A longitudinal study on the transmission dynamics of human Leishmania (Leishmania) infantum chagasi infection in Amazonian Brazil, with special reference to its prevalence and incidence

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Abstract This was a longitudinal study carried out during a period over 2 years with a cohort of 946 individuals of both sexes, aged 1 year and older, from an endemic area of American visceral leishmaniasis (AVL) in Pará State, Brazil. The object was to analyze the transmission dynamics of human Leishmania (Leishmania) infantum chagasi infection based principally on the prevalence and incidence. For diagnosis of the infection, the indirect fluorescent antibody test (IFAT) and leishmanin skin test (LST) were performed with amastigote and promastigote antigens of the parasite, respectively. The prevalence by LST (11.2%) was higher ($p<0.0001$) than that (3.4%) by IFAT, and the combined prevalence by both tests was 12.6%. The incidences by LST were also higher ($p<0.05$) than those by IFAT at 6 (4.7%×0.6%), 12 (4.7%×2.7%), and 24 months (2.9%×0.3%). Moreover, there were no differences ($p>0.05$) between the combined incidences by both tests on the same point surveys, 5.2%, 6.3%, and 3.6%. During the study, 12 infected persons showed high IFAT IgG titers with no LST reactions: five children and two adults developed AVL (2,560–10,120), and two children and three adults developed subclinical oligosymptomatic infection (1,280–2,560). The combined tests diagnosed a total of 231 cases of infection leading to an accumulated prevalence of 24.4%.

Introduction

American visceral leishmaniasis (AVL) is an anthropozoonosis, first recorded in Amazonian Brazil by Penna (1934) who, during an extensive study of postmortem necropsy tissues from individuals believed to have died from yellow fever in different parts of Brazil, diagnosed three cases of AVL in the State of Pará. Interestingly, all were from a forested area in that State, in the municipalities of Abaetetuba and Mojú where, in the following 2 years, other relevant information was obtained regarding the ecology and epidemiology of the disease. In 1937, Cunha and Chagas gave the name Leishmania chagasi to the causative agent of AVL, which was recently referred to as Leishmania (Leishmania) infantum chagasi Lainson and
Rangel 2005, and in 1938, Chagas et al. recorded eight more cases of AVL in the municipality of Abaetetuba and the presence of infected dogs. They found that the most common man-biting insect in and around the houses of infected individuals was the phlebotomine sandfly *Lutzomyia longipalpis* and suggested that it was the most likely parasite vector.

Unfortunately, following the premature death of Evandro Chagas in 1940, further observations regarding AVL in Amazonian Brazil were limited to a few clinical and epidemiological features regarding isolated cases in Pará State (Alencar 1962; Costa 1966). During some 46 years following Penna’s first report of the disease in this part of Brazil, a mere 32 cases were recorded, and this resulted in Amazonian AVL being regarded as an occasional and sporadic disease. A progressive change in the epidemiology, however, was clearly taking place. Thus, in only 2 years (1984–1985), a total of 135 cases were recorded on the outskirts of the town of Santarém, west of Pará State (Lainson et al. 1984), and SESPA (2004) recorded a mean of approximately 235 cases per year in this State in a recent 5 years period.

The reason for this continuing increase in AVL during recent years is not completely understood. The principal factor involved, however, is increasing deforestation, which results in the invasion of the peridomestic habitat of developing human communities by the sandfly vector *L. longipalpis*. Infestation of animal housing may be intense, and in the presence of a large population of dogs, which are highly susceptible to infection with *L. (L.) infantum chagasi*, these animals became a major source of human infection (Lainson 1988; Lainson and Rangel 2005).

Migration of nonimmunized individuals from other regions, improved diagnostic methods, and the increasing awareness of clinicians regarding the importance of including AVL in the differential diagnosis of febrile patients will continue to contribute the AVL increase (Lainson 1989).

