HUMAN TRYPANOSOME INFECTION AND THE PRESENCE OF INTRADOMICILE *RHODNIUS PALLESCENS* IN THE WESTERN BORDER OF THE PANAMA CANAL, PANAMA

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Abstract. An entomologic search was carried out to collect intradomicile triatomines in dwellings from rural communities in the western border of the Panama Canal, Panama. Sixty-nine triatomines were collected inside 20 houses of 67 houses investigated. *Rhodnius pallescens* was the only triatomine species found and included adults of both sexes and nymphs. A significantly high *Trypanosoma cruzi* (72.7%) and *T. rangeli* (40%) vector infection rate was detected. Blood meal analysis showed that 68% of *R. pallescens* had fed on humans. Human serologic analaysis and hemoculture performed on inhabitants from triatomine-infested houses showed that 32.1% (18 of 56) of the samples were trypanosome infected. Thirteen samples (23.2%) had antibodies against *T. cruzi*. Six of these seropositive samples were from children less than 15 years old. *Trypanosoma rangeli* was isolated in five hemoculture samples, all from children less than 11 years old. The epidemiologic implications of these findings in terms of human infection are discussed.

INTRODUCTION

Over the last decade most Southern Cone and Central America countries have achieved significant progress in controlling *Trypanososma cruzi* vector transmission.[1,2] The main Chagas disease vectors from these countries have a stable domestic cycle, a situation that facilitates its elimination by insecticide spraying of infested houses. However, this experience needs to be evaluated and adapted to countries such as Panama with a particular eco-epidemiologic situations regarding Chagas disease. The main vector in Panama, *Rhodnius pallescens*, has been found mainly in the sylvatic habitat, with sporadic presence inside dwellings.[3–6] This vector behavior complicates the vector traditional chemical control measures and therefore the control of the disease itself.

Rhodnius pallescens is also the vector of *T. rangeli*, a nonpathogenic trypanosome transmitted through the saliva of triatomines, which is also endemic in Panama.[7] Previous studies in the country have documented that *T. cruzi* and *T. rangeli* infections are normally circulating in the sylvatic ecotope in a balanced association with *R. pallescens* and with several species of mammal hosts in its natural niches.[6,8–11]

The recently launched National Chagas disease Control Program in Panama is in the process of defining appropriate strategies to reduce vectorial transmission. It is therefore essential to update data regarding the biology and behavior of Chagas disease vectors in the country. To collaborate with this initiative and to obtain a more complete panorama of the actual status of Chagas disease in Panama, we analyzed the presence of Chagas disease vectors in human dwellings and the potential risk for human trypanosome transmission in the studied rural areas.

MATERIALS AND METHODS

The results presented are part of a larger eco-epidemiologic study on Chagas disease currently taking place in two adjacent rural communities (Los Hules and Las Pavas) located on the western border of the Panama Canal in the Province of Panama (Figure 1). The number of houses in these localities is 160 with an estimated population of 732 people in the year 2,000. The overall living standard of most of its inhabitants is low and houses constructed of natural materials are not uncommon in the area. The original vegetation in this region consisting of tropical forest, has been partially destroyed for agricultural and livestock activities. Currently, there is a high prevalence of royal palm trees (*Attalea butyracea*), which is considered a natural ecotope of *R. pallescens*.[12]

An entomologic search to collect triatomines in the domestic habitat was conducted with community participation. At first, field trips were performed to elementary schools and houses from both communities to provide written and oral information about Chagas disease and to train local inhabitants in the identification of triatomine bugs and in their safety collection. Plastic containers with filter paper inside and with a *R. pallescens* picture adhered to its surface were distributed among trained local inhabitants for the collection of bugs inside the houses. Once a week the containers were collected and transported to the laboratory facilities for proper identification and to record the number, sex, stages, and house location. Homes where bugs were collected or whose inhab-
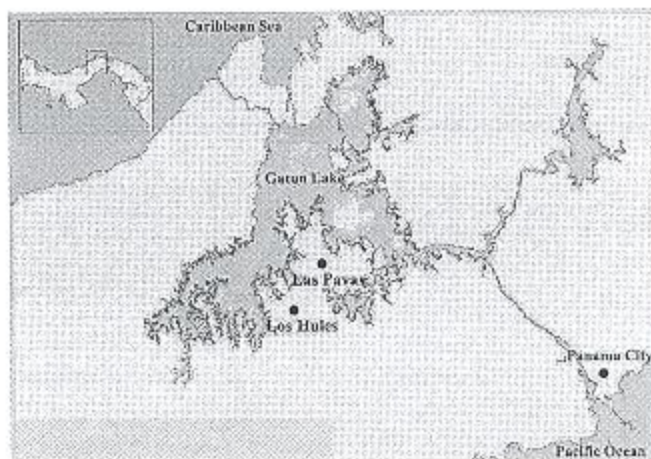

FIGURE 1. Panama Canal area showing the location of the studied sites. **Insert** shows Panama.

