

ECO-EPIDEMIOLOGICAL ASPECTS OF *Trypanosoma cruzi*, *Trypanosoma rangeli* AND THEIR VECTOR (*Rhodnius pallescens*) IN PANAMA

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SUMMARY

The eco-epidemiology of *T. cruzi* infection was investigated in the Eastern border of the Panama Canal in Central Panama. Between 1999 and 2000, 1110 triatomines were collected: 1050 triatomines (94.6%) from palm trees, 27 (2.4%) from periurban habitats and 33 (3.0%) inside houses. All specimens were identified as *R. pallescens*. There was no evidence of vector domiciliation. Salivary glands from 380 *R. pallescens* revealed a trypanosome natural infection rate of 7.6%, while rectal ampoule content from 373 triatomines was 45%. Isoenzyme profiles on isolated trypanosomes demonstrated that 85.4% (n = 88) were *T. cruzi* and 14.6% (n = 15) were *T. rangeli*. Blood meal analysis from 829 *R. pallescens* demonstrated a zoophilic vector behavior, with opossums as the preferential blood source. Seroprevalence in human samples from both study sites was less than 2%. Our results demonstrate that *T. cruzi* survives in the area in balanced association with *R. pallescens*, and with several different species of mammals in their natural niches. However, the area is an imminent risk of infection for its population, consequently it is important to implement a community educational program regarding disease knowledge and control measures.

KEYWORDS: American trypanosomiasis; Chagas disease; *Trypanosoma cruzi*; *Trypanosoma rangeli*; *Rhodnius pallescens*; Panama.

INTRODUCTION

The protozoan *Trypanosoma cruzi* is the etiological agent of Chagas disease, an incurable, chronic, and incapacitating condition afflicting millions of people in Central and South America²⁸. The disease is a zoonosis in which the parasite is transmitted to humans through the feces of blood sucking triatomine bugs. Because of a higher risk of exposure to infected triatomines, infection is primarily present among people who live under poor conditions in rural settlements. The extension of urban areas into neighboring forests, the original sylvatic ecotope of the vector, has resulted into the emergence of the infection to periurban areas. Furthermore, rural-urban migration of people has led to the urbanization of an originally rural disease⁷.

Ten different triatomine species have been described from Panama. However, the principal vectors of Chagas disease in the country are *Rhodnius pallescens* and *Triatoma dimidiata*^{12,13}. *R. pallescens* is also the vector of *Trypanosoma rangeli*, a non pathogenic trypanosome transmitted through the saliva of triatomines, that is endemic in Panama. Though with different geographic distribution and with different trypanosome infection rates, a previous study conducted three decades ago demonstrated that both vector species are highly prevalent in certain regions of the country²⁴. Nevertheless, there is a lack of recent data regarding triatomine prevalence and its natural infection rates with trypanosomes.

Triatomine species are highly adapted to their natural ecotope. A close association of *R. pallescens* and *T. dimidiata* with palm trees, particularly with the widely distributed "corozo royal palm tree", *Attalea butyracea* has been observed in Panama^{25,26}. Several natural mammal reservoirs for *T. cruzi* have been described in the country, opossums being considered responsible for the maintenance of the sylvatic cycle of the disease^{4,23}. It must not be overlooked that human activities in the forest interface and within the forested area, including deforestation, may lead to changes in the ecology of the vector, thus affecting the local epidemiology of Chagas disease in the area.

Chagas disease has been described in Panama since 1931¹⁴. A previous seroprevalence survey carried out by SOUSA & JOHNSON²⁴, showed that anti-*T. cruzi* antibodies were present in several parts of the country with a prevalence ranging from 3.1% to 21.5%. Since then, no information has been published on the prevalence of human trypanosomiasis in the country. However, evaluation of epidemiological data passively gathered from patients attending National Care Centers during the last decade, suggests an increased morbidity of Chagas disease¹⁵.

A significant reduction in *T. cruzi* transmission has occurred in certain South American Cone countries as a result of an aggressive insecticide spraying campaign to eradicate domiciliary vectors²². To what extent this experience can be extrapolated to countries with different eco-

epidemiological situations and where Chagas disease remains a persistent problem, as in the case of Panama, has not been evaluated.