Although the occurrence of AVL has increased drastically in recent years, there has been little field work in this region indicating the prevalence and/or incidence in terms of the symptomatic (acute AVL and subclinical forms) and/or asymptomatic infections. Such a study is, without doubt, of major importance for understanding more fully the dynamics involved in the transmission of *L. (L.) infantum chagasi* to man. In fact, the only available information regarding the interaction of the human immune response to infection with this parasite has come from the study of patients with AVL, an immune-suppressed condition which represents only the top of the iceberg in this interaction (Lainson and Shaw 2005; Silveira et al. 1997).

During the period 2000–2004, the mean annual incidence of AVL in the municipality of Barcarena, Pará State, north Brazil was 0.36:1,000 inhabitants (SESPA 2004): 67% were male patients, as well as 56% were under the age of 15 years old. In a locality known as Santana do Cafezal village, an annual average of 2.8 cases were recorded, indicating this area as a very suitable place in which to conduct a more elaborate study. This communication will comment the results of a longitudinal, prospective study of 946 individuals in Santana do Cafezal village over a period of 2 years (October/2003 to December/2005), using both the leishmanin skin test (LST) and the indirect fluorescent antibody test (IFAT) for trying to better understand the transmission dynamics of infection.

### Materials and methods

#### Study area

This study was carried out in the Santana do Cafezal village, which is situated on the banks of the river Cafezal, only 7 km from the administrative center of Barcarena municipality (01° 30′ S: 48° 37′ W), which is considered to be within the metropolitan region of Belém, Pará State, north of Brazil (Fig. 1). The climate is typically equatorial, with an average
temperature of 27°C and high humidity. The annual rainfall is in the region of 2,500 mm or more, with the period from January to June forming the principal rainy season. Following extensive destruction of the primary forest, the area now consists mainly of plantations, with occasional patches of developing secondary forest. Approximately 70% of the inhabitants occupy wooden houses in non-flood land, which is surrounded by secondary forest, while the rest live in the várzea, an area of low vegetation which is flooded twice daily by waters of the river Cafezal.

Study design and population

The population enrolled in this study consisted of a cohort of 946 individuals (almost 90% of total population), being 568 males and 378 females aged between 1 (minimum) and 89 (maximum) years old, with a median age of 20 years old, suggesting to be a relatively younger population. When the study began, the number of inhabitants in the area was estimated to be 1,064 (Instituto Brasileiro de Geografia e Estatística 2004). Moreover, since the present study was performed with the objective of obtaining a clear idea regarding the transmission dynamics of infection, it was necessary to follow the design and the planning of a prospective analysis on the prevalence and incidence of infection during a follow-up period over 2 years. Thus, the indirect fluorescent antibody test and the leishmanin skin test were chronologically used at the same time point of the prevalence and the incidence surveys, which allowed to measure these infection rates by each one of these tests, as well as these real infection rates using the combined results of both tests. In this way, the IFAT and LST were used for all individuals previously selected for the prevalence, and for the following three incidences, at 6, 12, and 24 months, these tests were only performed in those individuals that had been negative for both in the prevalence and in the prior incidence survey. Because of different reasons, however, such as holidays or travel, for example, there was a loss of almost 10% (94 individuals) of original sample during the 2 years of the follow-up period. In addition, the total population was also divided into three age groups, 1–10, 11–20, and ≥21 years, which consisted of 260, 218, and 468 individuals, respectively, with the subject of analyzing the age distribution of infection.

Clinical evaluation of infected individuals

It was also regarded that all individuals presenting any immune reaction either by LST and/or by IFAT would be clinically examined (a complete physical examination) in order to identify any signs and/or symptoms that could be recognized as the classical feature of AVL, as well as that of the subclinical oligosymptomatic infection; only those cases presenting typical feature of AVL received conventional antimony therapy as recommended by the Brazilian program of AVL control (Brasil 2003). The subclinical oligosymptomatic cases were, in principle, only followed up to confirm their spontaneously clinical resolution, as it was observed by a prospective study carried out in the neighboring State of Maranhão, in the northeastern region of Brazil (Gama et al. 2004).

