

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

José Rodrigues COURA (2), Henry Percy Faraco WILLCOX (2), Margarita ARBOLEDA NARANJO (3), Octavio FERNANDES (4) & Daurita Darci de PAIVA (4)

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 "piaçavas' lice", 48 (42.8%) knew the bugs from their work places being gatherers of piaçava fibers in rural areas and 19 (16.9%) said that have been bitten by bugs in their huts. 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 Pará¹⁹ in 1969. Since then several other cases have been notified in the States of Pará^{8,9,13,17,21,24,25,26}, Amapá^{12,18}, Amazonas^{11,22,23}, Maranhão²⁰ and Acre^{1,28} 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 surveys^{2,5} and in recent reviews^{3,4,7,26,27}. We also described an "attack" of a human population (piaçavas' workers) by wild triatomines (*Rhodnius brethesi*, MATTA, 1919)¹⁵ as a possible new mechanism for the transmission of Chagas' infection in the Amazon⁶.

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' in-

1) This work was supported by CNPq (Proc. 520287/93-2)

2) Departamento de Medicina Tropical, Instituto Oswaldo Cruz (FIOCRUZ), Av. Brasil 4365, 21045-900, Rio de Janeiro, RJ, Brasil.

3) Serviço de Saúde de Antioquia, Colombia

4) Universidade Estadual do Rio de Janeiro (UERJ), RJ, Brasil

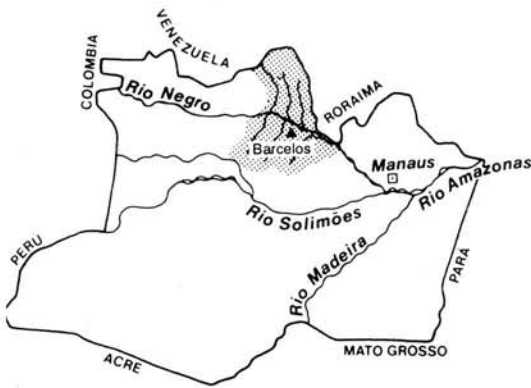


Fig. 1 - Location of Barcelos in the State of Amazonas

fection in the human population of the State of Amazonas²; at that time this result was considered to be due to a possible cross-reaction or "false-positive" phenomenon.

Considering the above-mentioned findings, the evidence of human positive serology for Chagas' infection¹⁰ and the occurrence of one acute case in a patient from Barcelos²³ (in the northern part of the State of Amazonas,

where we found 12.5% of the human population positive for anti-*T. cruzi* antibodies)⁵, 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 administrative districts of Novo Airão and Marãa, on the west by the administrative district of Santa Izabel do Rio Negro and on the north by Venezuela (with a latitude of 0°58'1" 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. 2 - Distribution of the dwellings investigated in Barcelos

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). Formolized culture forms of *T. cruzi* Y strain were used as antigen. The reactions were observed through a Leitz microscope (Dialux 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 V₁ to V₆). During

the interviews we showed a collection of *Triatoma*, *Panstrongylus* and *Rhodnius* to see if the patients could recognized 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

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

AGE GROUPS (years)	S E R O L O G Y		ECG AND CLINIC EXAM
	Performed	Positives(%)	Examined (%)
0-10	358	31(8.6)	24(77.4)
11-20	379	42(11.0)	32(76.2)
21-30	166	24(14.4)	14(58.3)
31-40	139	27(19.4)	14(51.8)
41-50	107	13(12.1)	11(84.6)
51-60	55	12(21.8)	05(41.6)
+ 60	82	21(25.6)	12(57.1)
TOTAL	1286	170(13.2%)	112(66%)

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 80 (12.7%) were positive, while in the second survey (1993) of 658 samples examined by the same technique 90 (13.7%) 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±20 years; 49.1% 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 *Rhodnius* during the interview 52 (46.4%) of the patients recognized the triatomines which they call "piaçavas' lice"; 48 (42.8%) knew the bugs from their work places being gatherers of piaçava fibers in rural areas and 19 (16.9%) 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 myocardium 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 suggestives 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 658 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 "piaçavas' 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 proportion of the people with positive serology for anti-*T. cruzi* antibodies recognized the triatomines shown as "piaçavas' lice", 42.8% knew the bugs from their work places, being gatherers of piaçava 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 piaçava fiber (*Leopoldinia piaçava*) is collected. All the piaçavas gatherers and their families with positive serology for anti-*T. cruzi* antibodies recog-

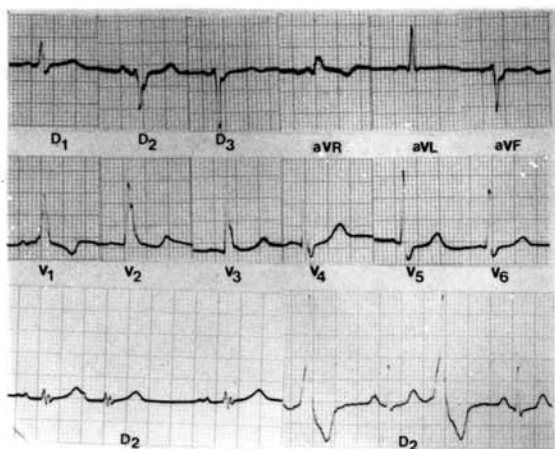


Fig. 3 - ECG alterations suggestive of Chagas' disease

nized the triatomines and a great deal of them mentioned that they have been bitten by these bugs, which show a link between *T. cruzi* infection and one's profession.

