Hindawi Publishing Corporation
Epidemiology Research International
Volume 2012, Article ID 138259, 7 pages
doi:10.1155/2012/138259

Clinical Study

Trypanosoma cruzi Infection in an Indigenous Kariña Community in Eastern Venezuela

Mariolga Berrizbeitia,[1,2] Dairene Moreno,[3] Brian J. Ward,[4] Erika Gómez,[2] Alicia Jorquera,[5] Jessicca Rodríguez,[1] Norys García,[1] Melfran Herrera,[6] Mercedes Marcano,[6] and Momar Ndao[4]

[1] *Postgrado en Biología Aplicada, Núcleo de Sucre, Universidad de Oriente, Cumaná, Estado Sucre 6101, Venezuela*
[2] *Instituto de Investigaciones en Biomedicina y Ciencias Aplicadas, Universidad de Oriente, Cumaná, Estado Sucre 6101, Venezuela*
[3] *Departamento de Bioanálisis, Núcleo de Sucre, Universidad de Oriente, Cumaná, Estado Sucre 6101, Venezuela*
[4] *National Reference Centre for Parasitology, Research Institute of the McGill University Health Centre (MUHC), Department of Medicine, Montreal General Hospital, 1650 Cedar Avenue, Room R3-R137, Montreal, QC, Canada H3G 1A4*
[5] *Centro de Investigaciones en Ciencias de la Salud, Núcleo de Anzoátegui, Universidad de Oriente, Barcelona, Estado Anzoátegui 6001, Venezuela*
[6] *Gerencia de Saneamiento Ambiental y Control de Endemias, Ministerio del Poder Popular para la Salud, Fundasalud Sucre, Carúpano, Estado Sucre 6150, Venezuela*

Correspondence should be addressed to Momar Ndao, momar.ndao@mcgill.ca

Received 6 March 2012; Revised 7 August 2012; Accepted 23 August 2012

Academic Editor: Percy Mayta-Tristán

We investigated the seroprevalence of *Trypanosoma cruzi* infection in an indigenous Kariña population in eastern Venezuela. A total of 175 serum samples were collected in the community of Piñantal during February 2009. Interviews targeting socioeconomic and environmental factors associated with the *T. cruzi* transmission were also conducted. Samples were evaluated using trypomastigote excreted/secreted antigens (TESAs) in an ELISA format. TESA-ELISA positive samples were confirmed by indirect haemagglutination (HAI) (Wiener). A nonsystematic collection of vectors was also undertaken. *T. cruzi* seroprevalence was 7.43% according to both assays, and the mean age of infected patients was 48.61 ± 10.40 years (range 34 to 73 years). The vector infection rate was 20.00% (2/10). *T. cruzi* seropositivity was associated with a history of triatomine bites, the ability to recognize the vector and poor knowledge about Chagas disease, but no associations were found with gender, house type, knowledge of how the disease is transmitted, or the presence of vectors or animals inside dwellings. To our knowledge, this is the first study of the seroprevalence of *T. cruzi* in an indigenous population in eastern Venezuela. All of the epidemiological variables required for the establishment of active vectorial transmission of *T. cruzi* were present in this community.

1. Introduction

The Southern Cone and Andean countries initiative proposed the reduction or interruption of the vectorial transmission of Chagas disease (CD) in several countries in Latin America such as Venezuela, Chile, Colombia, Uruguay, and regions of Brazil. This goal was achieved to the extent that some countries in South America have been declared free of the vectorial transmission of *T. cruzi* [1–3].

In Venezuela, the national Chagas Disease Control Program was started in 1966. This program reduced vectorial transmission through the use of insecticides, improvement of rural houses, education campaigns, and the screening of all public hospital blood banks for *T. cruzi*. In spite of these activities, several investigators raised the possibility that CD could reemerge in Venezuela [4, 5]. This fact has been demonstrated in several works: Añez et al. [6] carried out a multicentric study where they evaluated 310 patients

