

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 (Biolab) 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 positive reactions; and the strong correlation between our results and preliminary epidemiological data (such as the level of human contact with wild triatominae, known 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 CHAGAS¹¹ confirmed as *cruzi* trypanosomes found by Aben-Athar in *Saimiri sciurus* 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}.

in the region, nine of them infected with *T.cruzi* or "cruzi-like" trypanosomoses^{2, 3, 6, 8, 21, 26, 27, 28, 29}. 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.

At least 18 species of triatomine have been found

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 SUCAM (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 ²³ 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 Maraã, 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, 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 Amazon (Fig. 1).

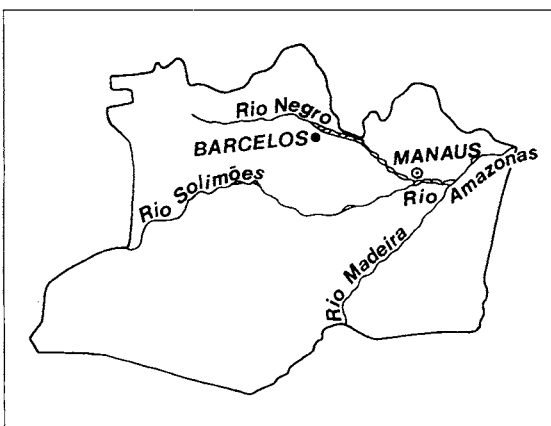


Fig.1 - Location of Barcelos in the State of Amazonas.

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 Marará (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 Marará, 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 ⁹ 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. Formolized 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 Marará.

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 Izabel and São Gabriel). However account must be taken of the significant levels of temporary inter-regional migration in the area.

TABLE
Serology for anti-*T.cruzi* antibodies in human population of Barcelos, Amazonas

Age Groups (Years)	Samples Examined (I F T)	Samples Positives N ^o	%
0 - 4	66	4	6.1
5 - 9	131	12	9.2
10 - 14	136	13	9.5
15 - 19	72	10	13.9
20 - 29	91	13	14.3
30 - 39	76	15	19.7
40 - 49	58	7	12.1
50 - 59	34	3	8.8
60 - 69	30	7	23.3
70 - over	16	5	31.2
TOTAL	710	89	12.5

I F T = Immunofluorescence test

The table shows a steady increase in positive serology for anti-*T.cruzi* antibodies in successive age-groups up to 39 years, followed by a drop between 40 and 59 and a resurgence after 60. However, considering the relatively small number of cases studied and of positives over 40, the variation may be an experimental artefact. If we re-arrange the age groups in three larger sets - 0-19, 20-49 and 50 and over, as in the graph (Fig.2) - we can see that the anti-*T.cruzi* antibody reaction is cumulative with age, possibly due to a longer exposure to the infective agent during the course of a life-time.

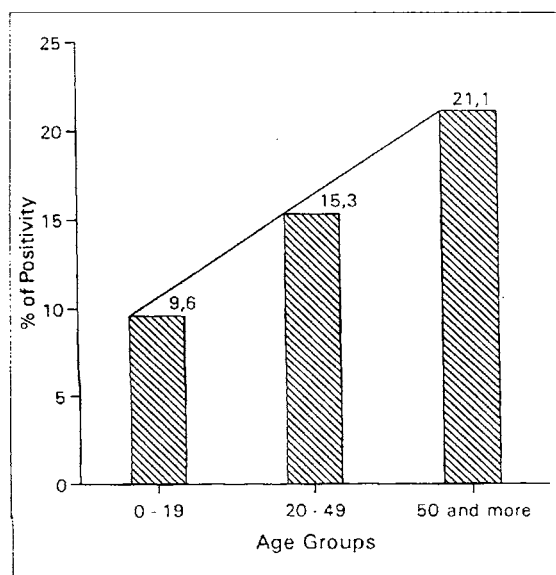


Fig.2 - Serological positivity for anti-*T.cruzi* antibodies by age groups

A cross-sectional study is presently being carried out in the same area to evaluate the epidemiological association between the presence of anti-*T.cruzi* antibodies in sera and the subjects' contact with sylvatic triatomines. We are also trying to isolate from the subjects, and so far we have succeeded in isolating one strain of *T.cruzi* from one of them, an eight-year-old boy (Pi-16) with positive serology for anti-*T.cruzi* antibody at a dilution of 1:320. He is able to recognize "Piaçava'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 ^{12, 13} 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 ³⁰ and six other serologically confirmed cases ²³ 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-*T.cruzi* antibodies found in this study does not necessarily signify that all the cases with positive serology have been 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 bugs (known locally as "Piaçava lice") and the isolation of one *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 *T.cruzi* by other tests (to be carried out in near future), using for example recombinant antigens ¹, lytic antibodies ²⁵, PCR amplification of minicircle kDNA ⁵ and isolation of *T.cruzi* by xenodiagnosis or hemoculture.

