Chagas Disease: from Bush to Huts and Houses. Is it the Case of the Brazilian Amazon?

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Two of the major problems facing the Amazon – human migration from the other areas and uncontrolled deforestation – constitute the greatest risk for the establishment of endemic Chagas disease in this part of Brazil.

At least 18 species of triatomines had been found in the Brazilian Amazon, 10 of them infected with Trypanosoma cruzi, *associated with numerous wild reservoirs. With wide-range deforestation, wild animals will perforce be driven into other areas, with tendency for triatomines to become adapted to alternative food sources in peri and intradomicilies.*

Serological surveys and cross-sectional studies for Chagas disease, carried out in rural areas of the Rio Negro, in the Brazilian Amazon, showed a high level of seropositivity for T. cruzi *antibodies. A strong correlation of seroreactivity with the contact of gatherers of piaçava fibers with wild triatomines could be evidenced.*

Key words: *Trypanosoma cruzi* - Chagas disease - seroprevalence - Brazilian Amazon

Chagas disease in the Brazilian Amazon has always been considered to be a sylvatic enzooty. Since 1924, when Carlos Chagas confirmed as *Trypanosoma cruzi* trypanosomes 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 (Barretto1964, Deane 1964).

At least 18 species of triatomine have been found in the region (Table I), 10 of them infected with *T. cruzi* or "*cruzi*-like" trypanosomes (Lent & Wigodzinsky 1979, Miles et al. 1981, 1983, Brazil et al. 1985, Barrett & Guerreiro 1991). 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 Amazon were stressed in reviews (Coura 1990, Coura et al. 1993, 1994b, Valente & Valente 1993), with 38 human cases described up to 1992. The national serological survey carried out by the Ministry of Health in Brazil from 1975 to 1980 (Camargo et al. 1984) showed a 1.88% seroprevalence 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 aforementioned findings, together with the evidence of human positive serology for Chagas infection (Ferraroni et al. 1977) and the occurrence of one acute case in a patient from the district of Barcelos (Souza-Lima et al. 1985) in the northern part of the State of Amazonas, we decided to carry out a multidisciplinary study in this area. Following our short review on the subject of Chagas disease in the Brazilian Amazon (Coura et al. 1994b), we herein present results of several studies.

MATERIALS AND METHODS

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

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TABLE I

Species of Triatominae found in the Brazilain Amazon

Belminus herreri	*Rhodnius nasatus*
Cavernicula lenti	*Rhodnius neglectus*[a]
Cavernicula pilosa	*Rhodnius paraensis*[a]
Eratyrus mucronatus[a]	*Rhodnius pictipes*[a]
Microtriatoma trinidadensis [a]	*Rhodnius prolixus*
Panstrongylus geniculatus[a]	*Rhodnius robustus*[a]
Panstrongylus lignarius[a]	*Triatoma maculata*
Panstrongylus rufotuberculatus [a]	*Triatoma rubrofasciata*
Rhodnius brethesi [a]	*Triatoma rubrovaria*

a: found infected with *Trypanosoma cruzi* or "*cruzi*-like"

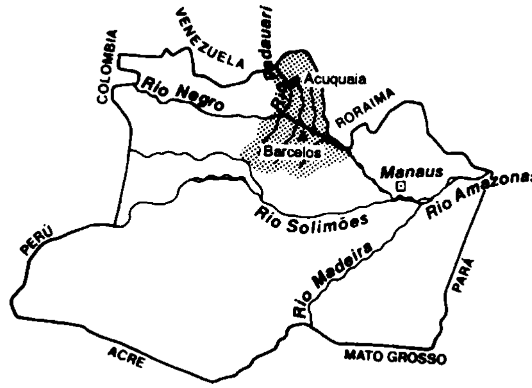

Fig. 1: location of Barcelos in the State of Amazon, Brazil.

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 up-river from Manaus, the capital of the State of Amazon (Fig. 1).

Surveys and samples - Three serological surveys for Chagas infection were carried out, respectively in 1991, 1993 and 1997 using conglomerate family samples from families residing in the town of Barcelos, employing indirect 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. In 1993 another 658 samples from the residents of 171 dwellings (one dwelling in every four) were examined. More recently, other 886 samples from 194 dwellings were analyzed (Fig. 2).

