Human Asymptomatic Infection in Visceral Leishmaniasis: A Seroprevalence Study in an Urban Area of Low Endemicity. Preliminary Results

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Abstract. Many aspects of the human asymptomatic visceral leishmaniasis (VL) remain not elucidated, and moreover, almost all the data come from highly endemic areas. The recent appearance of American VL (AVL) in the northeastern region of the state of São Paulo, Brazil, offered a good opportunity for further understanding. We present the preliminary results from a seroprevalence study on AVL in humans in Araçatuba, São Paulo. This was a cross-sectional survey on a random sample of the population (one-stage simple random sampling) in two areas, using rK39 dipstick tests. The sex ratios and age distributions in the two areas were comparable. Detectable antibodies were found in 23 subjects (20%) in area A1 and in 6 subjects (4.8%) in area A2. There was no significant difference in age distribution of seropositivity between the areas. We observed a difference in asymptomatic infection rates between the two areas, possibly associated with socioeconomic levels and transmission intensity.

INTRODUCTION

In Brazil, human visceral leishmaniasis (VL) occurs predominantly in the northeastern states (90% of all cases) but has urbanized and disseminated throughout the country since the 1980s, to reach southeastern urban areas.1 In 1998 and 1999, the first canine and human autochthonous cases were reported in Araçatuba in the northeastern part of the state of São Paulo,2 and the vector Lutzomyia longipalpis was reported for the first time in the region in 1997.3 L. longipalpis is only found in urban areas in the region.

The introduction of VL transmission in the region motivated an intensive control program using the traditional measures of infection detection in dogs, with culling of seropositive animals and insecticide spraying of households during the rainy season. In parallel, epidemiologic and control studies were undertaken to understand the epidemiology of VL in the region.

Because almost all data substantiating the existing control measures derive from areas of high-level transmission, there is a need to generate epidemiologic data on VL in this region, which is apparently an area of medium-level transmission.

Existing data in Brazil suggest that from 12% to 20% of infected humans may develop clinically overt disease, but this is linked to the prevalence of childhood malnutrition and possibly to other factors. Recent literature has correlated asymptomatic infection to clinical cases, thus suggesting occurrence in clusters and, at least in one study, implying that asymptomatic humans could constitute a reservoir.4,5

This study was conducted to obtain preliminary data on asymptomatic human VL infection in the urban area of Araçatuba and to identify possible contributory factors, such as sex, age, presence of dogs in the household, or human cases in the area.

This study also had the secondary objective of testing the usefulness of the K39 dipstick test for routine use in control programs. Although it is accepted that this test is not the most sensitive one for detecting asymptomatic cases, it is a fairly simple and practical test for field use and can be used in quick prevalence studies to support control programs. One advantage that it has over the standard skin test is that, unlike the latter, it is able to differentiate between mucocutaneous leishmaniasis (MCL) and VL, which is an important issue in this region, because MCL is common.6-7 This should be an important issue when planning control measures.

MATERIALS AND METHODS

Study design. The study consisted of a cross-sectional seroprevalence survey of a randomized population sample in Araçatuba, using rK39 dipstick tests. This study was approved by the Research Ethics Committee of the School of Medical Sciences of the State University of Campinas.

Study area. Araçatuba has 170,000 inhabitants, and 97.64% of this population lives in the urban area of the municipality. It is at an altitude of 398 m above sea level and is located 140 km from the Paraná River and the state of Mato Grosso do Sul, where canine and human VLs are endemic. It is a regional economic center, with adequate sanitation (100% of households have piped water and sewage systems) and an active economy based on sugar cane.8

The city is divided into eight areas for vector-borne disease control (i.e., five urban and three rural or semi-rural areas), and each area is divided into five sectors. According to Brazilian law, rural and urban areas within a municipality are defined by the municipality itself, but urban areas usually encompass populations living in areas that have urban infrastructure and urban economic activity. Two sectors, from different urban areas, were selected, named, and justified as follows: A1, this area had the highest number of confirmed human cases up to 2003 and has low socioeconomic status (Table 1); A2, this was where the first human VL case was detected, in 1999, and is an area with good socioeconomic status and a lower number of reported cases (Table 1).

The different socioeconomic levels in the two geographic areas were inferred according to municipality urban territorial tax.

