RISK FACTORS FOR *TRYpanosoma cruzi* HUMAN INFECTION IN BARINAS STATE, VENEZUELA

M. DORA FELICIANGELI,* MARIA J. SÁNCHEZ-MARTÍN, BENNY SUÁREZ, ROSALBA MARRERO, ANNHYMARIET TORRELLAS, ARTURO BRAVO, MEHUDY MEDINA, CINDA MARTINEZ, MAYELI HERNANDEZ, NEIDI DUQUE, JOSÉ TOYO, AND ROBERTO RANGEL

Facultad de Ciencias de la Salud, Centro Nacional de Referencia de Flebótomos y Otros Vectores, Universidad de Carabobo, Maracay, Venezuela; Dirección General de Salud Ambiental, Ministerio de Salud, Maracay, Venezuela; Coordinación Regional de Salud Ambiental y Contraloría Sanitaria, Ministerio de Salud, Barinas, Venezuela

**Abstract.** This study attempted to quantify the transmission of *Trypanosoma cruzi* in children less than 15 years of age in Barinas State, Venezuela and investigate risk factors for infection. Among 3,296 children, 4 (0.12%) were seropositive. The mother of one child also was also seropositive, which suggested that congenital transmission is a possible risk factor for Chagas disease in this area. Seroprevalence among the dwellers of 10 localities was 3.3%. *Rhodnius prolixus* was detected in 7 localities and in 8% of 125 dwellings. A multivariate logistic regression model showed that infection was associated with age, a dirt floor, and distance from houses to palm trees. The risk of infection is increased by the presence of adventitious sylvatic *R. prolixus* and transient or residual colonies. Insecticide spraying does not seem justified in this scenario, a finding that was also observed in other Latin American countries. New methods are therefore needed for Chagas disease control programs.

**INTRODUCTION**

Chagas disease is caused by *Trypanosoma cruzi* (Kineto-plastida: Trypanosomatidae), a protozoan with mammalian hosts that is transmitted to humans mainly by triatomine bugs (Hemiptera: Reduviidae). It is found exclusively in the New World, where it is distributed in 18 countries from Mexico to Argentina. The annual burden of this disease estimated by the World Health Organization in 2002 was 649,000 disability-adjusted life years and 13,000 deaths (http://www.who.int/tdr/diseases/chagacases/). Since its discovery in Brazil by Carlos Chagas, several national and international co-operative efforts have been launched towards the interruption of the transmission of Chagas disease. Evaluation of the impact of intervention activities are presently focused mainly on two aspects: detection of persistent active foci through infection in children as potential sentinel populations and detection of residual triatomine infestation in intervened areas and its association with seroprevalence.

In Venezuela, a long-term Chagas disease control program (ChDCP) of the Ministry of Health (MoH) was started in the 1950s and conducted until the end of the 1990s. The control strategies were based on vector control using residual insecticide spraying of rural houses and peridomestic shelters, the construction or improvement of the rural houses through an extensive national rural housing program that was started in 1958, and mandatory screening for *T. cruzi* infection in blood banks by enzyme-linked immunosorbent assay (ELISA) that was started in 1988. From 1952 to 1998, 1,638,605 kg of dieldrin, lindane, propoxur, fenitrothion, and deltamethrin and 16,857 liters of lambdacyhalothrin were used. By April 2000, 443,522 houses for > 2,400,000 inhabitants had been reconstructed in which cement block walls and zinc roofs replaced mud-walled palm-roofed huts. The long-term effects of the impact of the ChDCP on serologic results, electrocardiographic abnormalities, and clinical outcomes have been evaluated in prospective studies at the regional level and retrospective studies at national level and have shown important effects on disease transmission. These measures reduced overall seroprevalence from 44.5% to 9.2% and annual incidence of infection from approximately 10 per 1,000 people in the 1950s to 1 per 1,000 in the 1980s. However, analysis of epidemiologic data collected in 1991–2000 by the MoH has shown that despite this success, transmission has not been interrupted and may be increasing. However, it is not fully understood how transmission currently occurs, even after 40 years of control, because no studies that correlated human infection with *T. cruzi* with demographic, environmental, and entomological data have been recently conducted in Venezuela.

In this report, we present results of an epidemiologic study in Barinas, one of the states where several acute cases have been recently reported. We attempted to quantify transmission of *T. cruzi* by screening for antibodies to *T. cruzi* in children less than 15 years of age in a sample representative for this age group in this state. We also screened all people in households for seropositivity to *T. cruzi* in a sample of localities in an attempt to detect risk factors for infection.

