Integrate Study of a Bolivian Population Infected by *Trypanosoma cruzi*, the Agent of Chagas Disease

Simone Frédérique Brenière/+, Marie France Bosseno, François Noireau*, Nina Yacsik, Pascale Liegeard***, Christine Aznar****, Mireille Hontebeyrie*****

Institut de Recherche pour le Développement (IRD), UR 08 Pathogénie des Trypanosomatidae, 911 Av. Agropolis, BP 5045, 34032 Montpellier Cedex 1, France *Laboratório Nacional e Internacional de Referência em Taxonomia de Triatomíneos, Departamento de Entomologia, Instituto Oswaldo Cruz-Fiocruz, Rio de Janeiro, RJ, Brasil **Instituto Boliviano de Biología de Altura, La Paz, Bolivia ***Laboratoire d'Immunopathogenèse, Département d'Immunologie, Institut Pasteur, France ****UFR de Médecine, Université des Antilles et de la Guadeloupe, Cayenne, Guyane Française

A cross section of a human population (501 individuals) selected at random, and living in a Bolivian community, highly endemic for Chagas disease, was investigated combining together clinical, parasitological and molecular approaches. Conventional serology and polymerase chain reaction (PCR) indicated an active transmission of the infection, a high seroprevalence (43.3%) ranging from around 12% in < 5 years to 94.7% in > 45 years, and a high sensitivity (83.8%) and specificity of PCR. Abnormal ECG tracing was predominant in chagasic patients and was already present among individuals younger than 13 years. SAPA (shed acute phase antigen) recombinant protein and the synthetic peptide R-13 were used as antigens in ELISA tests. The reactivity of SAPA was strongly associated to Trypanosoma cruzi *infection and independent of the age of the patients but was not suitable neither for universal serodiagnosis nor for discrimination of specific phases of Chagas infection. Anti-R-13 response was observed in 27.5% only in chagasic patients. Moreover, anti-R13 reactivity was associated with early infection and not to cardiac pathology. This result questioned previous studies, which considered the anti-R-13 response as a marker of chronic Chagas heart disease. The major clonets 20 and 39 (belonging to* Trypanosoma cruzi *I and* T. cruzi *II respectively) which circulate in equal proportions in vectors of the studied area, were identified in patients' blood by PCR. Clonet 39 was selected over clonet 20 in the circulation whatever the age of the patient. The only factor related to strain detected in patients' blood, was the anti-R-13 reactivity: 37% of the patients infected by clonet 39 (94 cases) had anti-R13 antibodies contrasting with only 6% of the patients without clonet 39 (16 cases).*

Key words: Chagas disease - recombinant proteins - polymerase chain reaction - clones - Bolivia

Chagas disease is an endemic infection affecting many Latin American countries. Bolivia is considered one of the most endemic countries. The endemic area covers 80% of the country and, in 1985 more than one million people were infected (WHO 1991). The vectorial transmission of *Trypanosoma cruzi*, the agent of the disease, is mainly assured by *Triatoma infestans* which presents a *T. cruzi* infection rate of around 30% (WHO 1991). The vector control program is not yet expanded over all the country and, due to the large chagasic population, congenital transmission and transmission by blood transfusion are very abundant too (Azogue et al. 1985, Azogue & Darras 1995). High seroprevalences reported in blood donors indicate the magnitude of the human infection even in the towns outside the endemic regions (Carrasco et al. 1990, Landivar et al. 1992). The rural population has been considered as the main population at risk of infection, however, it was recently shown that vectorial transmission is occurring in suburbs of the main cities too (Revollo et al. 1997, Albarracin-Veizaga et al. 1999). Moreover, cardiac morbidity as well as digestive alterations remain important (Weinke et al. 1988, Brenière et al. 1989, Pless et al. 1992).

Recent developments in molecular biology applied to Chagas disease proposed new tools for a better knowledge of the natural human infection. PCR is proposed for sensitive detection of parasites in human blood (Moser et al. 1989, Avila et al. 1993, Wincker et al. 1997, Russomando et al. 1998). Several recombinant proteins allow specific and sensitive detection of antibodies directed against *T. cruzi*. Recombinant proteins have been proposed as stage or pathological specific markers while others should be relevant for cure criteria (Affranchino et al. 1989, Levin et al. 1989, 1991, Mesri et al. 1990, Aznar et al. 1995, Gomes 1997, Guevara et al. 1997).

