Risk profile for *Leishmania* infection in dogs coming from an area of visceral leishmaniasis reemergence

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ABSTRACT

Until the 1980s, visceral leishmaniasis was concentrated in poor rural areas of Brazil. The Vale do Rio Doce, located in the Southeastern Brazilian state of Minas Gerais, was an endemic area with high numbers of human and canine cases. Prophylactic measures adopted since the 1960s reduced the number of cases and the region became a 'controlled endemic' area. In the early 1990s, however, the program was interrupted, and the human disease reemerged in 2008. This cross-sectional study evaluated the prevalence and the risk profile of infection of dogs with *Leishmania spp* in this reemergence area of visceral leishmaniasis. Among a population of approximately 280,000 people, a total of 3835 dog owners were interviewed about socioeconomic conditions, housing, peridomicile features, and their dogs' characteristics and behavior. Blood samples were collected from 5822 dogs of an estimated canine population of 20,000 and anti-*Leishmaniasis* antibodies were identified using Dual-Path Platform and ELISA. We observed that 1282 of the 5822 dogs were seropositive for the protozoan indicating a seroprevalence of 22%. The risk factors associated with *Leishmania* infection in dogs were: non-paved backyard (OR 1.4; 95%CI 1.2–1.7); the presence of dry leaves and decaying fruit in the backyard (OR 1.3; 95%CI 1.1–1.5); medium-sized (OR 1.3; 95% 1.1–1.5) or big-sized dogs (OR 1.8; 95%CI 1.5–2.3); short-haired dogs (OR 1.8; 95%CI 1.5–2.1); dogs that slept in the backyard (OR 2.6; 95%CI 1.8–3.6) or in the balcony (OR 1.6; 95%CI 1.1–2.3); and history of canine visceral leishmaniasis in the household (OR 1.3; 95%CI 1.1–1.5). Our results suggest a strong reemergence of canine visceral leishmaniasis after the discontinuation of the control programs. Also, the observed risk factors reinforce the role of health education and environmental management measures to the effective control of the disease.

1. Introduction

Visceral leishmaniasis (VL) is a neglected disease with wide geographical distribution and high morbidity and mortality rates, especially in less developed countries (WHO, 2017). In South America and Europe, VL is caused by the protozoan parasite *Leishmania infantum* and is transmitted by phlebotomine sandfly vectors (Killick-Kendrick, 1999). The dog is the main urban reservoir and is usually capable of maintaining the disease in these areas (Fraga et al., 2012).

In Brazil, VL was initially described as an endemic disease occurring in rural areas of the country. However, since the 1980s the disease underwent a process of urbanization and territorial expansion. Currently, VL reaches 21 of the 27 Brazilian states with over 70% of the cases occurring in municipalities with more than 100,000 inhabitants (Werneck, 2016). In the 1950s, Deane's pioneer work in the Northeast of Brazil showed a predominantly rural epidemiological profile of VL (Deane and Deane, 1954). In 1966, a similar profile was reported for the Vale do Rio Doce region, which is located in the state of Minas Gerais, Southeast Brazil (Coelho and Falcão, 1966). An epidemiological survey conducted in the Vale do Rio Doce from 1965 to 1979 detected a high number of human and canine VL cases, mainly in rural areas (Magalhães et al., 1980). Health authorities initiated treatment of human cases before 1965. In the sequence, other prophylactic measures began to be adopted such as euthanasia of dogs that were parasitologically positive or that presented clinical manifestations suggestive of VL as well as insecticide spraying of domicile and peri-domicile

areas with dichlorodiphenyltrichloroethane (DDT) (Magalhães et al., 1980). These prophylactic measures reduced the number of VL cases over the assessed period, eliminating the disease from the region in 1978 and 1979 (Magalhães et al., 1980). After this time, the Vale do Rio Doce region was considered a 'controlled endemic' area for VL and became a model for the Brazilian Visceral Leishmaniasis Control and Surveillance Program (VLCSP) (Mayrink et al., 1979). However, in the early 1990s the program was interrupted, and the regular epidemiological surveillance of the area stopped. A few years later, new cases of canine VL (CVL) and the vector *Lutzomyia longipalpis* were detected in the region (Malaquias et al., 2007). In 2008, health officials reported new human cases in Governador Valadares, the main city on the Vale do Rio Doce region, suggesting the reemergence of human VL in the area (SINAN, 2016). Soon after, other studies provided compelling evidence confirming the reemergence of the disease in Governador Valadares (Barata et al., 2013; Tanure et al., 2015). The reemergence of VL may be due to the interruption of the VLCSP and by migration of the rural population to the urban areas coupled with unplanned city growth, high population densities, and environmental changes. These issues facilitate the spread of the disease with the emergence and reactivation of new cases (Arias et al., 1996; Desjeux, 2001; Werneck, 2008).