**MATERIALS AND METHODS**

**Study area.** Barinas is located in southwestern Venezuela (7°16'48"–9°05'00"N and 67°30'00"–71°49'00"W). It has an area of 35,200 km² (3.84% of Venezuela) and 624,508 inhabitants (2001 population census available from http://www.ine.org.ve). The mean ± SD temperature is 26 ± 3°C and the annual precipitation ranges from 1,300 mm to 1,500 mm. The predominant Holdridge life zones are a tropical dry forest in the eastern region and a subtropical wet forest in the western region. The main economic activity is agriculture and cattle breeding, although forestry was also an important economic activity in Barinas until extensive deforestation of 20,661 hectares between 1997 and 2000. The localities included in this study are rural settlements where people cleared forested areas for agriculture and raising cattle. The peridomestic area is characterized by chicken coops, cow-
sheds, and pigsties, typically between 5 and 20 meters from houses, which are dispersed. Most villages have a primary school, but the residents have to travel to larger villages for health care. Since the middle of the last century, the vector control program using residual spraying has been mainly directed on a large scale for malaria control and more recently for dengue control, which are the major disease concerns in this area. However, vector control activities of the ChDCP are limited to spraying localities where the presence of vectors is reported by the inhabitants.

This study was reviewed and approved by the Committee of Bioethics and Biosecurity of the Ministry of Science and Technology. The study was conducted between April 2003 and March 2004. Each locality was visited initially to inform residents about the objectives and activities of the study. Each household received educational leaflets describing Chagas disease (signs and symptoms), transmission patterns, and the role of vectors. Residents were also informed that all people seropositive for *T. cruzi* would receive appropriate medical care. All participants signed an informed consent form.

**Cross-sectional survey of children less than 15 years of age.** To obtain a random sample of children for serologic screening, localities selected had to conform to the following inclusion criteria: 1) localities must have had < 5,000 inhabitants (cut-off value used by the census office to register a locality as rural), 2) the sample must have been obtained from all municipalities in Barinas State, and 3) rural localities for all altitude ranges must have been selected from the referenced localities of the Instituto Geográfico Simón Bolívar (Caracas, Venezuela). Localities were classified into altitude categories at 100-meter intervals from 0 to 1,600 meters above sea level, the latter being the maximum altitude recorded for a locality in Barinas State. From those categories with more than 100 localities, 2% of the localities were randomly selected (most localities were between 0 and 200 meters). For those categories with less than 100 localities, 2 were randomly selected. This resulted in a total of 85 localities as shown in Figure 1. Most children (3,296) more than two years of age from the 85 selected localities were surveyed. This number was greater than the sample size of 2,122 children required to detect a seroprevalence of at least 0.5% on the basis of data obtained from the Ministry of Health (seroprevalence of 0.9% for antibodies to *T. cruzi* in persons 0–15 years of age in a total population of 239,228 children in Barinas State; http://www.ine.org.ve).

**Risk factors for *T. cruzi* infection.** To identify risk factors for *T. cruzi* infection, the population in 10 of 85 localities studied was screened, a questionnaire was completed by interviewing an adult resident, and direct observation of variables in each house were made. Variables recorded in the questionnaire were age, sex, total number of residents in the household, time of residence in the house (years), level of education level (illiterate, literate, primary education, secondary and greater), occupation (farmer, housewife, student, unemployed, other), time since last visit by the MoH personnel (< 6 months and > 6 months), time since last insecticide

![Figure 1](http://www.ajtmh.org)
spraying of the house by the MoH (≤ 12 months and > 12 months), residents’ reports of having seen (yes or no) triatomines in different areas of the house (bedroom, living room, kitchen, peridomestic area), and residents’ reporting of knowing about the presence or absence of triatomines in nearby houses. Variables recorded by direct observation of the interviewer were wall type (cement blocks, wood, mud and straw [adobe] or mud-stick [bahareque], palm, metal), wall plastering (unplastered, partially plastered, totally plastered), roof type (palm, zinc, metal), and floor type (cement, dirt). A house with one or more of the following components (dirt floor, walls of adobe or bajareque, wood, palm leaves, metal, and roof of palm leaves) was classified as a rancho, and a house with at least a concrete or cement walls block and a zinc roof or another of better quality was classified as a house. Other variables recorded were the size of the house, which was used to calculate the crowding index (no. of people in the houses/m² area of the house × 100), the presence or absence of animals in the house, the presence or absence and species of animals in the yard, the presence or absence and type of outbuildings such as a troja (a peridomestic outbuilding used for storage of household goods) in the peridomestic area, and the distance from the house to the nearest palm tree (< 30 meters, 31–60 meters, and > 60 meters). Other variables on knowledge and attitudes of people towards the disease were also investigated and these have been previously reported.19