Criteria for identification of human infection

The definition of a human case of infection with L. (L.) infantum chagasi was based on two immunological parameters: the IFAT and LST. Nevertheless, as the IFAT evidences the humoral response (susceptibility) and the LST the T-cell response (resistance; Awasthi et al. 2004), the definition of human infection with L. (L.) infantum chagasi was assumed to be the presence of reactivity to either one or both tests, aiming to ensure as much as possible the real diagnosis of all infected individuals in the endemic area. Moreover, with the objective of expressing the specificity of IFAT and LST, a scale of semi-quantitative results was used with scores varying from + to +++++, as follows: for the IFAT, serological titers of 80–160 and 320–640 (IgG) received + and ++, and those of 1,280–2,560 and 5,120–10,240 were given +++ and ++++, respectively; for the LST, exacerbated (very strong) reactions (≥16 mm) were regarded as +++, strong (13–15 mm) as ++, and weak (5–8 mm) as +. Thus, it was assumed that serological reactions with 80 (IgG) titer and intradermal reactions forming papules or indurations of ≥5 mm in diameter were regarded as the positive cut-off for the IFAT and LST, respectively (Lima et al. 2003; Silveira et al. 1991, 1998).

Immunological test procedures

The proceedings for LST were previously described in other studies on the American cutaneous leishmaniasis (Silveira et al. 1991, 1998). The antigen used in Santana do Cafezal, a village situated in an area where cutaneous and visceral leishmaniasis are potentially concomitant, although the ecology of study area is not well promising for the cutaneous disease, should have a high specificity for the visceral disease. Thus, to promote high specificity in LST, cultured promastigotes forms from the stationary phase (RPMI 1640 media) of a regional strain of L. (L.) infantum chagasi (MCAO/BR/2003/M22697/Barcarena, Pará State) were used. They were fixed in a merthiolate solution (1:10,000), with a final concentration of approximately 10 × 10⁶ parasites per milliliter. As control for the Leishmania antigen, 0.1 mL of the merthiolate solution (1:10,000) was intradermally used in the opposite forearm.
of each individual. In addition, as the Instituto Evandro Chagas (IEC) is an official laboratory linked to the Health Ministry of Brazil, all reagents prepared for human research are previously evaluated by a Quality Control Program before the use for humans.

IFAT was performed as proposed by Lima et al. (2003), who showed that amastigote antigen of L. (L.) infantum chagasi had a higher specificity and sensitivity than those with promastigote antigen of the same species and of Leishmania (Leishmania) major-like (Bio-Manguinhos, Rio de Janeiro, Brazil), as well as with amastigote-antigen of Leishmania (Leishmania) amazonensis. Briefly, amastigote antigens were impregnated in the IFAT slides by printing of small fragments of spleen and liver, in the case of L. infantum chagasi, and skin, in the case of L. amazonensis, from “hamsters” (Mesocricetus auratus) infected with these parasites. It has been shown that the crude amastigote antigen of L. (L.) infantum chagasi has the best specificity and sensitivity for IFAT diagnosis of human L. (L.) infantum chagasi infection. For diagnosis of canine visceral leishmaniasis, this procedure has also been used with higher specificity than IFAT and enzyme-linked immunosorbent assay (ELISA; Bio-Manguinhos, Rio de Janeiro, Brazil; de Jesus et al. 2003).

Data analysis

The data obtained were analyzed by the Bio-Estat 4.0 program (Ayres et al. 2004) and the $\chi^2$ and binomial tests were used for the significance of differences between the LST and IFAT surveys of infection with a confidence interval of 95% ($p$ value $<$ 0.05).

Results

All results regarding the prevalence, incidence, and the accumulated prevalence of infection as well as its distribution according to the age and the specificity of LST and IFAT may be seen in Tables 1, 2, 3, and 4. However, presented below is a brief description of these results.

