BOLETÍN
DE MALARIOLOGÍA
Y SALUD AMBIENTAL
Vol. XLV, Nº 2, Agosto-Diciembre, 2005

Molecular Identification of *Trypanosoma cruzi* in *Didelphis marsupialis* and *Rattus* spp. in an old endemic area of Chagas disease in Lara State, Venezuela

Hector De Lima*, Armando Rodríguez, Felix Flores, Wilmer Galindo, Jacinto Convit & Noris Rodríguez

During the period from October 1996 to October 1998, captures of mammals were made in two places with different ecological characteristics, La Matica and Tintinal, searching for reservoirs of *Trypanosoma cruzi*. A total of 513 animals, belonging to 10 different genera, were captured. Blood was extracted from them by cardiac puncture, to carry out examinations in fresh and culture in blood agar base medium. Xenodiagnosis was also carried out on a limited number of animals. Two different species were found naturally infected with *T. cruzi*, opossums (*Didelphis marsupialis*) and black rats (*Rattus* spp.). In *D. marsupialis* the presence of *T. cruzi* was determined in 9 of the 31 animals studied (29.03%). *Rattus* was found to be infected in 4.41 % (3/68). Five cultures (2 from *D. marsupialis* and 3 from *Rattus* spp.) were characterized as *T. cruzi* by means of the kDNA restriction pattern and hybridization with species-specific probes. When we studied the *D. marsupialis* according to the place of capture, we found that the infection in Tintinal was present in 57.14% (8/14) of the animals studied, while in La Matica it scarcely represented 5.88% (1/17). These differences were statistically significant. ($p < 0.05$). Finally, the inclusion of a "prevalence index of *T. cruzi* in reservoirs" is suggested, as an additional measure to predict the real situation of an endemic area.

Key words: *Trypanosoma cruzi*, *Didelphis marsupialis*, *Rattus*, Chagas disease, molecular biology, Venezuela.

INTRODUCTION

Chagas disease is an endemic zoonosis broadly distributed throughout the American Continent, representing an important health problem in 17 Latin American countries. About 100 million people of the Latin American population are at risk and 16-18 million people are estimated to be infected (WHO, 2002).

In Venezuela, the first case of Chagas disease was reported by Tejera (1919). Until 1980 it was considered one of the five principal causes of death, but from that year to this date it began to descend to positions of less importance, between the thirteenth and eighteenth, with an annual average of around 720 deaths according to reports issued by the Health and Social Development Ministry (MSDS, 1980-2000).

Instituto de Biomedicina, Esquina San Nicolás, San José, Apdo. Postal 4043, Caracas 1010A, Venezuela

* Autor de correspondencia: delimah@gmail.com

During these past decades, a control program based mainly on chemical vector control and house improvement has been conducted in Venezuela. This program has resulted in an important reduction of human seroprevalence, from 44.5 % during the period 1958-1968 to 9.2 % during 1988-1998, in the areas where the disease is considered endemic (Aché & Matos, 2001). However, the analysis of data of the last decade (Feliciangeli *et al*., 2003) and recent works (Añez *et al*., 2003) show that transmission was not interrupted and could now be increasing. The vector-control activities in Latin American countries, especially the Southern Cone Initiative (Argentina, Bolivia, Brazil, Chile, Paraguay and Uruguay), has permitted a reduction of new cases per year going from 700.000 new cases in 1983 to 200.000 new cases in 2000 (WHO, 2002) .

The etiological agent *Trypanosoma cruzi* (Kinetoplastida, Trypanosomatidae) can be detected over a wide area of America, from Southern United States (Latitude 42ºN) to the North of Argentina (Latitude 46ºS) coinciding with vectors (Hemiptera, Reduviidae, Triatominae) and reservoirs (seven orders

of mammals) distribution (WHO, 2002). The human disease has a minor extension in the continent. The use of isoenzymes (Ready & Miles, 1980) and analysis of the kDNA with restriction enzymes (Morel *et al*., 1980) have permitted to group the strains of *T. cruzi* in several groups of zymodemes or schizodemes, respectively, according to the technique used. These groups of strains have tried to correlate with their biological properties, clinical manifestations and epidemiological characteristics. Subsequently, with the use of ribosomal and mini-exon gene markers and randomly amplified polymorphic DNA (RAPD), have confirmed the existence of two groups of strains: *T. cruzi* I and *T. cruzi* II (Anonymous, 1999). In Venezuela, the few studies carried out in this sense suggest a predominance of *T. cruzi* of lineage I, both in patients and vectors (Añez *et al*., 2004; Feliciangeli *et al*., 2004); studies in mammals reservoirs have not been carried out to this date.