Cross-serological reactions with antibodies for cutaneous leishmaniasis, leprosy, tuberculosis and even *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

by xenodiagnosis any case of *T.rangeli* infection in patients from Barcelos with positive serology for *T.cruzi*.

Finally a cross-sectional study is now being carried out in the area to investigate the epidemiological, clinical, serological and parasitological correlations between human contact with sylvatic triatomines, positive serology for *T.cruzi* antibodies, isolation and comparison 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 reação 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 amostras de soro, 628 de pessoas residentes na sede do município, 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 infecção chagásica obtida no inquérito, as possibilidades de reações cruzadas e de testes confirmatórios das reações positivas e os dados epidemiológicos preliminares que indicam uma forte associação entre a infecção chagásica e o contacto da população com triatomíneos, conhecidos na área como "piolho de piaçava". 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 infecção chagástica.

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REFERENCES

1. ALMEIDA, E.; KRIGER, M.A.; CARVALHO, M.R.; OLEMANN, W. & GOLDENBERG, S. - Use of recombinant antigens for diagnosis of Chagas' disease and blood bank screening. *Mem. Inst. Oswaldo Cruz*, 85: 513-517, 1990.
2. ALMEIDA, F.B. - Triatomíneos da Amazônia. Encontro de três espécies naturalmente infectadas por trypanosoma semelhante ao cruzi, no Estado do Amazonas (Hemiptera, Reduviidae). *Acta amaz. (Manaus)*, 1: 89-93, 1971.
3. ALMEIDA, F.B. & MACHADO, P.A. - Sobre a infecção de *Panstrongylus geniculatus* pelo *Trypanosoma cruzi* em Manaus, Amazonas, Brasil. *Acta amaz. (Manaus)*, 1: 71-75, 1971.
4. ARBOLEDA, M.N. - *Aspectos epidemiológicos da infecção pelo vírus das hepatites B e Delta no município de Barcelos, Amazonas, 1991*. Rio de Janeiro, 1992. (Dissertação de Mestrado - Instituto Oswaldo Cruz)
5. AVILA, H.A.; PEREIRA, J.B.; THIEMANN, O. et al. - Detection of *Trypanosoma cruzi* in blood specimens of chronic chagasic patients by polymerase chain reaction amplification of kinetoplast minicircle DNA: comparison with serology and xenodiagnosis. *J. clin. Microbiol.*, 31: 2421-2426, 1993.
6. BARRETT, T.V. & GUERREIRO, J.C.H. - Os triatomíneos (Hemiptera, Reduviidae) em relação à doença de Chagas na Amazônia. In: VAL, A.L.; FIGLIUOLO, R. & FELDBERG, R. *Bases científicas para estratégia de preservação e desenvolvimento da Amazônia: fatos e perspectivas*. Manaus, Instituto Nacional de Pesquisas da Amazônia, 1991. p. 119-130.
7. BARRETT, M.P. - Reservatórios do *Trypanosoma cruzi* nas Américas. *Rev. bras. Malar.*, 16: 527-552, 1964.
8. BRAZIL, R.P.; SILVA, A.F.; ALBARELLI, A. & VALLE, J.F. - Distribuição e infecção de triatomíneos por *Trypanosoma cruzi* na Ilha de São Luiz, Maranhão. *Rev. Soc. bras. Med. trop.*, 18: 257-260, 1985.
9. CAMARGO, M.E. - Fluorescent antibody test for serodiagnosis of American Trypanosomiasis. Technical modification employing preserved forms of *Trypanosoma cruzi* in a slide test. *Rev. Inst. Med. trop. S. Paulo*, 8: 227-234, 1966.
10. CAMARGO, M.E.; SILVA, G.R.; CASTILHO, E.A. & SILVEIRA, A.C. - Inquérito sorológico da prevalência da infecção chagástica no Brasil, 1975-1980. *Rev. Inst. Med. trop. S. Paulo*, 26: 192-204, 1984.

