Seroprevalence of *Trypanosoma cruzi* Infection in French Guiana

Christine Aznar/+, Guy La Ruche, Stéphane Laventure*, Bernard Carme, Pascale Liegeard, Mireille Hontebeyrie****

Laboratoire Hospitalo Universitaire de Parasitologie et Mycologie, UFR de Médecine Antilles-Guyane and Centre Hospitalier Andrée Rosemon, Equipe EA 3593, BP 6006, Rue des Flamboyants, 97300 Cayenne, French Guiana *Laboratoire d'Entomologie Médicale, Institut Pasteur de la Guyane, Cayenne, French Guiana **Département de Biologie Structurale et Chimie, Institut Pasteur, Paris, France

A survey was carried out on 1487 individuals to assess the seroprevalence of Trypanosoma cruzi *infection in French Guiana. The overall prevalence of* T. cruzi *specific IgG was 0.5%. In multivariate analysis, residence in areas where housing is favorable for the presence of triatomine bugs was the only factor associated with the presence of* T. cruzi *antibodies. These results have implications for public health since blood donors are not routinely screened for* T. cruzi *infection in French Guiana.*

Key words: *Trypanosoma cruzi* - Chagas disease - seroprevalence - French Guiana

Chagas disease (American trypanosomiasis) is caused by the protozoan *Trypanosoma cruzi*, mainly transmitted to humans by blood-sucking triatomine bugs (Hemiptera, Triatominae) but also transmitted by blood transfusion from infected donors, and occasionally by transplacental mother-to-child transmission. It is estimated that 16-18 million people are infected in Latin America (WHO 1991). However, relatively few cases have been reported from the Guyanas (Guyana, Suriname, and French Guiana) (Luquetti & Schofield 2003).

In French Guiana, only 17 clinical cases of Chagas disease have been reported since 1939 (Floch & Tasque 1940, Beaudet et al. 1985, Esterre & Dedet 1987), including four cases of acute chagasic myocarditis diagnosed between 1994 and 1996 (Carme et al. 2001a). A serological study conducted in 1983-1985, using an indirect immunofluorescence test, showed that only one of 301 individuals (0.3%) had antibodies to *T. cruzi* (Esterre & Dedet 1987). We present here a cross-sectional study designed to assess the seroprevalence of *T. cruzi* infection in various areas of French Guiana.

MATERIALS AND METHODS

French Guiana (Figure) is located in the Amazonian forest between Brazil and Suriname. It is technically a Department of France and has the highest living standard in South America. In the 1990 census there were 114,678 inhabitants, and in 1999 were around 157,274 inhabitants. There is massive immigration from neighboring countries which is difficult to quantify, but it is estimated that illegal immigrants represent up to a third of the total population.

For this retrospective study, sera were selected randomly from several large banks of sera from "Centre National de Référence de Virologie et du Paludisme" (Collection Biological samples used for infectious disease surveillance) under the Ministry of Health. These sera are available without need for specific ethical clearance. Sera samples were stored at –80°C, without glycerol, for 3 to 4 years and were thawed once. These samples had originally been collected either for epidemiological studies on human T-cell leukemia/lymphoma virus type I, during routine testing of pregnant women, or during previous studies on viral hepatitis or arboviroses. The samples included most of the ethnic groups and regions of French Guiana. For each sample, basic demographic data included sex, age, place of current residence, and ethnic group (Table I). However, Cayenne and its surroundings was the only region where sera could be selected to include a sufficient number of each ethnic group; thus, the seroprevalence presented in this paper is calculated based on the respective percentages of ethnic groups observed in this city.