The Ministry of Health of the Republic of Panama is now in the process of defining a National Chagas disease Control Program, which includes free of cost diagnoses and treatment when necessary. Thus, there is an urgent need for recent information so that local authorities may plan new and effective strategies to achieve this goal. Control strategies will strongly depend on a better understanding of the biology of the insect population, as well as on all the various ecological aspects involved in the transmission of *T. cruzi* within the different regions of the country. An accurate assessment of *T. cruzi* and *T. rangeli* regarding specific hosts, reservoirs and vectors is considered an essential component for new control strategies.

The area selected for the present study is located in the central region of Panama, next to the Canal Reserve Forested Area. Previous studies conducted more than three decades ago, described this region as endemic for *T. cruzi* infection. Due to the increasing number of inhabitants, and therefore to the construction of new facilities, the area has experienced considerable ecological changes. The objective of the present study was to evaluate the present eco-epidemiological situation of *T. cruzi* infection within these particular sites. Data will provide recent information to the Panamanian government for effective control measures.

MATERIAL AND METHODS

Study site: A simple non-stratified random survey in 716 different houses (90% of the total), in the settlements of Buenos Aires (n = 368) and Agua Bendita (n = 348), was conducted as part of an eco-epidemiological study on Chagas disease carried out during 1999 and 2000. These two adjacent periurban neighborhoods within the Community of Chilibre are located on the Eastern border of the Panama Canal, 50 km North of Panama City, the capital city for the Republic of Panama. Buenos Aires and Agua Bendita had an estimated population of 6020 people in year 2000, considered to be 15% of the total population in Chilibre County. The first settlements were established 60 years ago, mostly for Panama Canal workers. Thirty years ago, a study in the Chilibre region described the domestic and peridomestic presence of *R. pallescens* with high natural trypanosome infection rates²⁴. No up-to-date information regarding the prevalence of triatomine natural infection rates or human trypanosomiasis in this region is available.

Eco-epidemiological information gathered from the survey contemplated the evaluation of Chagas disease risk factors, including the presence of vegetation and palm trees near houses, and the presence of domestic and sylvatic animals in the area. Other epidemiological data such as housing facilities, time of residency in the area, and knowledge about the disease and its vector were also investigated.

Vectors: Direct search for triatomines was made inside and around houses as well as in sylvatic ecotopes. Community participation was valuable for collecting triatomines within the domestic and peridomestic habitats, especially at night. Sylvatic triatomines were collected from dissected palm trees near the surveyed houses by our field workers. The number, stages and location of the collected triatomine were recorded. All hand-collected triatomines were taken to the laboratory facilities in plastic containers with filter paper for proper identification using a

standard reference key. Salivary glands and rectal ampoule content from collected specimens were placed on physiological saline and then microscopically examined for the presence of parasites. A combined intestine-rectum sample from trypanosome positive vectors was intraperitoneally inoculated into susceptible white mice (CFW). Twenty-one days post inoculation the animals were bled by cardiac puncture and the whole blood was cultured into modified Senekjie's biphasic medium overlaid with either saline or 10% FCS Scheinder's medium. Cultures were microscopically examined for the presence of trypanosomes for at least a 2-month period. Massive replication of the isolated parasites was achieved and lysates were stored at -20 °C for further characterization.

Trypanosome isolates: Isolated trypanosomes were characterized by means of isoenzyme electrophoresis on cellulose acetate membranes as previously described⁸. The following three systems were used: PGM 2.7.5.1 (phosphoglucomutase), ALAT 2.6.1.1 (alanine aminotransferase) and ASAT 2.6.1.1 (aspartate aminotransferase). Isoenzyme profiles were then phylogenetically grouped using the TAXAN PROGRAM (Information Resources Group, Maryland Biotechnology Institute, University of Maryland). Local reference strains for the present study were: H.I.R. 116-B-Clon 4 (*T. cruzi* reference strain for Panama) and T.r./HLN/75/c12 (*T. rangeli* reference strain for Panama).

Precipitin test: As epidemiological complementary information, feeding patterns of the insects blood sources were determined by the precipitin test^{2,3}.