Serologic methods. Two blood samples (each 1 mL) were obtained by micro-capillary from each person and placed on Whatman (Brentford, United Kingdom) no. 1 filter paper, kept dry in plastic bags, and transported to the Chagas Disease National Reference Laboratory (Maracay, Venezuela). Three serologic tests were used for detecting antibodies to T. cruzi: an indirect immunofluorescent test (IFAT),20 an indirect hemagglutination test (IHT),21 and an ELISA. Antigens for the IHT and ELISA were soluble protein extracts of T. cruzi produced at the Instituto de Medicina Tropical, Universidad Central de Venezuela (Caracas, Venezuela) according to the procedure of Mackelt.22 Cut-off points for each test were 1:32 for the IFAT, 1:32 for the IHT, and an optical density value of 0.30 for the ELISA. Samples were considered positive when a positive reaction was obtained in at least two of the three tests performed.

Entomologic methods. We collected triatomines in 125 of 166 houses in the 10 localities where all residents were sero-surveyed (Figure 1). The entomologic method used was collection by residents, which was most effective when compared with other passive and active search methods in a previous study in Portuguesa State (Feliciangeli MD, unpublished data). Pots were distributed to residents and they were instructed to collect any triatomines they found during a one-month period and place them in the pots. The pots were then collected by study personnel. Triatomine bugs were classified by sex and stage according to the procedures of Lent and Wygodzinsky.23 and feces was examined for T. cruzi infection. Positive slides were stained with Giemsa and parasites were identified by morphologic characteristics and confirmed by polymerase chain reaction using technique previously reported.24

Statistical analysis. Data were analyzed with STATA version 8 software (Stata Corp., College Station, TX). All continuous variables were transformed to ln (X) to normalize the data distribution. Univariate logistic regression analyses were performed for each of the variables recorded in the questionnaire to determine the odds ratios (ORs) of significant risk factors for infection (i.e., positivity to T. cruzi infection) after clustering by house (because several residents lived in the same house) and to identify risk factors associated with triatomine infestation in houses (presence of triatomines collected by residents) after clustering by locality (this generated robust standard errors). Multivariate stepwise logistic analyses initially included all variables with a P value < 0.1. Variables with P values > 0.05 were then removed stepwise from the maximal model until only significant variables remained. Finally, each excluded variable was added to this minimal model to confirm lack of explanatory power. When zero cases occurred in the referent categories of the exposure groups and ORs could not be calculated, such categories were excluded from the analysis; thus, the number of observations varied slightly between analyses.

RESULTS

Seropositivity in children less than 15 years of age. A total of 3,296 children (1,703 boys and 1,593 girls) less than 15 years of age who lived in 1,234 households were tested. The overall seroprevalence to T. cruzi was 0.12% (4 children positive: 2 boys 10 and 12 years of age and 2 girls 13 and 8 years of age). None of the children had history of travel outside the study area or blood transfusions. However, screening of their relatives for antibodies to T. cruzi showed that the mother of one of them was seropositive. All lived in localities at elevations less than 150 meters above sea level, 3 in the municipality of Pedraza and 1 in the municipality of Barinas.

Risk factors for infection. A total of 841 blood samples (93.0%) were collected from 904 people living in 166 houses in Socó and Quebradón (municipality of Cruz Paredes), Florida (municipality of Rojas), and San Rafael de Catalina, Cruz Verde, Potrerito, Salomé, Caimana, Rosalía, and Pionio (municipality of Pedraza) (Figure 1). Overall seroprevalence was 3.3% (28 of 841). Results of univariate logistic regression of the survey across the entire population are shown in Table 1 only for variables that resulted significant at P < 0.05. Risk or protective factors associated with T. cruzi infection in humans after adjusting for other possible confounding factors are shown in Table 2. Among the demographic variables (age, occupation, and level of education), only age (OR = 1.07) was a significant risk factor for seropositivity by both univariate (Table 1) and multivariate (Table 2) analyses. The youngest individual infected in these 10 localities was 8 years of age and the oldest was a woman 102 years of age.

