

Short communication

Seroepidemiological Study of Chagas Disease in the Southern Amazon Region of Ecuador

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Abstract: To determine the extent of *Trypanosoma cruzi* infection and/or transmission in the southern Amazon region of Ecuador, three indigenous communities in the provinces of Pastaza and Morona Santiago were serosurveyed. ChagatestTM, Immunocomb[®]II and immunofluorescent (IF) assays were used. Among the 385 inhabitants examined, nine (2.34%) were seropositive for *T. cruzi* infection. Of the nine positive sera, four (44.4%) fall in the 10–19, one each in the 20–29, 30–39 and 40–49, and two in the 50–59 age groups. These results suggested the possible existence of an autochthonous active *T. cruzi* transmission in the region and provide the first serological evidence for *T. cruzi* infection in the southern province of Morona Santiago bordering Peru. Further studies are needed in these Amazonian provinces to ascertain the spread of *T. cruzi* infection in the area.

Key words: *Trypanosoma cruzi*, Ecuador, Amazon, Chagas disease, seroprevalence

INTRODUCTION

Chagas disease is a chronic, systemic, parasitic infection caused by infection of the protozoan parasite *Trypanosoma cruzi* (Kinetoplastida: Trypanosomatidae). *T. cruzi* was reported for the first time in the Amazon region of Brazil in 1909. The disease affects about 8 million people in Latin American countries, of whom 30–40% either have or will develop cardiomyopathy, digestive megasyndrome, or both [1, 2]. It is a neglected infectious disease in the tropics and an emerging health problem in the developed nations of Europe and the USA [3]. The parasite is transmitted by blood-sucking insects of the subfamily Triatominae (Hemiptera, Reduviidae). There is currently no effective vaccine and disease control therefore relies on domestic vector elimination through application of residual insecticides [4].

In Ecuador, cases of Chagas disease have been confirmed in eight provinces, *i.e.*, Guayas, Manabí, El Oro,

Loja, Napo, Sucumbíos, Orellana and Pastaza [5–8]. It is estimated that 2.3 to 3.8 million people, out of the total Ecuadorian population of approximately 11 million, are at risk of infection by *T. cruzi* [5]. In 1991, sylvatic foci of Chagas disease were reported in Napo and Sucumbíos provinces, the first such cases in the Amazon region of Ecuador [9]. A further study in the Amazon region along the Napo River showed 15 of the 18 (83.3%) communities to be seropositive for *T. cruzi* infection, suggesting active transmission in an indigenous population in the northern Amazon region of Ecuador [6]. Moreover, another study described seroprevalence and risk factors for *T. cruzi* infection in the northern and central provinces of the Amazon region, including Sucumbíos, Napo, Orellana and Pastaza [7]. In 1997, Guevara *et al.* reported two cases of severe digestive pathology associated with chronic Chagas disease with megacolon, confirmed by polymerase chain reaction (PCR), in patients from the provinces of Loja and Morona Santiago [10]. However, the southern areas of the Amazon region of

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Ecuador, mainly composed of Pastaza and Morona Santiago provinces, have not yet been studied in detail for the presence and prevalence of *T. cruzi* infection.

The present seroepidemiological study, therefore, was conducted with a focus on Morona Santiago and neighboring areas in order to determine the status of the disease especially in the southern Amazon region of Ecuador. In this study, the inhabitants of three communities, *i.e.*, Arajuno, Yuwientsa and Makuma, located in the southern provinces of Pastaza and Morona Santiago, were examined by means of three serological tests. Our positive results indicate that *T. cruzi* infection and/or transmission is gradually spreading from the northeast to the southeast in the Amazon region of Ecuador, especially in Pastaza and Morona Santiago provinces, very close to the northwest Amazon region of Peru.

MATERIALS AND METHODS

Study area

The community of Arajuno is a rural settlement located in the eastern Ecuadorian province of Pastaza, while the communities of Yuwientsa and Makuma are situated in the southeastern jungle of Ecuador in the province of Morona Santiago (Fig. 1). A road was recently constructed into Arajuno, but Yuwientsa and Makuma remain accessible only on foot or by small aircraft. The entire area is a tropical lowland rainforest with a six-month rainy season (from November to May). The average temperature is 27°C (24–31°C). The population in the study areas consists almost entirely of members of the indigenous race “Shuar” with a small number of migrants. Agriculture and hunting are the main economic activities of the inhabitants.