Anti-T. cruzi antibodies assessment: During the first year of the study, blood samples were randomly collected from people living in the surveyed houses. Anti-*T. cruzi* antibodies were determined by Indirect Immunofluorescence (IIF). For the second year of the study, serological tests were performed only on residents from houses where triatomines were seen or captured. Positive sera samples were confirmed by ELISA (Wiener ELISA Chagas test) and Western Blot tests¹⁸. Additional searches for triatomines were carried out in these homes and peridomestic sites.

Statistical analysis: Statistical analyses were performed using the Epi Info 6 program. Significance of differences was verified using the Chi-square test.

RESULTS

A total of 716 houses in the Chilibre County, 368 from Buenos Aires and 348 from Agua Bendita were surveyed. Houses in these two areas accounted for 3122 people (1581 from Buenos Aires and 1541 from Agua Bendita). There was a mean of 4.4 people living in each house. Sex and age distribution was similar between the two studied sites. Mean residency in the area was 22 years.

The population's knowledge of Chagas disease and its vector was assessed. Half (51.8%) of the people surveyed recognized triatomines as vectors of Chagas disease transmission, however, no statistical significant difference was found between the two areas studied (54.9% Buenos Aires vs 48.5% Agua Bendita, p = 0. 22).

Housing facilities have been subject to constant improvement, especially during the last few years, thus affecting Chagas disease

epidemiology in the region. If the two study sites are considered as a whole, 86.4% (n = 619) of the total houses surveyed (n = 716) were made of cement with a galvanized roof, 10.1% (n = 72) were made of mixed construction materials and 3.5% (n = 25) were of natural material (palm thatch roof and cane walls) (Table 1). A significant difference was found between the housing facilities in the two studied sites (p = 0.00036). Buenos Aires had poorer housing conditions with a greater number of houses built of natural materials.

Table 1

Total number and type of housing facilities in two periurban neighborhoods, Community of Chilibre, Panama, 1999-2000

Area	Total	1*	2**	3***
Buenos Aires	368	302 (82.1%)	45 (12.2%)	21 (5.7%)
Agua Bendita	348	317 (91.1%)	27 (7.8%)	4 (1.1%)
Total	716	619 (86.4%)	72 (10.1%)	25 (3.5%)

1* Houses made of cement and galvanized roof; 2** Houses made of mixed materials (wood, galvanized or occasionally palm thatch roof); 3*** Houses made of natural materials (palm thatch roof and walls and dirt floor).

Most houses at both study sites (95%) were surrounded by haulm and secondary forest less than 20 meters from the house. Among the remaining secondary forest present in the area, *A. butyracea* was predominant. Since the principal vector of *T. cruzi* and *T. rangeli* in Panama, *R. pallescens*, is primarily associated with palm tree habitat²⁵, the proximity of houses to the *A. butyracea*, was evaluated as a risk factor²⁰. In Buenos Aires, 51.6% (190/368) and in Agua Bendita 31.3% (109/348) of the houses had palm trees less than 100 meters from the houses (Table 2). A significant difference was found when comparing the presence of palm trees near the houses at the two studied sites (p = 0.015).

Table 2

Presence of palm trees near houses in two periurban neighborhoods, community of Chilibre, Panama, 1999-2000

Area	Positive vs total number of houses	Distance from houses		
		<10m	20-100m	>100m
Buenos Aires	223/368 (60.6%)	40	150	33
Agua Bendita	138/348 (39.7%)	11	98	29
Total	361/716 (50.4%)	51	248	62

The role which sylvatic and domestic animals participate in the transmission of *T. cruzi* has not been well established at the study sites.

Table 3

Presence of sylvatic animals in two periurban neighborhoods, Community of Chilibre, Panama, 1999-2000

Area	Positive vs total number of houses	Sylvatic animals				
		Opossum	Mice	Bats	Monkeys	Other*
Buenos Aires	346/368 (94.0%)	240	250	294	62	203
Agua Bendita	324/348 (92.8%)	215	254	291	59	269
Total	670/716 (93.6%)	456	505	566	122	266

* Included armadillo, sloths and squirrels.

Sylvatic animals were common at both sites (Table 3). Opossums, considered to be the main reservoir host for *T. cruzi* in Panama, were among the most common sylvatic animals seen^{4,23}. Domestic animals were also frequent in both locations; dogs were predominant (62%), followed by fowls (55.1%) and cats (20.7%). Fowls were usually kept loose in the yard. No significant difference regarding domestic or sylvatic animal distribution between both study sites was observed.