The epidemiological cycle of *T. cruzi* also involves a broad variety of wild, peridomestic and domestic animals. More than 150 species belonging to 7 orders of mammals naturally infected, including man, have been registered.

The importance of the three cycles: wild, peridomestic and domestic is well known. In the domiciliary cycle, the most important reservoir is man itself, followed by rodents and dogs. In the peridomiciliary cycle the opossum, rodents and the dog would be important, while in the wild cycle the opossum and the armadillo would be important (OMS, 1991).

Marsupials, mainly those from the genus *Didelphis*, have been cited as one of the most important reservoirs of *T. cruzi* in different Latin American countries: Brazil (Grisard *et al*., 2000; Pinho *et al*., 2000; Ramirez *et al*., 2002), Mexico (Solis-Franco *et al*., 1997; Ruiz-Piña & Cruz-Reyes 2002), Venezuela (Gil de Soto 1971; Telford & Tonn 1982; Herrera & Urdaneta-Morales 1992), Argentina (Schweigmann *et al.*, 1999), Colombia (Travi *et al.*, 1994) and French Guiana (Raccurt, 1996; Dereure *et al.*, 2001). In the present study we identified by means of the kDNA restriction pattern and hybridization with species-specific probes, different isolates of *T. cruzi* obtained from synantropic animals and evaluated the domestic and peridomestic cycles of *T. cruzi*, and their possible relationship with the environmental characteristics of two nearby communities with different ecological characteristics.

MATERIALS AND METHODS

Study area

Captures were made in two small communities located in the Andres Eloy Blanco municipality of Lara State, Venezuela, La Matica and Tintinal (Fig. 1). The Andrés Eloy Blanco municipality located in the southern-eastern part of the Lara State, has an estimated population of 39,842 inhabitants, while the communities studied have an estimated population of 1,500 inhabitants each and their economy is based almost exclusively on agricultural work. La Matica (09º42'50''N, 69º41'53''W), with an average altitude of 1450 m a.s.l., and Tintinal (09º42'46''N, 69º43'13''W) with an average altitude of 1.150 m a.s.l. La Matica is located within a tropical rain forest (The Yacambu National Park), Tintinal is located in a tropical dry forest, and this determines that the average temperature is higher in Tintinal while pluviosity is greater in La Matica (Fig.1).

Animal trapping

The animals were captured five nights per month per communities over a period of two years from October 1996 to October 1998, trapped in home-made Tomahawk-like one door metal traps of two different dimensions, 19x5x5 and 26x9x9 inches, with the capacity to capture single small animals or several animals at the same time. Traps were baited with local fruits (banana, maize, etc.) set inside the houses, at 20 or 30 m from the house and in the cornfields, from 6 p.m. to 8 a.m. Twenty traps were used to capture an average of two animals per night; all animals were kept alive until they were studied.

The animals were classified according to the place where they were captured and subsequently according to sex, and divided in two age groups, young animals and adults, according to sexual maturity. The chi-square analysis was used to determine the degree of association between infection and factors studied.

Diagnostic methods

Blood samples were taken from the heart under anaesthetic (ether) conditions. Each sample was used for fresh microscopic examination, blood smears stained with Giemsa and culture in artificial culture medium. Xenodiagnosis was carried out using 5 triatomines of third or fourth instar nymphs of *Rhodnius prolixus.*

