Risk Profiles for *Leishmania infantum* Infection in Brazil

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Abstract. The aim of this study was to characterize risk profiles for *Leishmania infantum* infection in a population living in an area endemic for visceral leishmaniasis. A cohort study was conducted between January 2004 and December 2006 with the participation of 430 individuals living in the city of Teresina, northeast Brazil, who were initially negative for the Montenegro test. Data analysis was performed using the classification and regression tree method. The cumulative incidence (CI) of Montenegro's test conversion was 35% at 18-month follow-up. Eight different risk profiles for *L. infantum* infection were identified. The profile with the highest risk (CI = 75%) comprised individuals with less than 4 years of education who had never lived outside Teresina. The profile with the lowest risk (CI = 5%) included highly educated subjects who had owned a dog for 5 years or more and lived in areas that received some type of intervention. These results show that there is a high degree of complexity involved in the risk for *L. infantum* infection and point out the need of developing new studies to perform a comprehensive analysis focused on investigating the interrelation between risk factors rather than their isolated roles on the determination of infection levels in urban areas.

INTRODUCTION

Visceral leishmaniasis (VL) is widely distributed worldwide, occurring in Asia, Africa, Europe, and the Americas. It is estimated that approximately 0.2 to 0.4 million VL cases occur each year and nearly 90% of these cases are registered in a small number of countries: India, Bangladesh, Ethiopia, Sudan, south Sudan, and Brazil.¹ In the American continent, where the disease has already been described in 11 countries, about 90% of the cases are reported in Brazil.¹ Currently, it is considered one of the most important neglected diseases due to its high incidence and fatality rates (about 10%) in addition to being regarded an emergent condition in human immunodeficiency virus-seropositive individuals.^{1–3}

In Brazil, VL, also known as kala-azar and *barriga d'água*, is a zoonosis caused by the protozoan parasite *Leishmania infantum* and transmitted mainly by phlebotomine sandflies of the species *Lutzomyia longipalpis*. Since 1980, a marked increase in the number of VL cases has been observed in Brazil.⁴ Disease epidemics, that were previously limited to the rural environment, started to occur in large cities, where the disordered occupation of city outskirts, associated with inadequate sanitary conditions and low availability of public services, favored the adaptation and proliferation of leishmaniasis vectors. The result of this process is that the wild cycle of transmission is now present in the urban environment.⁵

The area most affected by VL in Brazil is the northeast region, accounting for almost 50% of cases reported in the country. From 1980 to the mid-2000s, incidence rates of VL increased not only in northeast Brazil but also in north, midwest, and southeast Brazil.⁴ In 2013, nearly 50% of the cases involved children from 0 to 9 years of age, and case-fatality rate was around 6%. Between 2011 and 2014, 13,638 autochthonous VL cases were reported in 1,460 Brazilian municipalities, with 70% of cases occurring in only 204

municipalities, including 10 state capitals and 56 cities with 100,000 inhabitants.⁶

The first large epidemics of VL in Brazil affecting urban areas occurred in Teresina, northeast Brazil, from 1981 to 1986, when almost a thousand cases of the disease were reported, most of them coming from recently created neighborhoods of migrant settlements.⁷ Subsequently, epidemics have been described in Natal and São Luís, northeast Brazil, and then the disease has spread to other areas in the country, including the south region.^{8,9}

The process of VL urbanization is relatively recent, and little is known about the characteristics of disease transmission in this environment, especially about the risk factors for *L. infantum* infection in urban areas. Studies on this topic may contribute to identify new intervention targets and to define which groups are at higher risk and thus should be given priority in control and clinical management actions.¹⁰ Thus, the aim of this study was to characterize risk profiles for *L. infantum* infection in a population living in an area endemic for VL.

MATERIALS AND METHODS

Study area. With a population of more than 800,000 inhabitants, Teresina, the capital city of the state of Piauí, northeast Brazil, has an area of 1,392 km² at the confluence of the rivers Parnaíba and Poti and is situated 72 m above sea level. About 94% of the population lives in the urban area, 47% of it comprises males, and the population density is 58,494 inhabitants/km².¹¹

The economy is heavily concentrated in the tertiary sector which includes government activities, commerce, and services. It has little economic diversification and a very significant portion of the population works in the informal sector. The average income of persons is low, corresponding to 50% of the average income of Brazilian capitals and 77% of the northeastern capitals (Prefeitura de Teresina, 2008).^{11,12}

Study design and participants' selection. This is a cohort study based on a cluster randomized trial¹³ that was carried out from January 2004 to December 2006 which evaluated the

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effectiveness, isolated and combined, of insecticide spraying and elimination of infected dogs on the incidence of human infection with *L. infantum* in Teresina, Brazil.