referred to a diagnostic center and reported 168 (54.19%) were seropositive for *T. cruzi*; of these, 75 (44.64%) were in the acute phase and 36.00% were children under 10 years old. Moreover, the serologic examination performed in 3,993 individuals from 75 rural areas in Venezuela showed 11.22% prevalence of *T. cruzi*, identifying 8.50% of the infections in children less than 10 years old. Therefore, the authors suggested the reemergence of Chagas disease in Venezuela due to the demonstration of active transmission found in children. In contrast, Rojas et al. [7] demonstrated seropositivity of *T. cruzi* in humans (1.57%) over 15 years old. Although they did not show active transmission in humans, they found 1.81% of dogs, aged 2-3 years, and 6-7 years infected by *T. cruzi* in Parroquia Xaguas, Urdaneta Municipality, Lara State. However, Rodríguez-Bonfante et al. [8] suggested active Chagas disease transmission in Andrés Eloy Blanco municipality for at least the last two decades because they found a seroprevalence of *T. cruzi* infection of 6.90% ($n = 60$), and in the seropositive individuals, 5 (8.33%) were under 10 years and 10 (16.66%) were under 20 years old. Also, they found *Rhodnius prolixus* and *Panstrongylus geniculatus* infected with *T. cruzi*: 20.00% and 5.00%, respectively. More recently, Bonfante-Cabarcas et al. [9] accomplished a study in 26 rural communities including 905 households, 2,156 humans, and 333 dogs in Lara State, Venezuela, finding a seroprevalence of *T. cruzi* infection in humans (7.24%) and canines (6.90%). In contrast with other regions in Venezuela, the authors suggested that the transmission of Chagas disease in this region has been interrupted in humans. They suggested control measures should be implemented for the presence of risk factors for disease transmission in the study area.

Feliciangeli et al. [10] revealed transmission of *T. cruzi* in children less than 15 years of age in Barinas State, (4/3296: 0.12%). The main vectors caught in Barinas were *Rhodnius prolixus* (2/37 infected), *Triatoma maculata* (2/3 infected), and 1 *Eratyrus mucronatus*. This epidemiological situation in Venezuela worsened when, in 2008 and 2009, school-based outbreaks of *T. cruzi* were reported among students and teachers in two different states. At the Andrés Bello municipal school (Chacao municipality, Caracas), 77 students, 25 school personnel, and 1 food handler were found to be *T. cruzi* infected from a total of 1,000 exposed individuals. One child died of acute myocarditis [11]. On April 5, 2009, the Minister of Health of Venezuela confirmed reports of a second outbreak of oral transmission of *T. cruzi* at the Romulo Monasterio state school (Chichiriviche de la Costa, Vargas state). A total of 47 students, 2 teachers, and 2 food handlers were infected by *T. cruzi* and 3 students died, the source of infection in both events (2008 and 2009) was contaminated guava juice [12] (Red de Sociedades Científicas de Venezuela 2009).

To our knowledge, there are no epidemiological studies that define the seroprevalence of CD in urban populations and also there are few works in blood banks in Venezuela. We demonstrated that seroprevalence in blood bank rates in Bolivar (0.62%) and Portuguesa states (5.47%) has not changed significantly during the past 12 years, and the great majority of those found to be positive were between 25 to 28 years of age [13, 14]. In contrast, investigations that focus on more rural areas in Venezuela have shown that vectorial transmission remains active in children under 15 years old [4, 8]. For example, in Barinas and Yaracuy states, seroprevalence rates ranging from 3.30 to 22.80% have recently been reported [4, 10]. However, *T. cruzi* seroprevalence rates in indigenous populations in Venezuela or even in Amerindians in Latin America are largely unknown. Herein, we report the rate of *T. cruzi* seropositivity and the epidemiological variables associated with the *T. cruzi* infection in the Kariña, an ethnic group that inhabits parts of the Anzoátegui, Bolívar, Monagas, and Sucre states in eastern Venezuela.

2. Methods

2.1. Study Area. The study was carried out in Piñantal (municipality Sucre, Raul Leoni parish), a small community located in the west part of the Sucre state about 73.80 km from Cumana (the state capital). Latitude: 10° 09′ 56.90″ N, longitude: 6.4° 18′ 17.58″ W, altitude: 336 m.a.s.l (Figure 1). This rural forested area is inhabited almost exclusively by an indigenous Kariña community with a total population of 764 inhabitants in 2007 as registered by the Community Council. The main economic activity is subsistence agriculture. Principal crops are corn, plantain, white bean, taro, pumpkin, yams, and cassava. Access to this community is difficult because a significant stretch of the route is gravel road. This fact determines that in times of rain. Piñantal is isolated from more developed communities.

