Preventive Veterinary Medicine 154 (2018) 47–53

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

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

Spatial and simultaneous seroepidemiology of anti-*Leishmania* spp. antibodies in dog owners and their dogs from randomly selected households in a major city of southern Brazil

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ARTICLE INFO

Keywords:
American cutaneous leishmaniasis
Seroprevalence
Risk factor
Kernel analysis
ELISA
L. (L.) amazonensis

ABSTRACT

Although leishmaniasis has been described as a classic example of a zoonosis requiring a comprehensive approach for control, to date, no study has been conducted on the spatial distribution of simultaneous *Leishmania* spp. seroprevalence in dog owners and dogs from randomly selected households in urban settings. Accordingly, the present study aimed to simultaneously identify the seroprevalence, spatial distribution and associated factors of infection with *Leishmania* spp. in dog owners and their dogs in the city of Londrina, a county seat in southern Brazil with a population of half a million people and ranked 18th in population and 145th in the human development index (HDI) out of 5570 Brazilian cities. Overall, 564 households were surveyed and included 597 homeowners and their 729 dogs. Anti-*Leishmania* spp. antibodies were detected by ELISA in 9/597 (1.50%) dog owners and in 32/729 (4.38%) dogs, with significantly higher prevalence ($p = 0.0042$) in dogs. Spatial analysis revealed associations between seropositive dogs and households located up to 500 m from the local railway. No clusters were found for either owner or dog case distributions. In summary, the seroepidemiological and spatial results collectively show a lack of association of the factors for infection, and the results demonstrated higher exposure for dogs than their owners. However, railway areas may provide favorable conditions for the maintenance of infected phlebotomines, thereby causing infection in nearby domiciled dogs. In such an urban scenario, local sanitary barriers should be focused on the terrestrial routes of people and surrounding areas, particularly railways, via continuous vector surveillance and identification of phlebotomines infected by *Leishmania* spp.

1. Introduction

Leishmaniasis is defined as a group of non-contagious infectious diseases caused by protozoans and vectored by *Leishmania* spp. and includes mainly visceral (VL) and cutaneous (CL) presentations; these diseases have worldwide distribution and are endemic in 88 countries on four continents (Alcântara et al., 2016). The World Health Organization (WHO) has classified leishmaniasis as a serious neglected global public health threat and a re-emergent disease due to a persistent increase in the number of cases, particularly over recent years, and as being among the most important infections worldwide due to its high capacity to produce chronic systemic damage and body deformities (WHO, 2010).

Overall, approximately two-thirds of the global estimated human CL incidence is concentrated in the top ten countries for human cases, including Afghanistan, Algeria, Brazil, Colombia, Costa Rica, Ethiopia,

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https://doi.org/10.1016/j.prevetmed.2018.02.011
Received 15 August 2017; Received in revised form 22 February 2018; Accepted 22 February 2018

Iran, North Sudan, Peru and Syria (Alvar et al., 2012). The Americas, where transmission of *Leishmania* spp. mainly occurs through female *Lutzomyia* sandfly vectors, is ranked second globally for numbers of human CL cases, with 66,941 recorded cases between 2003 and 2008. Over one-third (26,008) of the human CL cases in South America between 2003 and 2007 were reported in Brazil (Alvar et al., 2012).

Northern (46.36%) and northeastern (26.72%) Brazil accounted for most of the 19,395 CL cases registered in 2015 (the same period of the present study), followed by the central-western (15.22%), southeastern (9.14%) and southern (2.56%) regions ("TabNet Win32 3.0: Leishmaniose Tegumentar Americana – Casos confirmados Notificados no Sistema de Informação de Agravos de Notificação – Paraná," n.d.). Likewise, most of the 3289 VL cases in 2015 were reported in the northeastern (60.71%) and northern (15.76%) regions, followed by the southeastern (18.08%), central-western (5.28%) and southern (0.17%) regions, with the last region reporting only five cases (Fig. 01 , Supplementary material). Parana State in southern Brazil has historical records of CL incidence in 289/399 (72.43%) municipalities (Lima et al., 2002), along with the recent first-time occurrence of the vector *Lutzomyia longipalpis* (Santos et al., 2012) and the first autochthonous isolation of *L. (L.) infantum* in a dog (Thomaz-Soccol, 2013). Moreover, the first autochthonous human VL case in Paraná state was also recently reported at the triple border (Brazil, Paraguay and Argentina) located on the far-western region of the state (Trench et al., 2016). Finally, Londrina, the second-largest city of Parana State and where the present study was conducted, has been considered a highly endemic area for CL that is free of autochthonous VL to date (Alcântara et al., 2016) (Fig. 01, Supplementary material).

For non-human cases, prevalence of infected and reagent in dogs in Parana State may vary among regions (Castro et al., 2007) mainly due to differences in the *Leishmania* spp. detected (Hoffmann et al., 2013; Velasquez et al., 2006) and the phlebotomine species involved (Baum et al., 2013; Reis et al., 2011). Although canine CL has been long established worldwide, the spatial distribution and/or associated risk factors for the disease remain unclear, particularly in favorable and endemic areas for concurrent human and dog disease (Costa et al., 2016; Membrive et al., 2012).