Prevalence of human L. (L.) infantum chagasi infection in the Santana do Cafezal village

The infection prevalence of 11.2% (106 of 946) by LST was higher ($p$ < 0.0001) than that of 3.4% (32 of 946) by IFAT. However, among the 106 LST and 32 IFAT reactors, there were 18 individuals reacting to both tests together, giving 17% between those LST and 56.2% those IFAT reactors. Thus, these combined results (88 by LST, 14 by IFAT, and 18 by both) gave a real prevalence of 12.6% (120 of 946) for the community (Table 1).

Frequency according to sex, age, and LST and IFAT specificity of the human L. (L.) infantum chagasi infection prevalence in the Santana do Cafezal village

The distribution of 120 cases of infection showed no difference ($p$ > 0.05) between men (55.8%) and women (44.2%). The age distribution also showed no difference ($p$ > 0.05) between the younger age groups, with 19.2% of cases aged 1–10 and 25.8% aged 11–20 years old. However, when these rates were compared with that of the older age group, both were smaller ($p$ < 0.05); more than half (55%) of infected cases were ≥21 years old (Table 2). Regarding the LST and IFAT specificity, among the 106 LST reactors, 41.5% showed exacerbated reactivity, 14.1% strong, 19.8% moderate, and 24.6% weak. Thus, over half of the cases (55.6%) had marked (++/+ + +) immunological resistance to infection (Table 3). Among the 32 IFAT reactors, 21.8% showed low reactivity, 53.1% moderate, 18.8% strong, and 6.3% high reactivity (Table 4). Thus, 25.1% of cases had significant immunological susceptibility to infection. Of these, four (3.3%) were typical AVL cases (two children and two adults) with a prevalence of 0.42%, whereas another four (3.3%) cases (one adolescent and three adults) exhibited a subclinical oligosymptomatic infection.

Incidence of human L. (L.) infantum chagasi infection in the Santana do Cafezal village

The first incidence by LST, 4.7% (38 of 798), was higher ($p$ < 0.05) than that by IFAT, 0.6% (five of 798); only one case reacted to both tests representing 2.6% (one of 38) of LST and 20% (one of five) of IFAT reactors. Thus, a real incidence of 5.2% was found in the first period of 6 months (42 new cases to 798 non-infected individuals). The second

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### Table 1 Prevalence, incidence, and accumulated prevalence by LST and IFAT of human L. (L.) infantum chagasi infection in the Santana do Cafezal village, Barcarena municipality, Pará State, Brazil (October/2003–December/2005)

<table>
<thead>
<tr>
<th>Immunological procedure (%/n)</th>
<th>Surveys</th>
<th>Prevalence (n=946)</th>
<th>Incidence 1 (n=798)</th>
<th>Incidence 2 (n=724)</th>
<th>Incidence 3 (n=644)</th>
<th>Incidence 1st year (n=798)</th>
<th>Incidence 2nd year (n=644)</th>
<th>Accumulated prevalence (n=946)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LST</td>
<td>11.2/106</td>
<td>4.7/38</td>
<td>2.9/19</td>
<td>9.0/72</td>
<td>2.9/19</td>
<td>197 (20.8)</td>
<td>62 (6.5)</td>
<td>231 (24.4)</td>
</tr>
<tr>
<td>IFAT</td>
<td>3.4/32</td>
<td>0.6/5</td>
<td>2.7/20</td>
<td>3.1/25</td>
<td>0.8/5</td>
<td>6 (0.6)</td>
<td>2 (0.2)</td>
<td>8 (0.9)</td>
</tr>
<tr>
<td>LST/IFAT</td>
<td>12.6/120</td>
<td>5.2/42</td>
<td>6.3/46</td>
<td>11.5/88</td>
<td>3.6/23</td>
<td>231 (24.4)</td>
<td>231 (24.4)</td>
<td>231 (24.4)</td>
</tr>
</tbody>
</table>

