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Are opossums a relevant factor associated with asymptomatic *Leishmania* infection in the outskirts of the largest Brazilian cities?



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ABSTRACT

A population survey was conducted to explore the prevalence and factors associated with *Leishmania* infection in the Fercal region of the Federal District. The Fercal region is a group of neighborhoods in Brasília in which the first cases of visceral leishmaniasis were described. *Leishmania* infection was established by a positive leishmanin test. Although other tests were performed in the study (an immunochromatographic assay (Kalazar detect®) and a molecular assay), only the leishmanin skin test provided sufficient results for the measurement of the disease prevalence. Data on the epidemiological, clinical and environmental characteristics of individuals were collected along with the diagnostic tests. After sampling and enrollment, seven hundred people from 2 to 14 years of age were included in the study. The prevalence of *Leishmania* infection was 33.28% (95% CI 29.87–36.84). The factors associated with *Leishmania* infection according to the multivariate analysis were age of more than seven years and the presence of opossums near the home. Age is a known factor associated with *Leishmania* infection; however, the presence of wild animals, as described, is an understudied factor. The presence of opossums, which are known reservoirs of *Leishmania*, in peri-urban areas could be the link between the rural and urban occurrence of visceral leishmaniasis in the outskirts of largest Brazilian cities, as suggested by previous studies.

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Introduction

Visceral leishmaniasis (VL) is an expanding zoonosis in Brazil and Latin America. The infection has progressed from rural areas to the suburbs of large cities; there is a possibility of humans becoming infected in the urban areas of some of the largest cities in Brazil. The urbanization of VL is well described.¹ The first Brazilian cities to experience this phenomenon have experienced 40 years of VL endemicity,² and the first autochthonous cases of VL in Brasília, occurring in the Federal District, were described in 2005 by our group.³

In Brazil, several studies have failed to detect the factors associated with positive testing for *Leishmania*.⁴⁻⁸ A recent meta-analysis reviewed Latin-American publications, most of which were Brazilian, looking for factors associated with VL and described an association of older age, presence of dogs in the house, higher canine seropositivity in nearby areas, lower socioeconomic status, and highly vegetated areas with *Leishmania (L.) infantum* infection. The association of *Leishmania* infection and gender varied according to the test performed. The factors associated with seropositivity were not identical to those associated with delayed skin test reactivity.⁹ *Leishmania* infection was found to be significantly associated with variables such as living in the same house of a VL patient, having relatives with LV, or being over 23 months of age.¹⁰⁻¹³ Most of these studies were conducted in areas with a lengthy endemicity of VL. As noted by Belo et al., many studies assessing the risk factors for VL are questioned on the basis of a number of factors, such as the reliance on small or poorly representative samples, the strategies to avoid refusals or not describing the magnitude of losses, the use of combined diagnostic tests for diagnosing infections, and the control of confounding variables and issues related to the statistical analysis.

This study aims to describe the magnitude and factors associated with *Leishmania* infection in the area of Fercal, a conglomeration of neighborhoods in which we previously described the first human cases of VL in Brasília and from which most of the cases of the disease were reported between 2005 and 2010.

Patients, material and methods

This research was an observational, cross-sectional analytical study aiming to estimate the prevalence of *Leishmania* infection in the studied area.

Study area and population

The area identified as Fercal, is located in the administrative region of Sobradinho II in the outskirts of Brasília, Brazil. Between 2005 and 2010, this region contributed more than half of the cases of autochthonous VL in the Federal District. According to census, the population of Brasília was 2455.903 inhabitants in 2010, and Fercal estimated population was 32,000 people. Fercal comprises several communities separated by mountainous areas of residual forest and farms located in a area rich in vegetation and water sources. The

occupation of this region has gradually occurred for about 60 years, originating near a limestone factory. The study was conducted between 2007 and 2008 and covered the communities of Alto Bela Vista, Bananal, Boa Vista, Catingueiro, Córrego do Ouro, Curvas, Engenho Velho, Fercal I and II, Queima Lençol, Ribeirão-Pedreira, and Rua do Mato, all of which are in the Fercal region.

Sample size

Assuming that there were approximately 5000 residents within the chosen age range, an estimated 15% prevalence rate of infection, a precision of 5%, a sample size of 134 people would be necessary to be included in the study. Because this study would be the beginning of a follow-up study, the sample size was inflated by a factor of 5 and rounded to 700 participants. The number of subjects from each community was calculated taking into account the number of households in that community, so that 73 individuals from Alto Bela Vista, 132 from Bananal, 80 from Boa Vista, 20 from Córrego do Ouro, 65 from Curvas, 117 from Engenho Velho, 66 from Fercal I, 27 from Fercal II, 57 from Queima Lençol, 16 from Ribeirão-Pedreira and 47 from Rua do Mato were included.

Randomization

In each participating community the houses to be contacted were randomly selected. Only one participant from each house was included. Houses without individuals in the target age range or in case participation in the study was not accepted another house was randomized in the same community.

Inclusion criteria

The study included Fercal residents aged two to 14 years, who fulfilled the following inclusion criteria and agreed to participate in the study after approval from their parents or guardians. The following inclusion criteria were used: residing continuously for more than one year in the study area, no history of symptomatic leishmaniasis, and no contraindications to blood extraction or to administration of a leishmanin skin test (LST).

Laboratory tests

The leishmanin test: Montenegro's antigen contains a lysate of promastigotes of *L. (L.) amazonensis*, strain MHOM/BR/73/M2269, batch 01/07 manufactured by the Centro de Produção e Pesquisa de Imunobiológicos (CPPI) from the Instituto de Saúde do Paraná. For the test, 0.1 mL of the suspension was intradermally inoculated in the forearm. The reading was performed 48-72 h after the inoculation by measuring the diameter (in millimeters) of dermal induration with the ballpoint-pen technique. An induration equal or superior to 5 mm was considered positive. The immunochromatographic assay Kalazar detect® (InBios International, Seattle, WA) was performed on serum samples of the included participants. The assay is based on the color shift due to the presence of antibodies against the recombinant K39 antigen. The procedures were performed according to the manufacturer's indications.

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