Although some house characteristics variables (such as metal and palm walls) were significantly associated with the seroprevalence by univariate analysis (Table 1), only the presence of dirt floors remained significant in the multivariate model. The risk of infection with T. cruzi significantly increased among people living in houses with dirt floors (OR = 4.0) compared with those living in houses with cement floors. The presence of a palm tree within a 31–60-meter radius from the house (16.9% of the houses) reduced the probability of infection (OR = 0.1) compared with those living in a house with palm trees within a 30-meter radius (77.5% of the houses). However, this effect was not statistically significant at a greater distance, probably because of low numbers (5.5% of houses) (Table 2).
Risk factors associated with house infestation with triatomines. Triatomine bugs were collected by people in 7 of 10 localities and the house infestation index in the area was 8% (10 of 125). Residents reported that bugs entered houses frequently from the outside and were caught more frequently in close proximity to electric lights and TV screens and in bedrooms. A total of 41 Rhodnius prolixus, 3 Triatoma maculata, and 1 Eratyrus mucronatus were caught. On the 37 R. prolixus examined, 2 adults were positive for T. cruzi (5.4%), as well 2 of the 3 T. maculata adults.

Among the variables that were associated with the presence of triatomine bugs in the house by univariate analysis (Table 3), only number of residents, presence of a troja in the peridomestic area, and houses that had been visited less than 6 months earlier by MoH personnel or sprayed less than 12 months earlier by MoH personnel or sprayed less than 6 months earlier by MoH personnel or sprayed less than 6 months earlier by MoH personnel.
months earlier were predictive of bug infestation in the multivariate model (Table 4). However, there was no association between seropositivity and residents reporting infestation with triatomines in the house after controlling for possible confounding effect of other variables associated with seropositivity (age, type of floor, and distance of palm trees from the house) (OR = 1.24, 95% confidence interval = 0.037–4.11, P = 0.71).

**DISCUSSION**

This study quantified transmission of *T. cruzi* in children less than 15 years of age in Barinas State, Venezuela by serologic screening of a representative and randomly selected sample. A seropositive child whose mother was seropositive for *T. cruzi* may suggest congenital transmission in the absence of past and present infestations. However, this was observed in only one child in this study, and few children (0.12%) were infected. This value is less than that reported by the ChDCP (0.5%).

As observed in previous studies in Venezuela, Argentina, and Ecuador, the likelihood of being infected with *T. cruzi* increased with age because of the increased time of exposure and the life-long persistence of infection. The household structure (rancho versus house) and type of wall (palm, wood, mud or cement) favorable for triatomine infestation and colonization compared with the type of wall (cement blocks) unfavorable for these parameters were not predictors of seropositivity or triatomine infestation in Barinas State. This finding is contrary to what had been reported in studies in Venezuela. It appears that house structure per se does not seem to be the main risk factor for infection or infestation. The only house characteristic associated with infection was the type of floor. Living in a house with a dirt floor was a positive predictor for infection with *T. cruzi*, probably because it indirectly reflects the poor quality of life and hygiene in these houses. These factors are often associated with a large number of persons per households, which in this study was also significantly associated with the presence of bugs and is consistent with results of a previous study in Barinas.

The proximity of palm trees to houses was an explanatory factor for human infection with *T. cruzi* (e.g., when palm trees are located further from the house, one is least likely to find seropositive people living in that house), but there was no association between the distance from palm trees and *R. prolixus* infestation. This might be explained by the fact that although seropositivity is cumulative, house infestation was measured in a single cross-sectional survey. Conversely, adjusted odds of *R. prolixus* infestation increased considerably with the presence of a troja, which might represent a suitable peridomestic hiding place for this vector, which would then infest the house. Greater access to electricity in the rural villages, together with more accentuated phototropism, might be promoting active movement of bugs into the houses.

The presence of *R. prolixus* in houses was significantly associated with spraying by MoH personnel within the past 12

---

**Table 3**

Univariate analysis of risk factors associated with *Rhodnius prolixus* infestation in 125 houses of 10 localities in Barinas State, Venezuela

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>% positive for the risk factor</th>
<th>% with risk factor that were infested</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of residents (log transformed)</td>
<td>NA</td>
<td>NA</td>
<td>5.0 (2.5–9.8)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Crowding index (total residents/meter² house) × 100</td>
<td>NA</td>
<td>NA</td>
<td>1.2 (1.1–1.3)</td>
<td>0.001</td>
</tr>
<tr>
<td>Have residents seen triatomines in the bedrooms?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>59.2 (74)</td>
<td>4.1 (3)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>40.8 (51)</td>
<td>13.7 (7)</td>
<td>3.7 (0.9–14.8)</td>
<td>0.058</td>
</tr>
<tr>
<td>Presence of a troja in the peridomestic area</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>97.6 (119)</td>
<td>7.6 (9)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2.5 (3)</td>
<td>33.3 (1)</td>
<td>12.2 (1.0–146.0)</td>
<td>0.048</td>
</tr>
<tr>
<td>Time since last visit of MoH personnel</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>58.4 (73)</td>
<td>2.7 (2)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>≤ 6 months</td>
<td>22.4 (28)</td>
<td>21.4 (6)</td>
<td>9.7 (2.3–41.1)</td>
<td>0.002</td>
</tr>
<tr>
<td>&gt; 6 months</td>
<td>19.1 (24)</td>
<td>8.3 (2)</td>
<td>3.2 (0.5–21.5)</td>
<td>0.227</td>
</tr>
<tr>
<td>Sprayed by MoH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>68.0 (85)</td>
<td>2.4 (2)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>32.0 (40)</td>
<td>20.0 (8)</td>
<td>10.4 (1.9–57.0)</td>
<td>0.007</td>
</tr>
</tbody>
</table>