Table 4

Age and sex distribution as per microhabitat of *Rhodnius pallescens* collected in two periurban neighborhoods, Community of Chilibre, Panama, 1999-2000

<i>R. pallescens</i>	Intradomicile	Peridomicile	Palm trees	Total
Adults	32	27	324	383
Male	11	14	192	217
Female	21	13	132	166
Nymph stages	0	0	626	626
1 st	-	-	45	45
2 nd	-	-	212	212
3 rd	-	-	173	173
4 th	-	-	121	121
5 th	-	-	75	75
Not specified			100	100
Total	32	27	1050	1109

Sixty three palm trees were felled and totally dissected in search of triatomine bugs. Of the 48 palm trees from Buenos Aires, 75% (36/48) were found to be positive for triatomines, while in the area of Agua Bendita, 93.3% (14/15) were colonized by the insect. An overall of 50/63 palm trees were found to be positive for *R. pallescens* (infestation index rate = 79.4%). Two coconut palms trees were also examined, but no triatomines were observed. An additional palm tree specie, *Acrocomia aculiata*, resulted positive for *R. pallescens* nymph and adult stages, though few in numbers. Animals or animal nests were found in 68% (34/50) of the triatomine positive palm trees, 75% in Buenos Aires and 50% in Agua Bendita.

Of the 1109 triatomines collected for the present study during 1999 and 2000, 32 (2.9%) were collected inside houses, close to the light at night, while 27 (2.4%) were found crawling around the houses. The remaining 1050 collected triatomines (94.7%) were hand captured from palm trees (Table 4). All specimens were identified as *R. pallescens*, except for one identified as *Panstrongylus rufotuberculatus*, found resting on an outside wall of a house at the Buenos Aires study site. The latter is considered a sylvatic vector. Only adult triatomines were collected inside or around houses, no immature stages were found inside houses in either

of the two study sites. In contrast, all triatomine stages of *R. pallescens* were collected from palm trees, indicating a 100% colonization index rate (Table 4). Sex and age distribution of *R. pallescens* collected on palm trees were similar for both study sites.

Salivary glands from 380 *R. pallescens* were examined under the microscope for the presence of trypanosomes, 29 resulted positive (natural infection rate = 7.6%). Rectal ampoule content from 373 triatomines were also examined; active trypanosomes were seen in 168 of them (natural infection rate = 45%). Unfortunately, almost all triatomines collected inside and around houses at night by people from the community were too dry when handled by our team, therefore was not possible to assess infection in these specimens. Trypanosomes were recovered from 103 positive triatomines through mice inoculation and blood culture. Isoenzyme profiles proved that 85.4% (n = 88) of isolated trypanosomes were identified as *T. cruzi* and 14.6% (n = 15) as *T. rangeli*.

As a complement to epidemiological studies, blood meal analysis was performed in 829 *R. pallescens*. Of these, 466 (56.2%) contained sufficient blood for host identification. Mammalian feedings accounted for most of the blood sources (95.9%). Marsupials (opossums) were by far the preferential mammalian blood source (82.6%). Specimens feeding on bats (2.8%), sloths (0.8%) and man (0.4%) were also identified. Only 2.3% of all processed specimens fed on birds or reptiles and less than 2% fed on more than one animal (Table 5).

Table 5

Precipitin blood meal analysis on collected *Rhodnius pallescens* from two periurban neighborhoods, Community of Chilibre, Panama, 1999-2000

Blood source	Number	%
Mammalian		
Undetermined	43	9.2
Hominidae	2	0.4
Chiroptera	13	2.8
Didelphidae	385	82.6
Bradypodidae	4	0.8
Sub-total	447	95.9
Avian	6	1.3
Reptilian		
Undetermined	1	0.2
Sauria	3	0.6
Serpentelia	1	0.2
Double feeding		
Didelphidae + Myrmecophagidae	2	0.4
Didelphidae + Avian	5	1.1
Mammalian + Amphibian	1	0.2
Analyzed samples	466	
Insufficient or digested blood	363	
Total	829	