Fig. 1. Map of Ecuador, showing the three study areas: 1, Arajuno, Pastaza province; 2, Makuma, Morona Santiago province; 3, Yuwientsa, Morona Santiago province.

Study subjects

Prior to the study, the protocol of the epidemiological and laboratory study was approved by the Bioethics Committee of Universidad Central del Ecuador, Quito, Ecuador. Specific permission was granted in writing from the community leaders, and when necessary, the study plan was explained in their primary language. In addition, the subjects as well as parents of children voluntarily consented to participate in this study after receiving a detailed explanation of the procedures by our research members (AGG & MHC). A cross-sectional study was conducted in August 2007 in Arajuno and in June 2008 in Yuwienta and Makuma. The blood samples were obtained from the inhabitants living in the communities, irrespective of whether they were symptomatic or asymptomatic for Chagas disease. Children under the age of one year were excluded along with those who preferred not to participate.

Blood sampling

Blood collection was performed by venipuncture using disposable needles and vacuum 10 mL tubes (VACUETTE[®], Greiner bio-one GmbH, Austria). The samples were allowed to clot and then centrifuged for 15 minutes at 1000 g; the serum obtained was placed in a cryovial and stored at -20°C until use.

Serologic analysis

Blood samples were initially analyzed using an ELISA kit with *T. cruzi* recombinant antigens (Chagatest[™], Wiener lab, Argentina). Then, positives and suspected samples were subjected to an additional test (ImmunoComb[®]II, ORGENICS, Israel), as well as an immunofluorescent immunoassay (IFI) using cultured *T. cruzi* epimastigotes. Any sample testing positive on two or more of the individual assays was declared positive, according to WHO criteria [11]. *Chagatest[™] Protocol*

In brief, 200 μL diluent was added to each well of the supplied antigen-coated plate. This was followed by the addition of 10 μL of sample, including kit positive and negative controls to specific wells. The plate was covered and incubated at 37°C for 30 minutes. The plate was then washed five times with wash solution and incubated with 50 μL of conjugate per well for 30 minutes at 37°C . The wash step was repeated, and then 50 μL of substrate A was added to each well followed immediately by 50 μL of substrate B. The plate was then incubated for 30 minutes at room temperature and a drop of stop solution was added to each well. The plate was read at 450 nm ($1^{\circ}\lambda$) and 620–650 nm ($2^{\circ}\lambda$) in an ELISA reader (EIA 400 AT, Wittaker Bioproducts, Austria). According to the kit instructions, the optical density (OD) of negative controls should be below 0.150 and

positive controls should have an OD above 0.600. The samples were classified as positive, negative or doubtful. All the positive and doubtful samples were assayed again.

ImmunoComb[®] II, Chagas Ab Protocol

Briefly, 10 μL of the sera samples was added to a comb pre-impregnated with *T. cruzi* antigen and allowed to react for 10 minutes at room temperature. The comb's teeth were washed for two minutes and goat-human anti-IgG alkaline phosphatase conjugate was added and incubated for 10 minutes at room temperature. Washing solution was twice applied to the comb and a chromogenic substrate: 5-bromo-4-chloride-3-indol-phosphate (BCIP) and nitroblue tetrazolium (NBT) was added for 10 minutes. Positive results were based on distinguishable color development in both the test and control areas of each comb tooth.

Immunofluorescent Immunoassay (IFI) Protocol

All positive sera for the previous described tests were tested using an *in house* IFI assay. Sera previously identified as positive or negative for *T. cruzi* were used as the positive and negative controls, respectively. Using standard 12-well glass slides, 20 μL of *T. cruzi* epimastigotes ($1-2 \times 10^6$ parasites per 1 mL) was fixed in each well with 4% formaldehyde solution in phosphate buffered saline (pH 7.2 PBS) and left at room temperature for 24 hours. Afterwards, 30 μL of each sample diluted 1:40 in PBS was added to the corresponding well and incubated for 30 minutes at 37°C . Each sample was tested in duplicate. After incubation, the slides were washed three times with PBS, and 30 μL of goat anti-human IgG fluorescein conjugate (Sigma, St. Louis, MO) diluted 1:100 in Evans blue was added to the specific wells and incubated for 30 minutes at 37°C . Each slide was washed a final time with PBS, and a drop of 1:1 PBS/glycerol was added to each well before cover slide application. The slides were read with a fluorescence microscope under 100X power with oil immersion objective. Positive and negative controls were included for each assayed slide.