11. CHAGAS, C. - Infection naturelle des singes du Pará (*Crysotrix sciureus*) par *Trypanosoma cruzi*. *C. R. Soc. Biol. (Paris)*, 90: 873-876, 1924.
12. COURA, J.R. - The risk of endemic Chagas' disease in the Brazilian Amazon. *Rev. Soc. bras. Med. trop.*, 23: 67-70, 1990.
13. COURA, J.R.; JUNQUEIRA, A.C.V.; GIORDANO, C.M. & FUNATSU, I.R.K. - Chagas' disease in the Brazilian Amazon. I. A short review. *Rev. Inst. Med. trop. S. Paulo*, 36: 363-368, 1994.
14. COURA, J.R.; WILLCOX, H.P.F.; TAVARES, A.M. et al. - Aspectos epidemiológicos, sociais e sanitários em áreas do Médio Solimões. II. Estudo de dois bairros periféricos da cidade de Coari e quatro localidades do lago do Mamiá, Estado do Amazonas. *An. Acad. nac. Med.*, 153: 183-186, 1993.
15. DEANE, L.M. - Tripanosomídeos de mamíferos da região amazônica. I. Alguns flagelados encontrados no sangue de mamíferos silvestres do Estado do Pará. *Rev. Inst. Med. trop. S. Paulo*, 3: 15-28, 1961.
16. DEANE, L.M. - Animal reservoirs of *Trypanosoma cruzi* in Brasil. *Rev. bras. Malar.*, 16: 27-48, 1964a.
17. DEANE, L.M. - Tripanosomídeos de mamíferos da região amazônica. III. Hemoscopia e xenodiagnóstico de animais silvestres nos arredores de Belém, Pará. *Rev. Inst. Med. trop. S. Paulo*, 6: 225-232, 1964b.
18. DEANE, L.M. - Tripanosomídeos de mamíferos da região amazônica. IV. Hemoscopia e xenodiagnóstico de animais silvestres da estrada Belém-Brasília. *Rev. Inst. Med. trop. S. Paulo*, 9: 143-148, 1967.
19. DEANE, L.M. & DAMASCENO, R. - Tripanosomídeos de mamíferos da região amazônica. II. Tripanosomas de macacos da zona de Salgado, Estado do Pará. *Rev. Inst. Med. trop. S. Paulo*, 3: 61-70, 1961.
20. DEANE, L.M. & JANSEN, G. - Encontro do *Schizotrypanum cruzi* (Chagas, 1909) em marsupiais da espécie *Marmosa cinerea Desmarest*. *Brasil-méd.*, 53: 265-266, 1939.
21. DEANE, M.P. & DAMASCENO, R. - Encontro de *Panstrongylus lignarius* naturalmente infectado por tripanosoma do tipo cruzi e algumas notas sobre a sua biologia. *Rev. Serv. Saúde públ. Rio de J.*, 2: 809-814, 1949.
22. FIFE Jr., E.H. & MUSCHEL, L.H. - Fluorescent antibody technic for serodiagnosis of *Trypanosoma cruzi* infection. *Proc. Soc. exp. Biol. (N.Y.)*, 101: 540-543, 1959.
23. FERRARONI, J.J.; MELO, J.A.N. & CAMARGO, M.E. - Moléstia de Chagas na Amazônia. Ocorrência de seis casos suspeitos, autóctones, sorologicamente positivos. *Acta amaz. (Manaus)*, 7: 438-440, 1977.
24. FERREIRA, L.C. & DEANE, L.M. - Novo depositário silvestre do *Schizotrypanum cruzi* (Chagas, 1909): a Irará, *Toyra barbara*. *Brasil-méd.*, 52: 1159-1161, 1938.
25. KRETTLI, A.U. & BRENER, Z. - Resistance against *Trypanosoma cruzi* associated to anti-living trypomastigote antibodies. *J. Immunol.*, 128: 2009-2012, 1982.
26. LENT, H. & WYGODZINSKY, P. - Revision of the triatominae (Hemiptera, Reduviidae) and their significance as vectors of Chagas' disease. New York, *Bull. Amer. Museum nat. History*, (N. Y.), 163 (article 3), 1979.
27. MILES, M.A.; SOUZA, A.A. & POVOA, M. - Chagas' disease in the Amazona Basin. III. Ecotopes of ten triatomine bug species (Hemiptera, Reduviidae) from the vicinity of Belém, Pará State, Brazil. *J. med. Ent.*, 18: 266-278, 1981.
28. MILES, M.A.; ARIAS, J.R. & SOUZA, A.A. - Chagas' disease in the Amazon Basin. V. Periurban palms as habitats of *Rhodnius robustus* and *Rhodnius pictipes*, triatominae vectors of Chagas' disease. *Mem. Inst. Oswaldo Cruz*, 78: 391-398, 1983.
29. RODRIGUES, B.A. & MELLO, G.B. - Contribuição ao estudo da tripanosomíase americana. *Mem. Inst. Oswaldo Cruz*, 37: 77-90, 1942.
30. SOUZA LIMA, M.Z.; MIRANDA SANTOS, I.K.F.; SOUZA, A.A.A. et al. - Caso humano de infecção mista por *Trypanosoma cruzi* e organismos tipo *Trypanosoma rangeli* procedente de Barcelos, Rio Negro, Amazonas. In: CONGRESSO DA SOCIEDADE BRASILEIRA DE MEDICINA TROPICAL, 21., São Paulo, 1985. *Resumos*. p.44
31. PETANA, W.B. & WILLCOX, H.P. - *New data on the comparative serology for Chagas' disease*. In: *New approaches in american trypanosomiasis research*. Washington, PAHO, 1975. p.292-294. (Scientific Publication nº 318)

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