2.2. Epidemiological Study. We performed a descriptive cross-sectional study in cooperation with Environment Management and Endemic Diseases Control (Ministry of Popular Power for Health, Venezuela). We used a nonprobabilistic sampling method to evaluate infection by *T. cruzi* in the Piñantal community. This study was part of the "Control Program for Chagas Disease" at Sucre state, Venezuela. The study team visited the Piñantal indigenous community three times. First, we met members of the community to explain the importance of the study, to obtain informed consent from the participants or the parents/legal guardians of subjects <18 years of age, and to obtain samples. The individuals of this study voluntarily attended the study center. The samples were collected at the "Piñantal Basic School." Individuals walked to the school where the samples were taken. Epidemiological variables were evaluated by questionnaire to identify associated factors for *T. cruzi* infection including dwelling type, the ability to recognize the insect vector, history of recognized bites or blood transfusions, and the presence of animals inside or adjacent to the dwelling. Blood samples were permitted to clot at room temperature for 10 minutes, and afterward the sample was centrifuged at 1000 g for 10 minutes. Then serum was collected and stored in aliquots at −70°C until used for serologic testing.

2.3. TESA Proteins. TESA proteins from the *T. cruzi* Tulahuen strain were obtained from supernatants of infected

Vero cells as previously described [15]. Briefly, Vero cell monolayers grown to 65.00% confluence were infected with *T. cruzi* trypomastigotes (Tulahuen strain, National Reference Centre for Parasitology, Montreal, Canada) (1×10^9 parasites/mL/175 cm^2) and incubated at 37°C with 5.00% CO_2 for 4 days in Eagle's minimum essential media (EMEM; Wisent, St. Bruno, QC), supplemented with 1 M Hepes (1.00%) and gentamicin (0.10%) without fetal bovine serum (FBS) and phenol red. After four days of incubation, the infected monolayers were washed twice with the media and reincubated for 18–20 hours at 37°C in 5.00% CO_2 in complete media. Supernatants were then harvested and centrifuged at $2,800 \times g$ for 15 minutes at 4°C and filtered through a Millipore membrane (0.22 μm) (Bradford, MA). Supernatant proteins were concentrated ~32-fold (Amicon Ultra device: 30,000 MWCO, Bradford, MA) and either used immediately or stored at −80°C. Total concentrated TESA retained the high-molecular-weight polypeptide bands (150–170 kDa), which correspond to the most immunogenic antigens [16]. Protein content of the FBS-free TESA was quantified using the Micro-BCA protein assay reagent kit (Pierce Co, Rockford, IL).

2.4. TESA-Based EIA. The TESA-based EIA was performed as previously described [15]. Briefly, 96-well polystyrene plates (Immulon 2; Thermo Labsystems, Franklin, MA) were coated by incubating total TESA protein (1 μg/mL), at 4°C, overnight (100 μL/well) in 1 M sodium carbonate buffer (pH 9.6). Plates were washed four times with phosphate-buffered saline pH 7.40 (PBS, 0.01 M phosphate buffer, 0.14 M NaCl) containing 0.05% Tween 20 (PBST) (A&C, American Chemicals LTD, St-Laurent, QC), blocked for one hour at 37°C with PBS containing 5.00% skimmed milk (Parmalat, QC) and 0.10% Tween 20. Sera were diluted at 1 : 800 in the corresponding blocking solution (100 μL/well) and incubated for 1 hour at 37°C. These dilutions were selected to permit optimal differentiation between positive and negative control sera, as tested by checkerboard titration [15]. Assays were completed with an optimal dilution of HRP-conjugated goat, anti-human IgG (30 minutes at 37°C) (Perkin Elmer Life Science, Boston, MA, 100 μL/well), four washes with PBST, and a final incubation with 3.3′.5.5′-tetramethylbenzidine (TMB) (Serologicals Corporation, MA) (100 μL/well) for 10 minutes at room temperature. The reaction was stopped with 1N H_2SO_4 (50 μL/well). Optical density was measured at 450 nm using an automated EIA reader (Biotrak II ELISA reader, Amersham Biosciences, Cambridge, London). All experiments were performed in duplicate on different days and the data pooled. National Chagas Laboratory of Immunodiagnosis (NCLI) confirmed positive and negative controls were included on each plate. Results were accepted only when the coefficient of variation within and among plates was ≤15%, otherwise, the samples were tested again. The cutoff was determined by using the following calculation: cutoff = (mean OD of the negative control + 3 × standard deviation) × (mean OD of the positive control + mean OD of the negative control) (BIOSChile, Ingeniería Genética S.A., Santiago, Chile). All positive samples from the TESA-based EIA were confirmed by indirect haemagglutination (Wiener lab, Argentina).

Figure 1: Location of Piñantal community at Sucre state, Venezuela.

2.5. Entomological Surveys. Plastic containers were distributed to residents who agreed to collect all of the triatomines that they found inside or around their homes during a one-month period. Triatomine bugs were classified by sex and developmental stage according to [17]. Vector feces were obtained by abdominal compression, then diluted in normal saline solution and examined microscopically. Positive slides were stained with Giemsa, and parasites were identified using morphological characteristics.