Ten localities in seven neighborhoods in different regions of the city encompassing varied contexts regarding urbanization, vegetation cover, and patterns of disease transmission were selected for the study. All the selected localities had a history of recent occurrence of new cases of VL. Each of the ten localities was divided into blocks comprising an average of 60 households. Within each of the ten localities, four blocks were randomly selected, totaling 40 blocks. Blocks were randomly allocated to one of the following interventions for VL control: 1) vector control with the use of insecticides with residual effect in houses and annexes (area A), 2) monitoring of dog infection with the subsequent elimination of seropositive dogs (area B), 3) combination of the two interventions (area C), and 4) no intervention (area D). Interventions were delivered every 6 months, for three cycles, beginning in 2004.

Eligible participants were residents of selected blocks aged 1 year or above with no history of VL. One person from each household was randomly selected to undergo the Montenegro skin test (MST). If the selected individual was not at home or refused to participate, he/she was replaced with the youngest household member, this situation occurring in less than 5% of the visits. The baseline sample consisted of 1,106 participants, of which 408 (36.9%) had positive results for their first Montenegro test, one had a missing MST, and 697 had negative results. Among the latter, 430 (61.7%) were reexamined. Thus, the population of this study comprised 430 inhabitants from the ten localities who had negative results for their first MST (< 5 mm of induration) and underwent a second test 18 months after the first assessment.

Data collection and study variables. Data were collected at participants' households. On the first visit, participants answered a structured questionnaire about risk factors for *L. infantum* infection, which was supplied by a previously trained interviewer, and underwent the Montenegro test using 0.1 mL of leishmaniasis antigen injected intradermally. The antigen was prepared and provided by the Reference Center for Diagnostic Reagents (Bio-Manguinhos-FIOCRUZ, Rio de Janeiro, Brazil) using a *Leishmania amazonensis* strain. Test results were read 48–72 hours later, and subjects who showed negative results were visited again 18 months later to undergo a new test.

Exposure variables included a number of socioeconomic and demographic characteristics, as well as characteristics that described the household structure and the peridomestic environment. We obtained data on age, gender, educational level of the head of the household (< 4 or ≥ 4 years of education), history of migration (having lived outside Teresina), time of residence in the house (for how many years the subjects have been living in that house), number of people living in the house (number of household members), and history of VL in the household (whether some of the household members have already had VL). Information on the structure of the house in terms of roof lining (complete lining versus partial lining or no lining), walls (brick or another material), the fence around the house (masonry or other material), floor (cement or ceramic versus wood, soil, or clay), the system of water supply (tap water versus well or river water), and the type of sewer system (public sewer net-

work or septic tank versus rudimentary tank, ditch, or other alternatives). As for the peridomestic environment, the questionnaire asked about the presence of dogs and for those households with dogs, we also asked about the length of time the household owned that dog. On the basis of this information we derived a dichotomous variable: "no dog or owned a dog for less than 5 years" and "owned a dog for five or more years." We also asked about the presence of other animals in the house or in the peridomestic environment (such as chickens, pigs, cattle, horses, birds, and cats) and of the following items: kennel or chicken coop, warehouse or stove in the garden, and plants inside the house. Finally, it was also asked whether the participants used insecticide inside the house. Considering that the estimates of the effectiveness of all interventions were quite similar and non-significant after correction for bias due to selective loss to follow up,¹³ we decided to split to create a dichotomous variable indicating whether the area where the household was located had been allocated to receive some type of intervention in the background study: intervention area (area that received some type of intervention, i.e., areas A, B, and C) and nonintervention area (area that did not receive any intervention, i.e., area D). Study outcome was the cumulative incidence (CI) of *L. infantum* infection at 18 months as measured by Montenegro's test conversion.

Statistical analysis. Data analysis to identify risk profiles was performed by the classification and regression tree (CART) method. In this technique, the sample is subdivided through subsequent binary divisions with the purpose of creating subsamples less heterogeneous with regard to the incidence of VL ("nodes"). The algorithm selects the variable that better explains data variability within each node, and discriminative power decreases after each division. It is an easy-to-perform technique that allows for the detection of interactions between the variables and provides easily interpretable results, in addition to being an alternative to other regression and classification methods.^{14,15} In this study, it was defined that each "node" should have at least 40 observations (individuals) before splitting. Relative risks (RRs) and respective 95% confidence intervals (95% CI) of infection associated with each profile were estimated by using generalized linear models for binary data with a log link function. Statistical analyses were made using Stata 9.0 (STATA Corp., College Station, TX) and S-plus 4.5 (MathSoft Inc., Seattle, WA) software programs.

Ethical issues. This study protocol was approved by the Committee on Research Ethics of the Institute for Public Health Studies of the Federal University of Rio de Janeiro. Written informed consent was obtained from all adult subjects and from parents or legal guardians of child participants.

RESULTS

The CI of Montenegro's test conversion was 35% at 18-month follow-up.

