



## Anti-*Leishmania* antibodies in blood donors from the Midwest region of Brazil



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### ABSTRACT

The aim of the study was to detect asymptomatic infection by *Leishmania* sp. in blood donors. Serum samples (430) were tested by Immunofluorescent Antibody Test, and an interview with the blood donors was carried out. Antibodies were detected in 15.6% of samples. The variables associated with the infection were: origin of the donor, presence of builds, parks or squares, sick dog in the neighborhood, and neighboring with leishmaniasis. It was observed association between origin of donors and the presence of sick dog. It is important a careful screening of donors, due to the risk of infection through blood transfusion.

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## 1. Introduction

Visceral Leishmaniasis (VL) is a severe parasitic disease which affects between 200 and 400 thousand people worldwide, with annual incidence of approximately 20,000–30,000 deaths [1].

In Brazil, VL is a zoonosis caused by the protozoa *Leishmania infantum chagasi* and 90% of the all cases described in Latin America occur in this country. This disease is expanding geographically following urbanization processes and it is considered an emerging and reemerging disease in urban and rural areas [1]. VL is endemic in at least 21 Brazilian states, being considered a public health threat with outbreaks of difficult control [2,3]. In 2011 3894 cases of VL were confirmed, with a lethal index of

6.7%. In Mato Grosso do Sul this disease is reported in 56 out of 78 municipalities, with a high number of cases in Três Lagoas, Aquidauana and Corumbá. However, more than 50% of the cases were registered in the capital city of Campo Grande. In the last two years, an increase in confirmed cases (17%) was observed, but a reduction in the lethal index (42%) was observed as well [4].

Asymptomatic cases of VL have been reported in India [5], Iran [6], Italy [7], Iraq [8] and Brazil [9–13]. Based on this data, the risk of VL transmission by blood transfusion should not be ruled out. Considering several aspects such as severity of the disease, its evolution related to immunological status of the host, the immunosuppression of blood receptors, difficulties of control, epidemiological changes and the need to know aspects related to the transmission in different areas, more studies to evaluate the transmission risk of *Leishmania* sp. by blood transfusion are needed. Therefore, the aim of this study was to detect anti-*Leishmania* antibodies in blood donors in an endemic area in the Midwest of Brazil.

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## 2. Materials and methods

The study was carried out in the Hematology and Hemotherapy Institute of the state of Mato Grosso do Sul “José Scaff” – Hemosul of Campo Grande, MS, from April to November of 2011.

A total of 430 volunteers without history of infection by VL and clinically healthy were included in this study. All donors were submitted to an interview in order to obtain demographic, socioeconomic and epidemiological information. Patients who were seroreactive to *Trypanosoma cruzi* were excluded from the study. The present study received approval from the Ethics Committee in Human Research of the Universidade Federal de Mato Grosso do Sul (protocol nº1976 CAEE 0037.0.049.049-11).

Sera samples were evaluated by Immunofluorescent Antibody Test (IFAT) (Kit Instituto Biomanguinhos da Fundação Instituto Oswaldo Cruz – Rio de Janeiro), following the manufacturer's instructions. Samples were considered positive if IFAT titer  $\geq 1:80$ . Antigens utilized in this test were from *Leishmania major*-like obtained from cell culture. To perform this assay, the conjugate was titled and in each slide was included a negative and positive control. Slides were read by two independent laboratory technicians.

The statistical analyses were performed using Epi Info 3.5.3 (Centers for Diseases Control and Prevention, Atlanta/Georgia/USA) and BioEstat version 5.0 (Sociedade Mami-ruá, Belém/Pará/Brazil). The chi-square test, chi-square of tendency and Fischer's test were used to verify associations among the variables studied and prevalence ratios, which were calculated with confidence intervals of 95%. To estimate the adjusted prevalence ratios, the Cox regression was utilized (with time equal to 1 unit), using in the pre-selection variables with significance <20%. The variables included in the final model were defined by the backward selection.

## 3. Results

Antibodies against *Leishmania* were detected in 15.6% (CI 95%: range from 12.2% to 19%) out of 430 blood donors. In the population studied 70.2% were male, median age was 32 years (range 18–68), 65.3% were locals from Campo Grande, 96.3% were living in this city and 79.1% were from the urban area.

Associations among *Leishmania* sp. infection and gender, age, school level, family income, animals, vegetation in peridomicile and/or neighborhood, presence of dogs, and knowledge about the diseases were not observed. Significant associations were observed only with the variables listed in Table 1.

The variables associated to the infection under adjusted analysis were: origin of the blood donor (Campo Grande versus other urban areas) and the presence of sick dog in the neighborhood with skin lesions and/or limb paresis (Table 2).

## 4. Discussion

The present study reports for the first time infection by *Leishmania* sp. in blood donors from the Midwest region of

Brazil. Despite the difficulty in performing epidemiologic studies of this clinical form (asymptomatic infection), it is necessary to know its impact and importance in the epidemiology of parasites, in order to better understand the spread through transmission and the survival of the parasite in the carrier patient.

The seroprevalence of 15.6% found in this study was lower than those previously reported in the municipality of Três Lagoas/MS [11], where a prevalence of 36.4% among household members and contacts of carriers of VL was observed. However, the data from the present study confirms the presence of asymptomatic carriers of VL and serves as an alert to the possibility of these carriers acting as blood donors.

With respect to the group of blood donors, the prevalence observed in the present study was higher than that previously reported by Luz et al. [14] (seroprevalence of 9%) and Urias et al. [15] (5.5%) in Brazil, and in Spain (3.1%) and Italy (0.76%) [16,17]. However, in Greece and France similar prevalence were reported (15.2% and 13.4% respectively) [18,19].

The detection of antibodies against *Leishmania* reveals the induction of a humoral immune response, and is very useful in epidemiological surveys [20]. A positive reaction does not necessarily indicate active infection, but it may just be the result of a previous exposure to the parasite, especially in endemic regions [16,21]. This could explain the prevalence observed in this study, because the highest frequencies of reactive serum were observed in donors from the city of Campo Grande, MS, the main endemic area of the state, which during 2011 contributed with 57.14% of the cases reported in the state [4]. The origin of the blood donor remained associated to the infection after the multivariate analysis.

The presence of parks and squares around the homes of blood donors were associated to infection by *Leishmania* sp. This association may be justified by the fact that vegetation, often found in these locations, provide a suitable environment for the development of vectors of *L. infantum chagasi*, causing an increase in the incidence of the infection [22]. Another association observed (bivariate and multivariate analysis) in this study was between human infection and dogs with clinical signs of leishmaniasis (e.g., skin lesions, limb paresis). High susceptibility to *Leishmania* sp., intense cutaneous parasitism, as well as their close relationship with humans, makes the presence of seropositive dogs in human habitations a risk factor for infection by *L. infantum* [23].

The prevalence of infection was also higher among donors with a history of contact with people who died of leishmaniasis. Studies indicate that a history of VL on parents and neighbors is associated with higher rates of infection. Individuals living in the same residence with patients recently diagnosed with VL, have an increased risk for acquiring the disease of up to 26 times, confirming the role of these patients as reservoirs [11,24,25].

The risk of VL transmission by blood transfusion represents a major problem, due to the lack of a gold standard diagnostic method for the detection of asymptomatic infections. This risk has also been reported in Spain [16], Italy [17], France [19] and Brazil [15,26], with implications

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