AVANCES
EN CIENCIAS E INGENIERÍAS
COMUNICACIÓN/COMMUNICATION SECCIÓN/SECTION B

Epidemiological profile of Chagas disease in Southern coastal Ecuador: pilot study of the *Trypanosoma cruzi* infection in the vector *Triatoma dimidiata*

Perfil epidemiológico de la enfermedad de Chagas en la costa sur del Ecuador: estudio piloto de la infección de *Trypanosoma cruzi* en el vector *Triatoma dimidiata*

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Editado por/Edited by: Diego F. Cisneros-Heredia Ph.D.(c)
Recibido/Received: 03/05/2014. Aceptado/Accepted: 21/04/2014.
Publicado en línea/Published on Web: 19/12/2014. Impreso/Printed: 19/12/2014.

Abstract

Chagas disease is a prevalent and potentially fatal parasitic infection that affects millions of people, especially in Latin America. The causative agent of the disease is the protozoan *Trypanosoma cruzi*, transmitted mainly by blood-sucking insect vectors of the subfamily Triatominae. The objective of this study was to analyze the infection by *Trypanosoma cruzi* in the vector *Triatoma dimidiata* in a rural area of Southern coastal Ecuador. Triatominae searches were carried out in peridomestic habitats of households located in Bajada de Chanduy, a rural village in the border between Santa Elena and Guayas provinces. Feces and intestinal contents were extracted from the insects and observed in the microscope for the presence of mobile trypanosomes. Approximately 72% of the insects analyzed were infected with trypanosomes. This percentage reflects the occurrence of active foci of *T. cruzi* and the potential risk that this poses for people living in rural villages in the study area. The trypanosomes that were found infecting the triatomine bugs will be molecularly identified and a study of the genetic variability of the population of *T. dimidiata* collected at the study site will be carried out in the future. The results of all these analyses will provide a better understanding of the epidemiology of Chagas disease in rural Ecuador, which is an essential step in the development of control and prevention strategies for the transmission of this pathogenic parasite.

Keywords. Chagas Disease, *Trypanosoma cruzi*, *Triatoma dimidiata*, Ecuador.

Resumen

La Enfermedad de Chagas es una infección parasitaria prevalente y potencialmente fatal, que afecta a millones de personas, especialmente en América Latina. El agente causal de esta enfermedad es el protozoario *Trypanosoma cruzi*, que es transmitido por insectos hematófagos de la subfamilia Triatominae. El objetivo de este estudio es analizar la infección por *Trypanosoma cruzi* en el vector *Triatoma dimidiata* en un área rural de la costa sur del Ecuador. Las búsquedas de triatominos se llevaron a cabo en hábitats peridomésticos de viviendas ubicadas en Bajada de Chanduy, un pueblo rural en el borde entre las provincias de Santa Elena y Guayas. Las muestras de heces y contenido intestinal fueron extraídas de los insectos y observadas en el microscopio para detectar la presencia de tripanosomas móviles. Aproximadamente 72% de los insectos analizados estaban infectados con tripanosomas. Este porcentaje refleja la presencia de focos activos de *T. cruzi* y el potencial riesgo que esto implica para los habitantes de los pueblos en el área de estudio. Los tripanosomas que se encontraron infectando a los insectos triatominos serán identificados molecularmente y un estudio de la variabilidad genética de la población de *T. dimidiata* se llevará a cabo en el futuro. Los resultados de todos estos análisis contribuirán a un mayor conocimiento de la epidemiología de la Enfermedad de Chagas en las zonas rurales del Ecuador, lo que constituye un paso esencial en el desarrollo de estrategias de control y prevención para la transmisión de este parásito patógeno.

Palabras Clave. Enfermedad de Chagas, *Trypanosoma cruzi*, *Triatoma dimidiata*, Ecuador.

ISSN 1390-5384

Trypanosoma cruzi is a hemoflagellate parasite of mammals, and the causative agent of Chagas disease in humans. Chagas disease is a potentially fatal condition for which there is no vaccine or effective and safe treatment available. Chronic infection causes associated cardiac abnormalities in 30% and digestive and/or neurological disease in 10% of the cases [1]. Infection occurs when Triatominae insect vectors -commonly known as kissing bugs- feed on blood from an infected mammal, defecate close to the feeding wound, and the feces contaminated with *T. cruzi* enter the host's circulatory system through the wound or through mucosal tissues in the body. About eight million people are infected worldwide, primarily in Latin America, where *T. cruzi* is found in endemic rural areas of 21 countries in this region [1].

