SANTO INÁCIO REVISITED: PROTOZOAN DISEASES IN AN ISOLATED VILLAGE IN NORTHEASTERN BRAZIL AFTER TWENTY YEARS

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Abstract. The northeastern highlands of Brazil are endemic for several tropical diseases, especially American trypanosomiasis (Chagas’ disease) and schistosomiasis. Twenty years ago, we measured the seroprevalence of protozoan diseases in Santo Inácio, a village of approximately 1,000 inhabitants located 1,000 m above sea level. We detected small numbers of sera with antibodies against Trypanosoma cruzi and Toxoplasma gondii, and the area had a low prevalence both of American trypanosomiasis (3.54%) and toxoplasmosis (27.43%) compared with nearby Brazilian areas. This was attributed to a specific triatomine vector and local housing conditions. Twenty years later, we again determined the prevalences of both diseases and compared these results with those from Iraquara, a larger town with the same ethnic and social background but with a higher prevalence of rural activities. The incidence of Chagas’ disease in Santo Inácio showed the same low level, i.e., 3.78% (5 of 132) with only adult males affected in contrast with Iraquara, which had an incidence of 34.5%, but a low prevalence of only one of 22 among children up to 14 years of age. Santo Inácio maintained a low (25.8%) seroprevalence for toxoplasmosis. Housewives presented a higher incidence of toxoplasmosis during both periods, probably due to related risk factors. Cats were found less frequently in Santo Inácio than in Iraquara, which showed an incidence of 65.5% seropositivity for Toxoplasma gondii. These results suggest that the environmental conditions of Santo Inácio were preserved after 20 years, with a low incidence of these selected protozoan diseases.

The northeastern highlands of Brazil are endemic for several tropical diseases, especially American trypanosomiasis (Chagas’ disease) and schistosomiasis.² Twenty years ago, we conducted a survey of the seroprevalence of two selected protozoan diseases in Santo Inácio, a village of approximately 1,000 inhabitants located 1,000 m above sea level.² Three areas had specific characteristics of isolation, professions, and environmental conditions. The main activities are hunting, fruit picking, trading, and urban activities, without significant agriculture. During the first survey, we detected small numbers of sera with antibodies against Trypanosoma cruzi and Toxoplasma gondii, and the area had a low prevalence of both American trypanosomiasis (3.54%)³ and toxoplasmosis (27.43%).³ American trypanosomiasis is transmitted by a reduvid vector, and was notably frequent in this area, with more than 30% of the people affected.⁴ Population and housing conditions have been improved in Santo Inácio by vector control programs and also in Iraquara, a nearby city (45 miles away) that was used for comparative purposes.⁵ Toxoplasmosis is a protozoan disease that may be acquired by ingestion of food contaminated with oocysts from cat stools or by ingestion of cysts from the meat of infected animals.⁶ Usually benign, the infection of pregnant women results in severe fetal disease.⁷ The low incidence of both diseases observed in Santo Inácio was attributed to environmental isolation due to the highland location, or to a specific environmental effect on triatomine vector biology and local housing conditions.⁸ Few other characteristics were tested at that time. The study of these two protozoan diseases in isolated areas could reveal some biological or environmental conditions that might elucidate the disease patterns and transmission modes in areas with more complex environmental and social networks.

Twenty years after the first survey, we determined the seroprevalence of these two protozoan diseases and another disease not related to the environment, streptococcal infections, in the same area. We also compared these results with those obtained for Iraquara, a larger town with approximately 17,000 inhabitants located near Santo Inácio and having the same ethnic and social background. However, this town was not as environmentally isolated and had a higher prevalence of rural activities.

MATERIALS AND METHODS

Study sites and sampling. Santo Inácio is a small isolated village in the county of Gentio do Ouro, along the northern border of the Diamantina Highland in the state of Bahia, Brazil. Iraquara is a larger town on the southern border of the Diamantina Highland, located in a similar but less isolated environment.³ The location of the two towns is shown in Figure 1.

The study was conducted with the approval of the Public Health State Secretaries of both states involved (Bahia and São Paulo, Brazil). Blood samples or serum samples were collected in both areas during interviews conducted by one of us (RLC) after the subjects or their parents gave informed consent. Information on age, sex, housing, activity, and pedimiciliary animals was also obtained. In 1975, 113 blood samples from Santo Inácio were collected on filter paper, dried, adequately packed, and transported in ice bags to the Instituto Adolfo Lutz in São Paulo. In 1995, 242 blood samples were collected by venipuncture in both towns, and the sera was isolated, aliquoted, and transported in ice bags to the Instituto Adolfo Lutz. All samples were stored at −20°C until tested.

Specific antibody detection by ELISA. The presence of specific antibodies against T. cruzi was determined with a commercial assay (Embrabio, São Paulo, Brazil) using microplates adsorbed with an alkaline extract of T. cruzi epi-
mastigotes. Specific antibodies to Toxoplasma gondii were detected with an in-house ELISA based on a saline extract of tachyzoites of the RH strain of this parasite purified from the peritoneal exudate of previously infected mice. The tachyzoites were purified by differential centrifugation, freeze-thawed 10 times, and the protein content was determined in a Bradford assay. Microplates were absorbed with an antigen solution of 20 μg/ml, blocked with skim milk, and assayed with the sera. The bound antibody was visualized with specific anti-human globulin conjugates (Sigma, St. Louis, MO). The cut-off value of the reactions was determined using standardized negative sera, with a 99% confidence level.