The territorial case distribution of human CL, particularly in Brazil, chronologically began as sporadic rural cases followed by the spread of infection to settlements after forest devastation (Castro et al., 2007; Silveira et al., 1990), with the disease finally reaching urban settings near residual forest areas (Costa et al., 2016; Membrive et al., 2012). This change in the spatial transmission pattern has been associated with vector adaptation to the peri-domiciliary environment, which exponentially increased human exposure particularly in urban areas neighboring residual forests (Oliveira et al., 2004). The identification of areas with favorable environmental conditions for phlebotomine survival and *Leishmania* spp. spread has been crucial for disease control and prevention (González et al., 2015) since the transmission of both human CL and VL invariably occurs in such areas (Sevá et al., 2017).

Dogs have been considered reservoirs for *L. (L.) infantum* (the etiological agent of VL) and secondary hosts for *L.(V.) braziliensis* (the etiological agent of CL) in Brazil (Dantas-Torres, 2007). Dogs sharing the same intra-household environment with their owners is not only a concern for the animals but may also pose a public health threat (Reguera et al., 2016). Since epidemiological studies on leishmaniasis in urban areas have been solely focused on specific human populations (Pontello Junior et al., 2013), domestic and wildlife animals (Baneth et al., 2016; Belo et al., 2013; Otranto and Dantas-Torres, 2013; Sharifdini et al., 2016; Truppel et al., 2014) and disease vectors (Baum et al., 2015; Dorval et al., 2016) or have only been investigated at the same location at different times (Castro et al., 2007; Costa et al., 2016), the interpretation and analysis of epidemiological data have relied upon fragmented and non-connected data. Not surprisingly, a recent review concluded that of over 10 years of observational epidemiological research on human leishmaniasis in Brazil, only 18/283 (6.6%) studies have produced any level of evidence for scientific contribution (Trentini et al., 2014).

Despite leishmaniasis having been described as a classic example of a zoonosis requiring a comprehensive approach, to date, no study has been conducted on the spatial distribution of simultaneous seroprevalence of *Leishmania* spp. in dog owners and their dogs in households in urban settings. Accordingly, the present study aimed to simultaneously identify the seroprevalence, spatial distribution and associated factors of *Leishmania* spp. in dog owners and their dogs in an area endemic for CL and free of VL.

2. Materials and methods

2.1. Study area

Londrina (23°18′36″S and 51°09′46″W) is considered the county seat of a metropolitan area and is the second largest city in Parana State, southern Brazil. This city was selected for the present study due to its high proportion of urban area (97.72%), a high urban population density (543,003 habitants; ranked 18th) and high human development index (HDI = 0.841; ranked 145th) out of a total of 5570 Brazilian cities (IBGE, 2010). In addition, Londrina has been designated an endemic area for human cutaneous leishmaniosis (CL) and an area free of human visceral leishmaniasis (VL) (Fig. 01, Supplementary material). This city is located at 608 m above the sea level in the Atlantic forest biome and has a subtropical humid climate (Köppen classification: CFa), with average temperatures ranging from 15.6 to 27.5 °C, yearly average precipitation of 1.63 mm and average relative humidity of 71.10%. Five lakes and four city parks with open green areas were situated within the urban perimeter at the time of survey; these parks were mostly used for visitation and recreation. A railway used exclusively for cargo transportation (no passenger transportation) has been crossing the northern city, with the nearest train station located outside the urban area of Londrina. The Londrina population was highly concentrated at the time, with a total of 161,144/164,898 (97.72%) households situated within the urban area (IBGE, 2010).

2.2. Sample size and sampling

Calculations for the sampling size were designed using a freely available software (EpiInfo 3.5.2, CDC, Atlanta, GA, USA) as previously shown (Dean et al., 1990) with an initial population of 161,144 households and an expected 50% prevalence, 5% accuracy, and 95% confidence level, for a final minimum sampling size of 384 individuals with visits conducted only in urban households.

The method used to calculate the sample size was simple random for a known population and has been described as $n = (N \times p2 \times z2)/p2 \times z2 + (N - 1) \times E2$, were N is the size of population (this case 161,144), p is a prevalence assumed, z is a constant and E is the error. This method has assumed (1) the estimates can be produce a mean of the population, (2) in a sufficient big number of samples, the distribution of sampling means are similar to the normal distribution and the constant for normal can be used ($z = 1.96$). Assuming an error of 5% (convention), by a graphic simulation varying number of samples and prevalence, the more conservative scenario is assuming a 50% of prevalence. These calculations provide 384 samples to be collected for this study (Cochran, 2007).

An additional 20% safety margin was added to the sampling due to potential refusal to participate, dog aggression, sample clotting or hemolysis, or closed households and commercial properties, for a final sample size of 461 households. The calculated sample size (461) was divided for the 115 blocks resulting in 4 households per block and these households were randomly sorted using a commercially available software (BioEstat 3.0, Belém, PA, Brazil) (Ayres et al., 2007). Multiprofessional field teams were formed that randomly performed house-to-house visits following designs by the conglomerate and included