In Ecuador, it is estimated that 170,000 people are seropositive for *T. cruzi*, and 4,400 acquire the infection every year, resulting in 300 deaths from causes directly related to Chagas disease [2]. Most of the studies on Chagas disease in Ecuador have focused on a few provinces, namely Loja, Manabí, Guayas and El Oro, where some of the highest rates of *T. cruzi* prevalence have been reported [2]. These areas constitute the habitat of at least five species of triatomines, which are responsible for most of the vectorial transmission of Chagas disease in Ecuador [3]. *Rhodnius ecuadoriensis* and *Triatoma dimidiata* are the most important vectors in the country. It is believed that *T. dimidiata* was introduced from Central America and its presence is restricted to the coastal provinces of Guayas, Santa Elena, and Manabí. *R. ecuadoriensis* is also present in the Ecuadorian coast, commonly associated with palm trees, but it is not exclusively limited to this region [2]. In addition to occupying sylvatic habitats, this vector colonizes peridomestic and domestic areas. The transmission of *T. cruzi* is highly influenced by the invasion and adaptation of the kissing bugs to human dwellings [4].

It has been demonstrated that vector control is the most effective method of preventing *T. cruzi* infection and controlling the disease. Thus, more research on the biological interactions vector-parasite and the influence of environmental factors in parasite transmission is needed in order to establish effective and targeted control strategies. The presence of the non-pathogenic parasite *Trypanosoma rangeli*, which infects the same wild reservoir hosts and insect vectors of *T. cruzi*, and also occurs in the same geographic areas, could make parasite identification confusing and difficult and thus may interfere with the understanding of the epidemiology of Chagas disease in Ecuador. Therefore, the differentiation between *T. rangeli* and *T. cruzi* infections in vectors has a particular importance and a variety of epidemiological and medical implications [5]. For example, the presence of *T. rangeli* may explain why despite the high infection by *T. cruzi* in *T. dimidiata*, there is a low seropositivity in humans in Pedro Carbo (Guayas province, Ecuador) [6]. The morphological similarity and immunological cross-reactivity of *T. cruzi* and *T. rangeli* has prompted the development of molecular assays to differentiate these two parasites and to identify the several genetic strains of each species [7].

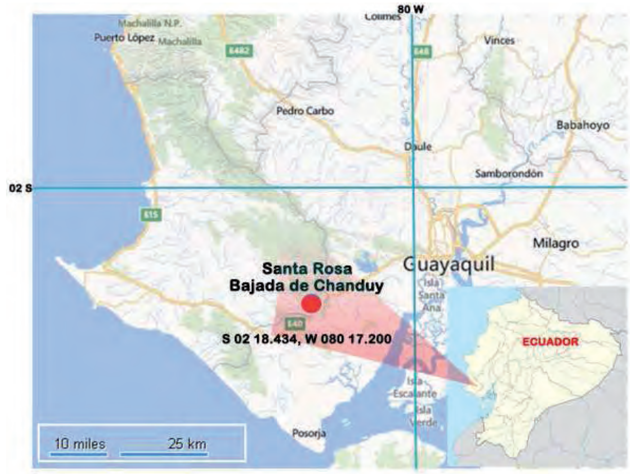

Figure 1: Study site. Rural village of Bajada de Chanduy.

The main objective of this study was to determine the infection by *T. cruzi* in Triatominae vectors in a village in the border of Santa Elena and Guayas provinces, Southern coastal Ecuador. Insect collection was carried out in the neighborhood of Santa Rosa, Bajada de Chanduy (S 02° 18.434, W 80° 17.200), in Santa Elena county (Fig. 1). The entomological searches were conducted by trained field personnel from the National Service for the Control of Diseases transmitted by Arthropod Vectors (SNEM) using the man-hour method [8] in peridomestic areas of houses that voluntarily agreed to participate in the study. The peridomestic sites that were searched included fire or construction wood, trash, tiles, and/or rocks as well as other potential places where triatomines' eggs, nymphs, and/or adults could be found. These areas are particularly important because they are usually close to sleeping/resting sites for domestic animals and also provide nesting places for wild animals (rodents, opossums, etc.). The neighborhood of Santa Rosa, Bajada de Chanduy, has a total of 60 houses. Thirteen houses of the village were closed at the moment of the visit, and the inhabitants of one house did not provide consent to the research team to look for triatomines in their household. Therefore, a total of 44 houses were thoroughly searched. Most houses were constructed with concrete, had wooden doors, and rooftops made of zinc. Additionally, most houses had either a front (or back) yard with piles of construction materials (bricks, tiles, wood, coal, reed, cement) and many had the presence of domestic animals (fowl, pigs, and dogs).

