CHAGAS' DISEASE IN THE BRAZILIAN AMAZON. II. A SEROLOGICAL SURVEY(1)

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SUMMARY

A serological survey, involving indirect immunofluorescence testing of blood sera samples, was carried out on the residents of one in every five dwellings in the town of Barcelos (in the northern part of the State of Amazonas, on the right bank of the Rio Negro, 490 Km from Manaus by river) and on the rural populations of the villages of Piloto and Marará (also on the right bank of the Rio Negro, 30 minutes by boat from Barcelos).

A total of 710 sera samples were tested, 628 from the resident population in the town of Barcelos, 35 from Piloto and 47 from Marará. The tests were carried out using human anti-gammaglobulin type IgG (Biobol) and antigen from formalized culture of T. cruzi Y strain. The sera were serially diluted from 1:40 to 1:320 in PBS 7.2.

Of the 710 samples examined 89 (12.5%) were positive for anti-T. cruzi antibodies: 2 of these (2.2%) at a dilution of 1:320; 12 (13.4%) at 1:160; 38 (42.6%) at 1:80; and the remainder at 1:40, giving a median serological dilution of 1:80.

The following questions are discussed: the high serological prevalence for Chagas’ infection found in our survey; the possibility of serological cross-reactions; the need for confirmatory tests for the positives reactions; and the strong correlation between our results and preliminary epidemiological data (such as the level of human contact with wild triatominae, know locally as “Piaçava’s lice”. We draw attention to the isolation by xenodiagnosis of one strain of T. cruzi from a patient with positive serology for Chagas’ infection.

KEYWORDS: Chagas’ disease; Serological survey; Brazilian Amazon.

INTRODUCTION

Chagas’ disease in the Brazilian Amazon has always been considered as to be a sylvatic enzootic. Since 1924, when CHAGAS (1) confirmed as cruzi trypanosomoses found by Aben-Athar in Saimiri sciureus monkeys from the State of Pará, several species of sylvatic animals - marsupials, chiroptera, rodents, edentates and primates - have been identified as T. cruzi reservoirs in the Amazon Region (7, 15, 16, 17, 18, 19, 20, 24).

At least 18 species of triatomine have been found in the region, nine of them infected with T. cruzi or “cruzi-like” trypanosomes (2, 3, 5, 6, 7, 11, 12, 13, 14, 15, 16, 17). Although there are no descriptions of domestic triatomines in the Brazilian Amazon it can not be definitively concluded from this that Chagas’ disease is not endemic in the Amazon, given the small number of existing studies, the vast scale of the region and the wide variety of intraregional differences.

The risk of endemic Chagas’ disease in the Brazilian

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Amazon were stressed in recent reviews 12,13,14, inclusive with 38 human cases described up to 1992. The national serological survey carried out by SUCA (now Fundação Nacional de Saúde) from 1975 to 1980 10 showed a 1.88% prevalence of positive serology for Chagas' infection in the human population of the State of Amazonas, although at the time this result was considered to be due to a possible cross-reaction or "false-positive" phenomenon.

Given the above-mentioned findings, together with the evidence of human positive serology for Chagas' infection 25 and the occurrence of one acute case of infection in a patient from the district of Barcelos 30 in the northern part of the State of Amazonas, we decided to carry out a multidisciplinary study. Following our recent short review on the subject of Chagas' disease in the Amazon, we herein present the preliminary results of this study.

METHODOLOGY

Location of the study area

The administrative district of Barcelos is located in the northern part of the State of Amazon, bordering in the east with the State of Roraima, in southeast and south with the administrative districts of Novo Airão and Marã©, in the west with the administrative district of Santa Isabel do Rio Negro and in north with Venezuela (latitude 0°58'1" south of the equator and longitude 62°56' west of Greenwich). The town of Barcelos, were 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 Amazon (Fig. 1).