Genetic variability of *T. cruzi* is now well documented. This parasite is composed of natural clones which present high genetic differences and recent studies demonstrate the existence of two principal lineage; regarding the nomenclature, and in agreement between scientists, two groups are now named, *T. cruzi* I and *T. cruzi* II (Tibayrenc 1995, Luquetti et al. 1999). In Bolivia, two major distinct monophyletic groups of clones have been identified in the domestic cycle and, they belong to *T. cruzi* I and *T. cruzi* II respectively (Brenière et al. 1998). These groups named clonet 20 and 39 can be detected directly in blood

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+Corresponding author. Fax: +33-4-6741.6330. E-mail: breniere@mpl.ird.fr
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by PCR and identified by hybridization with specific kDNA probes (Brenière et al. 1998).

We report here a transversal study of a cross section of an entire human population living in a highly endemic area in Bolivia (Cochabamba Department), selected at random, in order to determine the magnitude of the human infection combining together clinical, parasitological and molecular approaches including the determination of the *T. cruzi* clones. Each parameter was analyzed according to age and sex, and their relationships studied. The usefulness of each molecular tool was also examined and discussed.

MATERIALS AND METHODS

Studied area and patients - The 501 individuals examined were children, teenagers and adults all free of treatment and living in the village of Mizque (altitude 1,970 m) located in a highly endemic region for Chagas disease in Bolivia (Cochabamba Department). *T. infestans* was the only triatomine species collected in 100% of the dwellings and its *T. cruzi* infection rate was around 60%. In this area, insect control program had been implemented after the study. The community had 3,850 inhabitants. Blood samples were collected during 1994 and 1995 and the age of the patients ranged from 1 to 83 years old. Children and teenagers were mainly from elementary school and college and a written consent was obtained from parents. The adults were volunteers, born and residents of Mizque. The study included 281 children from whom serological and parasitological results were previously reported (Wincker et al. 1997).

Interpretation of the ECG tracings - The electrocardiographic test (ECG) was applied among 232 individuals selected at random from the 501 studied including 123 chagasic patients. ECGs were recorded in 12 standard derivations using a portable Hewlett Packard electrocardiograph. Two physician doctors coded each tracing (without serological information of the cases) according to the classification previously proposed and discrepancies were resolved by mutual agreement (Maguire et al. 1982). The ECG's showing atrioventricular block (AVB), incomplete and complete right bundle branch block (IRBBB and CRBBB), left anterior hemiblock (LAH), multifocal ventricular extrasystole (MVE), or junctional rhythm (JR) were considered abnormal. The following abnormalities were considered as borderline: atrial extrasystole, junctional extrasystole, unifocal and non repetitive ventricular extrasystole, and R waves in left precordial leads without pattern of ventricular strain in adults >35 years old. The other tracings were considered as normal ECG.

IgG antibodies anti-T. cruzi - Specific anti-*T. cruzi* IgG's were assessed in three assays except for five cases where only two tests were available**.** Immunofluorescence (IF) was performed at IBBA (Instituto Boliviano de Biología de Altura). Two ELISA assays were performed in two laboratories: IBBA (ELISA-IBBA) and Institut Pasteur Paris (ELISA-IP). The methodologies and the cut values of the three tests were previously described (Wincker et al. 1997, Aznar et al. 1997). ELISA values were expressed as ratio (optical density value of each sample/optical density value of cut value) for statistical analysis. The serological diagnosis of Chagas infection was based on the positivity or negativity of at least two out of three tests.

