Seroprevalence of *Trypanosoma cruzi* Infection in Schoolchildren and in Pregnant Women from an Amazonian Region in Orellana Province, Ecuador

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Abstract. Chagas disease is a parasitic disease caused by the protozoan parasite *Trypanosoma cruzi* and about 230,000 persons are estimated to be infected in Ecuador. However, limited studies have been performed in the Amazon region, on the eastern side of the country. We evaluated here the seroprevalence of *Trypanosoma cruzi* infection in 12 rural villages of the Loreto canton, Orellana Province in schoolchildren aged 5–15 years and in pregnant women. A total of 1,649 blood samples were tested for *Trypanosoma cruzi* antibodies by enzyme-linked immunosorbent assay and indirect hemagglutination, and discordant samples were tested by indirect immunofluorescence assay. We detected a seroprevalence of anti-*Trypanosoma cruzi* antibodies of 1.3% in schoolchildren aged 5–15 years, indicating the persistence of a constant and active vectorial transmission in the Loreto County and confirming the need of the implementation of nonconventional vector control. We also observed a seroprevalence of 3.8% in pregnant women, indicating a clear risk of congenital transmission. Further studies should help define this risk more precisely and implement current international guidelines for the diagnosis, treatment, and care of these cases.

INTRODUCTION

Chagas disease is a parasitic disease caused by the protozoan parasite *Trypanosoma cruzi* and transmitted principally by hematophagous triatomine bugs. It is endemic in the Americas, and international migrations are turning it into a global disease.¹ Following a brief acute phase characterized by a high parasitemia, most patients enter an asymptomatic chronic phase, but only 30–40% of patients develop a Chagasic cardiomyopathy and/or digestive megasindromes.² This symptomatic chronic phase has a very high morbidity, and the global burden of Chagas disease reaches 29,385,250 disability adjusted life years (DALYs) and generates health costs over \$23 billion,³ mostly in disease-endemic countries. In spite of this huge burden, it remains a highly neglected disease.^{4,5}

In Ecuador, estimates indicate that about 230,000 persons are affected, and both Chagasic cardiomyopathy and digestive forms of the disease have been observed.⁶ The seroprevalence of anti-*Trypanosoma cruzi* antibodies is highly variable among the regions and provinces of the country, ranging from < 1% up to 5–6%, with a national average of 0.7%. In the Amazon region, on the eastern side of the country, limited studies have been performed but a seroprevalence of up to 3% has been observed.⁷ The largest study in the Ecuadorian Amazon was performed among nearly 7,000 samples from 162 communities collected about 15 years ago and reported a seroprevalence rate of 2.4%.⁸ The highest seroprevalence of 3.4% was observed in the province of Orellana, and between 1.4% and 2.1% was reported in the other Amazonian provinces. The presence of anti-*Trypanosoma cruzi* antibodies was confirmed more recently in samples from eastern Orellana, in the Aguarico County⁹ as well as in the southern Amazon region, including the provinces of Pastaza and for the first time

Morona Santiago, with an overall seroprevalence of 2.34%.¹⁰ As a consequence, Ecuador is part of the Intergovernmental Initiative for the Surveillance and Prevention of Chagas Disease in the Amazon (AMCHA), which focuses on the environmental specificities of this region for disease control.

The main triatomine vectors of *Trypanosoma cruzi* in Ecuador are *Triatoma dimidiata* and *Rhodnius ecuadoriensis*, but several other species are considered secondary vectors.^{11,12} In particular, in the Amazon region, the most important triatomine vectors are *R. pictipes*, *R. robustus*, *Panstrongylus geniculatus*, and *Triatoma carrioni*^{7,12} and possibly *R. barretti*.¹³ These species seem to be rather intrusive in the domestic habitat as they are able to fly into houses from their breeding sites in palm trees and animal burrows and nests. However, domestic and peridomestic colonies have also been reported, suggesting some capabilities of these species to become domiciliated, possibly because of deforestation and other environmental changes,^{6,11,12} but further studies are needed to fully understand their potential adaptation to human habitat and define appropriate vector control interventions.¹⁴ Other species are also present, including *Triatoma venosa*, *Eratyrus mucronatus*, or *Cavernicola pilosa*, but may not be epidemiologically relevant for transmission of *Trypanosoma cruzi* to humans.^{6,11}

