MIXED DOMESTIC INFESTATION BY RHODNIUS PROLIXUS STÄL, 1859 AND PANSTRONGYLUS GENICULATUS LATREILLE, 1811, VECTOR INCrimination, AND SEROPREVALENCE FOR Trypanosoma cruzi AMONG INHABITANTS IN EL GUAMITO, LARA STATE, VENEZUELA

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Abstract. Mixed infestation of nymphs and adults of Rhodnius prolilxus Stål, 1859 and Panstrongylus geniculatus Latreille, 1811 was detected in 3 (15%) of 20 dwellings in El Guamito, an endemic focus of Chagas disease in Lara State, Venezuela. In one of the houses, both species were positive for Trypanosoma cruzi. 14.3% (R. prolilxus) and 20% (P. geniculatus). The overall infection rate in 143 of 352 R. prolilxus was 16.1%. Parasites isolated from R. prolilxus were identified as T. cruzi I by random amplified polymorphic DNA analysis. Dot–enzyme-linked immunosorbent assays of 36 R. prolilxus showed that 58.3% of the R. prolilxus had fed on humans. The gut contents of one fifth-instar nymph of P. geniculatus that was positive for T. cruzi also reacted with anti-human serum. A questionnaire was used to gather data on the demographic and socioeconomic characteristics of the population. An indirect immunofluorescent test, an indirect hemagglutination test, and an ELISA were used to detect the presence of antibodies against T. cruzi in 84 of 86 inhabitants and in 15.5% of people more than 20 years old. The relative risk (RR) of infection was greater in men than in women (RR = 1.61, 95% confidence interval = 0.54–4.80). Of the people more than 15 years old, 36.6% had no formal education. All respondents recognized triatomine bugs, but they did not relate them to Chagas disease transmission. A total of 85.7% of the houses were “ranches” suitable for the colonization of triatomine bugs. The possible domiciliation of P. geniculatus and the implications of competition with R. prolilxus for resources are discussed. Since there is no clear separation of food sources, abiotic factors such as microclimatic variation within houses may be critical to predict the outcome of the process of competition and potential domiciliation of this generally sylvatic species.

INTRODUCTION

Human Chagas disease is caused by the parasitic protozoan Trypanosoma cruzi (Kinetoplastida: Trypanosomatidae) that is mainly transmitted through fecal contamination by triatomine bugs (Hemiptera: Reduviidae). It is endemic in all Latin American countries (20°N–45°S), where approximately 80–100 million people are at risk and 11–12 million people are estimated to be infected.1

In Venezuela, a national Chagas Disease Control Program was undertaken in the 1960s with the aim of interrupting intradomestic transmission by vector control using residual spraying of dieldrin indoors and hexachlorocyclohexane outdoors and replacing the mud-walled, palm-roofed huts with doors and replacing the mud-walled, palm-roofed huts with

MATERIAL AND METHODS

Study area. The research was carried out in El Guamito (09°47′00″N, 69°20′14″W) in the Municipality of Iribarren, Lara State, Venezuela, a wet pre-mountain forest with an approximately average annual precipitation between 1,200 and 2,200 mm and an annual temperature range of 18–24°C.11 El Guamito is a small village of 25 houses scattered from 429–1043 m, sur-
rounded by a large variety of subsistence agriculture (coffee, banana, avocado, corn, beans) (75%), with secondary forest (10%) and primary forest (15%). Most of the houses are made of a structure of sticks plastered by a mixture of mud and straw, which is called “bahareque” in the colloquial language, with thatched or zinc roofs. Very few were constructed of sun-baked mud bricks, which are called “adobe” houses.

**Entomologic study.** Searching for triatomine bugs and natural infection with trypanosomatids. In response to the reported presence of Chagas disease vectors, an initial visit to El Guamito was carried out in November 2000. House searches were conducted using torches and fine forceps in cracks in walls, pictures on the walls, beds, and household goods in 10 houses. The finding in two houses of mixed infestations by *R. prolixus* and *P. geniculatus* led to subsequent visits for more extensive collections. The triatomine bugs were transported to the laboratory, where feces or gut contents were examined for the presence of trypanosomatids. The first group of positive fecal samples were fixed in methanol for five minutes, dried, and stained with 10% Giemsa. Additionally, a small amount of each sample was kept in lysis buffer (10 mM Tris, pH 8, 10 mM EDTA, 1 µg/µL of proteinase K) for a polymerase chain reaction (PCR). Inclusions and cultures were attempted as described later in this report.