Antibodies against SAPA recombinant protein - Anti-SAPA antibodies were investigated among the total population (except 23 missing cases) using the fusion glutathion S-transferase/shed acute phase antigen (GST/SAPA) previously purified from *T. cruzi* (Ibañez et al. 1987, Smith & Johnson 1988, Affranchino et al. 1989, Brenière et al. 1997). The current study included 266 sera from children previously tested and 212 additional ones from older patients (Brenière et al. 1997). Briefly, IgG antibodies were detected by ELISA, and SAPA optical density of each sample, was determined by the difference of values obtained by GST-SAPA-ELISA and GST-ELISA as control. The cut-off value for anti-SAPA was 0.38 (Brenière et al. 1997).

Antibodies against R-13 peptide - Sera from 469 individuals studied, selected at random, were tested twice for IgG anti-R-13 antibodies by ELISA (Aznar et al. 1995). In each test the samples were assayed in duplicate. The antigen was the synthetic peptide corresponding to the C-terminal sequence (EEEDDDMGFGLFD) of *T. cruzi* ribosomal P-protein (Aznar et al. 1995). Cut-off values were calculated, in each protocol, as the mean optical density values obtained from 60 control sera + 2 SD. Control sera were represented by 40 *T. cruzi* negative Bolivian sera and 20 sera from Caucasian individuals from a French blood bank.

PCR identification of clonet 20 and 39 - The recognition of clonet 20 and 39 was done by hybridization of the PCR products by clonet specific kDNA probes named 20 and 39 as previously described (Brenière et al. 1998). PCR was applied on 372 blood samples including 209 chagasic patients. Part of the sample was tested previously (Brenière et al. 1998).

Statistical tests - Statistics were computed by the Statix package. Prevalence rates of Chagas infection were calculated in different age classes and the correlation between prevalence and middle class age determined by the Pearson correlation test. χ^2 test was used to compare distributions between several categories and χ^2 Yates correction was applied when at least one of the expected values was < 5. The non parametric test of Kruskal-Wallis was applied to compare average values obtained for two categories.

RESULTS

Chagas infection - Two hundred and seventeen individuals out of 501 (43.3%) were positive in at least two of the three serological tests detecting total specific parasite IgG antibodies and were considered as chagasic patients. The accordance of the three tests was 94.7%. Three young children with negative serology were also classified as chagasic: two with evidence of *T. cruzi* parasitaemia by buffy coat and 1 with positive PCR (Wincker et al. 1997). One adult patient with negative serology was also PCR positive. Finally, a total of 221 patients were classified as chagasic in this study (44.1%). Chagas infection rates increased according to the age of patients from 11.8% to 94.7% (Table I, correlation coefficient = 0.93, $p = 10^{-4}$) and were not significantly different for male and female (40% and 48.6%, respectively; $\chi^2 = 3.29$, df = 1, p = 0.07).

TABLE I

Age-specific prevalence rates of Chagas infection

Age	Females				Males				Females and males			
(years)	N	Ch+	Ch-	% Ch+	N	Ch+	Ch-	% Ch+	N	Ch+	Ch-	% Ch+
1-5	17	3	14	17.6%	17	1	16	5.9%	34	4	30	11.8%
6-10	70	34	36	48.6%	150	63	87	42%	220	97	123	44.1%
11-15	62	27	35	43.5%	66	18	48	27.3%	128	45	83	35.2%
16-20	21	7	14	33.3%	17	10	7	58.8%	38	17	21	44.7%
21-25	4	1	3	25%	4	2	2	50%	8	3	5	37.5%
26-30	5	3	2	60%	9	6	3	66.7%	14	9	5	64.3%
31-35	9	8	1	88.9%	3	1	2	33.3%	12	9	3	75%
36-40	7	5	2	71.4%	6	3	3	50%	13	8	5	61.5%
41-45	6	5	1	83.3%	0	0	0		6	5	1	83.3%
> 45	11	10	1	90.9%	8	8	0	100%	19	18	1	94.7%
Total	212	103	109	48.6%	280	112	168	40%	492 [a]	215	277	43.7%

N: patient number; Ch+: chagasic patient; Ch-: no chagasic patient; *a*: 9 cases had missing age data