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itants reported their presence were further explored for triatomines by our field personnel.

Collected triatomines were completely macerated in 500 μL of phosphate-buffered saline (PBS) using sterile scissors. After centrifuging ($15,000 \times g$ for 10 minutes), the pellet was resuspended using a sterile wood applicator and the sample was centrifuged ($400 \times g$ for 5 minutes). The supernatant was collected and centrifuged ($15,000 \times g$ for 20 minutes). The parasite containing pellet was resuspended in 100 μL of PBS. A sample (10 μL) of this dilution was directly examined by microscopy for the presence of trypanosomes. In addition, DNA was extracted from this dilution using a commercial kit (Promega, Madison, WI) and used for the specific detection of *T. cruzi* and *T. rangeli* by a multiplex polymerase chain reaction (PCR).[13] The supernatant containing soluble proteins was used for blood meal identification by a dot-blot assay using polyclonal commercial antisera against IgG from chickens, mice (Jackson ImmunoResearch, West Grove, PA), and monkeys (MP Biomedicals, Irvine, CA), and monoclonal commercial antisera against IgG from dogs and humans (Sigma, St. Louis, MO).

Since opossums are considered the main reservoir of *Trypanosome* infection in Panama and no commercial antiserum is available, we produced antisera against whole IgG from a captured opossum using the standard methodology as previously described.[14] After obtaining informed consent, blood samples (5 mL) were collected by venipuncture from people living in houses where triatomines were captured. The serum was separated by centrifugation ($3,000 \times g$ for 5 minutes), and antibodies against *T. cruzi* was assessed by three serologic tests: an indirect immunofluorescence antibody test with a local *T. cruzi* isolate (Burunga strain) as antigen, a commercial recombinant enzyme-linked immunosorbent assay (ELISA) (ELISA Chagastest; Wiener Laboratory, Rosario, Argentina), and an immunoblotting technique with a crude epimastigote antigenic preparation derived from a Panamanian *T. cruzi* strain (Burunga).[15]

Samples were considered positive when they showed reactivity in at least two of the serologic tests used. Hemoculture of blood samples was performed as described by Vasquez and others,[16] and isolated trypanosomes were characterized by a multiplex PCR[13] coupled with hybridization using species-specific radiolabeled oligonucleotide probes.

RESULTS

Triatomine intradomicile collection performed by trained and motivated local inhabitants has represented a valuable strategy to capture sylvatic triatomines, which are more frequently found inside dwellings during the night, probably attracted by light or searching for food sources. Using this strategy, over a six-month period (June–December 2004), 69 triatomines were collected inside 20 of 67 houses searched in these rural communities. Most homes that were proved positive for triatomines were typical ranchos with thatched roofs and walls made with separate pieces of wood or cane that in most cases did not constitute an adequate barrier for insect invasion. All specimens were identified as *R. pallescens* (64 adults and 5 nymphs). The male:female ratio of adult *R. pallescens* was 0.56 (23:41). Unfortunately, 14 triatomines were dead and too dry when handled by our research team and

could not be tested for infection or host preference. Trypanosomes were detected in 15 (27.3%) of 55 adult *R. pallescens* evaluated by microscopy.

The PCR analysis conducted in the 55 adult specimens showed that 40 (72.7%) were infected with *T. cruzi* and 22 (40.0%) with *T. rangeli*. Twenty triatomines (36.3%) were infected by both parasites (Table 1). Twenty-five of 55 adult *R. pallescens* contained sufficient blood for host identification. We identified a host in 22 (88.0%) of these samples with the six IgG antibodies used (to opossums, mice, chickens, monkeys, humans, and dogs). Humans were the preferred host (17, 68.0%) followed by mice (2, 8.0%), opossums (2, 8.0%), and chickens (1, 4%). Only three (12%) of the samples could not be associated with one of the six hosts evaluated, and no mix meals were observed. Human serologic analysis and hemoculture performed on inhabitants from triatomine-infested houses showed that 18 (32.1%) of 56 samples were trypanosome infected (Table 1). Thirteen samples (23.2%) had antibodies against *T. cruzi*. Six (10.7%) of these seropositive persons were children less than 15 years old. Trypanosomes were isolated from five (8.9%) hemoculture samples, all from children less than 11 years old. Further molecular analysis showed that all isolated trypanosomes were *T. rangeli*. None of these *T. rangeli*-positive children by hemoculture had detectable antibodies against *T. cruzi* in the serologic tests used.

