CHAGAS' DISEASE IN THE BRAZILIAN AMAZON. III. A CROSS-SECTIONAL STUDY (1)

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SUMMARY

Two serological surveys for Chagas' infection were carried out, in 1991 and 1993, respectively, using a conglomerate family samples from the residents in the town of Barcelos (in the northern part of the State of Amazonas, on the right bank of the Rio Negro, 490 Km up-river from Manaus), using indirect immunofluorescent tests for anti-T. cruzi antibodies. In the first survey (1991), 628 blood samples from the residents of 142 dwellings were tested, showing positive in 12.7% for anti-T. cruzi antibodies and in 1993 an other 658 samples from residents of 171 dwellings showed positive in 13.7% of the tests, thus confirming the previous results.

From 170 individuals with positive serology for T. cruzi antibodies, 112 (66%) were interviewed and submitted to electrocardiographic and clinical examinations; 82 (73.2%) of them gave consent for xenodiagnosis. From the 112 interviewed 52 (46.4%) recognized the triatomines as "piquavas' lice"; 48 (42.8%) knew the bugs from their work places being gatherers of piqava fibers in rural areas and 19 (16.9%) said that have been bitten by bugs in their hats. Only 2 (2.4%) of 82 xenodiagnosis applied were positive for T. cruzi and 9 (8%) of the ECG had alterations compatible with Chagas' disease.

KEYWORDS: Chagas' disease; Cross-sectional study; Brazilian Amazon.

INTRODUCTION

The first humans cases of Chagas' disease in the Brazilian Amazon were reported in Belém do Pará in 1969. Since then several other cases have been notified in the States of Pará13,14,15,16,23,24,25,26, Amazonas,12,22,23, Maranhão15 and Acre24 with either clinical manifestations of acute disease or occasionally finding T. cruzi during tests for malaria parasites, blood cell counts and other routine blood examinations. Many others observations have not been published.

Although Chagas' disease has always been considered as an enzootic infection from wild animals and triatominae in the Brazilian Amazon, the risks of the disease becoming endemic in that area have been stressed, not only by the reports of some isolated human cases but also by serological surveys2,3 and in recent reviews4,5,16,27. We also described an "attack" of a human population (piquavas' workers) by wild triatomines (Rhodnius prolixus, MATTA, 1919)11 as a possible new mechanism for the transmission of Chagas' infection in the Amazon1.

The national serological survey carried out by SUCAM (now Fundação Nacional de Saúde - Ministry of Health in Brazil) from 1975 to 1980 showed the prevalence of 1.88% of positive serology for Chagas'.

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where we found 12.5% of the human population positive for anti-
*T. cruzi* antibodies), we decided to carry out this
cross-sectional study, including a new serological survey,
to evaluate the correlations of positive serology for anti-
*T. cruzi* antibodies with cases of human contact with wild
bugs, human infection from *T. cruzi* and eventually clinical
and electrocardiographic manifestation of Chagas' disease.

**METHODOLOGY**

**Location of the study area**

The 121,760 km² county of Barcelos the largest
administrative district in the State of Amazonas, is located
in the micro region of Rio Negro in the northern
part of the State, bordered on the east by State of
Roraima, on the southeast and south by the administrativa
districts of Novo Aripo and Marãa, on the west by
the administrative district of Santa Isaehel do Rio Negro
and on the north by Venezuela (with a latitude of 0°58'13"
south of the equator and longitude of 62°56' west of
Greenwich). The town of Barcelos, where this study was
carried out, is located on the right bank of the Rio Negro,
490 km by river from Manaus, the capital of the State of
Amazonas (Fig. 1).

![Fig. 1 - Location of Barcelos in the State of Amazonas](image)

**Domicílios investigados**

**BARCELLOS**

![Fig. 2 - Distribution of the dwellings investigated in Barcelos](image)
Surveys, samples and laboratory procedures

Two serological surveys for Chagas' infection were carried out, respectively in 1991 and 1993, using conglomerate family samples from families residing in the town of Barcelos, employing immunofluorescent tests for anti-*T. cruzi* antibodies. In the first survey (1991) 628 blood samples from the residents of 142 dwellings (one dwelling in every five) were tested and in 1993 another 658 samples from the residents of 171 dwellings (one dwelling in every four) were examined (Fig. 2). The tests were performed employing human anti-gammaglobulin type IgG (BioLab). Formalized culture forms of *T. cruzi* Y strain were used as antigen. The reactions were observed through a Leitz microscope (Diaplan model) with epi-illumination for immunofluorescence. The positive reactions were confirmed by enzyme-linked immunosorbent assay (ELISA).