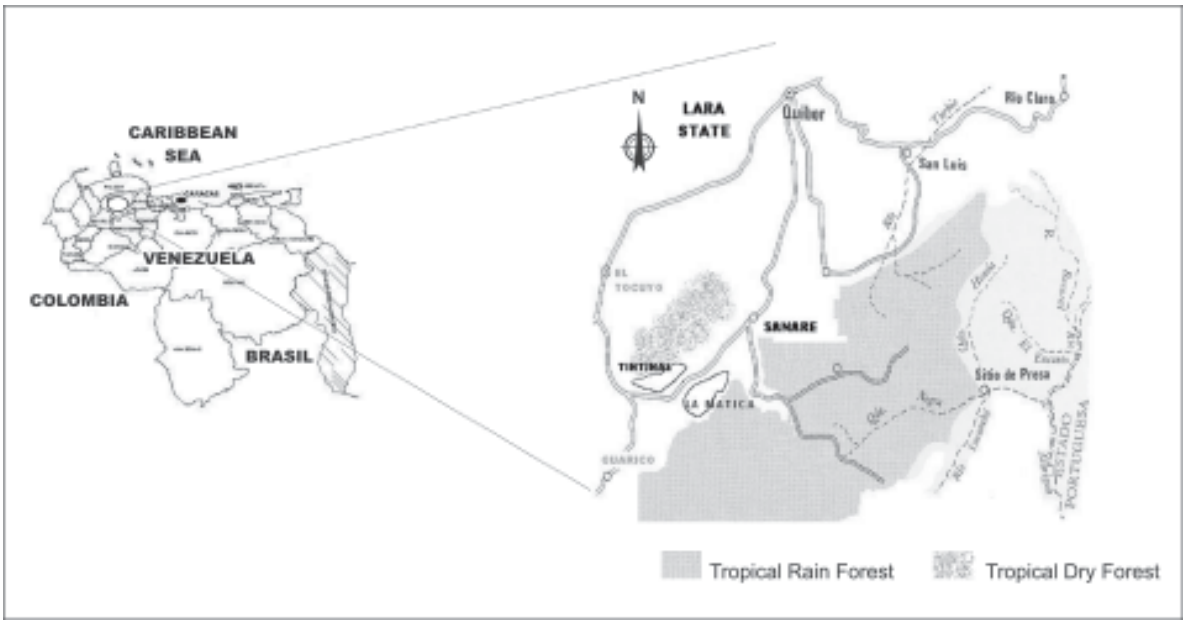

Fig. 1. Relative location of the study area.

T. cruzi parasites were identified based on the following criteria: (1) Cultures characterized by the kDNA restriction pattern and dot blot hybridization with species-specific probes; (2) Morphological study by using an immersion objective; (3) Xenodiagnosis; (4) Study of parasitemia in all positive cases, determined by fresh blood examinations.

Parasite isolation and culture

The parasites were isolated from blood and cultured in blood agar base medium (Difco), with 10% defibrinated rabbit blood plus 200 units of penicillin. Within seven days, samples of the culture were taken for microscopic examination. Negative cultures without contamination were kept for one month before discarding. When the culture was positive, rapid growth of the parasite was obtained in Petri dishes in the same medium. Parasites in the stationary phase were harvested by centrifugation at 5000 RPM for 10 min at 4ºC, and washed three times with sterile phosphate buffered saline, pH 7.4.

T. cruzi *strains pattern*

A strain obtained from the patient Elpidio Padron (EP), isolated in the State of Carabobo in the year 1967 by the School of Parasitology of the Carabobo University. Initially maintained in a mouse, subsequently adapted to grow in LIT (1977) medium, and then in blood agar base medium (Difco) with 10% defibrinated rabbit blood plus 200 units of penicillin (Boiso & Goitia, 1982).

kDNA isolation, digestion and hybridization

Kinetoplast DNA was isolated as previously described by Rodriguez *et al.* (1994). For schizodeme analysis, 5 µg of kDNA were digested with five units of Msp I endonuclease (Gibco-BRL). After digestion to completion, the digested products were separated by electrophoresis in 4.5-10% linear gradient polyacrylamide gels, run at 7 mAmp overnight. The gels were stained with silver nitrate and photographed. Dot blot hybridization was carried out as previously described (Barrios *et al.*, 1994). Pre-hybridization and hybridization were performed overnight at 42ºC in 50% formamide containing buffer. Filters were developed using Genius Systems (Boehringer-Mannheim) according to the instructions of the manufacturer.

RESULTS

Animals captured

A total of 513 animals, belonging to 2 orders and 10 different genera, were captured over a period of 2 years in the two study areas (Fig. 1), 482 were rodents and 31 marsupials. Infection by *T. cruzi* was detected in *D. marsupialis* (29.03 %) and *Rattus* spp. (4.41%) (Table I). Four methods were used to detect this infection

Table I. Prevalence of *Trypanosoma cruzi* by species captured in La Matica and Tintinal, Lara State, Venezuela.

Scientific name	Nº examined	Nº infected	% infection
Rodents			
Sigmodon hispidus	391	0	0.00
Rattus spp.	68	3	4.41
Other species of rodents			
Akodon sp., Rhipidomis sp., Oryzomys sp., Sciurus sp., Zygodontomys sp., Mus musculus,Proechimys sp.	23	0	0.00
Marsupials			
Didelphis marsupialis	31	9	29.03
Total	513	12	

(Table II). In the other animals captured and studied (Table I) no hemoflagellates were observed. Of 31 *D. marsupialis* captured, 17 were captured in La Matica and only 1 (5.88%) resulted infected with *T. cruzi*; the other 14 were captured in Tintinal and 8 (57.14%) of them resulted positive. This difference was statistically significant (Table III).

According to sex, in all opossums (males: 4/14, females: 5/17) no significant difference was observed in the frequency of infection. Regarding age (juveniles: 2/12; adults: 7/19) even though adults appeared to be more frequently infected, this difference was not significant ($p > 0.05$). Although all the infected *D. marsupialis* were captured during the rainy season (9/26) (Fig. 2) and none were detected during the dry season (0/5), this difference was not statistically significant ($p > 0.05$).