RESULTS

Of the 385 individuals examined, nine (2.34% of the study subjects) tested positive for two or more tests in the three separate trials. This included four of the 246 in Arajuno, Pastaza province; five of the 95 in Yuwienta, Morona Santiago province, and none of the 44 in Makuma, Morona Santiago province (Table 1). The seropositive cases were arranged by the age of subjects from Arajuno and Yuwienta. Of the nine seropositive subjects, four fall in the 10–19, one each in the 20–29, 30–39 and 40–49, and two in the 50–59 year age groups. Thus, the subjects in the 10–19 to 50–59 year age groups showed *T. cruzi* seropositivity ranging from 1.64% (1/61 tested) to 7.69% (2/26 tested)

Table 1. Seropositivity of inhabitants against *T. cruzi* infection by community

Community	Province	Subjects tested	Seropositivity (%)
Arajuno	Pastaza	246	4 (1.63)
Yuwientsa	Morona Santiago	95	5 (5.26)
Makuma	Morona Santiago	44	0 (0.00)

Table 2. Prevalence of *Trypanosoma cruzi* seropositivity according to age group

Age (Years)	No. examined	No. positives	Seropositivity (%)
1-9	72	0	—
10-19	95	4	4.21
20-29	52	1	1.92
30-39	61	1	1.64
40-49	49	1	2.04
50-59	26	2	7.69
60-69	20	0	—
70-79	9	0	—
80-89	1	0	—

(Table 2). Additionally, all the previously identified positive controls showed seropositivity for *T. cruzi* while none of the previously tested negative control sera showed *T. cruzi* positivity in any of the assays utilized.

DISCUSSION

The present study supports and expands the findings of previous studies [6, 7], providing serological evidence for *T. cruzi* infection in Arajuno, Pastaza province and in Yuwientsa, Morona Santiago province, and suggesting the further spread of Chagas disease into the southern areas in the Amazon region of Ecuador. The results presented here are the first report to demonstrate the possible existence of *T. cruzi* infection in the province of Morona Santiago, suggesting that the disease reaches farther south in the Amazon regions than previously reported.

It has been postulated that *T. cruzi* was introduced into the Amazon region due to the large migration of people from other provinces of the country, some of which are endemic for Chagas disease [6]. Within the last few years, Arajuno, a previously remote community accessible only by small aircraft, has been connected via roads to the larger town of Puyo, Pastaza province. The opening and development of Arajuno village has facilitated migration and trade in the area, which may be a possible cause for the introduction of *T. cruzi* infection. However, there is evidence of au-

tochthonous Chagas disease in humans from other provinces in the Amazon region of Ecuador [9]. The environment of Arajuno is ideal for the spread of *T. cruzi*, a fact that highlights the risk of possible transmission of *T. cruzi* in the region. Future studies involving the collection, identification, and examination of *T. cruzi* infection by the resident triatomine insect vectors in this area could thus yield valuable information. Our preliminary search for Triatomine bugs showed the presence of *Trypanosoma* positive *Panstrongylus herreri* (unpublished data), a significant *T. cruzi* vector in northern Peru [12], captured inside the house where one of the present seropositive subjects lives.

The findings in Yuwientsa, Morona Santiago province, where the majority of positive cases were encountered in youths ≤ 19 years of age except one among five tested in the 50-59 year age group, suggest the active transmission of *T. cruzi* in this region. However, the identification of vector species and their habitat preference in the area are still unknown. In this study, only two communities in the entire province of Morona Santiago were tested for *T. cruzi* infection. Considerable future studies are necessary therefore to ascertain the true extent of the disease in the area. The present limited number of samples, *i.e.*, only 44 subjects examined in Makuma, may account for the lack of evidence for *T. cruzi* existence in the area.

The present seroepidemiological study indicated that human *T. cruzi* infection is prevalent in the studied areas and revealed the active transmission of the parasite in the southern areas of the Amazon region of Ecuador. Further investigations in the surrounding Amazon areas as well as the less accessible regions further to the southeast are necessary to ascertain the full extent of Chagas disease in the regions, from both serological and vector-entomological points of view. In addition, a detailed investigation of domestic livestock and sylvatic reservoir mammals could yield valuable information concerning the transmission and/or spread of the disease in the areas. It is important to mention that the present *T. cruzi* seropositive subjects were treated appropriately according to the guidelines of the World Health Organization and the Ministry of Public Health of Ecuador.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest connecting the work reported in this paper.

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