**Identification of *T. cruzi*.** Parasites from *R. prolixus* were obtained by pulling out the digestive tract, mixing in two or three drops of physiologic saline, inoculating intraperitoneally into laboratory mice and subsequent blood culture, and/or direct culture of bug feces in diphasic blood agar medium. After isolation, the parasites were grown at 26°C in RPMI 1640 medium (GIBCO-BRL, Paisley, Scotland) supplemented according to the method of Miles.13 Genomic DNA was isolated and purified using the Nucleon DNA Extraction Kit (Amersham Pharmacia Biotech, Ltd., Bucks, United Kingdom) following the manufacturer’s instructions. The resulting DNA was suspended in 100 µL of TE buffer (10mM Tris-HCl, pH 7.2, 1mM EDTA). The isolates were identified and typed to *T. cruzi* group using the random amplified polymorphic DNA (RAPD) technique as reported by Carrasco and others.14 Alternatively, parasites were detected after extraction of genomic DNA directly from *P. geniculatus* feces using the Wizard Genomic DNA Purification System (Promega, Madison, WI), followed by amplification of a 280-basepair product, with specific primers derived from the sequence of a 480-bp fragment obtained by RAPD analysis with primer A2. This sequence has been shown to be species specific for *T. cruzi* infected. The sequences of the 480-bp morphic DNA (RAPD) technique as reported by Carrasco et al. (unpublished data). Parasite DNA was amplified in an MJ thermal reactor (PTC200; MJ Research, Inc., Waltham, MA). Amplified DNA products were analyzed by electrophoresis on agarose gels, stained with ethidium bromide, and photographed on an ultraviolet transilluminator.

**Blood meal identification by dot-ELISA.** Blood meal identification was carried out on triatomine bugs positive for *T. cruzi* (n = 17) and on a random sample of negative bugs (n = 20). The technique used was previously standardized for identification of blood meals in phlebotomine sand flies.15,16 We used IgG peroxidase antiserum conjugates against humans, mice, and some domestic animals (dog, chicken, pig, cow, and horse) (Sigma, St. Louis, MO). Serum dilutions in (phosphate-buffered saline [PBS] 1:100) from selected hosts were used as positive controls. Triatomine blood meal samples on filter paper were eluted individually in 100 µL of PBS (pH 7.4) at overnight at 4°C. After 24 hours, 5 µl of each eluate was transferred to small piece of nitrocellulose membrane in flexible polyvinyl chloride plates and incubated for one hour at 37°C. The plates were blocked with 150 µL of PBS, 1% bovine serum albumin for 30 minutes at room temperature and then washed three times with PBS, 0.05% Tween 20. The anti-IgG host-specific peroxidase conjugate was diluted in PBS, 0.05% Tween 20 according to previously determined ratios, mixed with 50 µL of each heterologous serum to reduce cross-reactivity and to enhance specificity, incubated for one hour at 37°C, and washed three times with PBS, Tween 20. Peroxidase substrate (4-cloro-naphthol) was added and incubated in the dark for 30 minutes. The reaction was stopped by washing with distilled water. The samples were considered positive if defined blue-purple spots developed on an antigen dot.

**Epidemiologic study.** With the aim of evaluating the prevalence of antibodies to *T. cruzi* among the population at risk, in May 2001 families of 21 houses gave informed consent for inclusion in a study according to a protocol reviewed and approved by the Committee of Bioethics of the Instituto de Altos Estudios Dr. Arnoldo Gabaldon of the Ministry of Health and Social Development. A questionnaire on demographic data (age, sex, occupation, education, time in the locality, knowledge of Chagas disease) structure of the houses, and peridomestic habitat was completed with the help of the householders. Finger prick blood samples were collected on Whatman (Clifton, NJ) no. 1 filter paper to screen for antibodies to *T. cruzi*. Samples were kept dry in plastic bags and transported to the Laboratorio de Chagas, Dirección de Salud Ambiental y Contraloría Sanitaria, Ministry of Health and Social Development (Maracay, Venezuela).