Sera were tested with a simple *T. cruzi* enzyme-linked immunoassay (ELISA) using antigen prepared from trypomastigotes of the Brener CL strain of *T. cruzi* (Aznar et al. 1997). Briefly, all samples were diluted 1:200 in reference to a previous study (Aznar et al. 1997). Samples were then assayed in duplicate and one negative and one positive control were included on each microplate (Nunc, Roskilde, Denmark). Fifty µl of a 1:1000 dilution of goat peroxidase-conjugated anti-human IgG (Sanofi Diagnostics Pasteur, Paris, France) were added to each well. After incubation, peroxidase was revealed with 50 µl of a 1/1 mixture of 2.2'-azino-di-3-ethyl-benzothiazoline sulfonate and hydrogen peroxide (ABTS Peroxidase Substrate System, Kirkegaard Perry Laboratories Inc., Gaithersburg, MD). Optical density (OD) was measured at 492 nm for peroxidase activity in an automated ELISA reader (Titertek Multiskan MCC/340 - Lab Systems, France). Sera were considered as positive when the mean of duplicate optical density values reached the mean value obtained from 68 negative controls + 3 standard deviations. Controls were chosen from among blood donors from mainland

+Corresponding author. Fax: +594-594-39-51-63. E-mail: christine.aznar@mdi-guyane.fr
Received 18 May 2004
Accepted 14 October 2004

Map of French Guiana (areas of study)

France (Aznar et al. 1997). The cut-off value for anti-*T. cruzi* IgG was determined as OD = 0.108.

Data were analyzed using Epi-Info and Egret software. The presence of *T. cruzi*-specific IgG (without specific IgM, data not shown) was considered to denote a chronic infection (Medrano-Mercado et al. 1996). From the results in the different areas of French Guiana, we extrapolated the global seroprevalence using the 1990 national census. Demographic factors related to *T. cruzi* infection were determined by univariate analysis using Chi-square or Fisher's exact test, with a significance level of 5%. Multivariate analyses using stepwise backward logistic regression were subsequently performed and strength of associations was estimated by the adjusted odd ratios (OR) with 95% confidence interval (CI).

RESULTS

A total of 1487 sera was tested for IgG antibodies to *T. cruzi* (40.3% from men and 59.7% from women, with a median age of 26 years, range 1 to 93). Of these sera, 324 (21.8%) were from patients living in the littoral plain or in Saint-Laurent, originally taken for diagnosis of dengue fever. The remaining 78.2% were taken from pregnant women or during epidemiological studies of populations living in the forest areas of the Oyapock or Maroni River regions.

The seroprevalence rates for *T. cruzi* antibodies ranged from zero to 7.3% according to place of residence, age class, and ethnic group (Tables I, II). Seroprevalence was below 3% except in three areas where the prevalence of antibodies was greater than 6%: in Antécume-Pata in the southern part of the Maroni river (2 of 33 sera, 6.1%), in Cacao in the forest area (3/41, 7.3%), in Camopi/Trois-Sauts in the southern part of the Oyapock river (6/82, 7.3%). Globally, the seroprevalence of *T. cruzi* antibodies extrapolated for the entire population of French Guiana was 0.5% (95% CI, 0.1-0.8%) (Table I).

Table II shows univariate comparisons of the demographic factors and the context of collection of sera between *T. cruzi* seronegative and seropositive patients. The only factors statistically associated with the presence of anti-*T. cruzi* IgG were residence in Camopi/Trois-Sauts, in Cacao or in Antécume-Pata. The prevalence of *T. cruzi* antibodies did not differ between males and females. Among 321 subjects with fever as the reason for sera collection, the prevalence of *T. cruzi* antibodies was not associated with Dengue fever (data not shown).

After multivariate analysis, the place of residence remained the only factor significantly associated with *T. cruzi* antibodies. The context of collection of sera (sera

TABLE I

Prevalence of IgG antibodies to *Trypanosoma cruzi* according to age class, ethnic group and area of residence, French Guiana, 1992-1998