Study population. Each block and house was numbered, and a census was conducted. Residents were asked to answer a questionnaire on how long they had lived in the area, what backyard vegetation they had, and how many dogs they had. Houses were selected through a random number table.
The Kalazar Detect dipstick test was used. This is an incidence rate of 15.3 cases per 100,000 inhabitants. If this represents 10% of the infected persons, we would have ~153 cases. Our study was a pilot study, because there are still many unanswered questions on VL asymptomatic infection prevalence rate and on the K39 dipstick test. Differences in proportions for categorical variables were analyzed using $\chi^2$, and $P < 0.05$ was considered statistically significant.

RESULTS

The data were collected from November 9 to 23, 2004. In area A1, there were 47 men and 78 women, with mean ages of 31.5 ± 20.97 years for the men and 30.04 ± 19.61 years for the women. In area A2, there were 50 men and 75 women, with mean ages of 44.84 ± 24.52 and 43.95 ± 21.79 years, respectively. The sex proportions were similar in the two areas. There was a significant difference between the sexes in the general population of Aracatuaba, and this difference was reflected in both study areas. The age distribution for the sexes differed between the two areas (Table 2).

There were 23 positive results (18.4%; 95% CI, 11.61–25.19%) in 15 homes in area A1 (9 men and 14 women). In area A2, there were six positive results (4.8%; 95% CI, 1.05–8.55%) in five homes (three men and three women). In one home with two positive residents, there was one HIV-positive resident; however, he tested negative. The case distribution by sex and age is shown in Tables 3 and 4. Patients with active clinically VL were not identified during the period of study.

The number of dogs in both areas was high: > 100 dogs in 136 homes. Over the preceding two years, 86 dogs had died because of laboratory-confirmed VL; however, no association between positive cases and presence of dogs was found in either area.

In A2, only two homes with positive results had dogs. One home had dogs over the preceding two years that had not died of VL, and the remaining homes had never had any dogs previously. In six homes, there were dogs with VL, but there was no evidence of human infection.

In A1, as in A2, no association was found with dogs. There were 17 positive results in homes that had no dogs, and of these, four had never had dogs and another four had had dogs with VL. In other homes that had dogs with VL over the preceding 2 years, there were no positive results. Another two
cases were in individuals who had or had had dogs, but without any evidence of VL. There were two individuals with positive tests in a home with dogs that died over the preceding 2 years. In 10 homes where there had been infected dogs over the preceding 2 years, there were no humans with positive results.

**DISCUSSION**

In areas of high endemcity, VL is mostly a childhood disease, particularly when transmission has been going on for many years. Araçatuba is an area where VL has recently appeared and presents low endemicity. In this particular region, with living conditions much better than what is usual in VL-endemic areas, the presence of symptomatic human cases is not a good indicator for the distribution of VL transmission. Therefore, the prevalence and distribution of asymptomatic cases seems to be a better alternative for understanding disease transmission and monitoring control efforts.

The data presented are the preliminary results from a study on the use of prevalence and distribution of asymptomatic human cases as an indicator for disease transmission in Araçatuba.

Normally in Brazil and other countries, VL occurs in children ≤ 5 years of age. In Araçatuba, the epidemiologic context seemed different, because clinical cases were occurring at all ages (children, teenagers, and adults) and solely in urban areas. There was no correlation between age and positive serology, as also found in an outbreak described in Salvador (northeastern Brazil), possibly because both areas were places with recent L. chagasi transmission.13 The same was found with asymptomatic cases: positive results were found in all ages.

The role of asymptomatic humans in VL transmission has recently been a subject for discussion,15 but that was not the intention of this study. Clinical cases of VL are concentrated in certain areas of Araçatuba, mostly on the poorer outskirts of the urban area. Our data suggest a socioeconomic gradient in the distribution of asymptomatic cases. This socioeconomic gradient has been found in other vector-borne diseases, such as dengue and Indian VL.13,14 The presence of dogs in the home does not seem to be a significant determinant of human infection, notwithstanding evidence to the contrary from other areas.15,16 Possibly the presence and number of dogs in the home is easily confounded with socioeconomic factors, such as household human density or a favorable environment for L. longipalpis breeding.

The data from this study suggest that human infection is more common than can be inferred from the occurrence of clinical cases. Further studies are needed before seroprevalence studies in humans can become a tool for VL control programs, although this seems promising.

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