2.6. Clinical Study. All subjects seropositive for *T. cruzi* according to the two serological assays employed were invited to participate in a clinical evaluation. Evaluations were performed at the Antonio Patricio Alcala Hospital (Cumana, Sucre state, Venezuela) by a cardiologist. Using a protocol that included clinical examination, an electrocardiogram, chest radiographs, and an echocardiogram, the seropositive subjects were classified in the different phases of Chagas disease according to [18].

2.7. Statistical Analyses. All statistical analyses were performed by a statistical software package (SAS version 17.0; SAS institute, Inc., Cary, NC, USA). To determine associated factors for *T. cruzi* infection, we used univariate analyses and chi square or Fisher Exact Probability tests were applied. P values < 0.05 were considered to be statistically significant. Odds ratio (OR) with a 95.00% confidence interval (IC95%) and a significance level of 0.05 was performed in the univariate analysis to determine the independent effect of the associations.

3. Results

3.1. Seroprevalence of T. cruzi Infection in Piñantal. Sera from a total of 175 individuals were tested (22.91% of the total

Table 1: Seroprevalence of *T. cruzi* infection of evaluated indigenous Kariña individuals, Piñantal community, Sucre state, Venezuela.

			TESA-ELISA			
Patients	Sex		Mean age	Mean	Range	Seroprevalence
(*n*)	F	M	(years) ± SD	OD ± SD	(min–max)	
Pos (13)	9	4	48.61 ± 10.40	1.95 ± 0.33	1.34–2.45	7.43%
Neg (162)	111	50	21.06 ± 14.96	0.09 ± 0.07	0–0.34	

F: female; M: male; OD: optical density; SD: standard deviation; Pos: positives; Neg: negatives; min: minimum; max: maximum.

Table 2: Epidemiological variables associated with *T. cruzi* infection in the indigenous Kariña community at Piñantal, Sucre state, Venezuela.

Associated factors	No.	Percentage of positives for the associated factors (*n*)	OR	CI 95%	*P*
(1) Sex					
Female	120	7.50 (9)	1.01	0.30–3.45	0.60
Male	54	7.40 (4)			
(2) Transfusion					
Yes	10	0	—	—	—
No	164	7.93			
(3) Recognized the vector					
Yes	104	11.54 (12)	9	1.14–70.88	*0.01
No	70	1.43 (1)			
(4) Knowledge about Chagas disease					
Yes	18	22.22 (4)	4.67	1.27–17.11	*0.03
No	156	5.77 (9)			
(6) Bite for the vector					
Yes	37	16.20 (6)	3.59	1.13–11.45	*0.03
No	137	5.11 (7)			
(7) Vectors inside of the dwellings					
Yes	71	7.04 (5)	0.90	0.28–2.87	0.86
No	103	7.77 (8)			
(8) Vectors around of the dwellings					
Yes	67	8.95 (6)	1.40	0.45–4.38	0.55
No	107	6.54 (7)			
(9) Kind of dwellings					
Consolidated	115	6.09 (7)	0.54	0.17–1.69	0.22
Nonconsolidated (rancho)	56	10.71 (6)			
(10) Vector associated with the disease					
Yes	20	15.00 (3)	2.49	0.62–9.94	0.18
No	151	6.62 (10)			

OR: odds ratio; CI: confidence interval, *P*: probability. Chi square was applied for associated factors: 3, 7, and 8, whereas Fisher Exact Probability test was used for the rest of associated factors, *significance.

population) of which 121 (69.15%) were females and 54 were males (30.85%). The mean age was 23.16 ± 16.36 years (range 6 months to 73 years). A total of 13 samples were positive in both EIA and HAI tests (7.43%), and 162 were negative (92.57%) (Table 1). There were no discordant samples. Of the infected individuals, nine were females, four were males, and all were more than 34 years old.

3.2. Results of the Entomological Surveys. The nonsystematic household searches of the Piñantal residents yielded only 10 triatomine bugs; most were caught inside the dwellings at night time, having been attracted to the house lights. All were adults and were identified as *Panstrongylus geniculatus.* Of these, 2/10 (20%) were positive for *Trypanosoma* sp. Flagellates could be visualized in the feces in the field by direct examination.

3.3. Clinical Findings. Of the 13 *T. cruzi* seropositive individuals, 8 participated voluntarily in the clinical evaluation. Of these, 87.50% (7/8 individuals) had no signs or symptoms associated with CD or abnormalities in their electrocardiograms, chest radiographs, or echocardiograms. They were thus classified as being in the indeterminate phase of the disease (Chagas stage I). One male patient (36 years old) was classified as Chagasic stage II. This patient manifested weakness, fatigue, and tachycardia: the electrocardiogram

revealed arrhythmia, and the chest radiographs showed an increase of the cardiac silhouette.