Studies done by MATTA¹⁶ and more recently by MASCARENHAS¹⁴ and by our group^{4,5} accompanied by BARRETT of the National Research Institute of Amazonia (INPA), have showed the contact the piaçava gatherers had with *Rhodnius brethesi* in the region of Rio Negro, State of Amazonas, where we recently described an attack on the human populations by this bug⁶.

Cross-serological reaction with antibodies from cutaneous, leishmaniasis, leprosy, tuberculosis and *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 of cutaneous leishmaniasis and leprosy in the state of Amazonas. In the present study we found only 4 cases of cutaneous leishmaniasis, 2 cases of leprosy and 2 of tuberculosis and we have not found by xenodiagnosis any case of *T. rangeli* infection. However the contact of the population with triatomines of the genus *Rhodnius* gives rise to great risk of transmission of that parasite.

Electrocardiograms of 112 patients with positive serology for anti-*T. cruzi* antibodies showed 9 (8%) alterations, 4 of them suggestive of chagasic miocardiopathy: 2 with right bundle branch block, one of these of the 3rd degree associated to left anterior hemiblock, one with A-V block of the first degree plus supraventricular extrasystoles and a disturbance of intraventricular stimulus conduction and one with the bigeminal extrasystoles (Fig.3). However, all the patients with electrocardio-

graphic alterations were over 60 years of age which makes it difficult to exclude an association with atherosclerosis. 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 atherosclerosis.

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 piaçava 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 "piolho da piaçava", 48 (42,8%) disseram existir em seus locais de trabalho, geralmente em piaçavais 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 compatíveis 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 patogenicidade, possivelmente por ser silvestre e ainda não adaptada ao homem.