Cardiac pathology - Table II summarized the characteristics of patients presenting abnormal ECG tracing (32 cases). The other 200 individuals had ECG tracing considered as normal. Among the 232 studied individuals, abnormal ECG was statistically associated with Chagas infection ($\chi^2 = 11.8$, df = 1, $p = 0.0006$) and this association was already present among individuals younger than 13 years ($\chi^2 = 5.41$, df = 1, $p = 0.02$) (not shown). Moreover, the prevalence of cardiac electric abnormalities was not significantly different among the following age groups: 13 to 18 years old, 19 to 35 years old and >35 years old groups ($\chi^2 = 0.38$, df = 2, $p = 0.83$). Only among adult patients (> 25 years old), ECG abnormalities were more common in men than in women (odd ratio = 4.4). No differences of anti-*T. cruzi* antibodies rates were observed between patients with or without cardiac abnormalities: the Kruskal-Wallis non parametric test was applied to the ELISA-IBBA and ELISA-IP ($p = 0.55$ and $p = 0.20$ respectively) and similarly for the IF test (titers ≤ 1/64 versus titers > 1/64; $\chi^2 = 3.3$, df = 1, $p = 0.07$). The most frequent ECG alteration was LAH (61.5% of patients) and the others were present in around 23-27%. More than one alteration was observed among 42% of patients. Finally, 21% of chagasic patients presented an abnormal ECG (26 cases of 123).

Anti-SAPA IgG response - The mean ratio of optical density values were 1.8 ± 1.14 and 0.31 ± 0.35 for chagasic and non chagasic individuals respectively. Anti-SAPA antibodies were shown in 72% of chagasic patients and were significantly associated with *T. cruzi* infection ($\chi^2 = 236.4$, df = 1, $p < 10^{-4}$). Among chagasic patients, anti-SAPA response was independent of sex ($\chi^2 = 3.76$, df = 1, $p = 0.052$) and ECG tracings ($\chi^2 = 0.01$, df = 1, $p = 0.91$). Moreover, similar optical density rates were observed for chagasic patients with abnormal and normal ECG (1.84 ± 1.18 and 1.62 ± 1.06 respectively; Kruskal-Wallis test, $p = 0.37$; not shown). Table III showed that anti-SAPA response was more frequent among chagasic patients < 13 years old than among older ($\chi^2 = 7.62$, df = 1, $p = 0.005$), but remained elevated among adult patients.

TABLE II

Chagas infection and ECG tracing of patients with abnormal ECG

Code	Age	Sexe	Chagas infection	ECG tracing	Interpretation of ECG [a]
Miz184	5	F	Yes	LAH	Abnormal
Miz009	6	M	No	LAH	Abnormal
Miz099	6	M	No	LAH, IRBBB	Abnormal
Miz076	6	M	Yes	LAH, IRBBB	Abnormal
Miz042	7	M	No	LAH	Abnormal
Miz015	7	M	Yes	LAH	Abnormal
Miz020	7	M	No	LAH	Abnormal
Miz041	7	F	Yes	LAH	Abnormal
Miz044	7	M	Yes	LAH	Abnormal
Miz103	7	M	Yes	LAH, IRBBB	Abnormal
Miz104	8	M	Yes	IRBBB	Abnormal
Miz018	9	M	Yes	LAH	Abnormal
Miz021	< 10	M	Yes	LAH	Abnormal
Miz454	12	M	Yes	AVB	Abnormal
Miz455	15	M	Yes	AVB	Abnormal
Miz413	15	F	Yes	LAH, SB	Abnormal
Miz406	16	F	Yes	LAH, CRBBB, SB	Abnormal
Miz426	>18	F	Yes	CRBBB	Abnormal
Miz458	24	F	Yes	AVB	Abnormal
Miz429	24	M	Yes	LAH	Abnormal
Miz467	29	M	Yes	AVB	Abnormal
Miz468	29	M	Yes	AVB, SB	Abnormal
Miz066	30	F	No	IRBBB, AVB	Abnormal
Miz486	30	M	Yes	LAH, SB	Abnormal
Miz433	36	M	Yes	CRBBB	Abnormal
Miz445	39	M	No	IRBBB	Abnormal
Miz431	50	F	Yes	LAH, SB	Abnormal
Miz506	56	F	Yes	LAH	Abnormal
Miz446	57	M	Yes	AVB	Abnormal
Miz471	57	M	Yes	LAH, SB	Abnormal
Miz466	66	M	Yes	AVB	Abnormal
Miz485	67	M	Yes	LAH	Abnormal