Interviews, clinical and electrocardiographic exams

In the surveys, two questionnaires were used, one residential, to evaluate the social, economic and sanitary conditions and another individual for anamnesis and clinical examinations.

The individuals with positive serology for *T. cruzi* antibodies were interviewed and submitted to clinical and electrocardiographic examination with the 12 classical standard leads (bipolar D1, D2, and D3, unipolar aVR, aVL and aVF and precordial V5 to V6). During the interviews we showed a collection of *Triatoma*, *Panstrongylus* and *Rhodnius* to see if the patients could recognize them.

Xenodiagnosis

All of the patients with positive serology for anti-*T. cruzi* antibodies, who gave the consent were submitted to xenodiagnosis with 40 4th stage nymphs. 20 of *T. infestans* and 20 of *P. megistus* (fasting for at least 20 days). The nymphs were distributed in four wooden boxes with ten nymphs each. Two boxes were applied to each inner forearm of the patients and left to feed for 30 min. The nymphs were again fed with chicken blood 23 days later and checked 45 days after being applied on the patients.

Feces from a pool of 2 or 3 nymphs were collected using slight abdominal pressure and deposited on slides containing one drop of PBS at pH 7.2, homogenized, covered with a 22x22 mm film and examined under a microscope which magnified their diameter 400 fold; if this was negative, the entire intestinal content was dissected, homogenized and examined using the same technique. Before checking for *T. cruzi* the hemolymph and the salivary glands of all nymphs were checked for *T. rangeli*.

Other tests

Other tests for confirmation of *T. cruzi* infection, such as Polymerase Chain Reaction (PCR) of kDNA, and

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**TABLE**

Distribution of the population examined and IFT positive for *T. cruzi* antibodies according to age groups

<table>
<thead>
<tr>
<th>AGE GROUPS (years)</th>
<th>SEROLOGY</th>
<th>ECg AND CLINIC EXAM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Performed</td>
<td>Positives(%)</td>
</tr>
<tr>
<td>0-10</td>
<td>358</td>
<td>31(8.6)</td>
</tr>
<tr>
<td>11-20</td>
<td>379</td>
<td>42(11.0)</td>
</tr>
<tr>
<td>21-30</td>
<td>166</td>
<td>24(14.4)</td>
</tr>
<tr>
<td>31-40</td>
<td>139</td>
<td>27(19.4)</td>
</tr>
<tr>
<td>41-50</td>
<td>107</td>
<td>13(12.1)</td>
</tr>
<tr>
<td>51-60</td>
<td>55</td>
<td>12(21.8)</td>
</tr>
<tr>
<td>&gt; 60</td>
<td>82</td>
<td>21(25.6)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>1286</td>
<td>170(13.2%)</td>
</tr>
</tbody>
</table>

IFT = Immunofluorescent test
biochemical and molecular characterization of trypanosomes isolated by xenodiagnosis are now being carried out.

RESULTS

In the first serological survey (1991), of 628 sera tested by the indirect immunofluorescence for anti-T. cruzi antibodies 1277% were positive, while in the second survey (1993) of 675 samples examined by the same technique 937% were also positive, thus confirming the previous results.

From the 170 individuals with positive serology for T. cruzi antibodies, 112 (66%) were interviewed and submitted to clinical and electrocardiographic examination, but only 82 (73.2%) of them consented to xenodiagnosis. The age group of the 112 interviewed patients varied from 2 up to 92 with an average of 27.5±9.20 years; 491% of them were male and 50.9% female. The table shows the distribution of the patients by age group and the results of serology.

When we showed a collection of Triatoma Panstrongylus and Rhodius during the interview 52 (46.4%) of the patients recognized the triatomines which they call "piagava's lice"; 48 (42.8%) knew the bugs from their work place being gatherers of piagava fibers in rural areas and 19 (15.3%) said that they have been bitten by the bugs in their huts in rural areas (the information from children younger than 10, was gathered from their parents).

No patient complained during anamnesis of symptoms suggestive of Chagas' disease but 5 (4.46%) showed a cardiac auscultation arrhythmia compatible with that disease.