Congenital transmission is emerging as the most important route of *Trypanosoma cruzi* transmission following vectorial transmission.^{15,16} Estimates from the World Health Organization suggest that there are 1,800,000 *Trypanosoma cruzi*-infected pregnant women in the Americas and over 14,000 cases of congenital transmission annually.¹⁷ In Ecuador, the same estimates suggest the presence of about 58,000 infected pregnant women in the country, and 800 cases of congenital transmission, but there have been no study clearly addressing this issue, so that little is known on the importance of congenital transmission in this country.

In this study, we evaluated the seroprevalence of anti-*Trypanosoma cruzi* antibodies in the Loreto County, Orellana Province in the Amazon region, and we focused on two critical populations: school children aged 5–15 years, which

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provide indication on active vectorial transmission, and pregnant women, to allow an assessment of the risk of congenital transmission.

MATERIALS AND METHODS

Study area and population. The study was carried out in the Orellana Province in eastern Ecuador, in the Loreto County, which has a total population of 21,163 inhabitants.¹⁸ The county has an average elevation of 320 m, and a rainy tropical climate, with an average temperature of 23.9°C and average rainfall of 3,284 mm/year. It is part of the Sumaco Biosphere Reserve, a United Nations Educational, Scientific and Cultural Organization-protected area of extensive biodiversity.

A total of 12 communities were included in the study, as part of the epidemiological surveillance activities from the National Institute for Research in Public Health (*Instituto Nacional de Investigación en Salud Pública*) and from the National Program for Control and surveillance of Arthropod Vector-Borne Diseases (*Servicio Nacional de Control y Vigilancia de enfermedades transmitidas por vectores artrópodos*). No informed consent was used as this work was part of the routine surveillance activities of the Ministry of Health. The villages included El Inca, Centro Huino, Bajo Huino, and Puerto Murialto, from the Puerto Murialto Parish; the villages of Puerto El Sol, Juan Pío Montúfar, San Francisco de Asís, Jumani Centro, and Chonta Cocha from the Loreto Parish; the village of Huiruno from the Avila Parish; and the village of 24 de Mayo from the 24 de Mayo Parish. These villages were selected based on previous reports of triatomine infestation. About 70% of the population of these villages is Kichwa (Quechua origin), with a minority of mixed ethnicity (*mezizos*, about 30%). Most are subsistence farmers, and the level of poverty is higher than the national average. The average education level reaches 7.4 years of school, whereas 8.6% of the population is illiterate.¹⁸

Following diffusion of the study in the communities through the local community leaders, schoolchildren aged 5–15 years were recruited in their respective schools. A total of 1,649 children were enrolled in the study in March and April 2012. Families of children's cases from the villages of Bajo Huino and El Inca were also invited to participate in the study, resulting in 25 additional samples.

For the study of pregnant women, recruitment was carried out in two hospitals, the Loreto Health Center (*Centro de Salud de Loreto*; 30 women), and the Orellana Provincial Hospital (*Hospital Provincial de Orellana*; 76 women), during March and April 2012. These hospitals receive patients from all surrounding villages of the province and provide primary and secondary care. The women were enrolled during their prenatal visit during pregnancy.