3.4. Demographic Results. The socioeconomic indicators in the population surveyed showed high levels of poverty in Piñantal; 66.90% of the inhabitants earned less than $100 per month. Most of the survey families practiced subsistence agriculture; feeding themselves from their own harvests and selling only the surplus. Although the majority of the houses belonging to the individuals that participated in the study were well constructed with cement floors (70.50%), brick walls (67.30%), and tin roofs (70.50%), we also found houses classified as "ranchos" with adobe (22.20%) or tin (3.50%) walls and dirt floors (20.50%). "Ranchos" are typically found in poor rural areas and are known to facilitate the colonization and establishment of triatomines, therefore promoting disease transmission. Nevertheless, in recent years, the presence of *T. cruzi* vectors has also been observed inside well-constructed brick houses even in urban areas, thus challenging the assumption that triatomines colonize only "ranchos" rather than "improved" houses [19, 20].

Overall, 18.30% of the study population was illiterate. Some (7.40%) had attended only kindergarten while 51.40% and 13.10% had attended primary and secondary school, respectively, and 5.10% had received no formal education (4.60% did not answer the question). The most frequent occupations in our study population were domestic activities, farming, and attending school. All the individuals evaluated stated that they have lived all their lives in Piñantal.

As regards to sanitary conditions, 82.20% of the individuals surveyed living in Piñantal do not have toilets in their homes and thus defecate outdoors. Furthermore, 56.30% do not have a potable water supply, which means they go to the nearby rivers in search of drinking water. Finally, 50.40% of the individuals interviewed affirmed that they had more than one animal (e.g., dogs, cats, chickens) that either lived either inside or close to homes.

3.5. Associate Factors for Infection. Among the variables associated with *T. cruzi* seropositivity were knowledge about CD, history of recognized triatomine bites, and ability to recognise the vector. No associations were found with gender, type of dwelling, knowledge of how the disease is transmitted, or the presence of vectors or animals either inside or close to the dwellings (Table 2).

4. Discussion

Infection by *T. cruzi* has been demonstrated over a period of 9 millennia in mummies evaluated from archaeological sites between Ilo, Peru to Antofagasta, Chile, and along South America's coastal area of the Atacama desert in southern Peru and northern Chile. The fact that 40.63% (115/283) of the mummies evaluated had a positive reaction (i.e., hybridized) with the probe for *T. cruzi* suggests the occurrence of a common infectious disease with an unchanging transmission mechanism that as a consequence demonstrates a static disease pattern over a period of 9,000 years. Initially, before human occupation in South America, the infection by *T. cruzi* appears exclusively in wild mammals, but, when the human being appeared in the southern Andean coastal area 9,500 years ago, the humans became part of the sylvatic cycle of Chagas disease that then evolved into domestic animals and the latter currently represents the most important cycle [21].

To our knowledge, there have been no previous investigations of Chagas disease in indigenous populations in eastern Venezuela. In this study, using a TESA-based ELISA that has been shown to be highly sensitive and specific [14], we found a high seroprevalence of antibodies against *T. cruzi* (7.43%) in the Kariña community Piñantal, Sucre state. However, the sample size required was not reached, so we applied a nonprobabilistic sampling method. It is very important to take in account the geographical area that is very difficult to access and and the resistance from the community. They were scared about the diagnosis, and they stated that they preferred not to know about their condition. The individuals evaluated indicated that they had always lived in that community. Therefore, the positive cases detected in this work are autochthonous and do not represent cases imported from other regions of the country. Of the 8 seropositive individuals who agreed to further investigations, seven had no evidence of cardiac involvement. This observation supports previous findings that most people with *T. cruzi* infection are asymptomatic [22].

Similar seroprevalence rates to those we observed in the current study (7.4%) have been reported for an indigenous population in Colombia (8.71%) [23] and 18 indigenous communities in Ecuador where 6.03% of the 1011 subjects studied were found to have antibodies against *T. cruzi*. In these latter communities, the seroprevalence rate was 4.8% among children under 5 years old [24]. In contrast, a very high prevalence rate (61%: 100 out of 164 individuals) has been reported among indigenous people belonging to the Toba, Wichi, and Pilaga ethnic groups in Argentina (Formosa Province) [25]. In our study, all of the seropositive individuals were over 34 years old, suggesting that there has been little active transmission of *T. cruzi* in this area for several decades. However, the presence of infected vectors in this community suggests a continued significant risk of vectorial transmission.