Only three houses from Santa Rosa, Bajada de Chanduy, had triatomines in their peridomestic sites. A total of 71 triatomines from different developmental stages were collected in these three houses (Fig.2). Sixty eight specimens were found in a pile of stored reed in the backyard of one house (12 adults, 56 nymphs). The backyard in this house had a few trees, dirt floor, and a

Variables	Houses searched		p value
	Non-infested houses (N=41)	Infested houses (N=3)	
Concrete wall			
-	11 (26.8)*	3 (100)*	
+	30 (73.2)	0	0.027
Wood wall			
-	35 (85.4)	0	
+	6 (14.6)	3 (100)	0.006
Brick wall			
-	35 (87.8)	3 (100)	
+	6 (12.2)	0	1
Zinc roof			
-	7 (17)	0	
+	34 (83)	3 (100)	1
Tile roof			
-	33 (80.4)	3 (100)	
+	8 (19.6)	0	1
Domestic animals			
-	30 (73.2)	0	
+	11 (26.8)	3 (100)	0.027
Hen house with chickens			
-	30 (73.2)	0	
+	11 (26.8)	3 (100)	0.027
Stored material			
-	11 (26.8	0	
+	30 (73.2)	3 (100)	0.562
Wood			
-	24 (58.5)	0	
+	17 (41.5)	3 (100)	0.086
Reed			
-	36 (87.8)	0	
+	5 (12.2)	3 (100)	0.004
Rock			
-	37 (90.2)	3 (100)	
+	4 (9.8)	0	1
Concrete			
-	36 (87.8)	2 (66.7)	
+	5 (12.2)	1 (33.3)	0.363
Metal			
-	38 (92.7)	3 (100)	
+	3 (7.3)	0	1
Trash			
-	26 (63.4)	2 (66.7)	
+	15 (36.6)	1 (33.3)	1
Proximity to vegetation			
-	29 (70.7)	0	
+	12 (29.3)	3 (100)	0.034
Altitude			
$\leq$ 40 mts	23 (56.1)	2 (66.7)	
>40 mts	18 (43.9)	1 (33.3)	1

*numbers in parenthesis are percentages
(+) presence of that variable
(-) absence of that variable

Table 1: Potential risk factors associated with house infestation by *T. dimidiata* in the neighborhood of Santa Rosa, Bajada de Chanduy.

Village	County	Infestation index (%)	Density	Crowding	Colonization index (%)
Bajada de Chanduy	Santa Elena	6.8	1.6	23.7	100

Table 2: Entomological indexes of *T. dimidiata* collected in Bajada de Chanduy.

Figure 2: Neighborhood of Santa Rosa, Bajada de Chanduy. The houses where *T. dimidiata* were found are shown as red circles (numbers outside the red circles refer to the total specimens collected in each house).

hen house full of chickens. This area also had trash and other stored materials (wood, zinc and trash). Additionally, two specimens (one adult and one nymph V) were found in a nearby house, and one specimen (nymph IV) was collected in one last house of the neighborhood of Santa Rosa (Fig. 2). These three triatomines were collected in piles of wood, reed and trash from the backyard of these dwellings. All three houses were located in an area of the village surrounded by abundant vegetation (Fig. 2). A Fisher's exact test was performed to determine if certain characteristics of the house or its surroundings were associated with the presence of triatomines. The variables analyzed were the following: altitude ($\leq$40 m or >40 m), housing structure (wood, brick or concrete walls, zinc or tile roofs), presence of animals, stored construction materials in peridomestic areas (reed, wood, concrete, rock, metal and trash), presence of a hen house with chickens, and proximity to vegetation (Table 1). There was a significant association between the presence of triatomines and wooden house walls (p=0.006), the presence of animals (p=0.027), the presence of a hen house with chickens (p= 0.027), stored reed (p=0.004) and the proximity to vegetation (p= 0.034) (Table 1). Additionally, the absence of concrete walls is also associated with house infestation (p=0.027). None of the other variables analyzed were significantly associated with the presence of triatomines (Table 1).

Infected triatomines	
Stage	**#**
Adult	10
Nymph V	5
Nymph IV	3
Nymph III	3
Total	21 (72.4%)

Table 3: Trypanosome-infected *T. dimidiata* by developmental stage.