$LST$ leishmanin skin test, $IFAT$ indirect fluorescent antibody test
incidence by LST, 4.7% (34 of 724), was higher \( p<0.05 \) again than that by IFAT, 2.7% (20 of 724); eight cases reacted to both tests consisting 23.5% (eight of 34) by LST and 40% (eight of 20) by IFAT. This association revealed a real incidence of 6.3% in the second period of 6 months (46 new cases to 724 non-infected individuals). The third incidence by LST, 2.9% (19 of 644), was also higher \( p<0.05 \) than that by IFAT, 0.8% (five of 644); only one case reacted by both tests representing 1.6% (one of 19) by LST and 20% (one of five) by IFAT; this combination given a real incidence of 3.6% following a period of 12 months in the second year of study (23 new cases to 644 non-infected individuals; Table 1).

These three incidence surveys at 6 (5.2%), 12 (6.3%), and 24 (3.6%) months revealed 111 new cases of infection, giving a real incidence of 11.5% for the first year, which was higher \( p<0.0001 \) than that of 3.6% found at the end of the second year study.

Frequency according to sex, age, and LST and IFAT specificity of the human \( L. (L.) \) infantum chagasi infection incidence in the Santana do Cafezal village

According to sex, the distribution of 111 new cases of infection showed no difference \( p>0.05 \) between men (47.7%) and women (52.3%). Moreover, the distribution according to age also revealed no difference \( p>0.05 \) between the younger age groups (1–10 × 11–20 years old), 29.7% and 25.3%, respectively, although both rates were lower \( p<0.05 \) than that (45%) of the older age group \( \geq 21 \) years old. However, in contrast to those rates found at the prevalence, the incidence of the younger age groups together were more than half (55%) of all cases recorded in the three incidence surveys, indicating that, among the new cases, most occurred in the two first decades of life, with almost 30% of cases in the first one (Table 2).

Regarding the LST and IFAT specificity, it was revealed that, among the 91 LST reactors, 36.3% exhibited exacerbated reactivity, 12% strong, 24.2% moderate, and 27.5% weak reactivity, indicating that, among the 111 new cases, there was no difference \( p>0.05 \) favoring exacerbated and strong LST reactors over moderate and weak LST reactors as occurred at the prevalence (Table 3). In contrast, among the 30 IFAT reactors, 40% showed low reactivity, 50% moderate, 6.7% strong, and 3.3% high reactivity, confirming that only three (10%) of new cases had humoral response compatible with AVL (Table 4). Of these, one case showed clinical feature suggestive of subclinical oligosymptomatic infection (febrile, asthenia, and adenopathy), while another two exhibited typical feature of AVL, who were diagnosed at the second incidence survey. Moreover, following 2 months, the first incidence, a 3-year-old girl who had showed low IFAT reactivity (80 IgG) with negative LST, developed to typical feature of AVL; these three AVL cases comprised an incidence of 0.37:1.000 inhabitants for the first year. During the second year, no recorded AVL cases occurred.

Accumulated prevalence of human \( L. (L.) \) infantum chagasi infection in the Santana do Cafezal village

Following these four surveys, there were diagnosed a total of 231 cases of infection (120 at the prevalence and 111 at

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**Table 2** Age distribution of human \( L. (L.) \) infantum chagasi infection at the prevalence, incidence, and accumulated prevalence surveys in the Santana do Cafezal village, Barcarena municipality, Pará State, Brazil (October/2003–December/2005)

<table>
<thead>
<tr>
<th>Age groups (^a)</th>
<th>Epidemiology surveys</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prevalence (%)</td>
</tr>
<tr>
<td>1–10</td>
<td>19.2</td>
</tr>
<tr>
<td>11–20</td>
<td>25.8</td>
</tr>
<tr>
<td>≥21</td>
<td>55</td>
</tr>
</tbody>
</table>

\(^a\) years old

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**Table 3** Distribution of leishmanin skin test specificity of human \( L. (L.) \) infantum chagasi infection at the prevalence, incidence, and accumulated prevalence surveys in the Santana do Cafezal village, Barcarena municipality, Pará State, Brazil (October/2003–December/2005)