DISCUSSION

Nearly 40 years ago, Pipkin reported a domiciliation trend of *R. pallescens* in rural localities near the studied area.[3] However, despite the public health implications of this finding, this study has not been followed-up and/or confirmed by other investigations. The capacity of *R. pallescens* to colonize human dwellings is a controversial biologic phenomenon that needs additional scientific evaluation. Since the earlier report, this area has been subjected to extensive ecologic changes. Deforestation is widespread and the land is now mainly used for livestock and agricultural activities. Furthermore, there are no recent data regarding Chagas disease prevalence and vectors in this area.

Currently, no organized preventive measures to control *T. cruzi* vector transmission in rural parasite-endemic areas are regularly carried out in Panama. The recognized sylvatic behavior of *R. pallescens* and the low number of Chagas disease cases passively detected by the health systems have probably conditioned the health authorities to minimize the need to implement expensive control measures. Previous entomologic studies in Panama have focused on *R. pallescens* collected

TABLE 1
Human trypanosomiasis and the presence of *Rhodnius pallescens* in 20 rural dwellings of the western border of the Panama Canal, Panama

	R. pallescens	Inhabitants
Total analyzed samples	55 (100%)	56 (100%)
Trypanosoma cruzi infected	40 (72.7%)	13 (23.2%)
T. rangeli infected	22 (40%)	5 (8.9%)
T. cruzi/T. rangeli infected	20 (36.3%)	0
Human blood meal positive	17/25 (68%)	–

from royal palm trees (*A. butyracea*), which is considered a natural ecotope of this vector.[12] In this sylvatic habitat, *R. pallescens* has showed a significant high infection rate with *T. cruzi* (at least 61%) and *T. rangeli* (at least 23%).[6,8] A zoophilic vector behavior has also been described with a preference for sylvatic mammals, mainly opossums.[6,9] However, the presence of triatomines in the domestic habitat and the risk of trypanosome transmission to the human population have been only partially explored in these previous studies.

Our results showed that the presence of triatomines inside dwellings in this area of Panama is a relatively common event. *Rhodnius pallescens* was the only species of triatomine found, including adults of both sexes and nymphs of third, fourth, and fifth instars. Of great epidemiologic concern is the fact that collected *R. pallescens* could have a high trypanosome transmission potential, as shown by the significantly high *T. cruzi* and *T. rangeli* infection rates (72.7% and 40%, respectively) detected by PCR. In addition, a clear preference for humans as a blood source was observed in the adult specimens (17 of 25, 68.0%). This anthropophilic behavior of the vector could reflect a clear attraction for humans as blood source or simply a higher availability of this host in the domestic habitat.

Of particular interest is the intradomicile collection of five *R. pallescens* nymphs, from which two (third and fourth instars) were infected with *T. cruzi*. Blood meal analysis showed that the *T. cruzi*-positive *R. pallescens* fourth instars had fed on humans. The presence of *R. pallescens* immature stages demonstrates the capacity of these species to thrive inside houses in this rural area. Also, the finding of a *T. cruzi*-infected nymph implies that this immature stage acquired the infection by feeding on infected humans or other domestic animal living in close proximity to the infested houses, as suggested by blood meal analysis. The proximity between royal palm trees and the houses in this region is a risk factor that should be considered in house infestations with *R. pallescens*.

Domiciliation of triatomines is a complex process that involves many factors such as local humidity, temperature, and food availability. Although we have not confirmed the complete intradomicile life cycle of the insect in this epidemiologic scenario, our results suggest that the housing characteristics in these rural areas are suitable for a progressive adaptation of *R. pallescens* to the human habitat. This behavior represents a growing risk for trypanosome transmission to exposed populations. Domiciliation of sylvatic triatomines has been recently reported in neighboring countries.[17,18] It is therefore necessary to carry out immediate preventive measures to interrupt vector *T. cruzi* transmission and to detect human infections in these localities near the western border of the Panama Canal.

The serologic and parasitologic results demonstrated that active trypanosome transmission is currently occurring in the human population in this area and that children are exposed early in life to the vector. Additionally, these findings demonstrate the vectorial capacity of *R. pallescens* and the high risk for Chagas disease in persons in this rural area. Studies are in progress to determine the human trypanosome infection rate in the entire population and to assess the clinical status of *T. cruzi*-seropositive persons.

Traditional chemical Chagas disease control methods are difficult to implement under this epidemiologic scenario.

Thus, we have initiated a dynamic and continuous education program through local schools and community leaders to provide information about the disease and the control measures, including the recognition and safe elimination of triatomines infesting houses.

Our results will have an effect on planning strategies to interrupt vectorial disease transmission. Efforts should focus on the recognition of areas infested with sylvatic triatomines in the process of adaptation to human domestic environments, such as the studied area. Clearly, infested houses and infected children deserved priority from the National Chagas Disease Control Program. However, to achieve a sustainable control of *T. cruzi* vector transmission, authorities will have to consider not only physical, chemical, and educational measures to avoid triatomine house invasion, but also preserve the delicate balance (vector-parasite-host) that is present in many localities with similar eco-epidemiologic conditions in central Panama.

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