Three serologic tests were used for detecting antibodies to *T. cruzi* as routinely applied at national level: 1) an indirect immunofluorescent test (IFAT),17 2) an indirect hemagglutination test (IHT),18 and 3) an ELISA (Code UM 2014; UMELISA Chagas-SUMA, Havana, Cuba). The IHT and ELISA antigens were soluble protein extracts of *T. cruzi* produced at the Instituto de Medicina Tropical, Universidad Central de Venezuela (Caracas, Venezuela) according to the method of Mackel.19 Cut-off points for each test were IFAT: 1:32, IHT: 1:32, and ELISA: 0.030 optical density values. Samples were considered positive when a positive reaction was obtained in two of the three tests.

**RESULTS**

**Entomologic and parasitologic results.** Among 20 houses inspected for triatomine bugs, 14 were found positive for *R. prolixus* (infestation index = 70%). Table 1 shows the number of specimens collected, instars, and results of examination for *T. cruzi*. A total of 352 *R. prolixus* were collected, of which 143 were examined. Twenty-three were found positive for *T. cruzi* (infection index = 16.08%). A total of 79.8% of the bugs (n = 281) were collected in one house (No. 2). Ninety-nine were examined and 15.15% (n = 15) were infected.
Table 2 shows the results of triatomine bugs catches in the three houses where a mixed infestation of two triatomine species was found and their examination for *T. cruzi*. A total of 36 *R. prolixus* and 11 *P. geniculatus* were collected (3.3:1.0). Only one *P. geniculatus* was positive for *T. cruzi* (11.1%), a fifth-instar nymph.

**Identification of *T. cruzi***. The PCR amplification of a 280-bp nuclear DNA fragment in feces from *P. geniculatus* indicated the presence of *T. cruzi* in the intestine of this triatomine bug, as shown in Figure 1. The pattern of bands obtained by RAPD of the parasites isolated from *R. prolixus* corresponded to *T. cruzi I* when compared with the TCI (Z1) reference strain WA250 cl10, as shown in Figure 2. It is noteworthy that both species of triatomine bugs were found in the same house.

**Blood meal identification**. The sample obtained from the stomach of the fifth-instar *P. geniculatus* that was positive for *T. cruzi* reacted with the human antiserum. Seventy-five percent of the samples obtained from *R. prolixus* (*n* = 27) were reactive and 25% (*n* = 9) not reactive. Twenty-one blood meals reacted only with human antiserum (58.3%), one with human and dog antisera (2.8%), one with human and mouse antisera (2.8%), and four with chicken antisera (11.1%). Among 15 *R. prolixus* positive for *T. cruzi*, 13 (86.7%) had fed on humans.

**Demographic and serologic results**. The demographic and socioeconomic indicators showed high levels of poverty in El Guamito where 85.7% of the dwellings were “ranchos” suitable for colonization by triatomine bugs. At the time of the study, 51 men and 35 women lived there, of whom 38.4% were less than 15 years old. A total of 36.6% of the people had no formal education. The most frequent occupations

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**TABLE 1**

Rhodnius prolixus collected, examined and positive for *Trypanosoma cruzi* in 20 houses in El Guamito, Lara State, Venezuela

<table>
<thead>
<tr>
<th></th>
<th>No. (%) collected</th>
<th>No. (%) examined</th>
<th>No. (%) positive for <em>T. cruzi</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>74 (21.0)</td>
<td>29 (39.2)</td>
<td>5 (17.2)</td>
</tr>
<tr>
<td>Females</td>
<td>78 (22.2)</td>
<td>42 (53.8)</td>
<td>8 (19.1)</td>
</tr>
<tr>
<td>Instar</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>66 (18.8)</td>
<td>26 (34.9)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>IV</td>
<td>62 (17.5)</td>
<td>19 (30.6)</td>
<td>3 (15.8)</td>
</tr>
<tr>
<td>III</td>
<td>57 (16.2)</td>
<td>18 (31.6)</td>
<td>7 (38.9)</td>
</tr>
<tr>
<td>II</td>
<td>14 (3.97)</td>
<td>8 (57.4)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>I</td>
<td>1 (0.28)</td>
<td>1 (100)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Total</td>
<td>352</td>
<td>143 (40.6)</td>
<td>23 (16.08)</td>
</tr>
</tbody>
</table>

**TABLE 2**

Mixed infestation by *Rhodnius prolixus* (*Rp*) and *Panstrongylus geniculatus* (*Pg*) in 5 of 20 houses in El Guamito, Lara State, Venezuela