Immunofluorescence assays. These tests were performed as previously described using an anti-human globulin conjugate. The cut-off value of diluted sera was monitored with standardized positive and negative sera in each reaction batch.

Hemagglutination assays. These assays were performed using an in-house reagent produced with goose erythrocytes adsorbed with T. cruzii epimastigote antigens, as previously described. Standardization was performed with appropriate clinically established negative and positive controls.

Streptococcal infection serology (streptolysin O) assays. Serologic analysis of the antibody response of the subjects in each area was done by measuring the response to streptococcal antigens, an air-borne infection with a high prevalence in Brazil. These tests were performed in unselected samples by measuring their response to streptolysin O.

Case infection definition. A serum sample was considered positive for American trypanosomiasis when at least two IgG antibody test results were positive, as recommended elsewhere. For toxoplasmosis, similar standards were used, and a sample was considered positive only when it showed positive results in both tests. Serologic evidence of past streptococcal exposure or disease was defined as a positive anti-streptolysin O sample.

Statistical analysis. Data were analyzed with the Epi Info 6.0 software package (Centers for Disease Control and Prevention, Atlanta, GA). Frequency analyses were performed with the chi-square test using Yates’ correction. Occasionally, small numbers of samples resulted in using Fisher’s exact test. Quantitative data were analyzed by the Student’s t-test or analysis of variance after testing for variance homogeneity, or by the Kruskal-Wallis test, if necessary. The level of significance was set at \( P < 0.05 \), with power of the tests at 0.90.

RESULTS

The demographic data and serologic frequencies of the populations studied are shown in Table 1. The incidence of Chagas’ disease in Santo Inácio remained at a low level, i.e., an incidence of 3.78% (5 of 132) with only adult males affected, in contrast with Iraquara, which showed an inci-

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### Table 1

Demographic and serologic data for the three populations studied

<table>
<thead>
<tr>
<th>Sample characteristics</th>
<th>Total</th>
<th>Santo Inácio</th>
<th>Iraquara</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic data</strong></td>
<td></td>
<td>1975</td>
<td>1995</td>
</tr>
<tr>
<td>No. of males</td>
<td>48</td>
<td>61</td>
<td>35</td>
</tr>
<tr>
<td>No. of females</td>
<td>75</td>
<td>71</td>
<td>75</td>
</tr>
<tr>
<td>Mean ± SD age, years</td>
<td>NA</td>
<td>30.0±2.0</td>
<td>30.9±1.6</td>
</tr>
<tr>
<td>&lt;18 years old</td>
<td>56 (42.4%)</td>
<td>28 (25.5%)</td>
<td></td>
</tr>
<tr>
<td>Rural occupancy (%)</td>
<td>NA</td>
<td>20 (15.2%)</td>
<td>28 (25.5%)</td>
</tr>
<tr>
<td>Occupancy in a house (%)</td>
<td>NA</td>
<td>31 (23.5%)</td>
<td>49 (44.5%)</td>
</tr>
<tr>
<td>Cats in the house of a patient*</td>
<td>14 (10.6%)</td>
<td>43 (39.1%)</td>
<td></td>
</tr>
<tr>
<td><strong>American trypanosomiasis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IIF/IHA/ELISA IgG</td>
<td>4 (3.5%)</td>
<td>5 (3.8%)</td>
<td>38 (34.5%)</td>
</tr>
<tr>
<td>≥18 years old</td>
<td>36 (27.4%)</td>
<td>34 (25.8%)</td>
<td>72 (65.5%)</td>
</tr>
<tr>
<td>&lt;18 years old</td>
<td>0</td>
<td>0</td>
<td>5 (4.5%)</td>
</tr>
<tr>
<td><strong>Toxoplasmosis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IIF/ELISA IgG</td>
<td>31 (27.4%)</td>
<td>34 (25.8%)</td>
<td>72 (65.5%)</td>
</tr>
<tr>
<td>≥18 years old</td>
<td>16.7%</td>
<td>50%</td>
<td></td>
</tr>
<tr>
<td>&lt;18 years old</td>
<td>0</td>
<td>5 (4.5%)</td>
<td></td>
</tr>
</tbody>
</table>

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*NA = not available; IIF = indirect immunofluorescence; IHA = indirect hemagglutination assay; ASLO = anti-streptolysin O.