<table>
<thead>
<tr>
<th>Immunological procedure</th>
<th>Epidemiology surveys</th>
</tr>
</thead>
<tbody>
<tr>
<td>LST intervals (mm)</td>
<td>Prevalence (%)</td>
</tr>
<tr>
<td>5–8</td>
<td>24.6</td>
</tr>
<tr>
<td>9–12</td>
<td>19.8</td>
</tr>
<tr>
<td>13–15</td>
<td>14.1</td>
</tr>
<tr>
<td>≥16</td>
<td>41.5</td>
</tr>
</tbody>
</table>

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**Table 4** Distribution of indirect fluorescent antibody test specificity of human \( L. (L.) \) infantum chagasi infection at the prevalence, incidence, and accumulated prevalence surveys in the Santana do Cafezal village, Barcarena municipality, Pará State, Brazil (October/2003–December/2005).

<table>
<thead>
<tr>
<th>Immunological procedure</th>
<th>Epidemiology surveys</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFAT intervals (IgG)</td>
<td>Prevalence (%)</td>
</tr>
<tr>
<td>80–160</td>
<td>21.8</td>
</tr>
<tr>
<td>320–640</td>
<td>53.1</td>
</tr>
<tr>
<td>1,280–2,560</td>
<td>18.8</td>
</tr>
<tr>
<td>5,120–10,240</td>
<td>6.3</td>
</tr>
</tbody>
</table>
the three incidence surveys), giving an accumulated prevalence of 24.4%. Moreover, the accumulated prevalence by LST, 20.8% (197 of 946), was higher (p<0.05) than that by IFAT, 6.5% (62 of 946; Table 1).

Frequency according to sex, age, and LST and IFAT

specificity of the human L. (L.) infantum chagasi infection accumulated prevalence in the Santana do Cafezal village

According to sex, the distribution of 231 cases of infection showed no difference (p>0.05) between men (53.2%) and women (46.8%), as well as in relation to all men, 21.6% (123 of 568), and women, 28.5% (108 of 378), in the sample examined. According to age, no difference (p>0.05) was found between the younger age groups (1–10 × 11–20 years old), 25.6% and 26.4%, respectively, although both rates were lower (p<0.05) than that (48%) of the older age group (≥21 years old), suggesting that a regular distribution of infection exists in the two first decades of life, with a progressive accumulation from the age 21 years old (Table 2).

Regarding the distribution of infection according to LST and IFAT specificity, it was found that, among the 197 LST reactors, 39.1% had exacerbate reactivity, 13.2% strong, 21.8% moderate, and 25.9% weak reactivity, indicating that more than half (52.3%) of infected individuals had significant immunological resistance against infection (Table 3). However, among the 62 IFAT reactors, 30.6% showed low reactivity, 51.6% moderate, 13% strong, and 4.8% high reactivity (Table 4), confirming that most IFAT reactors (82.2%) had low susceptibility to infection; among 51 cases with low IFAT reactivity, only one (1.9%) developed to active AVL.

Discussion

This represents the first longitudinal study carried out in Brazil which followed up a cohort of individuals with different ages regarding the transmission dynamics of human L. (L.) infantum chagasi infection by using two immunodiagnostic methods, LST and IFAT, simultaneously. As a result, this approach allowed to better estimate the real prevalence and incidence of infection in the endemic area, once the prior works regarding these epidemiology rates have used either a T-cell immune response assay (e.g., LST) or a humoral immune response assay (e.g., IFAT or ELISA, principally), which has raised some difficulties concerning a complete view of human immune response against the infection (Badaró et al. 1986; Caldas et al. 2001, 2002; Nascimento et al. 2005). In addition, these works have also been limited to the epidemiology of infection in children up to 15 years old, which underestimated the significance of older individuals in the epidemiology of infection. In the present study, however, it should be highlighted that the study population consisted of a stable community with a history of more than a century and composed of individuals within at least four generations. The inhabitants have thus had a long history of exposure to the transmission of L. (L.) infantum chagasi infection which, quite likely, might have stimulated the development of some degree of immunoprotection. Moreover, although a population of generally poor people, their diet consists largely of fish and basic agricultural products of fairly good nutritional value. For this reason, although there is some evidence suggesting that susceptibility to visceral leishmaniasis is strongly controlled by genetic mechanism (Peacock et al. 2002; Blackwell et al. 2004; Jamieson et al. 2007), it is equally possible that environmental and nutritional factors may also be influencing immune response.