IRBBB: incomplete right bundle branch block; CRBBB: complete right bundle branch block; LAH: left anterior hemiblock; AVB: atriventricular block; SB:sinusal bradicardia; *a*: classification according to Maguire et al. (1982)

TABLE III

Anti-SAPA antibody response among chagasic patients of different age

Age (years)	Anti-SAPA response Number of patients N	Negative	Positive
1-6	14	2	12 (85.7%)
7-12	98	21	77 (78.6%)
13-18	40	16	24 (60%)
19-35	20	8	12 (60%)
> 35	29	10	19 (65.5%)
Total	201	57	144 (71.6%)

SAPA: shed acute phase antigen; N: patient number

TABLE IV

Age-specific prevalence of anti-R13 antibody response among chagasic patients

Age (years)	Anti-R13 response Number of patients N	Negative	Positive
1-6	7	4	3 (42.8%)
7-12	44	25	19 (43.2%)
13-18	40	32	8 (20%)
19-35	19	16	3 (15.8%)
> 35	27	23	4 (14.8%)
Total[a]	137	100	37 (27%)

a: two chagasic patients (< 13 years) with positive anti-R13 response were not included in table because their exact age is unknown.

Anti-R-13 IgG response - Twice ELISA assessed anti-R-13 antibodies and the accordance between both assays was of 81.2%. Individuals presenting disagreement were discarded from the analysis and finally, 381 individuals were included. Anti-R-13 antibodies were exclusively detected in chagasic patients (39 patients among 142 tested) demonstrating a strong association between anti-R-13 response and *T. cruzi* infection ($\chi^2 = 73.1$, df = 1, $p < 10^{-4}$). Anti-R-13 antibodies response among chagasic patients, was not related to ECG abnormalities ($\chi^2 = 0.98$, df = 1, $p = 0.32$) and sex ($\chi^2 = 0.01$, df = 1, $p = 0.93$). Moreover, the anti-R-13 antibodies ratios were not different between the five chagasic patients with abnormal ECG (mean = 2.1 ± 0.9) and the 12 patients with normal ECG (mean = 2.55 ± 1.2), $p = 0.53$, (Kruskal-Wallis test). Surprisingly, the percentage of chagasic patients with anti-R-13 antibodies was different according to the age (Table IV, χ^2 Yates = 11.30, df = 4, $p < 0.05$). It is worth noting that more chagasic patients < 13 years old (41.4%) than older patients > 12 years old (17.4%) presented a positive response ($\chi^2 = 10.05$, df = 1, $p = 0.0015$). Furthermore, the antibody ratio was significantly increased among the younger population (< 13 years old, m = 3.3 ± 1.9; > 12 years old, m = 2.3 ± 1.4, $p = 0.009$, Kruskal-Wallis test).

Detection of clonet 20 and 39 in patient bloods - PCR detection of clonet 20 and 39 was applied among 372 individuals including 209 chagasic patients. PCR was positive (presence of 320 bp amplified band) in 83.8% of chagasic patients, and its prevalence was independent of the ECG tracing ($\chi^2 = 1$, df = 1, $p = 0.31$) and sex ($\chi^2 = 0.29$, df = 1, $p = 0.59$). Higher percentage of positive PCR was observed in young patients < 13 years than in older ($\chi^2 = 16.72$, df = 1, $p < 10^{-4}$).