Samples

We received small quantities of blood sera collected by vein puncture for another study 4 from 628 residents of the town of Barcelos and from 82 inhabitants of the rural communities of Piloto and Mararã (located approximately 30 minute by boat from the town of Barcelos), given a total of 710 sera samples. In Barcelos, conglomerate family samples were taken from one in every five dwellings, aiming at coverage of approximately 20% of the population in all age groups. Due to absences, refusals or collection problems, samples were in fact obtained from only 17.8% of the resident population. In Piloto and Mararã, respectively, 35 and 47 samples were obtained.

Laboratory procedures

The blood samples were centrifuged and sera stored at minus 20° until use. The sera were examined using indirect immunofluorescence technique of FIFE & MUSCHEL 22 as modified by CAMARGO 7 and PETANA & WILLCOX 31. The sera were serially diluted from 1:40 to 1:320 in PBS 7.2.

The tests were carried out employing human anti gammaglobulin type IgG (Biolab), at a dilution 1:100. Formalized culture forms of T. cruzi Y strain were used as antigen. The reaction was observed through a Leitz microscope (Dialux model) with epi-illumination for immunofluorescence.

RESULTS

Of the 710 samples examined 89 (12.5%) were positive for anti-T. cruzi antibodies; 2 of these (2.2%) at a dilution of 1:320; 12 (13.4%) at 1:160; 38 (42.6%) at 1:80; and the remainder at 1:40, given a median serological dilution of 1:80. The serological positivity for Chagas' infection was similar in the population of Barcelos, Piloto and Mararã.

Of the 89 study subjects with positive serology for anti-T. cruzi antibodies, 57.5% were natives of the Barcelos district, 18% came from Santa Isabel do Rio Negro, 4.5% from São Gabriel da Cachoeira and the remainder from other localities in Amazonas or in other Brazilian states. Thus 80% of the positives came from the Rio Negro area (Barcelos, Santa Isabel and São Gabriel). However account must be taken of the significant levels of temporary inter-regional migration in the area.

Fig.1 - Location of Barcelos in the State of Amazonas.
A cross-sectional study is presently being carried out in the same area to evaluate the epidemiological association between the presence of anti-\textit{T. cruzi} antibodies in sera and the subjects’ contact with sylvatic triatomsines. We are also trying to isolate from the subjects, and so far we have succeeded in isolating one strain of \textit{T. cruzi} from one of them, an eight-year-old boy (Pi-16) with positive serology for anti-\textit{T. cruzi} antibody at a dilution of 1:320. He is able to recognize “Piçaava’s lice” and confirms that he has been bitten by this insect several times when he was living with his family at Curuduri river region.

**DISCUSSION**

The recent attention given to the likelihood of endemic Chagas’ disease in Brazilian Amazon \textsuperscript{11,12} and the results of the national serological survey for Chagas’ infection, which shows a serologic prevalence of 1.88\%, together with the presence of one acute case \textsuperscript{30} and six other serologically confirmed cases \textsuperscript{21} from Barcelos, constitute ample justification for the present study and for the research project that we are developing in the locality.

The high level of positive serology for anti-\textit{T. cruzi} antibodies found in this study does not necessarily signify that all the cases with positive serology have been infected with \textit{T. cruzi}. None the less, the study shows strong epidemiological and serological correlations, such as previous contact of the positive cases with wild bugs (known locally as “Piçaava lice”) and the isolation of one \textit{T. cruzi} strain from a patient with positive serology. These data strongly suggest that significant proportion of the serologically positive cases will be confirmed to be infected with \textit{T. cruzi} by other tests (to be carried out in near future), using for example recombinant antigens \textsuperscript{1}, lytic antibodies \textsuperscript{21}, PCR amplification of minicircle kDNA \textsuperscript{5} and isolation of \textit{T. cruzi} by xenodiagnosis or hemoculture.

