Veterinary Parasitology: Regional Studies and Reports 7 (2017) 40–44

Contents lists available at ScienceDirect

Veterinary Parasitology: Regional Studies and Reports

journal homepage: www.elsevier.com/locate/vprsr

Original Article

Serological evidence of canine exposure to arthropod-borne pathogens in different landscapes in Rio de Janeiro, Brazil

Liliane Maria Valentim Willi [a,*], Flavya Mendes-de-Almeida [a], Celeste da Silva Freitas de Souza [b], Tainá Laeta [c], Jonimar Pereira Paiva [d], Marcia Gonçalves Nobre de Miranda [e], Fabiana Batalha Knackfuss [f], Norma Labarthe [a,g]

[a] *Universidade Federal Fluminense, Faculdade de Veterinária, Rua Vital Brazil Filho 64, 24230340 Niterói, RJ, Brazil*
[b] *Laboratório de Imunomodulação e Protozoologia, Instituto Oswaldo Cruz, Fiocuz, Av. Brasil 4365, 21040360 Rio de Janeiro, RJ, Brazil*
[c] *Programa de Pós-Graduação em Geografia, Departamento de Geografia, Instituto de Geociências, Universidade Federal do Rio de Janeiro, 21941972 Rio de Janeiro, RJ, Brazil*
[d] *Departamento de Medicina e Cirurgia Veterinária, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, 23890000 Seropédica, RJ, Brazil*
[e] *Médica Veterinária, Vet Ypiranga, 22231120 Rio de Janeiro, RJ, Brazil*
[f] *Escola de Ciências da Saúde, Universidade do Grande Rio, 25071202 Duque de Caxias, RJ, Brazil*
[g] *Fundação Oswaldo Cruz, Av. Brasil 4365, 21040360 Rio de Janeiro, RJ, Brazil*

ARTICLE INFO

Article history:
Received 18 March 2016
Received in revised form 25 November 2016
Accepted 28 November 2016
Available online 05 December 2016

Keywords:
Anaplasma
Ehrlichia
Heartworm
Leishmania
Canine parasites

ABSTRACT

Arthropod-borne infections are dependent on environmental conditions, and several combinations of natural and human-related variables play an important role in vector populations as well as the life cycle of agents carried by the arthropods. The top 5 canine arthropod-transmitted agents, *Dirofilaria immitis*, *Leishmania* spp., *Ehrlichia canis*, *Anaplasma phagocytophilum*, and *Borrelia burgdorferi* infect unprotected animals without propensity. The purpose of this study was to determine the prevalence of these parasite species in three different landscape settings (sandbanks, plains and mountains) along a 60-km line. During a 6-month period, blood samples were collected from dogs (>12 months old) living in the different settings. Prevalence of *D. immitis* was determined by modified Knott test and ELISA. Prevalence of *E. canis*, *A. phagocytophilum*, and *B. burgdorferi* was determined by ELISA, and *Leishmania* spp. by ELISA, indirect immunofluorescence, and immunocromatographic assays. *D. immitis* was most prevalent in the sandbank (68.9%) as well as *Leishmania* spp. (34.5%), and tick-transmitted agents, *A. phagocytophilum* and *E. canis* in the plains (61.7%). *B. burgdorferi* was not detected. Depending on the resources for arthropods present in regions, dogs are likely to be exposed to different arthropod-borne parasites and should receive preventives tailored to the risk of infection in the region in which the dog resides.

1. Introduction

Dirofilaria immitis, *Ehrlichia canis*, *Anaplasma phagocytophilum*, *Borrelia burgdorferi* and *Leishmania* spp., are important arthropod-transmitted pathogens of medical and veterinary concern (Labarthe et al., 2003; Dantas-Torres, 2008; Bowman et al., 2009; Villeneuve et al., 2011; Cardoso et al., 2012). It is known that *D. immitis* is one of the most important nematodes in veterinary medicine due to the high numbers of infected domestic and wild animals (Labarthe et al., 2002; AHS, 2014). This parasite is vectored by several culicidae species that may present hemi-synanthropic (e.g., *Ochlerotatus scapularis* and *Aedes taeniorhynchus*) or synanthropic (e.g. *Culex quinquefasciatus*) behavior (Labarthe et al., 1998). Tick-borne pathogens such as *E. canis*, *A. phagocytophilum*, and *B. burgdorferi* are the cause of important diseases for humans and domestic or wild animals (Little et al., 2014; Sykes, 2014). *Rhipicephalus sanguineus*, known as the brown dog tick, is their principal vector species in Brazil (Labruna and Pereira, 2001).