Table V summarized the hybridization results of positive PCR products (163 cases), and negatives ones (33 from randomly selected chagasic patients and 43 from non chagasic individuals as control) with probes 20 and 39, after their transfer on nylon membranes. Part of the sample (composed of 137 children < 10 years old) was previously tested, and the additional results confirmed the major prevalence of clonet 39 over clonet 20 in the blood of chagasic patients of any age in this population. Clonet 39 was detected in 77.8% of patients > 18 years old and only 11.1% presented double infections with clonet 20. Six PCR

TABLE V

Identification of clonets 20 and 39 of *Trypansosoma cruzi* by specific probes in PCR-amplified human bloods

Patients	Total no.	Hybridization with: Probe 20 only No.	%	Probe 39 only No.	%	Probes 20 & 39 No.	%	Neither with probe 20 nor 39 No.	%
Chagasic patients with PCR +									
Total	160	4	2.5%	109	68.1%	26	16.3%	21	13.1%
1-6 years	9	1	11.1%	5	55.6%	3	33.3%	0	0%
7-12 years	84	1	1.2%	57	67.9%	14	16.7%	12	14.3%
13-18 years	32	1	3.1%	23	71.9%	6	18.8%	2	6.3%
19-35 years	13	1	7.7%	11	84.6%	1	7.7%	0	0%
> 35 years	22	0	0%	13	59.1%	2	9.1%	7	31.8%
Individuals with PCR -									
Total	76	0	0%	5	6.6%	1	1.3%	70	92.1%
Chagasic patients	33	0	0%	4	12.1%	1	3%	28	84.8%
No chagasic individuals	43	0	0%	1	2.3%	0	0%	42	97.7%

negative samples presented a positive hybridization of the 320 bp band with probe 39 only (five cases) and with both probes (one case); all these patients had a positive serology except one (Table V). Moreover, no significant association of a particular clonet or mixed infection with ECG tracing or sex were observed. The anti-R13 response was analyzed according to the clonets detected in blood patients and significant differences were observed: 37% of the patients infected by clonet 39 (94 cases) had anti-R13 antibodies contrasting with only 6% of the patients (16 cases) without clonet 39 (χ^2 Yates = 4.64, df = 1, p = 0.031).

DISCUSSION

In the present report we established the seroprevalence of *T. cruzi* infection among a population living in a Bolivian endemic region, in the village of Mizque (Cochabamba Department). Serodiagnosis was determined by three serological tests which presented high concordance even if different *T. cruzi* strains were used as source of antigens in the two ELISA tests and if the tests were processed independently in two different laboratories. Moreover, PCR confirmed the infection in around 84% of the seropositive patients. Positive PCR was found in 3 over 167 seronegative individuals among whom one presented circulating parasites. This patient, 8 years old, was probably in an early acute phase of infection, when the specific IgG response is not yet established. The two others cases may be considered as false positive PCR, but a singular situation has been previously described in Bolivia where cases of positive xenodiagnosis with negative serology were reported (Brenière et al. 1984). High sensitivity of PCR using kDNA primers has been demonstrated in different studies performed with Bolivian populations in endemic areas (Wincker et al. 1997, Antas et al. 1999). Seroprevalence increased regularly with the age of the patients as previously observed in a study of 140 individuals in a rural community of Cochabamba department (Pless et al. 1992). Taken together, these results show that *T. cruzi* active transmission occurs at any age in those rural endemic areas.

In the current study, chagasic patients were 3.8 times more likely to have had abnormal ECG tracing than non chagasic individuals. This strong association of conduction abnormalities and *T. cruzi* infection was also observed in two rural communities of Cochabamba and Santa Cruz departments. However, differences of cardiomyopathy prevalence were observed between the three studies, ranging from 10% to 30% (Weinke et al. 1988, Pless et al. 1992). Cardiomyopathy was first investigated among 131 adult chagasic patients originating from different Bolivian endemic areas and living in La Paz for at least 5 years (average time spend in La Paz: 10 years), where vectorial transmission is absent. Similar prevalence of cardiopathy was found among adults in Mizque population (23.5%), showing a possible absence of relationships between endemicity and cardiomyopathy.

In Mizque, ECG abnormalities were also associated with seropositivity among young patients and its prevalence was not increased among older patients. This finding is not common, only one another study of Brazilian young patients mentioned this association (Andrade et al. 1998). This suggest a particular rapid development of pathology in such areas that may be due to the strain of parasite and prompt us to strongly recommend the treatment of young seropositive children. Men appeared more sensitive than women did to the development of cardiomyopathy measured by ECG abnormalities, in agreement with a previous study (Barretto et al. 1993). Moreover, in a wild rodent experimental model, results showed that gonadal hormones play a role in control of parasitemia (do Prado Junior et al. 1998). Nevertheless, data are not sufficient in humans to indicate a worse prognostics of Chagas disease associated with sex and influence of sex needs to be further investigated.