Figure 1 shows risk profiles for *L. infantum* infection identified with the CART method. From this "tree," 53 individuals were excluded who had missing values in any of the variables. For the 377 subjects included, it is possible to identify eight different "risk profiles," that is, groups of individuals with different characteristics and having varied probabilities of infection (shaded squares).

FIGURE 1. Classification and regression tree model for risk of *Leishmania infantum* infection, Teresina, Brazil, 2004–2006.

The profile with the highest risk for infection (profile 8, CI = 75.0%) comprised individuals with less than 4 years of education who had never lived outside Teresina. Individuals with the same educational level but who had lived outside the city (profile 5) showed a 42.5% risk of infection. The profile with the lowest risk (profile 1, CI = 5.0%) included highly educated subjects who had owned a dog for 5 years or more and lived in an area that received some type of intervention. Highly educated females aged 30 years or over who lived in a nonintervention area (profile 7) had a probability of infection of 57.8%, similar to that observed in males with the same educational level and living in the same areas

(profile 6, CI = 59.0%). The probability of infection was 33.3% among highly educated subjects living in intervention areas who had no dogs or owned a dog for less than 5 years and whose household had three members or more (profile 4). Conversely, the probability of infection was 14.8% for highly educated individuals living in intervention areas but whose household had less than three members (profile 2). Finally, highly educated females aged below 30 years and living in nonintervention areas (profile 3) showed a 25.0% risk of infection.

In Table 1, using RRs to measure the association between profiles and *L. infantum* infection, it was observed that the

TABLE 1
Cumulative incidence of *Leishmania infantum* infection, RR, 95% CI, and *P* values according to risk profiles, Teresina, Brazil, 2004–2006

Profile	Characteristics	Incidence (%)	RR (95% CI)	<i>P</i> value
1	≥ 4 years of education + intervention area + had owned a dog for ≥ 5 years	5.0	1.0	–
2	≥ 4 years of education + intervention area + had no dogs or owned a dog for < 5 years + number of household members < 3	14.8	2.96 (0.35–24.52)	0.314
3	≥ 4 years of education + nonintervention area + female gender + age < 30 years	25.0	5.00 (0.64–39.05)	0.125
4	≥ 4 years of education + intervention area + had no dogs or owned a dog for < 5 years + number of household members ≥ 3	33.3	6.66 (0.97–45.49)	0.053
5	< 4 years of education + had lived outside Teresina	42.5	8.51 (1.22–59.15)	0.030
6	≥ 4 years of education + nonintervention area + male gender	59.0	11.81 (1.69–82.37)	0.013
7	≥ 4 years of education + nonintervention area + female gender + age ≥ 30 years	57.8	11.57 (1.65–81.25)	0.014
8	< 4 years of education + had never lived outside Teresina	75.0	15.00 (2.18–102.74)	0.006

CI = confidence interval; RR = relative risk.

risk of infection was 15 times as high in profile 8 compared with the profile that had the lowest risk (profile 1, reference profile) ($P = 0.006$). Both profiles 6 and 7 showed an 11-time ($P = 0.013$ and $P = 0.014$, respectively) risk of infection, while in profiles 5 and 4, this risk was nearly 8 times ($P = 0.030$) and 6 times higher ($P = 0.053$) than that of the reference profile (profile 1).

DISCUSSION

The CI of *L. infantum* infection at 18 months was 35% in this study, a value much higher than those found in similar studies. In a research conducted in Vila de Santana do Cafezal, northeast Brazil, the incidence of the disease at the end of 1 year was 11.5%, as measured by Montenegro's test conversion rates.¹⁶ In Raposa, northeast Brazil, Caldas and others¹⁷ found a 10.8% incidence of asymptomatic infection in children younger than 5 years over a 7-month follow-up. At least two facts may explain the high infection rates observed in this study. First, the areas selected in this study were considered as having moderate or high rates of VL transmission. Second, the interval between Montenegro tests in our study was a little longer, consisting of 18 months.

In Table 1, a comparison of risk profiles 5 and 8 shows that, in poorly educated individuals, the probability of infection was higher among those who had never lived outside Teresina (two-sample test of proportion, $P = 0.01$). This result is different from what would be expected, because, when arriving in the city, immigrants occupied peripheral regions and recently created neighborhoods characterized by an inadequate sanitation infrastructure and the marked presence of domestic animals that favors the accumulation of organic material and the proliferation of VL vectors. Migration was implicated in creating favorable conditions for the occurrence of the first VL epidemic in Teresina in the 1980s.⁷ According to Silva and others,¹⁸ migration is a favorable factor for VL urbanization, especially if occurring toward the outskirts of large cities. Therefore, based on the data found, it is possible to assume that the lower risk for infection among migrants reflects the conditions of the region they occupied. In other words, specific situations may have led these individuals to settle in places with a lower density of phlebotomine sandflies and thus with lower transmission rates. However, these aspects could not be directly addressed in this study. Hence, more than representing a vulnerable condition due to their migrant status, this variable, for poorly educated individuals, may only indicate the environment where they live, which may be more or less favorable to transmission, since all subjects, either being migrants or not, started this study under the same susceptibility conditions, that is, with a negative Montenegro test.