Similarly, *Leishmania* spp. have been included in the list of the top 5 parasites that affect an incalculable number of domestic, synanthropic, or wild animals, and is considered one of the most prevalent neglected human infections. It is believed that approximately 200,000 to 400,000 new cases of human visceral leishmaniasis occur annually, with >90% of these cases occurring in 6 developing countries, including Brazil (WHO, 2015). From 0.7 to 1.3 million new cases of cutaneous leishmaniasis are reported annually, with approximately 95% of cases occurring in the Americas, the Mediterranean basin, the Middle East, and Central Asia. In Brazil, both cutaneous (caused by *Leishmania braziliensis*) and visceral forms are endemic (Aguilar et al., 1987), and outbreaks are usually related to disorderly land occupation (Kawa and Sabroza, 2002; Aguiar et al., 2014).

The occurrence of infected dogs with *D. immitis* or tick-borne parasites is high in tropical and subtropical areas and vectors are prevalent throughout the year in those areas (Labarthe et al., 1997; Labarthe et al., 2002; Genchi et al., 2009; Willi et al., 2012; Little et al., 2014). In

* Corresponding author.
E-mail address: liliwillimonteiro@hotmail.com (L.M.V. Willi).

http://dx.doi.org/10.1016/j.vprsr.2016.11.003
2405-9390/

Brazil *D. immitis* infections are known to be frequent in coastal areas where nature is better conserved, providing better conditions for development of culicidae (Labarthe et al., 2014). Similarly, as with the other vector-borne pathogens, the distribution of *Leishmania* spp. depends on vector populations, and therefore, the expansion of phlebotomies sand flies habitats is directly related to the distribution of leishmaniasis (Killick-Kendrick, 1999; Maia-Elkhoury et al., 2008). Undoubtedly, the environment plays an important role in the occurrence of vector-borne pathogens, since it is strictly related to the development of their vectors. Therefore, the present study assessed the prevalence of *D. immitis*, *E. canis*, *A. phagocytophilum*, *B. burgdorferi* and *Leishmania* spp. in dogs living in different landscape settings in a Brazilian tropical area.

2. Materials and methods

2.1. Ethical aspect and study area

This study was approved by the *Comissão de Ética no Uso de Animais – CEUA* of the *Fundação Oswaldo Cruz* (protocol number: LW-33/11). This study was conducted from August 2011 to January 2012 in 3 different landscape areas of the state of Rio de Janeiro (22° 54′ S 43° 10′ W), Brazil. The landscapes were located along a 60-km line from a sandbank section to the mountain region in the eastern area of the state of Rio de Janeiro.

In the sandbank area (sea level) (Site 1), one locale was between the lagoon and the seashore and the other at the opposite margin of the lagoon. In the plains (50 m above sea level) (Site 2), the area studied was between the sandbank and the mountain regions. Finally, in the mountain landscape, 2 locales were included, one at 140 m and the other at 840 m above sea level (Site 3) (Fig. 1). The main features considered for all 3 landscapes were distance from the coast, land use, altitude, human population density, and environmental conservation status. The landscape margins and surface features were determined by visual analysis using Google Earth. Locations where dogs were sampled were acquired by accessing the American global navigation satellite system (GNSS) using a global position system receiver (GPSMAP 62 receiver, GARMIN). Acquired locations were subsequently processed in ArcGIS 10 software.

In the sandbank area, human population density was intermediate and conserved areas were scarce. The locale between the lagoon and the seashore consists basically of homes that are devoid of public water supply, sewage treatment, and paved streets, although public power had recently been installed. The houses had an unfinished aspect, with filthy yards and no trees. At the opposite margin of the sandbank region, houses were brick and well-finished. Roads were partially paved, and public power, water supplies, and public sewage treatment were the norm.

In the plains, the human occupation was dense, leading to intense destruction of natural resources in the region. The population in the area was expanding as people were abandoning urban settings and moving to these more rural areas. Streets were generally unpaved and houses were unfinished; however, public power and water supplies were available, but there was no public sewage treatment.