Our findings are in contrast with Añez et al. [26] who conducted an evaluation in individuals from a Yukpa ethnic community in western Venezuela. Although the sample size was similar to our work ($n = 173$), they found a higher *T. cruzi* infection in the indigenous population (13.90%). Moreover, 25% of the seropositives were under 10 years old, and all the positives had a nonsymptomatic infection. These results contrast with our findings since all the infected patients in the present work were over 34 years old and only 1 individual was classified through clinical examination as chagasic type II.

In this study, we have shown invasion of Piñantal houses by wild triatomine species. The triatomines captured inside or around of the dwellings were adults of *P. geniculatus*. The

inhabitants informed us that these vectors were attracted to the lights during the night. In contrast with other studies in Venezuela, however, we were not able to detect the domiciliation of *P. geniculatus* [27]. These authors reported mixed household infestations of *Rhodnius prolixus* and *P. geniculatus* adults and nymphs at El Guamito, Lara State, Venezuela. *P. geniculatus* has even been collected in households in Caracas (the Venezuela capital) and in the neighboring states of Miranda and Vargas. In these studies, 76.12% of *P. geniculatus* collected were infected with *T. cruzi* of which 60.21% (53/88) had a positive reaction to human antiserum (i.e., had fed on humans). Moreover, 41% of the vectors that had fed on humans were also positive for *T. cruzi* [28]. Although we could not demonstrate the domiciliation of *P. geniculatus* with our limited vector collection in Piñantal, the observations of flagellates in the triatomine guts and the presence of these infected bugs in human dwellings represent a risk of vectorial transmission of *T. cruzi* for the individuals living in this area.

Combining our serologic and epidemiologic data, we found significant associations between *T. cruzi* infection and poor knowledge about CD in general and the ability to recognize the vector. Other studies have shown a relationship between *T. cruzi* infection and lack of adequate understanding about Chagas disease. For example, a study done in Honduras revealed that 57.80% of infected individuals evaluated knew little or nothing about this parasitic infection. In fact, lack of knowledge is well known as a major obstacle in the fight against CD [29]. On the other hand, the majority of the seropositive subjects in our study (12/13 versus 92/162: P value < 0.05) stated that they could recognize the vectors and had seen them inside or close to their dwellings. Thus, vector recognition appears to be quite a good indication of association for acquiring CD (Table 2). These results are in agreement with a study carried out in Guaranis, Bolivia, where 98.00% of the population studied recognized the CD vector [30]. Although the type of housing has historically been linked with the risk of acquiring CD [31], our findings of an absence of such association are in agreement with other recent work in Venezuela [19].

5. Conclusions

The current epidemiological situation for CD in Venezuela is alarming. Our own work [32] and that of others [11, 33, 34] demonstrate the continued presence of CD in many regions of the country. The recent school-based outbreaks of oral transmission are eloquent reminders that this infection will always be present in wild reservoirs. Indigenous communities often live in close proximity to such wild reservoirs and typically have poor access to high-quality diagnostic and treatment facilities. These facts put such communities at particular risk of acquiring CD. In conclusion, we have shown CD seroprevalence in an indigenous community in Venezuela. We identified infected vectors in this same community. The conditions in which the people of Piñantal live as well as their lack of knowledge about this illness and its transmission place this community at very high risk for CD. Control strategies that specifically target communities such as Piñantal should be developed and implemented as quickly as possible.

Acknowledgments

The authors thank Dr. Herbert Tanowitz (Albert Einstein College of Medicine, New York) for providing the *T. cruzi* strains. This work was supported by Health Canada grant no. HT070-010033 and partially supported by a Grant from FONACIT (Misión Ciencias) no. 2007001425 and Grant no. CI-2-040102-1417-08 from Research Commission, Núcleo de Sucre, Universidad de Oriente, Venezuela to Dr. M. Berrizbeitia.

References

[1] M. Lorca H, A. García C, M. I. Bahamonde M, A. Fritz M, and R. Tassara O, "Serological certification of the interruption of the vectorial transmission of Chagas disease in Chile," *Revista Medica de Chile*, vol. 129, no. 3, pp. 264–269, 2001.

[2] A. Moncayo, "Progress towards interruption of transmission of chagas disease," *Memorias do Instituto Oswaldo Cruz*, vol. 94, no. 1, pp. 401–404, 1999.

[3] A. C. Silveira and M. C. Vinhaes, "Elimination of vectorial transmission of Chagas disease in Brazil," *Medicina*, vol. 59, pp. 97–102, 1999.

