J Vector Borne Dis 52, March 2015, pp. 23–29

Genetic variability of *Trypanosoma cruzi* TcI isolates from rural and urban areas of Venezuela

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ABSTRACT

Background & objectives: Several studies have demonstrated genetic heterogeneity in populations of *Trypanosoma cruzi* that allowed the identification of six different discrete typing units (DTU) classified as TcI, TcII, TcIII, TcIV, TcV and TcVI. Furthermore, some characterization studies have described genetic variability within TcI isolates from endemic regions. The objective of the present study was to analyze Venezuelan *T. cruzi* isolates, obtained from triatomine-vectors, mammal-hosts including infected humans, detected in both rural and urban areas from diverse geographic origins.

Methods: Molecular characterization of 44 Venezuelan *T. cruzi* isolates, obtained from triatomine-vectors, mammalian hosts and human patients from both rural and urban areas of different geographic origins, were carried out. Samples were analyzed by PCR amplification of the intergenic region of the mini-exon gene, 24Sα rDNA and 18S rDNA, followed by sequencing of the amplification products.

Results: The TcI amplification pattern was found in 42 out of 44 (95.5%) isolates; a TcIII strain and one possible TcIV were also found. The sequence analysis of the TcI Venezuelan isolates showed genetic variability among them. Urban isolates formed a homogeneous group, with differences in their sequences, when compared to rural isolates.

Interpretation & conclusion: The results showed genetic heterogeneity in Venezuelan TcI strains, probably in response to different environmental conditions.

Key words Genetic variability; mini-exon; TcI; *Trypanosoma cruzi;* Venezuela

INTRODUCTION

Trypanosoma cruzi, the etiological agent of American Trypanosomiasis or Chagas disease, affects about 10 million people and 25 million are at risk in Latin America[1]. The human pathology includes an acute phase, followed by the chronic phase with an unpredictable clinical course, ranging from no symptom to a severe disease with cardiovascular compromise and/or digestive alterations that could cause death[2]. In Venezuela, number of studies suggest an active transmission and re-emergence of the disease[3-4].

Several investigations based on biochemical and genetic markers showed that *T. cruzi* strains are highly polymorphic and consist of a variety of parasite subpopulations, with biological, biochemical, immunological and genetic heterogeneity observed in their triatomine-vectors, reservoir hosts and people living in risk areas where the kinetoplastid is endemic[5].

T. cruzi populations have been classified into six discrete taxonomic units (DTUs), named as TcI, TcII, TcIII, TcIV, TcV and TcVI based on different molecular markers and biological features[6-8]. Although, *T. cruzi* I was considered a homogeneous DTU, genetic variability within *T. cruzi* I has been reported in recent years[9-11].

Several authors associated the parasite variability with differences in the biological cycle, tissue invasion, virulence, clinical profiles, geographic distribution, *etc*. The molecular epidemiology based on the genetic typing of *T. cruzi* isolates from different sources may be useful to understand the variability of this parasite and its possible relationship to the clinical and epidemiological characteristics of the disease[12-13].

In Venezuela, some studies revealed TcI, TcIII and TcIV in human beings, triatomine bugs, and other mammals[14-17]. In the present study, 44 Venezuelan *T. cruzi* isolates, obtained from triatomine-vectors, mammalian

hosts including infected humans, detected in both rural and urban areas from diverse geographic origins, were analyzed.