	Prevalence of antibodies (%)	
	Nr tested	IgG
Age class (years)		
< 10	102	1.0
10-19	275	0.0
20-29	502	0.6
30-39	304	0.7
> 39	260	1.5
Unknown	44	6.8
Ethnic group		
Native American	416	1.7
Brazilian	160	0.0
Caucasian	62	1.6
Chinese	16	0.0
Creole	120	0.0
Haitian	56	0.0
Hmong	79	2.6
Bush-Negro	440	0.7
Others	34	0.0
Unknown	104	0.0
Residence [a]		
Littoral plain		
Cayenne and surrounding	170	0.0
Macouria/Tonnégrande	27	0.0
Kourou/Sinnamary/Iracoubo	70	0.0
Maroni river region		
Awala-Yalimapo	35	0.0
Javouhey/Mana	36	0.0
Saint-Laurent	34	2.9
Apatou	97	0.0
Papaïchton	183	0.5
Maripasoula	487	0.4
Twenké	30	0.0
Antécume-Pata	33	6.1
Oyapock river		
Camopi/Trois-Sauts	82	7.3
Saint-Georges	162	0.0
Forest area		
Cacao	41	7.3

a: prevalence observed, except for Cayenne where prevalence is calculated taking into account the percentage of ethnic groups in this area.

TABLE II

Demographic factors and context of collected sera in relation with anti-*Trypanosoma cruzi* IgG in French Guiana, 1992-1998: univariate analysis

	Prevalence of anti-*T. cruzi* IgG in presence or absence of factor		
Factor	Presence	Absence	p
Male	1.0	0.8	0.66
Age > 30 years	1.2	0.4	0.11
Ethnic group [a]			
Native Americans	1.7	0.6	0.06
Caucasians	1.6	0.8	0.43
Haitians	0.0	0.9	0.60
Place of residence [a]			
Antécume-Pata	6.1	0.8	0.032
Cacao/Roura	5.1	0.8	0.044
Camopi/Trois-Sauts	6.2	0.6	< 0.001
Cayenne and surrounding	0.0	1.0	0.20
Macouria/Tonnégrande	0.0	0.9	0.79
Sera collected for fever	0.6	0.9	0.43
Sera collected before 1995	0.8	0.9	0.66

a: anti-*T. cruzi* IgG were not associated with other ethnic groups and places of residence.

collected for fever, year of collection) was not associated with *T. cruzi* antibodies. The presence of anti-*T. cruzi* IgG was independently associated with living in Camopi/Trois-Sauts (adjusted OR = 21.8, 95% CI = 5.7-83.0, p < 0.001), in Antécume-Pata (adjusted OR = 21.4, 95% CI = 3.8-121.3, p < 0.001) and in Cacao (adjusted OR = 18.0, 95% CI = 3.2-101.1, p = 0.001).

DISCUSSION

Our study suggests that 0.5% of the general population in French Guiana – approximately 785 people – may have *T. cruzi* specific IgG antibodies. Such a seroprevalence is 1.75 times higher than that previously observed in French Guiana (Esterre & Dedet 1987), and is similar to seroprevalence levels recorded for children in Brazil and Uruguay prior to the Southern Cone Initiative against Chagas disease (WHO 1997, 1998, Schofield & Dias 1999).

By contrast, very few cases of clinical Chagas disease have been observed in French Guiana during the last 50 years (Carme et al 2001b). Several hypotheses could explain this paradox. Firstly, our survey could have overestimated the actual seroprevalence of *T. cruzi* infection. However, the method that we used, ELISA, offers a high reliability and is concordant with other serological methods such as direct agglutination test or indirect immunofluorescence antibody test (Añez et al. 1999) and PCR (Brenière et al. 2002). Furthermore, *T. cruzi* trypomastigote extract, with almost no cross-reactivity with other protozoan infections (Aznar et al. 1997), has been shown to be an excellent reagent for detecting the infection in areas of low prevalence, and appears to be better than epimastigote extracts.