[4] N. Añez, G. Crisante, and A. Rojas, "Update on chagas disease in Venezuela—a review," *Memorias do Instituto Oswaldo Cruz*, vol. 99, no. 8, pp. 781–787, 2004.

[5] M. D. Feliciangeli, D. Campbell-Lendrum, C. Martinez, D. Gonzalez, P. Coleman, and C. Davies, "Chagas disease control in Venezuela: lessons for the Andean region and beyond," *Trends in Parasitology*, vol. 19, no. 1, pp. 44–49, 2003.

[6] N. Añez, G. Crisante, A. Rojas et al., "La cara oculta de la enfermedad de Chagas en Venezuela," *Boletín de Malariología y Salud Ambiental*, vol. 43, no. 2, pp. 45–57, 2003.

[7] M. E. Rojas, P. Várquez, M. F. Villarreal et al., "An entomological and seroepidemiological study of Chagas' disease in an area in central-western Venezuela infested with *Triatoma maculata* (Erichson 1848)," *Cadernos de Saude Publica*, vol. 24, no. 10, pp. 2323–2333, 2008.

[8] C. Rodríguez-Bonfante, A. Amaro, M. García et al., "Epidemiology of Chagas disease in Andrés Eloy Blanco, Lara, Venezuela: triatomine infestation and human seroprevalence," *Cadernos de Saude Publica*, vol. 23, no. 5, pp. 1133–1140, 2007.

[9] R. Bonfante-Cabarcas, C. Rodriguez-Bonfante, B. O. Vielma et al., "Seroprevalence for *Trypanosoma cruzi* infection and associated factors in an endemic area of Venezuela," *Cadernos de Saúde Pública*, vol. 27, no. 10, pp. 1917–1929, 2011.

[10] M. D. Feliciangeli, M. J. Sánchez-Martín, B. Suárez et al., "Risk factors for *Trypanosoma cruzi* human infection in Barinas State, Venezuela," *American Journal of Tropical Medicine and Hygiene*, vol. 76, no. 5, pp. 915–921, 2007.

[11] B. A. de Noya, Z. Díaz-Bello, C. Colmenares et al., "Large urban outbreak of orally acquired acute chagas disease at a school in Caracas, Venezuela," *Journal of Infectious Diseases*, vol. 201, no. 9, pp. 1308–1315, 2010.

[12] Red de Sociedades Científicas de Venezuela, "Enfermedad de Chagas, a 100 años de su descripción y descubrimiento del *Trypanosoma cruzi*," 2009, http://www.ovsalud.org/doc/Notiepi2.pdf.

[13] A. Aché, "Prevalence of human infections by *Trypanosoma cruzi* in blood banks in Venezuela," *Revista do Instituto de Medicina Tropical de Sao Paulo*, vol. 35, no. 5, pp. 443–448, 1993.

[14] M. Berrizbeitia, M. Ndao, J. Bubis et al., "Field evaluation of four novel enzyme immunoassays for Chagas' disease in Venezuela blood banks: comparison of assays using fixed-epimastigotes, fixed-trypomastigotes or trypomastigote excreted-secreted antigens from two *Trypanosoma cruzi* strains," *Transfusion Medicine*, vol. 16, no. 6, pp. 419–431, 2006.

[15] M. Berrizbeitia, M. Ndao, J. Bubis et al., "Purified excreted-secreted antigens from *Trypanosoma cruzi* trypomastigotes as tools for diagnosis of Chagas' disease," *Journal of Clinical Microbiology*, vol. 44, no. 2, pp. 291–296, 2006.

[16] M. Nakazawa, D. S. Rosa, V. R. A. Pereira et al., "Excretory-secretory antigens of *Trypanosoma cruzi* are potentially useful for serodiagnosis of chronic Chagas' disease," *Clinical and Diagnostic Laboratory Immunology*, vol. 8, no. 5, pp. 1024–1027, 2001.

[17] H. Lent and P. Wygodzinsky, "Revision of the triatominae (Hemiptera, Reduviidae) and their significance as vectors of Chagas'disease," *Bulletin of the American Museum of Natural History*, vol. 163, pp. 125–520, 1979.

[18] J. Puigbo, H. Giordano, C. Suarez et al., *Clinical Aspects in Chagas Disease. Actualizaciones en la Enfermedad de Chagas*, Buenos Aires, Organismo Oficial del Congreso Nacional de Medicina, 1992.

[19] N. Añez, G. Crisante, and H. Parada, "Nuevos casos agudos de enfermedad de Chagas en el occidente de Venezuela," *Salus*, vol. 11, pp. 87–90, 2007.