To understand the *L. infantum* infection profile and add strategic information to strengthen VL control in an endemic area, it is necessary to identify the risk factors associated with the canine and human infection (Desjeux, 2004). A systematic review and meta-analysis conducted in Brazil identified the risk factors associated with CVL (Belo et al., 2013). However, information about additional factors involving canine transmission and the risk profile of reemergence areas remain unknown.

The current study evaluated characteristics related to the household and the backyard environment, the socioeconomic status of the dog owners, and the animal care adopted by them. We aimed to identify the risk factors associated with canine *Leishmania* spp. infection in an urban area where VL reemerged and became endemic years after the interruption of the activities of the disease control program.

2. Materials and methods

2.1. Ethical statement

The study was approved by the Committee of Ethics in Animal Experimentation of the Universidade Federal de Ouro Preto, Minas Gerais, Brazil (protocol no. 18/2014). All procedures followed the guidelines set forth by the Brazilian Animal Experimental College (federal law number 11794). Dog owners were informed of the research objectives and signed an informed consent before data and sample collection.

2.2. Study area

The cross-sectional study was conducted in 2014 and 2015 in the municipality of Governador Valadares, (18°51′12″ S–41°56′42″ W), located in the Eastern region of the Minas Gerais state, within the Vale do Rio Doce region (Fig. 1). According to the Instituto Brasileiro de Geografia e Estatística (IBGE, 2017), the human population of this area is 279,665. The canine population is estimated to be approximately 20,000 animals, according to the Centre for Zoonosis Control of Governador Valadares, the main city in the Vale do Rio Doce region. In the year 2014 and 2015, seven and 17 cases of VL were reported respectively in the municipality (DATASUS, 2017).

2.3. Study design

The following parameters were used for sample size calculation: a canine population of 20,000; a prevalence of CVL between 25 and 30%;

95% confidence interval (CI); and estimated precision of 2%. The appropriate sample size for the study was of approximately 1600 animals. The study was conducted in three areas of the Governador Valadares and evaluated several types of environmental conditions such as residences very close to one another, residences located on hills and slopes, residences near small areas of forest, households near the river, and commercial buildings. Approximately 1600 dogs were collected in each of the three areas. The fieldwork was done in collaboration with the Governador Valadares Municipal Health Service, and data were collected during the canine survey census conducted by the health agents in 2014 and 2015. The studied areas were selected by convenience and were chosen because at that moment (2014) a canine survey was beginning in these areas. A total of 3835 households were included in the study, and 5822 dogs from the selected houses were sampled.

2.4. Data collection

Health agents of the Governador Valadares were properly trained to perform the interviews with the dog owners using a previously tested questionnaire. Information was collected regarding the presence of previous cases of CVL, the spraying of insecticide by the VLCSP, and the characteristics of the domicile and any adjacent backyards. In addition, the following information was collected about the dogs: age, gender, size, hair length, vaccination and veterinary checkups, behavior (habits related to the place where the dog slept and spend most of its time), and clinical data. The hair length was defined according to the breed, i.e., Border Collies were classified as long-haired while Dobermans were classified as short-haired. Mongrel dogs were classified according to the breed they resembled the most.

2.5. Sample collection

Approximately 5 mL of venous blood was collected from the radial vein of large dogs or from the jugular vein of medium- and small-sized dogs using 25 × 7 mm needles and sterile syringes. The material was sent to the Serology Laboratory of the Centre for Zoonosis Control of Governador Valadares.

2.6. Serological tests

Each sample was tested using the diagnostic protocol established by the Brazilian Ministry of Health and VLCP that recommend the use of Dual-Path Platform (DPP), for the screening of infected dogs, and ELISA to confirm the positive results (Ministério da Saúde, 2011).

The DPP® (Bio-Manguinhos, Rio de Janeiro, RJ, Brazil) was performed using 5 μL plasma and two drops of buffer placed sequentially to the same sample well (#1). After 5 min four drops of buffer were placed into another well (#2). The readings were performed after 10 min. In the presence of *L. infantum* antibodies, an immune complex is formed in the test band and a brown line is visualized. In the absence of specific antibodies, no line is visible in the test band. In both cases, the running buffer continues to migrate along the strip, and produce a similar brown line in the control band. The results were considered positive when two lines were visualized.

The ELISA was carried out using the EIE-LVC® kit (Bio-Manguinhos, Rio de Janeiro, RJ, Brazil) that employs a soluble antigen from promastigote forms of *L. major- like* (ELISA-L. major-like). This serological analysis was performed according to manufacturer's instructions. The serum titration was 1:100, and the conjugate titration was 1:1000. The absorbance value was measured at 450 nm in an automatic micro-ELISA reader.

2.7. Statistical analysis

The collected information and the generated results were double