Of the 68 *Rattus* captured 3 were infected with *T. cruzi* (4.41%); all were detected by hemoculture. The infected *Rattus* were captured in La Matica (3/53), while in Tintinal no positive animals were captured (0/15). This difference was not statistically significant (Table III). Classification according to sex, age and capturing season (rainy or dry) did not provide any important data.

Microscopical examination

A very low parasitaemia was observed in fresh blood of *D. marsupialis* (1 parasite per 50 fields, 40X), with a morphology compatible with *T. cruzi* after Giemsa staining and observing them with an immersion objective (100X). In *Didelphis* no hemoflagellates of other species were observed.

22 of the 68 *Rattus* presented abundant hemoflagellates in fresh blood examined which, after microscopic observation of the smear previously stained with Giemsa, were identified as *T. lewisi*. No hemoflagellates morphologically compatible with *T.*

Table II. *Trypanosoma cruzi* in wild animals as detected by diferent diagnostic methods.

	Fresh blood		Stain smear		Culture		Xenodiagnosis		All methods	
Scientific name	Positive	%	Positive	%	Positive	%	Positive	%	Positive	%
D. marsupialis	5/31	16.12	5/31	16.12	5/31	16.12	1/12	8.33	9/31	29.03
Rattus spp.	0/68	0	0/68	0	3/68	4.41	0/12	0	3/68	4.41

Table III. Prevalence of *Trypanosoma cruzi* by place of capture of *Didelphis marsupialis* and *Rattus* spp., Lara State, Venezuela

	Didelphis marsupialis (a)			*Rattus* spp. (b)		
Localidad	Nº examined	Nº infected	% infection	Nº examined	Nº infected	% infection
1. La Matica	17	1	5.88	53	3	5.66
2. Tintinal	14	8	57.14	15	0	0.00
Total	31	9	29.03	68	3	4.41

(a) 1-2: $p < 0.05$; (b) 1-2: $p > 0.05$

cruzi were observed in these rats. Mixed infection was evidenced only in *Rattus* by molecular biology techniques, not only between *T. cruzi* and *T. lewisi* but also between the latter and different species of *Leishmania* (De Lima *et al.*, 2002; De Lima *et al.*, 2003).

Molecular biology techniques

Five cultures (2 *Didelphis*, 3 *Rattus*) were used for the taxonomic identification of parasites. The restriction patterns of five isolates after digestion with Msp I restriction enzyme are shown in Fig. 3. Samples from one to five have similar restriction patterns with *T. cruzi* (EP). These results were confirmed after dot blot hybridization with *T. cruzi* (EP) kDNA probe labeled with digoxigenin. The results are shown in Fig. 4, a high hybridization signal was observed in samples 7, 8, 9, 10 and 11, corresponding to the same samples in Fig. 3.

DISCUSSION

T. cruzi is a protozoan with a high capacity to parasite a great variety of mammals, especially opossums and rodents. Rodents and marsupials are major sources of *T. cruzi* infection for several triatomine species (Rocha e Silva *et al.*, 1975).