*NA = not applicable; OR = odds ratio; CI = confidence interval; troja = a peridomestic outbuilding used for storage of household goods; MoH = Ministry of Health.

---

**Table 4**

Explanatory factors of *Rhodnius prolixus* infestation in 125 houses of 10 localities in Barinas State, Venezuela

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of residents (log transformed)</td>
<td>5.0 (1.3–18.8)</td>
<td>0.021</td>
</tr>
<tr>
<td>Presence of a troja in the peridomestic area</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>6.0 (1.5–24.3)</td>
<td>0.011</td>
</tr>
<tr>
<td>Sprayed by MoH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>10.2 (1.8–57.0)</td>
<td>0.007</td>
</tr>
</tbody>
</table>

*OR = odds ratio; CI = confidence interval; troja = a peridomestic outbuilding used for storage of household goods; MoH = Ministry of Health.
months. There was also evidence of early house reinfection post-intervention, possibly from sources located in palm trees, which are relevant in the control of infestation.

This study identified a new epidemiologic situation in Barinas State in which the risk of infection was the result of adventitious specimens from sylvatic R. prolixus populations and the presence of low transient or residual domestic colonies. Although a high proportion of houses in the study area were ranchos, their walls are made of boards, which do not have cracks in which bugs can hide and do not favor an high bug population growth rate, as observed in ranchos with adobe and bahareque walls (mud and straw or mud-stick walls). Molecular studies showed that specimens from houses, peridomestic habitats, and palm trees in the area were indistinguishable. Moreover, wing geometric morphometry also showed that the residual domestic population of R. prolixus might play a role in village reinfection after a control campaign, but more importantly, sylvatic subpopulations play a significant role in reinfection. Therefore, because the domestic transmission cycle per se with abundant triatomine colonies in this area is not the predominant situation, widespread residual insecticide spraying does not seem to be justified. The Chagas disease elimination program in Venezuela appears to have been compromised by reinvasion of houses by sylvatic R. prolixus bug populations.

Continuous invasion of adventitious vectors compared with the classic intradomestic cycle of Chagas disease, which is easier to control, is not geographically restricted to Venezuela and is a matter of concern in the Brazilian Amazon, Ecuador, and Costa Rica. Thus, in areas with such transmission, the basis of Chagas disease control programs must be reviewed because interruption of transmission, as indicated by the Southern Cone initiative and the Andean and Central America initiatives, is not sustainable with traditional costly programs. Pyrethroid-impregnated curtains or bed nets or use of more resistant wire gauze in windows and doors in well-constructed houses might be implemented to prevent contact between persons and sylvatic bugs. However, health promotion is essential to ensure community acceptance and participation for long-term sustainability of control intervention strategies.

Acknowledgments: We thank Diarmid Campbell-Lendrum and Clive Davies for valuable discussion in sample design, and the personnel of the MoH in all Demarcaciones and the families in the 85 communities of Barinas State for their helpful collaboration.

Financial support: This study was supported by the Ministry of Science and Technology, Venezuela (FONACIT-Agenda Salud 20000008) and the Wellcome Trust (Project no. 062984/Z/00Z).

Authors’ addresses: M. Dora Feliciangeli, Maria J. Sánchez-Martín, Benny Suárez, Rosalba Marrero, Annymariet Torrellas, and Arturo Bravo, Facultad de Ciencias de la Salud, Centro Nacional de Referencia de Flebótomos y Otros Vec- tores–BIOMED, Universidad de Carabobo, Sede Aragua, Apartado 4873, Maracay, Venezuela, Telephone: 58-243-242-5822, Fax: 58-243-242-5333, E-mail: mdora@movistar.net.ve, spinicrassa@yahoo.com.

REFERENCES