The electrocardiograms showed alteration in 9 (8%) of the 112 patients. Four had ventricular extrasystoles: isolated, frequent or bigeminal. One patient showed supraventricular extrasystoles, first degree atrioventricular block and intraventricular delayed stimulus conduction. Two patients presented right bundle-branch block, one of them of the first degree and the other of the 3rd degree, associated with the left anterior hemiblock which is very suggestive of Chagas' disease. Finally one showed a disturbance of the ventricular repolarization and another had inactive electrical zones and an elevation of the S-T segment suggestive of a myocardial lesion of the left ventricle. In summary we found the following ECG alterations in nine patients:

a) Disturbance in the stimulus formation - 5 times;

b) Disturbance in the stimulus conduction - 3 times;

c) Primary alteration of T wave and S-T segment - 2 times;

d) Electrical inactive zones - one time.

Fig. 3 illustrates the electrocardiographic alterations findings suggestive of Chagas' disease.

From 82 xenodiagnosis performed on patients with positive serology for T. cruzi antibodies, only 2 (2.4%) were positive. The exams of the hemolymph and salivary glands were negative for T. rangeli in all bugs.

Polymerase chain reaction (PCR) of kDNA performed up to now on the blood of 30 patients, was positive for T. cruzi in 3 (10%) of the cases.

The T. cruzi strains isolated by xenodiagnosis were difficult to be adapt in mice. They have a very long prepatent period and they do not kill the mice; strains grew very slowly in culture media.

DISCUSSION

The results of the first serological survey for anti-T. cruzi antibodies performed in a sample of 628 sera collected in 1991 showed a positive result in 12.7% of the tests and these were confirmed in a second survey of 675 samples collected in 1993 which showed positive 13.7% of the time.

The high level of positive serology for anti-T. cruzi antibodies found in this study does not necessarily signify that all the cases with positive serology are actually infected with T. cruzi. None the less, the study shows strong epidemiological and serological correlations, such as previous contact of the positive cases with wild triatomines (known locally as "piagava's lice"), isolation of T. cruzi by xenodiagnosis and positivity or PCR of kDNA of T. cruzi in some cases. This data strongly suggest that significant proportions of the serological positive cases will be confirmed to be infected with T. cruzi.

A large portion of the people with positive serology for anti-T. cruzi antibodies recognized the triatomines shown as "piagava's lice", 42.8% knew the bugs from their work places, being gatherers of piagava fibers in rural areas and 16.9% said that they have been bitten by the bugs in their huts. On the other hand none of those interviewed recognized the existence of the bugs in their houses in the town of Barcelos, but only in rural areas where the piagava fiber (Leopoldinia piagava) is collected. All the piagava gatherers and their families with positive serology for anti-T. cruzi antibodies recog-
graphic alterations were over 60 years of age which makes it difficult to exclude an association with atheroeclesclerosis. It is important to stress that of the 12 patients older than 60 which were studied, 9(75%) had ECG alterations, which indicate a possible association of Chagas' disease and heart atheroeclesclerosis.

Finally we conclude that Chagas' infection in the studied area may be transmitted by wild bugs in the rural areas or in the boats during transportation of piáçava fibers. The circulating strain of T. cruzi is of low virulence and pathogenicity and not yet adapted to man in the domestic cycle.

RESUMO

Doença de Chagas na Amazônia brasileira. III - Um estudo seccional

Dois inquéritos sorológicos para a infecção chagásica foram realizados, respectivamente em 1991 e 1993, em amostras por conglomerado familiar na população da cidade de Barcelos, utilizando-se a reação de imunofluorescência indireta para anticorpos anti-T. cruzi. No primeiro inquérito de 628 amostras de sangue de residentes em 142 domicílios, 12,7% foram positivas para anticorpos anti-T. cruzi; no segundo inquérito de 658 amostras de sangue de residentes em 171 domicílios, 13,7% foram positivas, confirmando os resultados anteriores.

De 170 indivíduos com sorologia positiva para infecção chagásica 112 (66%) compareceram para entrevista e para exame clínico e eletrocardiográfico. Destes 82 (73,2%) submeteram-se ao xenodiagnóstico. Dos 112 entrevistados, 52 (46,4%) reconheceram o triatomíneo como “piolo da piáçava”, 48 (42,8%) disseram existir em seus locais de trabalho, geralmente em piáçavais na área rural e 19(16,9%) disseram já terem sido picados pelo inseto. Dos 82 xenodiagnósticos aplicados, 2 (2,4%) foram positivos para T. cruzi. Apenas 9 (8%) dos 112 ECG realizados tinham alterações comparáveis com a doença de Chagas.

As evidências demonstram que a infecção chagásica na área tem características profissionais, é transmitida pelo contato com triatomíneos silvestres e que a cepa de T. cruzi circulante é de baixa virulência e pathogenicidade, possivelmente por ser silvestre e ainda não adaptada ao homem.

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