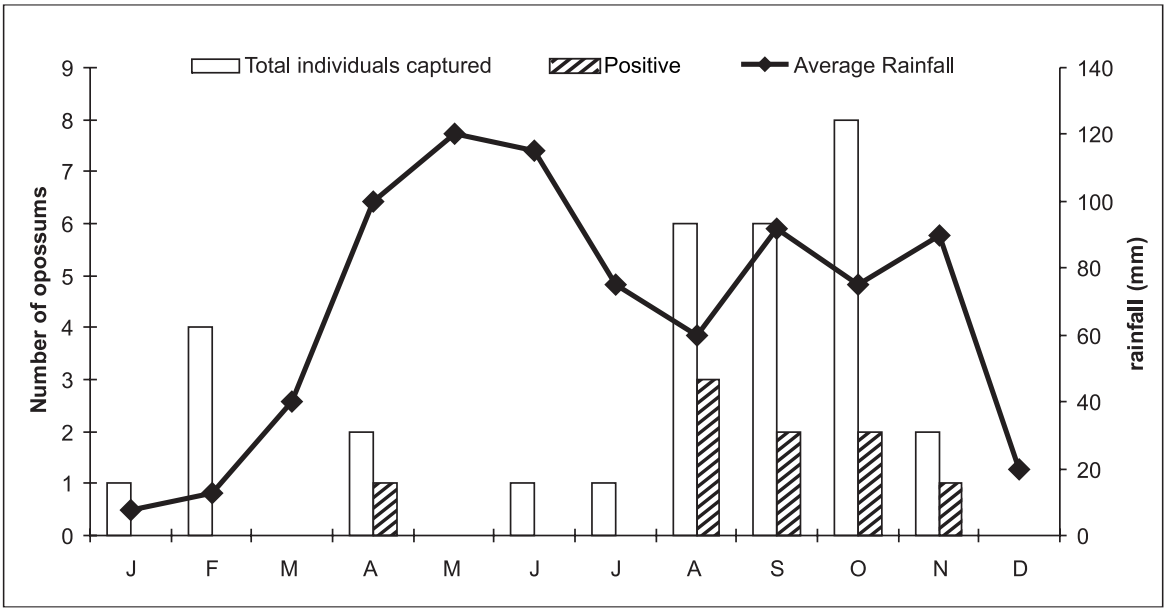

Fig. 2. Monthly distribution of *Didelphis marsupialis* captured and infected by *T. cruzi* in La Matica and Tintinal, Lara State, Venezuela, in relation to the rainfall.

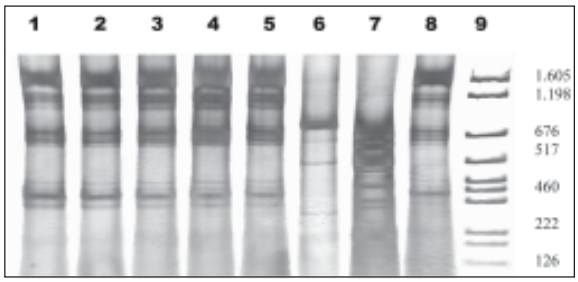

Fig. 3. Restriction pattern after digestion with Msp I restriction enzyme. Order of the samples: lane 1: 6E; lane 2: 10C; lane 3: 5C; lane 4: 19B; lane 5: 18B; lane 6: *Leishmania braziliensis* (LTB300); lane 7: *L. amazonensis* (PH8): Lane 8: *Trypanosoma cruzi* (EP); lane 9: Molecular weigh markers (pGEM DNA Markers, Promega). Samples identified with letters "B" and "E" correspond to *Rattus spp*. While those identified with "C" correspond to *Didelphis marsupialis*.

The study of each of the elements of the *T. cruzi* cycle is important and, therefore, to know the reservoirs implied in the persistence of the parasite in a determined area is important in order to establish control measures. In Venezuela, few studies have been carried in this respect. Gil de Soto (1971) demonstrated the importance of *D. marsupialis* in the region of Zipanare, Zulia State. Telfort and Tonn (1982) carried out a study in the Venezuelan plains, where they demonstrated the infection by *T. cruzi* in 10 different species of wild mammals, including *D. marsupialis* as the most important. They demonstrated in the rural villages of San Jorge and Finca Coromoto, the infection of *D. marsupialis* in 64.74% (90/139) and 33.33% (29/87) respectively. Herrera & Urdaneta-Morales (1992) reported the infection of *D. marsupialis* by *T. cruzi* in 25% (6/24), in an urban area of Caracas.

The same authors (1997) reported the infection of *Rattus rattus* by *T. cruzi* in 16.66% (2/12), also in the urban area of Caracas. In all these studies the morphology and biological behavior were used for the identification of the parasites, while in this work we used molecular biology techniques for this purpose. Our results indicate that parasites isolated from *Didelphis* and *Rattus* are genetically homogeneous among them, with minor heterogeneity with the *T. cruzi* EP strain (human strain) in a faint band below 460 bp; this could be due to the differences in the age of the cultures. The *T. cruzi* EP strain has been in artificial culture medium for many years with its consequent adaptation to this condition. However, no differences were observed in the hybridization with species specific probes confirming a high homology between our animal isolates and the human strain EP of *T. cruzi*. It was not possible to establish lineage of *T. cruzi* I or II given that our restriction patterns were made with the Msp I enzyme, and in previous publications patterns were made with Eco RI. (Morel *et al.*, 1980; Muñoz *et al.*, 1994).

Fig. 4: Hybridization with species specific probes for *Trypanosoma cruzi* (EP). Samples identified with letters "B" and "E" correspond to *Rattus spp*., while those identified with "C" correspond to *Didelphis marsupialis* and with "A" correspond to *Sigmodon* sp. Lb: *Leishmania (V.) braziliensis* (LTB300); Lm: *L. (L.) amazonensis* (PH8); Tc: *T. cruzi* (EP).