<table>
<thead>
<tr>
<th></th>
<th>Rp</th>
<th>Pg</th>
<th>Rp</th>
<th>Pg</th>
<th>Rp</th>
<th>Pg</th>
</tr>
</thead>
<tbody>
<tr>
<td>House no.</td>
<td>5</td>
<td>11</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>5 (2)</td>
<td>3 (3)</td>
<td>2 (0)</td>
<td>1 (1)</td>
<td>3 (3)</td>
<td>1 (0)</td>
</tr>
<tr>
<td>Females</td>
<td>2 (0)</td>
<td>1 (1)</td>
<td>3 (2)</td>
<td>2 (2)</td>
<td>1 (1)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Instar</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>1 (0)</td>
<td>7 (5)</td>
<td>3 (2)</td>
<td>2 (2)</td>
<td>1 (1)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>IV</td>
<td>2 (0)</td>
<td>2 (0)</td>
<td>2 (0)</td>
<td>2 (0)</td>
<td>2 (0)</td>
<td>2 (0)</td>
</tr>
<tr>
<td>III</td>
<td>3 (3)</td>
<td>2 (2)</td>
<td>1 (1)</td>
<td>2 (2)</td>
<td>1 (1)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>II</td>
<td>3 (2)</td>
<td>2 (2)</td>
<td>1 (1)</td>
<td>2 (2)</td>
<td>1 (1)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>I</td>
<td>10</td>
<td>2</td>
<td>21 (3+)</td>
<td>5 (1+)</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

*Values in parentheses are number of bugs dissected. + = no. of bugs positive for *Trypanosoma cruzi*. 

**FIGURE 1**. Polymerase chain reaction amplification with primers Tc83F and Tc83R of a 280-basepair (bp) DNA fragment from feces of triatomine bugs. Lane 1, *Rhodnius prolixus* from house 11; lane 2, *Panstrongylus geniculatus* from house 11; lane 3, *R. prolixus* from house 1; lane 4, degraded product; lane 5, purified DNA of *Trypanosoma cruzi* obtained from *R. prolixus* from house 11.

**FIGURE 2**. Random amplified polymorphic DNA profiles obtained with primer A2 of *Trypanosoma cruzi* stocks isolated from the intestine of *Rhodnius prolixus*. Lane M, 1-kb molecular mass marker; lane 1, *R. prolixus* from house 11; lane 2, *R. prolixus* from house 1; lane Z3 = reference strain CAN II; lane Z1 = reference strain WA250 cl10B; lane Z2 = reference strain Esmeraldo cl3. Values on the left are in basepairs.
were domestic activities (29.1%), farmers (37.2%), and students (8.1%).

All but two men were screened for antibodies to *T. cruzi*. The overall seroprevalence was 15.5% (nine men and four women) and all were more than 30 years old. No significant difference by sex was found. However, the relative risk (RR) of infection was greater in men than in women (RR = 1.61, 95% confidence interval = 0.54–4.80). All infected women were housekeepers and all of the men were farmers.

All of the heads of households said that they recognized triatome bugs: 14 (82.4%) said they had seen them inside houses. Additionally, 10 (58.8%) had seen them in the peri-domestic habitats. However, 78.9% said they knew nothing of Chagas disease, although 89.5% recognized the bugs as a problem for their family.

A total of 61.1% of the families had lived in the houses they occupied for more than 10 years. However, the rest were children of older inhabitants who had married and constructed their own houses in the same village. Eight houses had bugs and seropositive occupants, six houses had bugs but no seropositive occupants, three houses had no bugs but seropositive occupants, while three houses had no bugs and no seropositive occupants. Fisher’s exact one-tailed test showed no significant difference among the groups ($P = 0.5743$).

### DISCUSSION

The village of El Guamito in Lara State is a long established focus of Chagas disease in Venezuela, as demonstrated by the distribution of the presence of antibodies to *T. cruzi* among its inhabitants, which were detected only in adults, the majority of whom had lived all their life in El Guamito. The bias in relative risk towards men could be interpreted as the result of outdoor transmission, probably due to contamination of men with the urine or feces of *Didelphis marsupialis*, natural reservoir of *T. cruzi*, which are commonly hunted for food. However, because of the maintenance of poor dwelling conditions, which are highly favorable for the presence of *R. prolixus*, and because of the presence of *T. cruzi* in these vectors feeding on humans and dogs, recent infections cannot be excluded. The additional presence of *P. geniculatus* also infected with *T. cruzi* in a pre-imaginal stage and the fact that it had fed on humans confirms that not only *R. prolixus* but also *P. geniculatus* is involved in an active domestic cycle.