[20] M. J. Sanchez-Martin, M. D. Feliciangeli, D. Campbell-Lendrum, and C. R. Davies, "Could the Chagas disease elimination programme in Venezuela be compromised by reinvasion of houses by sylvatic *Rhodnius prolixus* bug populations?" *Tropical Medicine and International Health*, vol. 11, no. 10, pp. 1585–1593, 2006.

[21] A. C. Aufderheide, W. Salo, M. Madden et al., "A 9,000-year record of Chagas' disease," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 7, pp. 2034–2039, 2004.

[22] N. Añez, G. Crisante, A. Rojas et al., "Detection and significance of inapparent infection in Chagas disease in western Venezuela," *American Journal of Tropical Medicine and Hygiene*, vol. 65, no. 3, pp. 227–232, 2001.

[23] A. Corredor Arjona, C. A. Alvarez Moreno, C. A. Agudelo et al., "Prevalence of *Trypanosoma cruzi* and *Leishmania chagasi* infection and risk factors in a Colombian indigenous population," *Revista do Instituto de Medicina Tropical de Sao Paulo*, vol. 41, no. 4, pp. 229–234, 1999.

[24] M. Chico H, C. Sandoval, A. Guevara E et al., "Chagas disease in Ecuador: evidence for disease transmission in an indigenous population in the Amazon region," *Memorias do Instituto Oswaldo Cruz*, vol. 92, no. 3, pp. 317–320, 1997.

[25] M. Galván, A. R. Fabre, J. M. Alonso et al., "Impacto de la enfermedad de Chagas en comunidades aborígenes de la provincia de Formosa—Argentina," http://www1.unne.edu.ar/cyt/2003/comunicaciones/03-Medicas/M-027.pdf.

[26] N. Añez, R. Atencio, Z. Rivero et al., "Chagas disease inapparent infection in asymptomatic individuals from a Yukpa ethnic community in western Venezuela," *Boletín de Malariología y Salud Ambiental*, no. 2, pp. 167–175, 2011.

[27] M. D. Feliciangeli, H. Carrasco, J. S. Patterson, B. Suarez, C. Martínez, and M. Medina, "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," *American Journal of Tropical Medicine and Hygiene*, vol. 71, no. 4, pp. 501–505, 2004.

[28] H. J. Carrasco, A. Torrellas, C. García, M. Segovia, and M. D. Feliciangeli, "Risk of *Trypanosoma cruzi* I (Kinetoplastida: Trypanosomatidae) transmission by *Panstrongylus geniculatus* (Hemiptera: Reduviidae) in Caracas (Metropolitan District) and neighboring States, Venezuela," *International Journal for Parasitology*, vol. 35, no. 13, pp. 1379–1384, 2005.

[29] W. B. Petana, "Educational approach in the control of Chagas' disease," *Bulletin of the Pan American Health Organization*, vol. 9, no. 4, pp. 300–305, 1975.

[30] J. Verdú and M. Ruiz, "Control del Chagas en comunidades Guaraníes: conocimiento y hábitos higiénicos dentro del proyecto de mejoramiento de viviendas en Bolivia," *Gaceta Sanitaria*, vol. 172, no. 2, pp. 166–168, 2003.

[31] M. D. Starr, J. C. Rojas, R. Zeledon, D. W. Hird, and T. E. Carpenter, "Chagas' disease: risk factors for house infestation by *Triatoma dimidiata*, the major vector of *Trypanosoma cruzi* in Costa Rica," *American Journal of Epidemiology*, vol. 133, no. 7, pp. 740–747, 1991.

[32] M. Berrizbeitia, B. J. Ward, J. Bubis et al., "85-kDa protein of *Trypanosoma cruzi* purified by affinity chromatography used in the multiple antigen binding assay (MABA) for the diagnosis of *T. cruzi* infection in a Venezuelan rural community," *Parasitology Research*, vol. 106, no. 5, pp. 1127–1134, 2010.

[33] I. Sandoval, N. Añez, E. Villegas, and J. V. Scorza, "Persistencia de la transmisión de la enfermedad de Chagas sin colonización por el vector conocido, en localidades controladas de Venezuela," *Revista de la Sociedad Venezolana de Microbiología*, vol. 23, pp. 166–168, 2003.

[34] L. Traviezo and R. Bonfante, "Estudio seroepidemiológico de la enfermedad de Chagas en la localidad de Caballito, municipio Simón Planas, estado Lara. Venezuela," *Parasitol Latinoam*, vol. 59, pp. 46–54, 2004.