A comparison of profiles 2 and 4 showed that, among highly educated people living in areas that received interventions against VL who had no dogs or owned a dog for less than 5 years, the risk of infection was higher in individuals whose household had more members (two-sample test of proportion, $P = 0.05$), which indicates that, in this specific scenario, a greater number of individuals in the household may lead to an increased risk for infection. A possible explanation for this finding would be that this variable is acting

as a proxy of socioeconomic status, that is, a high number of household members reflects more unfavorable economic conditions. However, alternative explanations should not be ruled out, such as the fact that the higher concentration of people may have attracted a greater number of sandflies. A similar result was found in the study conducted by Cavalcante¹⁹ who observed that the presence of more than four household members was associated with 80% increase in the incidence of *L. infantum* infection. Nevertheless, the study by Lima²⁰ did not find a significant association between the number of household members and the presence of human infection as measured by the Montenegro test.

It is interesting to notice that, in an area in which interventions are being implemented, having a dog for 5 years or more (risk profile 1) was associated with a lower probability of infection (5%) as compared with profiles 4 and 6 in which not owning a dog or owning a dog for less than 5 years was associated with a combined incidence of infection of 31% (data not shown). Explaining such seemingly counterfactual results is only speculative, as the information on the infection status of the dog was not obtained in our study. Therefore, the variable "length of time the household has owned the dog" might be conceived as a proxy for the probability of canine infection. This probability, however, would depend on the existence or not of control measures being implemented. With no interventions, one would suppose that the longer the dog being in an area endemic for VL, the greater the risk of being infected. Conversely, in an area in which interventions are being implemented, infected dogs would be removed, and those present in the house for a longer period are those that have not been removed and, consequently, would have a higher probability of being noninfected.

In the risk profile 6, highly educated males from nonintervention areas showed a 59% risk of infection (which was 11 times higher than that of the reference profile 1). Such a high risk in individuals from a highly educated subgroup, a feature they share with those of the profile that had the lowest incidence (profile 1), may result from the fact that they lived in areas where no intervention was performed.¹⁶ This result highlights that, in the absence of control measures, a higher educational level would not be able to reduce the risk of infection, particularly in males, who are more exposed to infection during leisure-time activities.²¹

Profile 7 shows that highly educated older females who lived in nonintervention areas had a high risk for infection (CI = 57.8%). Women aged 30 years or over would be at higher risk than their younger counterparts, since they would be more exposed to risk factors, considering that phlebotomine sandflies have nocturnal habits. In addition, although belong to a highly educated group, these women lived in nonintervention areas, reinforcing the fact that having a higher educational level was not enough to reduce the risk for *L. infantum* infection in an area where no control measures were implemented.

The association between high levels of education and lower risk of infection has been reported from a systematic review with meta-analysis based on seven individual-level and three ecological-level studies.²² Education might affect the risk of infection in two ways. First, because it is closely linked to disease control, since low educational level undermines the comprehension and application of control measures, such as household spraying with insecticides and bednet use,

making individuals more vulnerable to infection.^{23–26} Second, because education is a proxy for many other variables that might increase peridomestic conditions that favor transmission, such as the ownership of animals, poor household conditions, poverty, and lack of urban services.^{6,21,23,27–30} It should be noted, however, that findings from profiles 6 and 7 reveal that a higher educational level alone was not able to reduce the risk of infection in settings favorable to the maintenance of the transmission cycle.

As for study limitations, it is worth emphasizing that the data used here come from an intervention study that was specifically designed to evaluate the effectiveness of different strategies to control VL in the city of Teresina and not to assess risk profiles for *L. infantum* infection. Furthermore, this study had a significant loss to follow up, since 38.2% of the individuals with negative results in the first Montenegro test did not undergo a second test. This loss led to a reduction in sample size, which certainly influenced its power to detect significant associations. However, the greatest concern with regard to these losses is related with the possibility that they were not random and may have caused distortions due to selection bias. Nonetheless, there was no great variability between participants and losses to follow up in terms of socio-demographic characteristics and implementation of actions to control VL, which suggests that these losses did not lead to this bias (Supplemental Table 1).

Despite study limitations, results show that there is a high degree of complexity involved in the risk for *L. infantum* infection and point out the need of developing new studies to perform a comprehensive analysis focused on investigating the interrelation between risk factors rather than their isolated roles on the determination of infection levels in urban areas.

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