With regards to a comparison between the two immunodiagnostic methods, it was found that LST infection rates were always higher (p<0.05) than those of IFAT, either in the prevalence (11.2% × 3.4%) or in the three incidences, at 6 (4.7% × 0.6%), 12 (4.7% × 2.7%), and 24 months (2.9% × 0.3%) of study, which indicated that among all infected individuals (231), the great majority (85%) had a consistent immunological character of resistance against the infection (Jeronimo et al. 2007). This finding might also explain the low prevalence (0.42%) and incidence (0.37 cases of 1,000 inhabitants) of acute AVL found in this study.

In any comparison, however, between the T-cell and humoral responses of human L. (L.) infantum chagasi infection, it must be regarded that results may be strongly influenced by different methodological proceedings. There are some prospective studies carried out in the northeastern region of Brazil, in Bahia, and Maranhão states, principally, which have used different antigens (soluble extract, crude antigen, and a recombinant protein (rK39) of L. (L.) infantum chagasi and a soluble extract of L. (L.) amazonensis) for determining either the prevalence or the incidence of infection by either LST or enzyme-linked immunosorbent assay. Thus, as the diagnosis of infection is not limited to a single type of immune response, it is evident that both prevalence and incidence have underestimated the true epidemiology of infection in that region (Badaró et al. 1986; Caldas et al. 2001, 2002; Nascimento et al. 2005).

On the other hand, in an old focus of visceral leishmaniasis in Sicily, Italy, where the disease is due to *Leishmania (Leishmania) infantum*, a promastigote antigen of this parasite was used for LST diagnosis of infection in differently age individuals, which showed a prevalence of 16.6% (Pampiglione et al. 1975). This most closely resembles the result of the present study (11.2% by LST alone) and suggests similar transmission dynamics for
the two subspecies \textit{L. (L.) infantum chagasi} and \textit{L. (L.) infantum infantum}.

The diagnosis of infection based on both LST and IFAT assays, simultaneously, allowed to find a prevalence of 12.6\%, which showed to be a new finding for Brazil. Regarding distribution of cases by sex, the result of 55.8\% men and 44.2\% women showed no significant difference. It was also of interest that the youngest age groups of 1–10 and 11–20 years old showed similar infection rates of 19.2\% and 25.8\%, respectively, being both smaller than that (55\%) of the older age group (≥21 years old). The prevalence progressively increased with age, a fact also characteristic of human infection with \textit{L. (L.) infantum infantum} in Sicily, Italy, which is only indicating that older people have had more time of exposure to infection than younger people (Pampiglione et al. 1975).

The specificity of the two tests showed that, among 106 LST reactors at the prevalence, 56.6\% had an important degree of hypersensitivity, suggesting that a considerable number of individuals might have received repeated bites of infected sand flies over a long period. This should be considered in any future vaccination control program. On the other hand, it was noted that, among 32 IFAT reactors, only 25\% had shown a susceptibility profile to \textit{L. (L.) infantum chagasi} infection through their high IFAT titers ranging from 1,280 to 10,240 (IgG). Thus, the great majority (75\%) had shown low IFAT reactivity from 80 to 640 (IgG), indicating that only few cases with humoral response had some degree of predisposition for developing AVL, which was confirmed in only 12.5\% (four cases) of IFAT reactors.

