an urban tick that depends on human dwellings for its free-living developmental stages (LABRUNA et al., 2000; LABRUNA & PEREIRA, 2001). The absence of *R. sanguineus* in Lábrea, as shown by our canine examinations during the field study, was corroborated by the owners' reports that they have never seen ticks crawling on walls in their homes, which is a typical behavior of *R. sanguineus* s.l. engorged stages (LABRUNA & PEREIRA, 2001). The populations of *R. sanguineus* s.l. have been considered to be widespread all over Brazil, including different urban areas of the Amazon region (LABRUNA et al., 2005; SERRA-FREIRE, 2010). For this reason, the current situation of Lábrea should be considered an exception for the occurrence of *R. sanguineus*, and consequently, for the tick-borne agents *E. canis, A. platys,* and *B. vogeli.* It will be interesting to perform new surveys in Lábrea in the coming years, in order to check if *R. sanguineus* s.l. (and associated diseases) has been introduced in the region.

The Amazonian region is considered to have a great diversity of ticks, which is primarily associated with its rich vertebrate fauna (ARAGÃO, 1936; LABRUNA et al., 2005). Notably, ticks were seldom observed on the dogs of the present study. It was very interesting to hear from the riverines that their dogs usually became infested by ticks when they visited low plateau forest areas. In fact, all six dogs that were found infested by *Amblyomma* ticks had visited low plateau forest areas. Apart from 26 dogs that lived in Indian villages, the remaining 40 rural dogs were owned by riverines that lived in the Várzea or Igapó, areas that have flooded ground during at least half of the year. Because long submersion in water is lethal for eggs and engorged stages of many tick species (SMITH, 1973; PAULA et al., 2005; CANÇADO et al., 2006), it is likely that no tick species would be able to complete a life cycle on the ground of Várzea and Igapó lands. Additionally, these areas also become less attractive for many land mammals that usually forage on low plateau areas. This physical condition certainly turns the Várzea and Igapó landscapes less suitable for ticks, reflecting in the absence of ticks on dogs that did not visit low plateau areas. This scenario was corroborated by the riverines, who reported that their dogs (and themselves; data not shown) usually get tick-infested while visiting low plateau areas, where they usually go for hunting. Low exposure to wild ticks in the present study would explain the absence of *Hepatozoon*-infected dogs, because this protozoan has been associated with *Amblyomma* spp. ticks in Brazil (FORLANO et al., 2007; DEMONER et al., 2013).

Overall, only 6.1% (6/99) of the sampled dogs were seroreactive to one or more *Rickettsia* species. Because dogs are suitable sentinels for SFG rickettsiosis (SANGIONI et al., 2005; HORTA et al., 2007), it can be inferred that the human population of Lábrea (at least among the sites sampled in the present study) is at low risk of acquiring tick-borne spotted fever rickettsiosis. Indeed, this condition is also associated with low human and canine exposure to wild ticks (genera *Amblyomma* and *Haemaphysalis*), which carry all tick-borne *Rickettsia* species currently known to occur in Brazil (LABRUNA et al., 2011).

While testing canine blood for *Anaplasmataceae* agents, we were originally searching for tick-borne agents of the genera *Anaplasma* and *Ehrlichia*. However, we only amplified *Wolbachia* DNA from 27 dogs. While these results were surprising at first sight, they were technically acceptable because the genus *Wolbachia* belongs to the family *Anaplasmataceae* (DUMLER et al., 2001). The *Wolbachia* DNA sequences generated in this study were all identical to each other, as well as 100% identical to the available sequences of the *Wolbachia* endosymbiont of *D. immitis*, the filarial agent of canine heartworm. While many filarial species have been shown to be 100% infected by *Wolbachia* endosymbionts, it was shown that filarial nematode and *Wolbachia* phylogenies are concordant; i.e., each infected filarial species has a specific *Wolbachia* genotype (CASIRAGHI et al., 2001). Therefore, once we observed that the *Anaplasmataceae* DNA sequences corresponded to the *D. immitis-Wolbachia*, we presumed that these dogs were infected by *D. immitis*. Then, samples were further tested by a PCR targeting filarial DNA, which indicated that 44.4% of the dogs were infected by filarial nematodes, including the 27 that were PCR-positive for *Wolbachia*. We randomly selected filarial DNA from 12 dogs to be DNA sequenced, which revealed that they all corresponded to *D. immitis*. Under these circumstances, the 44 filarial PCR-positive dogs were presumed to be infected with *D. immitis*.

The transmission of infective stages of *D. immitis* to dogs involves a number of Culicidae mosquitoes, such as *Culex* spp., *Anopheles* spp., and *Aedes* spp. (DANTAS-TORRES & OTRANTO, 2013), which are abundant in the basin of the Purus River (NATAL et al., 1992). Infection by *D. immitis* was shown to be widespread in the study region because all sampled areas, regardless of landscape type, or being urban or rural, contained infected dogs. Interestingly, while the prevalence of infected dogs was 18.2% in the urban area, it varied from 40 to 83.3% among the rural areas (Table 3). Higher canine infection rates in rural than in urban areas were also reported in another study in the state of Amazonas, where Silva et al. (2008b) found 4.3 and 42.3% of urban and rural dogs, respectively, infected by *D. immitis*. Similarly to the present study, the rural dogs sampled by Silva et al. (2008b) were mostly owned by riverines; therefore, higher canine infection rates in rural than urban areas could be related to the closer proximity of large water collections because riverines' homes are typically over water during the 6-month flooding period every year. These results could also be related to

the distinct species composition of Culicidae mosquitoes among urban and riverine communities. Interestingly, lower prevalence (2 to 25%) has usually been reported in non-Amazonian sites of Brazil (ALVES et al., 1999; LABARTHE & GUERRERO, 2005), whereas rates of 32.4-53.4% were reported in the eastern Brazilian Amazon (GARCEZ et al., 2006; FURTADO et al., 2009). Further studies are warranted to verify the possible epidemiological conditions that make the Amazon region more favorable for higher incidence of canine heartworm infection. Finally, *D. immitis* has been treated as a zoonotic agent, which can induce pulmonary, subcutaneous, or ocular lesions in humans (DANTAS-TORRES & OTRANTO, 2013). However, such zoonotic potential could be related to strain variation or vector species, conditions yet to be investigated (DANTAS-TORRES & OTRANTO, 2013). Further studies are needed to evaluate the zoonotic potential of *D. immitis* in the Purus River region, where the human filarial parasite *Mansonella ozzardi* (Manson) is also prevalent (MEDEIROS et al., 2009).

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