Serological testing. About 3 mL blood was taken from each participant in vacutainer tubes, labeled with code IDs and maintained refrigerated at 4°C until serum was prepared. Serum samples were then processed for the detection of anti-*Trypanosoma cruzi* IgGs by enzyme-linked immunosorbent assay (ELISA), using Wiener Chagatest recombinant V3.0 test and Wiener indirect hemagglutination (HAI) test (Wiener, Rosario, Argentina). In case of discrepancy between the two tests, samples were tested using an indirect immunofluorescence (IIFIA) test (Imuno-Con Chagas, Wama, Sao Carlos, Brazil). All tests were run according to the instructions of the

manufacturers. Samples were considered positive if reactive with at least two tests.

Triatomine collections. Triatomine infestation was evaluated by timed manual search in four houses from El Inca in both domestic and peridomestic habitats.^{19–21} This was performed by two trained researchers. In addition, two palm trees in close proximity of the village of Juan Pío Montúfar, Loreto Parish, were completely dissected and searched for triatomines.

Data analysis. Prevalence was calculated for the different villages as well as according to sex and age of the children, and was expressed as percentage with its 95% confidence interval (CI). Comparisons between groups were performed by χ^2 tests. Maps of the geographic distribution of the seroprevalence were elaborated in QGIS 1.7.²²

RESULTS

Serosurvey in schoolchildren. The 12 studied villages had a total population of registered schoolchildren of 1,792, of which we were able to collect a total of 1,649 blood samples (92%) indicating a very good level of participation in the study. All samples were tested for *Trypanosoma cruzi* antibodies by ELISA and HAI, and discordant samples were tested by IIFA. We obtained a total of 13 samples reactive by ELISA and HAI, and nine samples reactive by ELISA and IIFA, which gave a total of 22 positive samples for *Trypanosoma cruzi* antibodies, corresponding to a global seroprevalence of 1.3% (22/1,649; 95% CI = 0.9–2.0) in children aged 5–15 years in this region.

The seroprevalence varied from 0% to 3.7% among the study villages, but these differences were not statistically significant ($\chi^2 = 11.7$, $df = 11$, $P = 0.38$; Table 1), suggesting a rather homogenous distribution of *Trypanosoma cruzi* infection and transmission in the region (Figure 1). The highest seroprevalences were observed in the villages of El Inca (3.7%, 95% CI = 1.3–10.3), Chonta Cocha (3.2%, 95% CI = 1.2–8.9), and Bajo Huino (3.1%, 95% CI = 1.2–7.6). On the other hand, no anti-*Trypanosoma cruzi* antibodies were detected in the villages of Alto Huino, Centro Huino, and Puerto el Sol, most likely because of the small size of the school children population in these villages (40, 25, and 25, respectively).

The youngest positive child was 5 years old, and the oldest 15 years old. There was a slightly higher prevalence in older children (10/817, 1.2% versus 12/820, 1.4%, for 5–10 and 11–15 years old, respectively), but this was not significantly different ($\chi^2 = 0.17$, $df = 1$, $P = 0.67$). Similarly, there were no significant differences in the anti-*Trypanosoma cruzi* antibody seroprevalence between girls and boys (12/757, 1.6% versus 10/892, 1.1%, respectively, $\chi^2 = 0.67$, $df = 2$, $P = 0.41$) (Table 1).

The three families from Bajo Huino and the two from El Inca with a positive child were further studied. One older sister (aged 22 years) was found positive in El Inca, all other family members were negative. Congenital transmission may thus be discarded for these five cases, and vector-borne transmission is thus very likely.

Serosurvey in pregnant women. The serosurvey of pregnant women included a total of 106 women, of which four resulted positives for *Trypanosoma cruzi* antibodies, corresponding to a seroprevalence of 3.8% (95% CI = 1.5–9.3) in this population. One woman was from the village of Juan Pío Montúfar and one from San José de Payamino (Figure 1),

TABLE 1
Seroprevalence of *Trypanosoma cruzi* antibodies in children aged 5–15 years in Loreto County

