



Canine visceral leishmaniasis: Incidence and risk factors for infection in a cohort study in Brazil



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ABSTRACT

Zoonotic visceral leishmaniasis in Brazil is caused by *Leishmania infantum* parasites and is transmitted by sand flies of the Phlebotominae family. Dogs are the main urban reservoirs and represent the major source of contagion for the vectors. Studies have shown that most infected dogs are polymerase chain reaction-positive months before seroconversion. Herein, we describe a cohort study designed to identify the incidence of and risk factors for *L. infantum* infection as detected by polymerase chain reaction-restriction fragment length polymorphism. To determine the risk factors for infection, we conducted a baseline canine survey ($n = 1443$) from which dogs were selected for the cohort study ($n = 282$) involving three evaluations over the course of a 26-month follow-up period. Serology, molecular tests, and a structured questionnaire were used. The risk factors for infection were identified by means of the Cox regression model. The overall infection incidence was 5.8 per 100 dog-months (95% confidence interval 5.1–6.5). Increased risk of infection was associated with the presence of previous cases of canine visceral leishmaniasis in the domiciles (hazard ratio [HR] 1.4; 95% confidence interval [CI] 1.1–1.8) and unplastered house walls (HR 3.6; 95% CI 1.6–8.1). These risk factors suggest that insecticide spraying in cracks and crevices in unplastered walls can reduce biting rates within and around homes. Furthermore, our results demonstrate that the Visceral Leishmaniasis Control and Surveillance Program should adopt environmental management measures in homes with previous cases of canine visceral leishmaniasis, because these homes are more likely to maintain the transmission cycle.

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1. Introduction

Visceral leishmaniasis is caused by *Leishmania donovani* or *L. infantum*, protozoan parasites that are transmitted to human and animal hosts by the bite of phlebotomine sand flies (Chappuis et al., 2007). Depending on whether or not a reservoir host is present, there are two basic types of epidemiological cycles: zoonotic, generally caused by *L. infantum*, and anthroponotic, generally caused by *L. donovani* (Palatnik-de-Sousa et al., 2001). Dogs are the main urban reservoirs of *L. infantum* and represent the major source of contagion for the vectors by virtue of the high prevalence of infection and intense cutaneous parasitism (Molina et al., 1994; Giunchetti et al., 2006).

Canine visceral leishmaniasis (CVL) is present in approximately 50 countries, mainly in South America and the Mediterranean region (Solano-Gallego et al., 2009; Dantas-Torres et al., 2012). Epidemiological studies in endemic areas have demonstrated that the prevalence of infection as determined by molecular techniques (50–80%) is higher than the seroprevalence (10–30%) (Solano-Gallego et al., 2009; Wang et al., 2011). A cohort study conducted by Oliva et al. (2006) showed that most of the study animals were polymerase chain reaction (PCR)-positive months before seroconversion. The CVL infection rate depends on several factors including the length of the transmission season, the vector density, the susceptibility of the dog population, dog behavior, the degree of exposure to vectors, and dog owners' attitudes toward prevention (Baneth et al., 2008; Dantas-Torres et al., 2012). Preventing the expansion and urbanization of zoonotic visceral leishmaniasis (ZVL) requires that the risk factors associated with human and canine infection be identified. Some cross-sectional serological surveys have suggested that canine susceptibility to infection is associated with dog size, fur length, age, and housing conditions (Franca-Silva et al., 2003; Almeida et al., 2009; Galvez et al., 2010; Cortes et al., 2012). During a previously reported cross-sectional study carried out in an urban area of Brazil (Belo Horizonte, southeastern Brazil), we evaluated the prevalence of and risk factors associated with *L. infantum* infection in dogs, as identified by means of a molecular test (polymerase chain reaction-restriction fragment length polymorphism, PCR-RFLP) (Coura-Vital et al., 2011b). Some studies have evaluated the risk factors associated with *L. infantum* infection in dogs, however few have used a cohort studies (Belo et al., 2013), which is the most appropriate observational design to establish causal inference. Recently in cohort study we demonstrated that dogs with PCR positive for *L. infantum* showed approximately twice the risk of seroconversion as those that were PCR negative (Coura-Vital et al., 2013). Herein, we report the results of a cohort study designed to identify the incidence of and risk factors for *L. infantum* infection as identified by PCR-RFLP; we evaluated the domiciliary and peridomiciliary environments, the socioeconomic status of the owners, the type of care provided for the animals, and specific animal behaviors.

2. Methods

2.1. Baseline survey (cross-sectional study)

The rationale for and organization of the study and the data collection methods have been described elsewhere (Coura-Vital et al., 2011b). Briefly, a cross-sectional study was carried out in the northwest sanitary district (36,874 km²) of Belo Horizonte in 2008. According to a census conducted by the Brazilian Institute of Geography and Statistics, the human population of this area was 331,362 in 2000. The canine population comprised 20,883 animals in 2008, according to the Zoonosis Control Management. At the time of this study, the seroprevalence in Belo Horizonte (7.6%) was similar to that in the northwest sanitary district (7.8%) (PBH, 2007). With an expected CVL positivity in the study area between 5% and 10%, a confidence interval (CI) of 95%, and an estimated precision of 1.5%, the required sample size for the study was approximately 1500 animals. A total of 918 households were visited, and 1443 dogs were included in the baseline survey (Coura-Vital et al., 2011b).

2.2. Follow-up cohort study

We then conducted a follow-up cohort study consisting of three evaluations. Evaluation I was conducted 10 months after the baseline survey (April 2009), and a total of 282 seronegative/PCR-negative dogs were enrolled. This number of dogs was similar to the number of PCR-positive dogs in the cross-sectional study. Households with seronegative/PCR-negative dogs were selected by proximity to the seronegative/PCR-positive dogs. The dogs were selected from 222 owners, all of whom were interviewed; all dogs were clinically examined, and blood was collected by venipuncture. Evaluation II was conducted 16 months after the baseline survey (October 2009), and 225 dogs were tested. Evaluation III was carried out 26 months after the baseline survey (August 2010), and 178 dogs were tested. All the dogs included in evaluations II and III were subjected to the same procedures used in evaluation I.

To evaluate loss trends during the cohort study, we compared the sex, size, and fur length of the included dogs with the same variables for the unincluded dogs in each evaluation.

2.3. Data and sample collection

A trained research team interviewed the owners of the study animals using a previously tested structured questionnaire that sought information regarding (i) the owner's knowledge about the human disease (i.e., form of transmission and clinical signs of human visceral leishmaniasis); (ii) the owner's knowledge about the vector (characteristics and presence in the domicile and peridomicile); (iii) the owner's knowledge about the reservoir host (epidemiological importance of the host, clinical signs of CVL, and dog care); (iv) the owner's socioeconomic characteristics (per capita/family income and schooling); (v) the characteristics of the domicile, annexes, and surroundings (i.e., structure of roof, floor, and walls; number of rooms, including

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