During the first year of the study serology by IIF demonstrated that 7 (3.1%) of 222 samples randomly collected from people living in the

study area, presented antibodies against *T. cruzi*. Positive samples included five females and two males older than 28 years with serum titers ranging from 1:40 to 1:160. People sampled during the second year of the study were those living in houses where triatomines had previously been captured inside or around the house, and thus considered to be at high-risk. A total of 151 samples were collected from both neighborhoods, 135 samples from Buenos Aires and 16 samples from Agua Bendita. Seropositivity by IIF was detected in 7 (5.2%) people from Buenos Aires and in one (6.2%) person from Agua Bendita, indicating an overall seroprevalence of 5.3%. However, when these positive IIF samples (n = 8) were evaluated by ELISA using recombinant antigen and Western blot analysis, only three samples were positively confirmed, thus lowering the seroprevalence to 1.9%.

DISCUSSION

The process of increasing urbanization and expansion of agricultural frontiers into sylvatic areas might generate new public health problems in Panama. The migrating population is at high risk for contracting a vector borne disease, such as Chagas disease. Both study sites are next to the Panama Canal Reserve Primary Forest and were once part of this reserve. The presence of a great number of wild animals in the area is in fact, an indicator of the proximity to primary forest. The area is undergoing significant ecological changes. Primary forest has been destroyed due to constant human migration and colonization.

Our findings from collected triatomines confirmed that despite all changes in the ecotope, the previous reports indicating that *R. pallescens* is the triatomine most frequently found in Central Panama continue to be valid^{12,13}. *R. pallescens* is considered sylvatic, we have not found any evidence demonstrating that this insect has become domiciliated in Panama. More than 30 years ago, a previous study, however, showed evidence of possible domiciliation of *R. pallescens* in certain locations of Central Panama¹⁷. The domiciliation trend of this triatomine has not been recently evaluated in areas with poor housing facilities, although it is an important issue to resolve due to the potential epidemiological risk for transmission of the infection to people living in infested houses. For the present study, most triatomines were hand collected from palm trees (n = 1050), their natural sylvatic ecotope. We did find *R. pallescens* inside (n = 33) and/or around the surveyed houses (n = 27), but no nymph stages were found. It is well known that triatomine species can be attracted at night to visible and UV light sources. Thus, considering the close proximity of palm trees to houses, domestic and peridomestic finding of triatomines in dwellings from this area may simply be an occasional or chance event.

Despite all changes in the area we found, in agreement with a previous report²⁴, a high natural trypanosome infection rate in *R. pallescens* collected from peridomestic and sylvatic habitats (palm trees). *T. cruzi* was found in most of the isolated trypanosomes (85.4%), whilst *T. rangeli* was identified in 14.6 % of the samples. Though the use of three different isoenzyme systems was sufficient to separate *T. cruzi* from *T. rangeli*, further biochemical and molecular studies are in progress to identify possible *T. cruzi* variants found in the area. Results from this work will be published later.

Results from the present study confirmed that *R. pallescens* is a zoophilic vector, feeding mainly from sylvatic mammals. As in previous reports, we also found that opossums are the preferred blood source of

R. pallescens, and more likely continues to be the reservoir host responsible for maintenance of the sylvatic cycle of *T. cruzi* in the area.

More than 20 years ago, triatomines examined by blood meal analysis from three rural communities in central Panama province, also demonstrated their preference for wild mammals as blood source, although in that report slightly less than half of the collected triatomines had fed on humans³. In the present study, using the same procedure, hominidae feeding was only detected in two insects. The abundance of sylvatic animals in the area has contributed to maintain *R. pallescens* within its own natural ecotope, because blood sources from wild animals are sufficiently abundant to meet their requirements. On the other hand, the continuous improvement of housing facilities observed in these areas has probably limited potential triatomine domiciliation.

A small number of huts with thatched roofs remain in the area. However, most of the houses in these communities are now constructed of materials which are not suitable for triatomine breeding and survival. This means that an important control of Chagas disease transmission in the area might have arisen spontaneously with the social evolution of people and better overall living standards.

Despite the close proximity of houses to palm trees highly colonized by *R. pallescens* infected with *T. cruzi*, the seroprevalence in humans was relatively low (less than 2%), specially when IIF positive samples were further tested with two additional serological assays, as recommended by the World Health Organization (WHO). Serological tests for *T. cruzi* infections are considered highly sensitive, but their specificity has been questioned due to antigenic cross-reactivity with other parasites, such as *Leishmania* and *T. rangeli*^{11,18,21}. Both trypanosome species (*T. cruzi* and *T. rangeli*) are circulating in the area, and cutaneous leishmaniasis is also endemic, suggesting a high possibility for false-positive results when a single serological test is used.