The collected insects were placed in labeled containers and transported to the entomology laboratory (LEMMT) at San Francisco University for further analysis. The following entomological indexes were calculated: infestation index, density, crowding, and colonization index [9]. All the collection procedures in the field were performed by the SNEM personnel following the standard guidelines for vector handling. Feces and intestinal contents were extracted in the laboratory from each triatomine. The rest of the body was preserved in 96% ethanol and stored at -20°C for further molecular analysis. A small volume of each of the intestinal samples and/or feces was observed under the microscope to detect living trypanosomes and the rest was stored in TE buffer at -20°C until further use.

T. dimidiata vectors were found in 6.8% of the 44 houses that were inspected. The triatomine density was 1.6 insects per house (Table 2). The vector crowding was 23.7 insects per infested house. Nymphs of *T. dimidiata* were found in 100% of the infested houses.

From the 71 triatomines collected, feces and intestinal contents were extracted from 29 specimens (13 adults and 16 nymphs) and observed under the microscope. It was not possible to obtain samples for microscopy from the remaining specimens and thus the presence of trypanosomes in those insects will be analyzed with molecular techniques. Mobile trypanosomes were detected by microscopy in 21 of 29 specimens (Table 3). The infected triatomines are described in Table 3 according to developmental stage. Taking into account only the total number of specimens that were studied by microscopy (29 triatomines), the infection rate of these vectors is 72.4% (95% CI= 52.8-87.3). This percentage evidences an active presence of *T. cruzi* in the village of Bajada de Chanduy, where the human population may be at risk of contracting the parasite, and therefore there is a need for the implementation of vector control strategies.

As it was mentioned before, it was not possible to search all the houses in the neighborhood of Santa Rosa due to unpredictable factors (N= 44, total= 60). Thus, the total number of houses where the vectors were found (N=3) may be over or underestimated. This also has implications in the previous analysis of the domestic and peridomestic determinants for house infestation by *Triatoma dimidiata*. Even though the wooden house walls, the presence of animals, the presence of chickens in a corral, stored reed, and the proximity to vegetation, were significantly associated with the presence of triatomines, it cannot be concluded that these are risk factors given the low number of infested houses (N=3). However, various studies in Latin America have shown a positive association between house infestation by triatomines and poor sanitary conditions, presence of animals (rodents, chicken, dogs), construction material storage and proximity to vegetation [10–13]. The study of these determinants of invasion is crucial for a long-lasting control of the vector population and can help explain the variability in infestation between houses of the same village.

It is important to note that 68 of the 71 triatomines collected in Bajada de Chanduy were found exclusively in one house (house 2) and only three specimens were found in the rest of the houses (houses 5 and 6) (Fig. 2). The fact that nymphs were found in all the infested houses indicates that the insects are actively breeding and the population most likely growing. As triatomines colonize the peridomicile and move closer to domestic animals and people, the risk for *T. cruzi* infection in humans increases dramatically. Since all the specimens in house 2 were collected from a pile of reed located in the backyard, it is possible that this is the primary source of triatomines in this village. However, according to the owners of the house, that material was brought into their property from elsewhere. It is not clear whether the triatomines came imported with the reed or if a local population of vectors colonized the habitat provided by that material after it came into the house. The genetic analysis of the vectors collected will provide additional information that will help to elucidate the origin and structure of this particular triatomine population. The genetic study of this population of *T. dimidiata* will allow us to compare its genetic similarity with other local and foreign populations and to determine the geographic distribution of these vectors as well as their immigration status. Knowledge of the biology and genetics of the most prevalent vector species, their infection status (*T. cruzi, T. rangeli* or both) and dispersal is essential for the understanding of the transmission dynamics of Chagas disease and the implementation of adequate strategies for vector control.

Acknowledgements

This study was supported by a Chancellor's Grant from San Francisco de Quito University. We thank the National Service for the Control of Diseases Transmitted by Arthropod Vectors (SNEM) for facilitating the search and collection of the insects used in the study. We also thank David Salomon, David Guevara, Daniela Zuñiga, and Antonia Castells from the undergraduate program in Biotechnology (USFQ), as well as Ana María Salinas and Melina Rodriguez from the Masters program in Microbiology (USFQ), for their help with the field work. Finally, we thank Pablo Endara from the Health Science Department (USFQ) for his guidance and support with the statistical analysis.

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