Village	Boys	Girls	Total
Bajo Huino	1/68 (1.5% [0.3–7.8])	3/63 (4.8% [1.7–13.1])	4/131 (3.1% [1.2–7.6])
El Inca	1/37 (2.7% [0.6–13.8])	2/44 (4.5% [1.4–15.1])	3/81 (3.7% [1.3–10.3])
Centro Huino	0/10 (0% [0–28.5])	0/13 (0% [0–23.1])	0/23 (0% [0–14.2])
Alto Huino	0/23 (0% [0–14.2])	0/15 (0% [0–20.6])	0/38 (0% [0–9.0])
Puerto Murialdo	1/108 (0.9% [0.2–5.0])	1/108 (0.9% [0.2–5.0])	2/216 (0.9% [0.3–3.3])
Puerto El Sol	0/12 (0% [0–24.7])	0/10 (0% [0–28.5])	0/22 (0% [0–14.8])
Chontacoc	2/48 (4.2% [1.3–14.0])	1/46 (2.2% [0.5–11.3])	3/94 (3.2% [1.1–8.9])
Juan Pío Montúfar	1/82 (1.2% [0.3–6.5])	1/71 (1.4% [0.3–7.5])	2/153 (1.3% [0.4–4.6])
San Francisco Asís	2/203 (0.9% [0.3–3.5])	1/125 (0.8% [0.2–4.3])	3/328 (0.9% [0.3–2.6])
Jumandi Centro	0/28 (0% [0–11.9])	1/22 (4.5% [1.1–21.9])	1/50 (2.0% [0.5–10.4])
Huiruno	1/84 (1.2% [0.3–6.4])	1/58 (1.7% [0.4–9.1])	2/142 (1.4% [0.4–5.0])
24 de Mayo	1/189 (0.5% [0.1–2.9])	1/182 (0.5% [0.1–3.0])	2/371 (0.5% [0.2–1.9])
Total	10/892 (1.1% [0.6–2.0])	12/757 (1.6% [0.9–2.7])	22/1,649 (1.3% [0.9–2.0])

Data are presented as *n* positives/*N* analyzed (% [95% confidence interval]). There were no significant differences between boys and girls ($\chi^2 = 0.67$, *df* = 2, *P* = 0.41), nor among villages ($\chi^2 = 11.7$, *df* = 11, *P* = 0.38).

and two from the village of Pimampiro, from the county of Sachos further north of Loreto County. There was no statistical difference in the seroprevalence observed between the two maternities (2/30; 6.7%, 95% CI = 2.0–21.4 versus 2/76; 2.6%, 95% CI = 0.8–9.1, for the Loreto Health Center and for the Orellana Provincial Hospital, respectively, $\chi^2 = 0.87$, *df* = 1, *P* = 0.35).

Triatomine search. The timed-manual search for triatomine bugs in four houses from El Inca was negative as no bugs were collected. On the other hand, the dissection of two palm trees close to houses in Juan Pío Montúfar resulted in the collection of six adult triatomines, four corresponding to *R. pictipes*, two to *R. robustus*.

DISCUSSION

In this study, we evaluated the seroprevalence of anti-*Trypanosoma cruzi* antibodies in the Loreto County, Orellana

Province in the Amazon region, and we focused on two critical populations: schoolchildren aged 5–15 years and pregnant women. We detected an overall seroprevalence of 1.3% in schoolchildren aged < 15 years, indicating the presence of active *Trypanosoma cruzi* transmission in these communities. The seroprevalence in these children even reached over 3% in several communities and was significant even in the youngest age group (1.2% in 5–10 years old), suggesting an important incidence rate even in recent time (at least 0.12% per year). In two of the villages, congenital transmission could be discarded, and vectorial transmission is thus the most likely, although oral transmission may also be possible.