The RAPD analysis showed that the triatome bugs were infected with *T. cruzi I* (Z1). These results are in agreement with those obtained with many other isolates found circulating in domestic and sylvatic cycles in different localities in Portuguesa State in west-central Venezuela, which were also identified as *T. cruzi I*.

*Panstrongylus geniculatus* is a sylvatic triatome bug widespread in 16 Latin American countries, which is commonly associated with the armadillo *Dasypus novemcinctus* and only occasionally enters houses when attracted by light. It has been frequently reported to be infected with *T. cruzi*, and in Brazil mainly with *T. cruzi Z3*. The potential for domiciliation of *P. geniculatus* was suggested by Valente and others in the Amazon Basin of Brazil, where hundreds of specimens were found infesting pig sties adjacent to human dwellings and specimens were collected from 10 houses, in one of which it repeatedly attacked people. Although no human infections were detected, *T. cruzi I* (Z1) was isolated from bugs, pigs, and opossums collected in the area. This indicates the need for a program to control Chagas disease in the Amazon Basin. In northcentral Venezuela, 20 specimens of *P. geniculatus* of different stages were found in a house in Hoyo de la Puerta, Miranda State, associated with *Rattus rattus* living in a cavity inside the house. However, no investigation for *T. cruzi* infection was done. In both of these cases, no other triatome species was found associated with *P. geniculatus* nymphal stages, indicating that *P. geniculatus* successfully colonized the houses.

The present work in El Guamito, Lara State, Venezuela seems to be the first report of mixed infestation of *R. prolixus* and *P. geniculatus* adults and nymphs. Mathematical models, such as the Lotka-Volterra equations, have been used to hypothesize what would happen when two species live together in the same habitat. It is accepted that competition for food and space would occur, which might give rise to transient coexistence with eventual competitive exclusion to the point of the replacement of one species by the other. The logistic model predicts that competing species can co-exist only if interspecific competition is relatively weak compared with intraspecific competition. The competitive exclusion principle (CEP), as derived by Gause, states that two species that occupy the same habitat cannot also occupy the same ecological niche. Any two species that occupy the same niche will compete with each other to the detriment of one of the species, which will thus be excluded. The CEP assumes a number of conditions that must be met for it to be obtained. Among the most important, 1) the environment must be spatially uniform, 2) the environment must be temporally constant, 3) time must be sufficient to allow exclusion, and 4) species must have the opportunity to compete.

An example of transient co-existence of *T. dimidiata* and *R. ecuadoriensis* in Ecuador has been reported (Abad-Franch F, unpublished data). The ability of *Triatoma infestans* to replace *T. sordida* in a laboratory experiment is supported by observations of the spreading of *T. infestans* in domestic ecotopes in Bolivia, and is evidence of the successful replacement of one triatome species by another better adapted species after a period of stability of the human population, followed by significant migration. What is the case for *R. prolixus* and *P. geniculatus*? Theoretically, it could be argued that they could coexist indefinitely if there is a distinction between their niches, i.e., *P. geniculatus* exploiting the humid dusty burrow-like areas in the house (perhaps feeding only on domestic animals, e.g., dogs and/or chickens or pigs), leaving *R. prolixus* to occupy the drier parts and feeding on humans. However, the identification of the blood meal in one *P. geniculatus* collected in El Guamito indicates that this would not be the situation. Nevertheless, *P. geniculatus* and *R. prolixus* have different relative humidity requirements, which are higher for *P. geniculatus*. This factor may be critical in modulating the outcome of the process of competition between these two species and the domestication of this sylvatic species. In El Guamito, it was observed that the three houses where *P. geniculatus* was found shared similar features: all were made of adobe bricks rather than “bahareque”, and piles of adobe were found inside and outside the houses.

In conclusion, the follow-up of invasion of houses by *P. geniculatus* in the field, the study of the association of its
presence with the features of the niches occupied, and experimental simulation in the laboratory are necessary to confirm and predict if incipient domiciliation of *P. geniculatus* may give rise to successful domestic colonization.

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