Laboratory procedures - The blood samples were centrifuged and sera stored at -20° until use. The sera were examined using indirect immunofluorescence technique of Fife and Muschel (1959) as modified by Camargo (1966) and Petana and Willcox (1975). The sera were serially diluted from 1:40 to 1:320 in PBS at pH 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 ob-

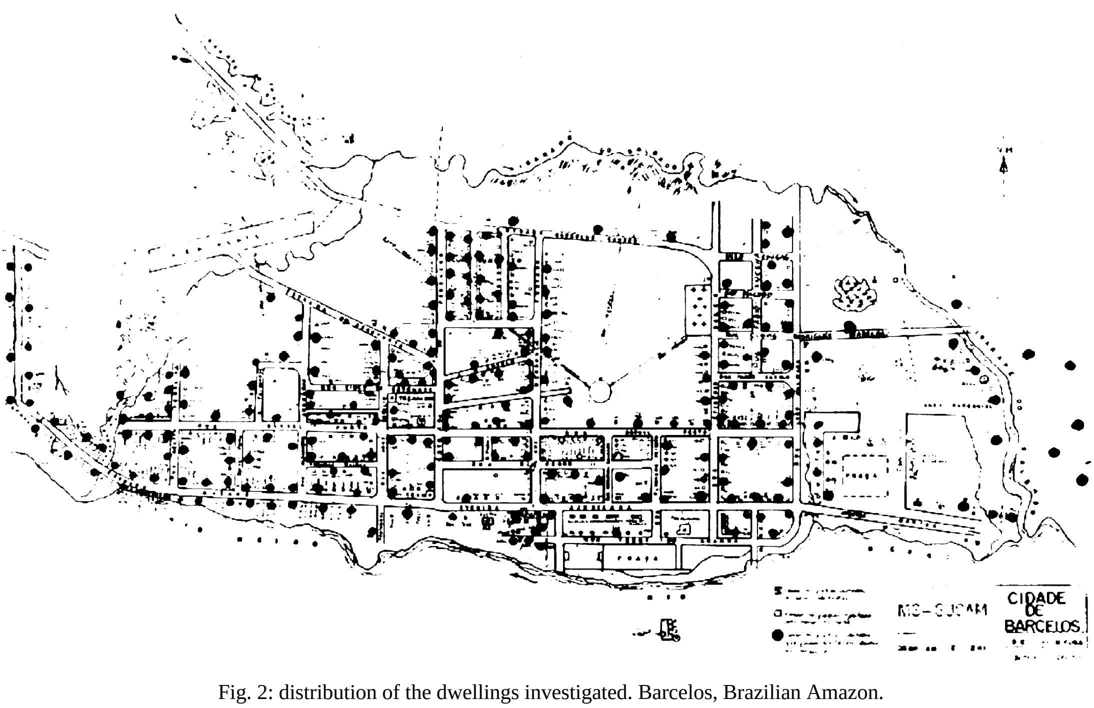

Fig. 2: distribution of the dwellings investigated. Barcelos, Brazilian Amazon.

served through a Leitz microscope (Dialux model) with epi-illumination for immunofluorescence. The positive reactions were confirmed by enzyme-linked immune absorbent assay (ELISA). In the last survey (1997), a screening test for *T. cruzi* based on agglutination reactions of coloured polymer particles (ID-PAGIA™, DIAMED), sensitized by three distinct *T. cruzi* peptides was used. The reactions were confirmed by indirect immunofluorescence, conventional and recombinant ELISA (CRA & FRA).

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 D_1, D_2 and D_3 , unipolar aVR, aVL and aVF and precordial V_1 to V_6). 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 two or three 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 - Polymerase chain reaction (PCR) amplification of the variable region of the minicircle molecule, one component of the mitochondrial genome (kDNA) of *T. cruzi* was performed as previously described in 30 patients that showed to be seropositive in the first survey (Britto et al. 1995). Molecular typing of two distinct *T. cruzi* strains isolated by xenodiagnosis was carried out by analyzing the intergenic transcribed spacer of the mini-exon gene (Fernandes et al. 1998a,b).

RESULTS

In the first serological survey (1991), of 710 sera tested by the indirect immunofluorescence for anti-*T. cruzi* antibodies, 89 (12.5%) were positive. In the second survey (1993), of 658 samples examined by the same technique 90 (13.7%) were also positive. Finaly in the third survey (1997), of 886 sera analyzed by the agglutination test, 117 (13.2%) were reactive, thus confirming the results obatined in the previous surveys. Table II 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 206 (20.7%) of the patients recognized the triatomines which they call "piaçavas' lice", 139 (67.5%) knew the bugs from their work places being gatherers of piaçava fibers in rural areas and 62 (30%) said that they have been bitten by the bugs in their huts in rural areas (the information from children younger then 10, was gathered from

TABLE II

Seroprevalence of chagasic infection in Barcelos, distributed by age in 1991, 1993 and 1997

Age (years)	Seroprevalence					
	1991		1993		1997	
	Tested N	Positive (%)	Tested N	Positive (%)	Tested N	Positive (%)
0 – 10	197	7.6	248	5.6	215	12.1
11 – 20	208	11.7	143	13.3	255	25.4
21 – 30	91	14.3	91	9.9	139	15.7
31 – 40	76	19.7	58	8.6	100	11.3
41 – 50	58	12.1	42	7.1	69	7.8
51 – 60	34	8.8	34	11.8	43	4.9
> 60	46	22.2	42	24.0	65	7.4
Total	710	12.5	658	13.7	886	13.2

their parents). Considering the group that described a previous attack of the triatomine, 25.8% were seropositive for *T. cruzi* antibodies against 11.7% of the non bitten people (p=0.03).