This study was developed in two rural areas considered, in previous decades, endemic for Chagas disease. All the *D. marsupialis* were captured in the peridomiciliary area (distance less than 30 m from the houses) and even though the greatest number of captures (17) was made in La Matica, in Tintinal 57.14% (8/14) of the *D. marsupialis were* found infected by *T. cruzi*. In La Matica the infection was only observed in 5.88% (1/17). These differences were statistically significant. This higher rate of infection that we observed in the tropical dry forest, may be explained by the lack of foods that could force opossums to adopt as their main food, the very triatomines that live in their nests. The rats considered in this study were captured intradomiciliary, but only 3 animals infected with *T. cruzi* were from La Matica, which represents 5.66% (3/53). The rats (15) captured in Tintinal did not present infection by *T. cruzi*, statistically there were no differences. These animals present at least two characteristics that make them strong candidates as Chagas disease reservoirs: (1) High rate of infection, and (2) living in intimate contact with human beings. In the communities studied and in accordance with the places where they were captured, the opossum could be considered an important element in the peridomiciliary cycle while rats would be important in the domestic cycle.

All the infected animals, both *Didelphis* and *Rattus*, were captured in the rainy season; this may be attributed to temporary abundance of vector populations during this season. Chagas disease,

originally considered a zoonosis, progressively became a public health problem as humans invaded areas with ecological characteristics that favored the development of the disease. At the beginning, the invasion of these habitats by humans was in socio-economic-cultural conditions which broadly favored coexistence with the triatomine vectors of the disease. Subsequently, with the introduction of insecticides and improvements made in the houses, there was a reduction in the interaction between man and the triatomine vectors, reaching an important reduction in the indexes of prevalence of the disease, and in some areas even disappearing. In the decade between 1950 and 1960, the endemic area in Venezuela represented approximately 750,000 km^2 while at the present time it is estimated to be around 365,000 km^2 (Aché & Matos 2001).

This invasion by man permitted the development, in addition to the wild cycle, of two other cycles, the peridomestic and domestic, which apart from perpetuating the existence of the parasite, allowed a more intimate coexistence of man with the triatomine vectors.

In the areas where this study was carried out, no recent cases of Chagas disease have been reported. This was evidenced by a serological survey that we performed during the same period, (data not shown), where 12.35% (11/89) positive serologies were observed (indirect hemagglutination), but all the cases were in persons over 55 years of age.

The present study demonstrated the existence of *T. cruzi* in synanthropic animals (*Rattus* and *D. marsupialis*). For this reason we believe that in these communities the risk of infection by *T. cruzi* is latent or in a kind of equilibrium or temporary control of the disease where man is no longer part of the cycle, but any environmental change that breaks this equilibrium may lead to an epidemic.

The interruption of the transmission of Chagas disease in Venezuela (Aché & Matos 2001) has been evaluated throughout the past five decades by following-up on these factors: (a) house infestation index; (b) house infection index; (c) house density index; (d) seroprevalence; and (e) blood donor prevalence.

We believe that greater emphasis should be given to the studies of reservoirs, including the introduction of a "prevalence index of *T. cruzi* in reservoirs", especially domestic and synanthropic, that will allow us to know the real situation of an area being studied, since the reduction of the indexes of house infestation, house infection, house density and seroprevalence, although undoubtedly good indicators of the transmission interruption in an area, are not indicating the real potentiality of the same in the reappearance of any epidemic situation.

The "prevalence index of *T. cruzi* in reservoirs" would be a complementary indicator of those traditionally used, since with it we would cover all the elements implied in the biological cycle of the disease.

IDENTIFICACIÓN MOLECULAR DE *Trypanosoma cruzi* EN *Didelphis marsupialis* Y *Rattus* spp. EN UNA ANTIGUA ÁREA ENDÉMICA DE ENFERMEDAD DE CHAGAS EN EL ESTADO LARA, VENEZUELA

RESUMEN

En el período comprendido entre octubre 1996 y octubre 1998, se realizó la captura de mamíferos sinantrópicos en dos comunidades con características ecológicas diferentes, La Matica y Tintinal, para la identificación de reservorios de *Trypanosoma cruzi*. Un total de 513 animales, pertenecientes a 10 diferentes géneros, fueron capturados. Por medio de punción cardíaca se extrajo sangre para realizar examen en fresco y cultivo en medio agar sangre. Xenodiagnóstico fue realizado a un número limitado de animales. Dos diferentes especies fueron encontradas naturalmente infectadas con *T. cruzi*, *Didelphis marsupialis* y *Rattus* spp. En *D. marsupialis* la presencia de *T. cruzi* fue determinada en 9 de los 31 animales estudiados (29.03%). *Rattus* se encontró infectado en 4.41 % (3/68). 5 cultivos (2 de *D. marsupialis* y 3 de *Rattus* spp.) fueron caracterizados como *T. cruzi* por medio del uso de enzimas de restricción (Msp I) e hibridización con sondas especie específicas. Cuando nosotros estudiamos el *D. marsupialis* de acuerdo con el lugar de captura, encontramos que la infección en Tintinal estaba presente en 57.14% (8/14) de los animales estudiados, mientras en La Matica escasamente representaba 5.88% (1/17). Esta diferencia es estadísticamente significativa ($p < 0.05$). Finalmente, la inclusión de un "índice de prevalencia de *T. cruzi* en reservorios" es sugerido, como una medida adicional para predecir la situación real de un área endémica.