In the mountain landscape, the balance of nature was conserved, and human density was the lowest of the 3 regions. The 140-m point was set in a small village surrounded by Atlantic forest and some rural properties, where only the principal streets were paved, public power and water supplies were present, but sewage treatment was not. The 840-m point was a rural village with unpaved streets, water supply from local sources, and poor public power supply, situated in Atlantic forest.

2.2. Animals and blood sampling

Dogs (n = 333) estimated to be at least one year of age were used in this study. The study aimed to evaluate as many dogs as possible in a given area. To be included in the research, dogs had to have lived within the study area for at least 6 months and owners had to complete an Informed Consent Form giving permission for the dog's participation in the study. Whole blood samples were collected from each dog through the puncture of the cephalic vein and stored in sterile plastic tubes containing anticoagulant (EDTA). Afterwards, each sample was divided in an aliquot of whole blood and from the other was obtained the plasma.

2.3. Laboratorial procedures

Whole blood samples were used to detect the presence of microfilariae by using a modified Knott test (Newton and Wright, 1956). In addition, plasma samples were tested for the presence of *D. immitis*, *E. canis*, *A. phagocytophilum*, and *B. burgdorferi* antigen using a commercial ELISA test (SNAP 4Dx®, IDEXX Laboratories, Maine, USA) following the manufacturer's instructions. Dogs were considered infected with *D. immitis* when microfilariae or antigens were detected and with *E. canis*, *A. phagocytophilum*, or *B. Burgdorferi* when antibodies were detected.

The detection of *Leishmania* spp. infection was performed by the ELISA (EIE Leishmaniose Canina – BioManguinhos/Fiocruz, Rio de Janeiro, Brazil) and immunofluorescence antibody test (IFAT) (Leishmaniose Canina - BioManguinhos/Fiocruz, Rio de Janeiro, Brazil). In addition, an immunocromatographic assay (TR DPP® canine visceral leishmaniasis - BioManguinhos/Fiocruz, Rio de Janeiro, Brazil) was performed. Dogs were considered positive for cutaneous leishmaniasis by ELISA and IFAT and for visceral leishmaniasis when tested positive by ELISA, IFAT and TR DPP.

2.4. Data analysis

Data were entered into EPI INFO 2000 data forms generated for this study, and data entry was verified for accuracy by 2 researchers. Non-parametric analysis was performed by chi square or Fisher's exact tests.

3. Results

After receiving owners´ consent, dogs were tested for *D. immitis* infection and tick-borne parasite seroprevalence; however, only 56.7% (189/333) could be tested for seroprevalence to *Leishmania* spp. because the ethical permission limited the volume of blood that could be collected. Therefore, only surpluses could be used.

The overall prevalence of the pathogens studied was 37% (123/333) for *D. immitis* infection; 46.8% (156/333) for tick-borne parasite seroprevalence and 27% (51/189) for *Leishmania* spp. seroprevalence. It is important to note that *B. burgdorferi* was not detected in the dogs tested (Table 1).

In the sandbank area, the most prevalent parasite was *D. immitis* (68.9%; 115/167) when compared with tick-borne parasites (43.7%; 73/167; $x^2 = 21.465$; df = 1; $P < 0.0001$) or *Leishmania* spp. (34.5%; 39/113; $x^2 = 32.128$; df = 1; $P < 0.0001$). There was no difference in the seroprevalence between tick-borne pathogens and *Leishmania* spp. ($x^2 = 2.376$; df = 1; $P = 0.1564$) (Table 1).

In the plains, tick-borne pathogens had a significantly higher prevalence (61.7%; 37/60) compared with *D. immitis* infection (0/60; $x^2 = 53.494$; df = 1; $P < 0.0001$) or *Leishmania* spp. (22.2%; 10/45; $x^2 = 16.181$; df = 1; $P = 0.0001$). In this landscape a significant difference between *D. immitis* infection and *Leishmania* spp. seroprevalence was observed ($x^2 = 14.737$; df = 1; $P = 0.0005$) (Table 1).

In the mountain area, the seroprevalence of tick-borne pathogens was highest (43.4%; 46/106) compared with the prevalence of *D. immitis* infection (7.5%; 8/106; $x^2 = 35.880$; df = 1; $P < 0.0001$) or *Leishmania* spp. (6.4%; 2/331; $x^2 = 14.383$; df = 1; $P = 0.0003$). No difference in *D. immitis* infection and *Leishmania* spp. was detected ($x^2 = 14.383$; df = 1; $P = 0.8523$) (Table 1).