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.

From 156 xenodiagnosis performed on patients with seroreactive for *T. cruzi* antibodies, only 3 (1.9%) were positive for the protozoa. The exams of the hemolymph and salivary glands were negative for *T. rangeli* in all bugs used in the xenodiagnostic assays.

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

Of the three *T. cruzi* isolates obatined after the xenodiagnostic procedure, one strain was difficult to adapt in mice and was lost during cultivation. They all show a very long pre-patent period and do not kill mice; furthermore strains grow very slowly in culture media. The two remaining isolates were submitted to the mini-exon gene typing assay, which revealed that they belong to the lineage of *T. cruzi* that is usually found in the sylvatic environment. Interestingly, one isolated presented an insertion in the mini-exon gene non-transcribed spacer that is a molecular marker for zimodeme III (Miles et al. 1980, Fernandes et al. 1998b).

DISCUSSION

The high level of seropositivity 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*. Nonetheless, the study shows strong epidemiological and serological correlations, such as previous contact of the positive cases with wild triatomines, isolation of *T. cruzi* by xenodiagnosis and positivity of 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 seropositive individuals for anti-*T. cruzi* antibodies recognized the triatomines shown as "piaçavas' lice", 20.7% knew the bugs from their work places, being gatherers of piaçava fibers in rural areas and 30% of those 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 piassava)* is collected (Fig. 3). All the piaçavas gatherers and their families with positive serology for anti-*T. cruzi* antibodies recognized the triatomines and a great number 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 Matta (1919, 1922) and more recently by Mascarenhas (1991) and by our group (Coura et al. 1993, 1995), have shown the contact the piaçava gatherers had with *Rhodnius brethesi* in the region of Rio Negro, State of Amazonas, where we described an attack on the human populations by this bug (Coura et al. 1994a).

Cross-serological reaction with antibodies from cutaneous leishmaniasis, leprosy, tuberculosis and *T. rangeli* could be a possibility in some cases. In the present study we found only four cases of cutaneous leishmaniasis, two cases of leprosy and two 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, as we have shown (Coura et al. 1996), by diagnosing three human cases in 86 hemocultures in the Brazilian Amazon.

Fig. 3: piaçavas fibers collected and stored for transportation.

Electrocardiograms of 112 seropositive patients for anti-*T. cruzi* antibodies showed nine (8%) alterations, four of them suggestive of chagasic miocardiopathy. However, all the patients with electrocardiographic alterations were over 60 years of age which makes it difficult to exclude an association with atheroesclerosis.

Uncontrolled deforestation and colonization in the Amazon are currently amongst the great concerns of (thinking) citizens in our country, and give rise to great international speculation regarding the possibility of exponential increase in these activities (Fearnside 1982) . Several studies on the matter have been carried out recently and will not be discussed here due to lack of space. With wide-ranging deforestation, wild animals will perforce be driven into other areas, with a tendency for triatominae to become adapted to alternative food sources in peri and intra-domiciliary areas. Barretto (1967), in his study of *T. cruzi* reservoir hosts and vectors, gives a painstaking analysis of the consequences of modifying natural foci. Forattini (1980) and Aragão (1983) have carried out datailed studies of the origin of and preadaptation to domiciliary activity of triatomines in Brazil, corroborating with our assertion.

Finally, uncontrolled migratory movements from the south, southeast, northeast and west-central areas towards the Amazon, increased during recent years, have been responsible for maintaining and exacerbating various endemic parasitic diseases in Brazil, such as malaria. In the case of Chagas disease, although its adaptation mechanisms are slower, it poses one the most serious threats of transposition of an endemic disease into the Amazon; the effects of this will become evident during the next century unless the following precautions are taken: (a) reduction and control of deforestation, particularly on the periphery of population centres; (b) constant surveillance of domestic adaptation of wild triatominae and transposition of triatominae and domestic reservoir hosts from endemic areas to the Amazon; (c) formulation of a global policy for the settlement and colonization of the Amazon, which would simultaneously preserve the ecology of the area, while promoting its socioeconomic development.

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