Palabras claves: *Trypanosoma cruzi*, *Didelphis marsupialis*, *Rattus*, Chagas disease, biologia molecular, Venezuela.

ACKNOWLEDGEMENTS

To the community of La Matica for the support given during our field work. Also to Ms. Irene Gamundi for the translation, and Dr. Marian Ulrich for the corrections and critical reading of the manuscript. Financial Support: World Bank/Venezuelan government, projects Ven/96/002/021-024

REFERENCES

Aché A. & Matos A. J. (2001). Interrupting Chagas disease transmission in Venezuela. *Rev. Inst. Med. Trop. São Paulo*. **43**: 37-43.

Anonymous, (1999). Recommendations from a Satellite Meeting. International Symposium to Commemorate the 90th Anniversary of the Discovery of Chagas Disease. April 11-16, 1999. Rio de Janeiro, Brazil. *Mem. Inst. Oswaldo Cruz.* **94**: 429-432.

Añez N., Crisante G., Rojas A., Diaz N., Añez-Rojas N., Carrasco H. *et al.* (2003). La cara oculta de la enfermedad de Chagas en Venezuela. *Bol. Mal. Salud Amb.* **43***:* 45-57.

Añez N., Crisante G., da Silva F.M., Rojas A., Carrasco H., Umezawa E.S., *et al*. (2004). Predominance of lineage I among *Trypanosoma cruzi* isolates from Venezuelan patients with different clinical profiles of acute Chagas' disease. *Trop. Med. Int. Health*. **9**: 1319-1326.

Barrios M. A., Rodríguez N., Feliciangeli D. M., Ulrich M., Telles S., Pinardi M. E. *et al.* (1994). Coexistence of two species of Leishmania in the digestive tract of the vector *Lutzomyia ovallesi*. *Am. J. Trop. Med. Hyg.* **51**: 669-675.

Boiso J. F. & Goitia A. M. (1982). Estudio del cultivo de *Trypanosoma cruzi* en medio LIT modificado. *Acta Cient. Venez.* **33**: 488-451.

De Lima H., De Guglielmo Z., Rodríguez A., Convit J. & Rodríguez N. (2002). Cotton Rats (*Sigmodon hispidus*) and Black Rats (*Rattus rattus*) as possible reservoirs of *Leishmania* spp. in Lara State, Venezuela. *Mem. Inst. Oswaldo Cruz.* **97**: 169-174.

De Lima H., Rodríguez N., Borges R. & Convit J. (2003). *Trypanosoma (Herpetosoma) lewisi* in *Rattus* spp from La Matica, Lara State, Venezuela and the possible relationship with zoonotic diseases. *Bol. Dir. Malar. San. Amb.* **43**: 37-43.

Dereure J., Barnabe C., Vie J. C., Madelenat F. & Raccurt C. (2001). Trypanosomatidae from wild mammals in the neotropical rainforest of French Guiana. *Ann. Trop. Med. Parasitol.* **95**: 157-166.

Feliciangeli M.D., Campbell-Lendrum D., Martínez C., González D., Coleman P. & Davies C. (2003). Chagas disease control in Venezuela: lessons for the Andean Region and beyond. *Trends Parasitol.* **19**: 44-49.

Feliciangeli M.D., Carrasco H., Patterson J., Suarez B., Martinez C. & Medina M. (2004). Mixed domestic infestation by *Rhodnius prolixus* stal, 1859 and *Panstrongylus geniculatus* Latreille, 1811, vector incrimination, and seroprevalence for *Trypanosoma cruzi* among inhabitants in El Guamito, Lara state, Venezuela. *Am. J. Trop. Med. Hyg.* **71**: 501-505.

Gil de Soto S. T. (1971). *Didelphis marsupialis*, Linnaeus 1758, su importancia como reservorio de *Trypanosoma cruzi* en la región de Zipanare (estado Zulia, Venezuela). *Kasmera.* **4**: 5-44.

Grisard E. C., Carvalho-Pinto C. J., Scholz A. F., Toma H. K., Schlemper B.R. Jr & Steindel M. (2000). *Trypanosoma cruzi* infection in *Didelphis marsupialis* in Santa Catarina and Arvoredo Islands, southern Brazil. *Mem. Inst. Oswaldo Cruz.* **95**: 795-800.

Herrera L. & Urdaneta-Morales S. (1992). *Didelphis marsupialis*: a primary reservoir of *Trypanosoma cruzi* in urban area of Caracas, Venezuela. *Ann. Trop. Med. Parasitol.* **86**: 607-612.