Characteristics of Chagas disease in Panama are different from those clinical manifestations seen in most South American countries. There is no record of megatube inductions, parasitemia in man is of relatively short duration and the chronic phase seems to be more benign²³. In fact, during the two years of the study there were few reports of people with clinical manifestations associated to Chagas disease. It is worth mentioning that Chagas disease assumes its mildest form in countries such as Panama, where vectors of the disease, especially *R. pallescens*, have high *T. rangeli* infection rates, a parasite thought to be entirely non-pathogenic to humans. For endemic regions in Panama, *T. rangeli* is more frequently found in human blood than *T. cruzi*²³. In this sense, recent data have shown that complete or partial immunity against *T. cruzi* can be obtained in animal models previously immunized with low virulent *T. cruzi* strains^{5,9} or with *T. rangeli*^{1,29}. The fact that more than one *T. cruzi* strain^{10,16} and even more than one *Trypanosoma* species^{6,23} can be transmitted by *R. pallescens* in Panama makes this possibility feasible. Undoubtedly, this hypothesis needs to be further evaluated and other aspects responsible for the large spectrum of Chagas disease manifestations, such as host genetic factors and local *T. cruzi* isolates virulence, should also be taken into consideration.

Our results demonstrate that in the area under study, *T. cruzi* survives

in a balanced association with *R. pallescens* and with several species of mammals in its natural niches. However, ecological changes such as deforestation may cause mortality amongst the sylvatic vertebrate hosts, leading to poor nutritional status of their associated triatomines and inducing the insect to seek other blood sources in new habitats. Samples of domiciliation in periurban and intradomiciliary areas, in which sylvatic triatomines had become adapted to alternative food sources, have been recently described in neighboring countries^{19,27}.

Under the above described ecological conditions, human *T. cruzi* transmission within the Chilibre Community is low. The fact that 50% of the population is aware of the presence of the vector, and that there is a tendency to kill the bug as soon as it is seen flying into the house, may have contributed to control triatomine colonization of dwellings. However, taking into consideration that the population is at real and imminent risk of infection, it is important to implement a community educational program, regarding both the disease and control measures. Periodic entomological surveillance to monitor vector population and possible domestic triatomine foci are also necessary.

RESUMO

Generalidades do *Trypanosoma cruzi*, do *Trypanosoma rangeli* e do seu vetor (*Rhodnius pallescens*) no Panamá

A epidemiologia da infecção do *T. cruzi* foi investigada na margem oriental do canal do Panamá, na região central da República do Panamá. A informação obtida durante o estudo avaliou fatores de risco da doença de Chagas nesta área. Entre 1999 e 2000, 1110 triatomíneos foram coletados: 1050 triatomíneos (94,6%) em palmeiras, 27 (2,4%) em habitats periurbanos e 33 (3,0%) no interior de casas. Todos os espécimens foram identificados como *R. pallescens*. Não havia nenhuma evidência de domiciliação do vetor. O exame de glândulas salivares de 380 *R. pallescens* revelaram taxa de infecção natural por *Trypanosoma* de 7,6%, mas o conteúdo da ampola rectal de 373 triatomíneos mostrou 45% de positividade. Os perfis de isoenzimas em *Trypanosomas* isolados demonstraram que 85,4% (n = 88) eram *T. cruzi* e 14,6% (n = 15) eram *T. rangeli*. A análise da refeição de sangue de 829 *R. pallescens* demonstrou comportamento zoofílico do vetor, sendo os gambás a fonte preferencial de sangue. Soroprevalência nos seres humanos de ambos locais de estudo foi menos que 2%. Nossos resultados demonstram que *T. cruzi* sobrevive na área em associação equilibrada com *R. pallescens* e com diversas espécies diferentes de mamíferos em seus nichos naturais. Entretanto, a área é um risco eminente de infecção para sua população, pelo que é importante executar um programa educacional na comunidade a respeito das medidas, do conhecimento e do controle da doença.

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