† \( P < 0.01 \) versus Santo Inácio 1995 samples.
FIGURE 2. Distribution of serologic titers for the diseases investigated in samples from both localities in the last sampling period. Samples were considered nonreactive when the titer was < 20 in the indirect immunofluorescent (IIF) assay and the indirect hemagglutination assay (IHA) for Trypanosoma cruzi, ≤ 40 in the IIF assay for Toxoplasma gondii, and < 250 in the anti-streptolysin O (ASLO) assay. Statistical significance was determined by analysis of variance of log-transformed titers. Sto. = Santo.

dence of 34.5%, mainly among adults, without a sex preference. The housing conditions of the two areas were similar, and vector transmission was controlled in Iraquara, resulting in a low prevalence among children < 14 years of age, (only 1 of 22). For toxoplasmosis, Santo Inácio maintained a low prevalence, with only 25.8% of the people being seropositive. Interestingly, housewives had a higher incidence of this disease, as observed in the previous survey, suggesting that risk factors related to this activity must be present. Another interesting fact was the prevalence of cats in the home, which was less frequent in Santo Inácio than in Iraquara, which showed a 65.5% frequency of individuals seropositive for Toxoplasma gondii, similar to other areas in Brazil. Exposure and antibody response to streptococcal disease was similar in both areas, as determined by the detection of anti-streptolysin O antibodies. These results suggest that the environmental conditions of Santo Inácio were preserved after 20 years, with a low incidence of these two protozoan diseases.

Another phenomenon observed was the lower titers of specific antibodies that were found in sera from Santo Inácio compared with those from Iraquara, as shown in Figure 2. As can be clearly seen for American trypanosomiasis, the samples from Santo Inácio had lower antibody titers than those from Iraquara. This pattern was also seen with anti-Toxoplasma antibody titers, but was absent for the anti-streptolysin O titers, showing that the immune response to an external agent was similar in both populations, without di-
versity of immune responses in these areas. Thus, the lower specific titers obtained could be attributed to lower exposure to these protozoan agents in Santo Inácio.

**DISCUSSION**

The serologic survey of two selected protozoan diseases in two diverse ecotypes in northeastern Brazil showed that this could be used as prevalence indicator and occasionally as an incidence predictor. The immunofluorescence assay was useful for both toxoplasmosis and Chagas’ disease, allowing the comparison of results obtained 20 years apart. We also used two other confirmatory techniques, indirect hemagglutination and/or an ELISA, to show that the data were reliable and accurate. The prevalence of streptococal exposure or disease, estimated by the anti-streptolysin O tests, demonstrated that this air-borne diseases was similarly transmitted at both localities with an adequate and similar immune response in both populations, showing that no bias due to population immunity or genetic background was likely.

Our data show that the environmental conditions related to lower transmission of two protozoan diseases were maintained in Santo Inácio, a small village in the central plains of Brazil, in spite of 20 years of public health improvements. Initially attributed to regional characteristics of triatomine bugs, the low incidence of Chagas’ disease is not adequately explained by this fact, but it is reasonable to assume that the small numbers of infected persons and better housing conditions significantly reduced transmission in this locality independent of the period of study. Another important fact was the stability of the population, which despite improvement in transportation, was generally composed of native inhabitants without significant migration, with a consequent reduced contact of vectors with infected persons. Since the climatic and soil conditions of Santo Inácio are relatively arid, with rocky and sandy soil, few people live in the rural area, with most leaving the urban area only for hunting or other activities. The lack of significant agricultural activities reduces the time of exposure to biting by triatomines. Despite having no statistical significance, only men were found seropositive for Chagas’ disease, a characteristic of occupational transmission, a pattern not observed 20 years ago when both sexes were affected.

The lower incidence of toxoplasmosis seropositivity was also interesting because this disease is not transmitted by an insect vector, and infection probably occurs by consumption of food contaminated with oocytes from cat feces or with cysts from previously infected tissue. The arid conditions of Santo Inácio may impair the environmental resistance of oocytes in cat feces, since they usually need humid and fresh soil for maturation and some running water for flotation and dissemination, conditions rarely found in the soil of this region. It is interesting to note that in countries with desert environments, the incidence of toxoplasmosis is quite diverse, ranging from 21% to more than 80%. Another significant fact was the lower prevalence of cats in Santo Inácio observed recently. This was attributed both to cultural conditions, since the population usually eats hunted animals, among them wildcats, or to the relatively low availability of food for these animals that usually feed in peridomestic areas. The prevalence of cats as a source of infection source was also found to be related to seropositivity in other studies.

In Iraquara, the prevalence of both protozoan diseases was higher and similar to that found in other areas of Brazil for Chagas’ diseases or toxoplasmosis. Iraquara also had a low incidence of new cases of Chagas’ disease, with only one young person infected. However, the long-lasting characteristic of this disease maintains its high prevalence and impairs adequate control in blood banks. This lower incidence could be attributed both to better education and to improvement in vector control that was implemented nationwide in the 1970s. The prevalence of toxoplasmosis could be attributed to the prevalence of cats in houses in this town, which was much higher than in houses in Santo Inácio, as determined through the questionnaire given to the inhabitants of the houses investigated. Most of the serologic data obtained in Iraquara were similar to those for other towns in Brazil. This probably indicates intense human migration related to the agricultural work market, which facilitates transmission of Chagas’ disease, despite the current intense vector control in the area. It is interesting that specific ecologic and social conditions could maintain a low incidence of two protozoan diseases in an area for 20 years.

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