Alternatively, it may be that Chagas disease is significantly under-diagnosed in French Guiana, possibly as a result of frequent asymptomatic forms of *T. cruzi* infection or recent infections with clinical symptoms too mild to be recognized (Medrano-Mercado et al. 1996). Our results could therefore signify the emergence of a public health problem in French Guiana and the number of clinical cases could increase in the future. Indeed, our results indicate highest seroprevalence among people living in rural rain-forest villages (Upper-Maroni, Upper-Oyapock, Cacao) with traditional housing (wood and/or palms). Clinical cases in these areas may well go undiagnosed because of the basic level of health facilities there and because the Hmong and the Native American groups often have different heath-seeking behaviors than metropolitan or Creole French ethnic groups. Active detection campaigns should be implemented to establish the true prevalence of Chagas disease in areas of French Guiana with poor medical facilities. Potential vectors such as *Rhodnius pictipes* and *R. robustus* are recorded from such areas, and we have also found these species, and *Panstrongylus geniculatus*, infected with *T. cruzi* in urban areas of Cayenne and surrounding villages (Aznar C., unpublished observation). These observations lead us to further investigate the presence of infected vectors and domestic or wild reservoirs in rural and urban areas of French Guiana, and also indicate a possible risk of blood transfusional transmission in French Guiana since French law does not require any serological screening of blood donors for this infection.

ACKNOWLEDGMENTS

To Antoine Talarmin and Jean-Louis Sarthou for their support; to Alejandro Luquetti for helpful advice; to M Nacher and CJ Schofield for critical revision of the manuscript.

REFERENCES

Añez N, Carrasco H, Parada H, Crisante G, Rojas A, Gonzalez N, Ramirez JL, Guevara P, Rivero C, Borges R, Scorza JV 1999. Acute Chagas disease in Western Venezuela: a clinical, seroparasitologic, and epidemiologic study. *Am J Trop Med Hyg 60*: 215-222.

Aznar C, Liegeard P, Mariette C, Lafon S, Levin MJ, Hontebeyrie M 1997. A simple *Trypanosoma cruzi* enzyme-linked immunoassay for control of human infection in nonendemic areas. *FEMS Immunol Med Microbiol 18*: 31-37.

Beaudet B, Esterre P, Jacquet-Viallet P, Ganty J, Dedet JP 1985. Un cas de cardiopathie chagasique chronique autochtone en Guyane française. *Bull Soc Path Exot 78*: 56-63.

Brenière SF, Bosseno MF, Noireau F, Yacsik N, Liegeard P, Aznar C, Hontebeyrie M 2002. Integrate study of a Bolivian population infected by *Trypanosoma cruzi*, the agent of Chagas disease. *Mem Inst Oswaldo Cruz 97*: 289-295.

Carme B, Aune I, Nguyen G, Aznar C, Beaudet B 2001a. Four cases of acute chagasic myocarditis in French Guiana. *Am J Trop Med Hyg 64*: 162-163.

Carme B, Aznar C, Pradinaud R 2001b. Absence of a proven resurgence of Chagas disease or cutaneous leishmaniasis in French Guiana over the last two decades. *Ann Trop Med Parasitol 95*: 623-625.

Esterre P, Dedet JP 1987. Situação atual da doença de Chagas na Guiana Francesa. *Rev Soc Bras Med Trop 20*: 139-142.

Floch H, Tasqué P 1940. Un cas de maladie de Chagas en Guyane française. *Pub Inst Pasteur Guyane Fr Territ Inini 2*: 1-4.

Luquetti A, Schofield CJ 2003. Chagas disease in the Guyanas. *Proceedings ECLAT-AMCHA International Workshop on Chagas Disease Surveillance in the Amazon Region*, Universidad de Los Andes, Bogota, p. 28-29.

Medrano-Mercado N, Luz MR, Torrico F, Tapia G, Van Leuven F, Araujo-Jorge TC 1996. Acute-phase proteins and serologic profiles of chagasic children from an endemic area in Bolivia. *Am J Trop Med Hyg 54*: 154-161.

Schofield CJ, Dias JCP 1999 The Southern Cone Initiative against Chagas disease. *Adv Parasitol* 42: 1-27

WHO 1991. Control of Chagas disease. *WHO Technical Report Series 811*.

WHO 1997. Chagas disease. Interruption of transmission. *WHO Wkly Epidemiol Rec 72*: 1-5.

WHO 1998. Chagas disease. Interruption of transmission. *WHO Wkly Epidemiol Rec 73*: 1-4.