We also observed a seroprevalence of 3.8% in pregnant women, which is as expected higher than that of school children. These cases were notified to the Provincial Health Authorities in Orellana city for follow-up and treatment of the mother and child, but no follow-up by the research team was performed. Nonetheless, these data suggest that there is a

FIGURE 1. Distribution of *Trypanosoma cruzi*-seropositive children in Loreto County. The insert shows the map of Ecuador with the province of Orellana in darker gray. The main map shows the location of the study villages with the size of the gray/red circles proportional to observed seroprevalence of anti-*Trypanosoma cruzi* antibodies, which is also indicated for each village. White circles indicate villages where no seropositives were detected. Diamonds indicate the location of *Trypanosoma cruzi*-seropositive pregnant women.

significant risk of congenital transmission and *Trypanosoma cruzi*-associated reproductive complications in this region.²³ Based on a total number of births in the Loreto County of 255 per year, about 10 newborns from a *Trypanosoma cruzi*-infected mother may be expected per year. Further studies should help define more precisely the risk of congenital transmission for these newborns, and implement current international guidelines for their diagnosis, treatment, and care.^{16,24}

Taken together, our results are very consistent with previous studies in the Amazonian region of Ecuador, although no cases were previously reported for the Loreto County in the large study by Grijalva and others.⁸ This may have been due to the small sample used for this region in the previous work ($N = 135$ samples). However, compared with neighboring regions, the seroprevalence we observed is indeed similar to that reported in recent studies,²⁵ and does not seem to have changed much over the past 15 years.^{6–8} Together with the lack of significant differences among villages we observed, this suggest a rather constant, homogenous, and persistent *Trypanosoma cruzi* transmission over this time in the region. This transmission had been qualified as continuous and low-intensity transmission, and generating a hypoendemic pattern by previous authors.⁷

Triatomine species responsible for *Trypanosoma cruzi* transmission in the Ecuadorian Amazon are known to belong principally to *R. robustus* and *R. pictipes* species.^{11,12} These present very limited colonization of houses, and our limited active search in the village of El Inca is in agreement with a lack of a high infestation/colonization of dwellings by triatomine bugs. These species are indeed rather considered sylvatic, but adult bugs occasionally fly to enter houses to feed on humans,^{6,11,12} and our detection of both species in palm trees close to a village support this scenario of transient intrusion by these triatomines. Risk factors for infestation are still poorly understood, but appear to include housing quality and structure, as seropositive patients are more likely to live in houses with walls of open or mixed type construction and made of materials such as bamboo cane and wood boards and have thatched roof.⁸ These triatomines are also believed to be attracted by artificial light,²⁶ which may thus be an additional risk factor for infestation as observed for other species.²⁷ Further studies aimed at a deeper understanding of these determinants of infestation may help focus vector control intervention on those determinants. Indeed, this behavior poses a challenge for vector control, as conventional insecticide spraying is of limited efficacy against intrusive triatomines as observed in other settings.²⁸ This appears confirmed by the lack of association between intradomiciliary insecticide spraying and the seropositivity of inhabitants against *Trypanosoma cruzi* in the Amazon region.⁸ Thus, alternative vector control intervention, such as the use of insect screens,^{29,30} need to be considered to reduce or prevent human–vector contact in these conditions.

In conclusion, we detected a seroprevalence of anti-*Trypanosoma cruzi* antibodies of 1.3% in schoolchildren aged 5–15 years, indicating the persistence of a constant and active vectorial transmission of *Trypanosoma cruzi* in the Loreto County of the Ecuadorian Amazon region. Although this transmission has been considered as low compared with regions of higher incidence, it remains highly significant, and nonconventional vector control should be implemented to stop triatomine intrusion into houses and vector–human con-

tact. We also observed a seroprevalence of 3.8% in pregnant women, indicating a clear risk of congenital transmission. Further studies should help define this risk more precisely and implement current international guidelines for the diagnosis, treatment, and care of these cases, as well as effective vector control interventions.

Received December 16, 2014. Accepted for publication April 22, 2015.

Published online August 17, 2015.

Financial support: This work was funded in part by the Proyecto Prometeo of the Secretaria de Educación Superior, Ciencia, Tecnología of Ecuador to ED.

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