With regards to the incidence, it should be emphasized that the differences found in the three surveys at 6 (5.2\%), 12 (6.3\%), and 24 (3.6\%) months were not of any statistical significance \((p>0.05)\), which suggested that the infection transmission during the 24 months may have stabilized and was without oscillation. Nevertheless, if the incidence had been evaluated in two periods of 12 months, the first would be 11.5\% and the second 3.6\%, thus showing that in the first year there was a higher rate of transmission \((p<0.05)\) than that occurred in the second year. This may, in part, have an explanation in the results of control measures involving the elimination of IFAT-positive dogs in the study area. This not only decreased the canine infection rates from 43.8\% in the first year to 29\% in the second (Pereira et al. 2006), as well as may have also decreased human infection rates from 11.5\% in the first year to 3.6\% in the second. Thus, although it is not the subject to discuss the merits of culling seropositive dogs, advocated by the Brazilian Ministry of Health (Brasil 2003), these figures seem to support this type of control measure.

The distribution by sex among the 111 new cases of infection was 47.7\% men and 52.3\% women, with no significant difference \((p>0.05)\). When these cases were analyzed by age group, it was noted that the youngest age groups of 1–10 and 11–20 years old had shown no significant difference \((p>0.05)\) in their infection rates of 29.7\% and 25.3\%, respectively, but that both rates were lower than that 45\% of the older age group of ≥21 years old. When combined, however, the infection rates of the two young groups totaled 55\% of the incidence, indicating that, contrary to the prevalence profile, these cases were more frequent in the young groups and occurred principally in the children of 1–10 years (almost 30\%). This finding correlates well with previous observations in the Brazilian Amazon region which have shown that transmission of \textit{L. (L.) infantum chagasi} to man is principally intra-domiciliary or peri-domestic, where children are particularly vulnerable (Lainson and Rangel 2003, 2005; Lainson and Shaw 2005; Silveira et al. 1997).

With regards to the LST in determining the incidence, there was no significant difference between 48.3\% with exacerbated or strong positive reactions and 51.7\% with moderate or weak reactions. This contrasted with the predominance of very strong reactions (56.6\%) found in the prevalence survey, which suggests that the LST reactivity might be influenced by the exposure time of persons to the successive inoculation of the parasite by repeated bites of infected sandflies and indicates that, in any vaccination program, it is advisable to give repeated doses at regular intervals in order to maintain a good level of immunoprotection.

Among the 30 IFAT reactors, 90\% presented moderate to weak positive results (80–640 IgG) and a low susceptibility to infection, although 2 months following the first incidence survey, one of these cases with a negative LST and a positive IFAT (80 IgG) reactions had evolved to typical AVL. Among the remaining 10\% with high IFAT reactions (≥1,280 IgG), two cases were typical acute AVL, and another had a subclinical oligosymptomatic infection; all seven AVL cases diagnosed in this study (four at the prevalence and three during the incidence surveys) have received antimony therapy in accordance with the Brazilian AVL control program (Brasil 2003), while the subclinical oligosymptomatic cases have only been clinically followed up for confirming their self-healing. These findings have also confirmed that IFAT using \textit{L. (L.) infantum chagasi} amastigote antigen is very efficient for determining human susceptible infection with this parasite (Lima et al. 2003).

In spite of a loss of 94 individuals from the original sample (946 individuals) of this study, it was possible to show that the accumulated prevalence of 20.8\% by LST was higher than that of 6.5\% by IFAT, indicating a LST/IFAT ratio of 3.2:1. In other words, there were three times more resistant individuals than susceptible in the endemic area. Moreover, when all LST and IFAT diagnosed cases
were considered together, there was found an accumulated prevalence of 24.4% for the 2 years study.

In conclusion, the above commented results have raised speculations regarding the possibility that either the control measures or certain environmental or ecological factors may have influenced the incidence of human *L. (L.) infantum chagasi* infection transmission in the endemic area of this study, leading this infection rate from 11.5% in the first year to 3.6% in the second year, although it has been recorded that most (55%) cases have occurred in the two first decades of life, principally in first one (almost 30%), confirming prior findings on the transmission of this parasite in the Amazon region of Brazil.

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