Cross-serological reactions with antibodies for cutaneous leishmaniasis, leprosy, tuberculosis and even \textit{Trypanosoma rangeli} could be a possibility in some cases, but in general these diseases are not particularly common in the area. On the contrary, the area has the lowest prevalence for cutaneous leishmaniasis and leprosy in the State of Amazonas. The prevalence of tuberculosis is similar to that found in other parts of Amazon. On a recent on-going study we have not found

**TABLE**

<table>
<thead>
<tr>
<th>Age Groups (Years)</th>
<th>Samples Examined (IFT)</th>
<th>Samples Positives N\textsuperscript{o}</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 4</td>
<td>66</td>
<td>4</td>
<td>6.1</td>
</tr>
<tr>
<td>5 - 9</td>
<td>131</td>
<td>12</td>
<td>9.2</td>
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<tr>
<td>10 - 14</td>
<td>136</td>
<td>13</td>
<td>9.5</td>
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<tr>
<td>15 - 19</td>
<td>72</td>
<td>10</td>
<td>13.9</td>
</tr>
<tr>
<td>20 - 29</td>
<td>91</td>
<td>13</td>
<td>14.3</td>
</tr>
<tr>
<td>30 - 39</td>
<td>76</td>
<td>15</td>
<td>19.7</td>
</tr>
<tr>
<td>40 - 49</td>
<td>58</td>
<td>7</td>
<td>12.1</td>
</tr>
<tr>
<td>50 - 59</td>
<td>34</td>
<td>3</td>
<td>8.8</td>
</tr>
<tr>
<td>60 - 69</td>
<td>30</td>
<td>7</td>
<td>23.3</td>
</tr>
<tr>
<td>70 - over</td>
<td>16</td>
<td>5</td>
<td>31.2</td>
</tr>
<tr>
<td>TOTAL</td>
<td>710</td>
<td>89</td>
<td>12.5</td>
</tr>
</tbody>
</table>

IFT = Immunofluorescence test
by xenodiagnosis any case of *Tran
geli* infection in
patients from Barcelos with positive serology for *T.
cruzi*.

Finally a cross-sectional study is now being car-
ried out in the area to investigate the epidemiological,
clinical, serological and parasitological correlations
between human contact with sylvatic triatomines, posi-
tive serology for *T. cruzi* antibodies, isolation and com-
parison of *T. cruzi* strains from men and bugs, and clinical
repercussion of human infection.

**RESUMO**

**Doença de Chagas na Amazônia brasileira. II.**

**Inquérito sorológico**

Foi realizado um inquérito sorológico através da rea-
ção de imunofluorescência indireta em uma amostra
de soro sanguíneo da população residente em uma em
cada cinco casas habitadas na sede do município de
Barcelos, localizado no norte do Estado do Amazonas,
na margem direita do Rio Negro, a 490 km de Manaus
por via fluvial e na população das localidades rurais de
Piloto e Marará, localizadas também na margem direita
do Rio Negro, a 30 minutos por via fluvial da sede do
município de Barcelos.

No total foram utilizadas no inquérito 710 amo-
stras de soro, 628 de pessoas residentes na sede do munici-
pio, 35 residentes em Piloto e 47 em Marará. Foi
utilizada anti-gamaglobulina humana do tipo IgG (Bio-
lab) e como antígeno cultura formolizada da cepa Y do
*T. cruzi*. Os soros foram diluídos em PBS pH 7.2 a partir
de 1:40 até 1:320.

Das 710 amostras de soro examinadas 89(12,5%)
foram positivas para anticorpos anti-*T. cruzi*; 2(2,2%)
com títulos de 1:320, 12(13,4%) com títulos de 1:60,
38(42,6%) com títulos de 1:80 e os demais com títulos
de 1:40, portanto com a mediana dos títulos sorológicos
de 1:80.

São discutidos a alta prevalência sorológica para
infeção chagásica obtida no inquérito, as possibili-
tades de reações cruzadas e de testes confirmatórios
das reações positivas e os dados epidemiológicos preli-
ninares que indicam uma forte associação entre a
infeção chagásica e o contacto da população com tria-
tomíneos, conhecidos na área como "piolho de piaça-
va". Salienta-se o isolamento por xenodiagnóstico de
uma cepa de *T. cruzi* de um dos pacientes do inquérito,
com sorologia positiva, com título de 1:320 para
infeção chagásica.

**ACKNOWLEDGMENTS**

The authors are grateful to the Instituto de Me-
dicina Tropical de Manaus and to the Universidade do
Amazonas for the use of their facilities.

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Received for publication on 27/06/1994. Accepted for publication on 13/10/1994.