ACKNOWLEDGMENTS

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

REFERENCES

1. BARATA, J.M.S.; ROCHA, R.M.; RODRIGUES, V.L.C.C. & FERRAZ Fº, A.N. - Primeiro caso autóctone de tripanosomíase americana do Estado do Acre, Brasil, e sua correlação com as cepas isoladas do caso humano e de triatomíneos silvestres da área. *Rev. Saúde públ. (S. Paulo)*, 22: 401-410, 1988.
2. CAMARGO, M.E.; SILVA, G.R.; CASTILHO, E.A. & SILVEIRA, A.C. - Inquérito sorológico de prevalência da infecção chagásica no Brasil, 1975-1980. *Rev. Inst. Med. trop. S. Paulo*, 26: 192-204, 1984.
3. COURA, J.R. - The risk of edemic Chagas' disease in the Brazilian Amazon. *Rev. Soc. bras. Med. trop.*, 23: 67-70, 1990.
4. COURA, J.R.; ARBOLEDA NARANJO, M. & WILLCOX, H.P.F. - Doença de Chagas na Amazônia brasileira. *Rev. Soc. bras. Med. trop.*, 26(suppl. 2): 15-17, 1993.
5. COURA, J.R.; ARBOLEDA NARANJO, M. & WILLCOX, H.P.F. - Chagas' disease in the Brazilian Amazon. II-A serological survey. *Rev. Inst. Med. trop. S. Paulo*, 37: 103-107, 1995.
6. COURA, J.R.; BARRETT, T.V. & ARBOLEDA NARANJO, M. - Ataque de populações humanas por triatomíneos silvestres no Amazonas: uma nova forma de transmissão da infecção chagásica? *Rev. Soc. bras. Med. trop.*, 27: 251-253, 1994.
7. 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.
8. CRESCENTE, J.A.; VALENTE, S.A.; VALENTE, V.C. & ARAUJO, J.A. - Ocorrência de 4 casos agudos de doença de Chagas na Vila Icoaraci-PA. *Rev. Soc. bras. Med. trop.*, 25(suppl.): 29, 1992.
9. DOREA, R.C. - Doença de Chagas na Amazônia: aspectos epidemiológicos regionais e considerações a propósito de um caso pediátrico. *Hiléia méd. (Belém)*, 3: 81-109, 1981.
10. 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.
11. FRANÇA, M.S.; FRADE, J.M.; KONAZUGAWA, K. & ALMEIDA, F.B. - Doença de Chagas: primeiro caso autóctone na Amazônia Ocidental, Amazonas, Brasil. *Acta amaz. (Manaus)*, 10: 759-762, 1980.
12. LACERDA JR., K.G.; FARIA, J.I.M.; MATTE, J.R. et al. - Doença de Chagas. Registro do primeiro diagnóstico feito no homem no Território Federal do Amapá. In: CONGRESSO DA SOCIEDADE BRASILEIRA DE MEDICINA TROPICAL, 10., Curitiba, 1974. **Programa e sumários.** (res. 32).
13. LAINSON, R.; SHAW, J.J.; FRAHIA, H.; MILES, M.A. & DRAPER, C.C. - Chagas' disease in the Amazon Basin. I-*Trypanosoma cruzi* in silvatic animals, triatomine bugs and man in the State of Pará, north Brazil. *Trans. roy Soc. trop. Med. Hyg.*, 73: 193-204, 1979.
14. MASCARENHAS, B.M. - Triatomíneos da Amazônia: sobre o habitat e algumas considerações comportamentais de *Rhodnius brethesi* Matta, 1919 (*Hemiptera Reduviidae*: Triatominae) na Região do Médio Rio Negro, Amazonas. *Bol. Mus. para. Emilio Goeldi, Serv. Zool.*, 7: 107-116, 1991.
15. MATTA, A. - Um novo redúvdeo do Amazonas, *Rhodnius brethesi* n.sp. *Amazonas med.*, (Manaus), 2: 93-94, 1919.
16. MATTA, A. - Sobre o gênero *Rhodius* do Amazonas. *Amazonas med. (Manaus)*, 5: 161-162, 1922.
17. RODRIGUES, I.R.C.; SOUZA, A.A. & VALENTE, S.A.A. - Novo caso autóctone de doença de Chagas no Estado do Pará. In: CONGRESSO DA SOCIEDADE BRASILEIRA DE PARASITOLOGIA, 9., Fortaleza, 1985. **Resumos.** p.22
18. RODRIGUES, I.R.C.; SOUZA, A.A.; TERCEROS, R. & VALENTE, S. - Doença de Chagas na Amazônia. I- Registro de oito casos autóctones em Macapá. *Rev. Soc. bras. Med. trop.*, 21: 193-197, 1988.
19. SHAW, J.; LAINSON, R. & FRAHIA, H. - Considerações sobre a epidemiologia dos primeiros casos autóctones de doença de Chagas registrados em Belém, Pará, Brasil. *Rev. Saúde. públ. (S. Paulo)*, 3: 153-157, 1969.
20. SILVA, A.R.; MENDES, J.R.B.; MENDONÇA, M.L.; CUTRIM, R.N. & BRASIL, R.P. - Primeiros casos agudos autóctones da doença de Chagas no Maranhão e inquérito soroprevalência da população. *Rev. Soc. bras. Med. trop.*, 18: 269-270, 1985.
21. SILVEIRA, P.T.; DIAS, M.G.V.; PARDAL, P.P.; LOBÃO, A.O. & MELO, G.B. - Novo caso autóctone de doença de Chagas registrado no Estado do Pará, Brasil. *Hiléia méd. (Belém)*, 1: 61-62, 1979.
22. SOUZA LIMA, M.Z.M.; ARAUJO FILHO, N.A.; ALECRIM, M.G.C. et al. - Forma aguda da doença de Chagas no Amazonas (caso autóctone). In: CONGRESSO DA SOCIEDADE BRASILEIRA DE MEDICINA TROPICAL, 18., Ribeirão Preto, 1982. **Resumos.** p.A-3.
23. SOUZA LIMA, M.Z.M.; 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.
24. SOUZA, A.A.A.; PARDAL, P.P.O.; VALENTE, S.A.A. et al. - Doença de Chagas no Estado do Pará: aspectos clínicos e laboratoriais de 4 novos casos autóctones. In: CONGRESSO BRASILEIRO DE PARASITOLOGIA, 11., 1989. **Anais.** p.10.
25. VALENTE, S.A.; SOUZA, A.A.A.; MELO, P.R.R. et al. - Considerações epidemiológicas sobre um novo caso agudo de doença de Chagas observado no Estado do Pará. In: CONGRESSO DA SOCIEDADE BRASILEIRA DE MEDICINA TROPICAL, 25., Florianópolis, 1989. **Resumos.** p.171 (res.149).
26. VALENTE, S.; SOUZA, A. & VALENTE, V. - Doença de Chagas na Amazônia: situação atual e perspectivas. *Rev. Soc. bras. Med. trop.*, 25 (suppl.): 29, 1992. (res.063).
27. VALENTE, S.A.S. & VALENTE, V.C. - Situação atual da doença de Chagas na Amazônia. *Rev. Soc. bras. Med. trop.*, 26 (suppl.29): 68-70, 1993.
28. VALENTE, S.A.S.; VALENTE, V.C.; SILVA, F.M. et al. - Registro de doença de Chagas agudo em Sena Madureira, Estado do Acre. *Rev. Soc. bras. Med. trop.*, 27 (suppl.1): 169, 1994.

Recebido para publicação em 06/03/1995

Aceito para publicação em 08/06/1995