SAPA antigen response has been related to acute phase of Chagas disease when parasites are abundant in blood (Affranchino et al. 1989, Frasch & Reyes 1990, Levin et al. 1991). The previous work in Mizque showed that anti-SAPA response was strongly associated with Chagas disease in young patients but not discriminative between initial and later stages of Chagas infection (Brenière et al. 1997). The current result showed that in older patients (> 13 years old) this response is still elevated (60%) and this rate is maintained among 29 studied patients >35 years old (65.5%). As the intracellular parasite multiplication has been proposed to induce high anti-SAPA antibody production, we compared the rates of these antibodies in patients with and without positive buffy coat test and PCR and no significant differences were found (data not shown). Higher SAPA reactivity was also recently suggested in symptomatic than in asymptomatic cases, but such association was not observed in Mizque sample (Lorca et al. 1992, 1993). These results and others definitively do not favor the common use of SAPA antigen for diagnosis of infection (lower sensitivity and specificity than conventional serology) neither for infection stage marker (among chronic patients 69% present anti-SAPA antibodies). Nevertheless, SAPA molecule may be used to discriminate patients with *T. cruzi* and *T. rangeli* infections as proposed (Vergara et al. 1992, Saldana et al. 1995).

First results showed that anti-R-13 antibodies were absent in other protozoan infections such as leishmaniasis, African trypanosomiasis and malaria (Levitus et al. 1991). Moreover, anti-R-13 antibody response appeared related to the cardiac form of chronic Chagas disease (Levitus et al. 1991, Aznar et al. 1995). In the present sample anti-R-13 response was absent in non-chagasic population and its prevalence was not significantly different between asymptomatic patients and patients with ECG abnormalities (18.4% and 29.4% respectively). Moreover, the anti-R13 reactivity was much lower than previously reported in chronic patients. A possible explanation of varying results is that the studied groups are not comparable. Most of the previously examined populations were selected in a clinical setting contrasting with the examination in this work of an entire population living in an endemic area selected at random. Mechanisms of pathogenicity of Chagas disease are not well understood and autoimmunity, including increased levels of autoantibodies, was considered to be involved (Kierszenbaum 1999). The production of autoantibodies against the human ribosomal P protein may well result from the mimicry between

host protein and *T. cruzi* antigens. Furthermore, the observed discrepancies between the levels of anti-R-13 in different human populations may reflect strain genetic variability of this antigen, which has not been yet studied. The significant difference in anti-R-13 reactivity between patients infected by clonet 39 and patients where clonet 39 was not detected in blood, favors the hypothesis of strain dependence of the anti-R-13 antibodies production. Surprisingly, equal frequencies of anti-R-13 reactivity were observed in young and older patients, a result not yet described which indicates that autoimmune response should be involved early during the infection.

Previous results showed similar frequencies of clonet 20 and 39 in vectors collected in Mizque and these clonets were equally detected in blood patients during the acute phase (Brenière et al. 1998). The present results indicated that in later infection stages, clonet 39 is mainly detected in patients at any age. Indeed, clonets 20 and 39 belong to different groups of *T. cruzi* (*T. cruzi* I and *T. cruzi* II) which present high genetic distances between them (Brenière et al. 1998). These high genetic differences should be related to biological variability as virulence or variable susceptibility to the immune response (control of parasitemia) developed after the acute phase of human infection. Consequently, strain genetic differences may influence parasitemia in man. This important observation can explain discrepancies of PCR sensitivity between studies carried out in different endemic areas. In Bolivia and Brazil, in areas where *T. cruzi* II stocks are abundant, PCR technique presented high sensitivity contrasting with areas of Amazonian Basin and Mexico where only *T. cruzi* I stocks circulate (Wincker et al. 1994, 1997, Zingales et al. 1998, Pacheco et al. 1998).

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