Herrera L. & Urdaneta-Morales S. (1997). Synanthropic rodent reservoirs of *Trypanosoma (Schizotrypanum) cruzi* in the valley of Caracas, Venezuela. *Rev. Med. Trop. São Paulo.* **39**: 279-282.

Ministerio de Sanidad y Desarrollo Social (1980-2000). *Anuario de Epidemiología y Estadística Vital*, Venezuela.

Morel C., Chiari E., Camargo E. P., Mattei D. M., Romanha A. J. , & Simpson L. (1980). Strains and

clones of *Trypanosoma cruzi* can be characterized by pattern of restriction endonuclease products of kinetoplast DNA minicircles. *Proc. Natl. Acad. Sci.* U S A. **77**: 6810–6814.

Muñoz S., Lorca M., Munoz P. & Solari A. (1994). Highly homogeneous *Trypanosoma cruzi* populations in a low endemic region for Chagas disease: importance in the pathogenesis of Chagas disease in Chile. *Rev. Med. Chil.* **122**: 1231-1238.

OMS-Organización Mundial de la Salud (1991). *Control de la enfermedad de Chagas*. Serie de Informes Técnicos 811, Ginebra, 102 pp.

Pinho A. P., Cupolillo E., Mangia R. H., Fernandes O. & Jansen A. M. (2000). *Trypanosoma cruzi* in the sylvatic environment: distinct transmission cycles involving two sympatric marsupials. *Trans. R. Soc. Trop. Med. Hyg.* **94**: 509-514.

Raccurt C.P. (1996). *Trypanosoma cruzi* in French Guinea: review of accumulated data since 1940. *Med. Trop.* **56**: 79-87.

Ramírez L. E., Lages-Silva E., Alvarenga-Franco F., Matos A., Vargas N., Fernandes O. *et al*, (2002). High prevalence of *Trypanosoma rangeli* and *Trypanosoma cruzi* in opossums and triatomids in a formerly-endemic area of Chagas disease in Southeast Brazil. *Acta. Trop.* **84**: 189-98.

Ready P.D. & Miles M.A. (1980). Delimitation of *Trypanosoma cruzi* zymodemes by numerical taxonomy. *Trans. R. Soc. Trop. Med. Hyg.* **74**: 238-242.

Rocha e Silva E. O., Andrade J. C. R. & Lima A. R. (1975). Importancia dos animais sinantrópicos no controle da endemia chagásica. *Rev. Saú. Públ. São Paulo.* **9**: 371-381.

Rodríguez N., Guzmán B., Rodas A., Takiff H., Bloom B. & Convit J. (1994). Diagnosis of cutaneous leishmaniasis and species discrimination of parasite by PCR and hybridization. *J. Clin. Microbiol.* **32**: 2246-2252.

Ruiz-Pina H. A. & Cruz-Reyes A. (2002). The opossum *Didelphis virginiana* as a synanthropic reservoir of *Trypanosoma cruzi* in Dzidzilche, Yucatan, Mexico. *Mem. Inst. Oswaldo Cruz.* **97**: 613-20.

Schweigmann N.J., Pietrokovsky S., Bottazzi V., Conti O., Bujas M.A. & Wisnivesky-Colli C. (1999). Prevalence of *Trypanosoma cruzi* infection in opossum (*Didelphis albiventris*) in Santiago del Estero, Argentina. *Rev. Panam. Salud. Pública.* **6**: 371-377.

Solis-Franco R. R., Romo-Zapata J. A. & Martínez-Ibarra J. A. (1997). Wild reservoirs infected by *Trypanosoma cruzi* in the ecological park "El Zapotal", Tuxtla Gutierrez, Chiapas, México. *Mem. Inst. Oswaldo Cruz.* **92**: 163-164.

Tejera E. (1919). La Trypanosomose américaine ou maladie de Chagas au Venezuela. *Bull. Soc. Path. Exot.* **12**: 509-513.

Telfort Jr S.R. & Tonn R. (1982). Dinámica de *Trypanosoma cruzi* en poblaciones de un reservorio primario, *Didelphis marsupialis*, en los llanos altos de Venezuela. *Bol. Of. Sanit. Panam.* **93**: 341-364.

Travi B. L., Jaramillo C., Montoya J., Segura I., Zea A., Goncalves A. *et al.* (1994). *Didelphis marsupialis*, an important reservoir of *Trypanosoma (Schizotrypanum) cruzi* and *Leishmania (Leishmania) chagasi* in Colombia. *Am. J. Trop. Med. Hyg.* **50**: 557-565.

WHO (2002). *Second Report of the WHO Expert Committee, Control of Chagas Disease*.Technical Report series 905, Geneve.

Recibido el 24 de Julio 2005
Aceptado el 26 de Octubre 2005