MATERIAL & METHODS

Parasite isolates

A panel of 44 Venezuelan *T. cruzi* isolates, from rural and urban areas, including both domestic and peri-domestic transmission cycles, were studied (Table 1). Caracas City, the states of Cojedes and Guárico are in the central region of Venezuela, whereas Anzoátegui state is in the northeastern part. Five *T. cruzi* isolates, previously characterized, were also included in the analysis, three TcI and two TcV. Parasites were cultured in liver infusion tryptose (LIT) liquid medium and harvested by centrifugation; parasite pellets were stored at –70°C until use. The kinetoplastids were obtained from mammal blood and vec-

Table 1. Molecular characterization of the *Trypanosoma cruzi* isolates by PCR

S.No.	Isolates	Host	Locality	Habitat	DTU
1.	MDID/VE/1984/Dm28c	*Didelphis marsupialis*	Guárico	Rural	TcI[a]
2.	MHOM/VE/2007/EP	*Homo sapiens*	Guárico	Rural	TcI[a]
3.	MHOM/VE/2007/EP6c	*Homo sapiens*	Guárico	Rural	TcI[a]
4.	MHOM/PA/2007/LH31	*Homo sapiens*	Paraguay	Rural	TcV[a]
5.	MHOM/PA/2007/LH32	*Homo sapiens*	Paraguay	Rural	TcV[a]
6.	TMAC/VE/2007/LH1	*Triatoma maculata*	Anzoátegui	Rural	TcI
7.	TMAC/VE/2007/LH4	*Triatoma maculata*	Anzoátegui	Rural	TcI
8.	TMAC/VE/2007/LH5	*Triatoma maculata*	Anzoátegui	Rural	TcI
9.	TMAC/VE/2007/LH6	*Triatoma maculata*	Anzoátegui	Rural	TcI
10.	TMAC/VE/2007/LH12	*Triatoma maculata*	Anzoátegui	Rural	TcI
11.	TMAC/VE/2007/LH13	*Triatoma maculata*	Anzoátegui	Rural	TcI
12.	TMAC/VE/2007/LH19	*Triatoma maculata*	Anzoátegui	Rural	TcI
13.	TMAC/VE/2007/LH20	*Triatoma maculata*	Anzoátegui	Rural	TcI
14.	TMAC/VE/2007/LH23	*Triatoma maculata*	Anzoátegui	Rural	TcI
15.	TMAC/VE/2007/LH26	*Triatoma maculata*	Anzoátegui	Rural	TcI
16.	TPRX/VE/2007/LH2	*Rhodnius prolixus*	Anzoátegui	Rural	TcI
17.	TPRX/VE/2007/LH3	*Rhodnius prolixus*	Anzoátegui	Rural	TcI
18.	TPRX/VE/2007/LH10	*Rhodnius prolixus*	Anzoátegui	Rural	TcI
19.	TPRX/VE/2007/LH18	*Rhodnius prolixus*	Anzoátegui	Rural	TcI
20.	TPRX/VE/2007/LH21	*Rhodnius prolixus*	Anzoátegui	Rural	TcI
21.	TPRX/VE/2007/LH22	*Rhodnius prolixus*	Anzoátegui	Rural	TcI
22.	TPRX/VE/2007/LH25	*Rhodnius prolixus*	Anzoátegui	Rural	TcI
23.	TPRX/VE/2007/LH27	*Rhodnius prolixus*	Anzoátegui	Rural	TcI
24.	TPRX/VE/2007/LH28	*Rhodnius prolixus*	Anzoátegui	Rural	TcI
25.	MDID/VE/2007/LH7	*Didelphis marsupialis*	Anzoátegui	Rural	TcI
26.	MDID/VE/2007/LH9	*Didelphis marsupialis*	Anzoátegui	Rural	TcI
27.	MDID/VE/2007/LH24	*Didelphis marsupialis*	Anzoátegui	Rural	TcI
28.	MDID/VE/2007/LH14	*Didelphis marsupialis*	Anzoátegui	Urban	TcI
29.	MDES/VE/2007/LH33	*Desmodus* sp	Anzoátegui	Rural	TcI
30.	MDES/VE/2007/LH43	*Desmodus* sp	Anzoátegui	Rural	TcI
31.	MCAN/VE/2007/LH8	*Canis familiaris*	Anzoátegui	Rural	TcIV[b]
32.	TGEN/VE/2007/LH35	*Panstrongylus geniculatus*	Caracas	Urban	TcI
33.	TGEN/VE/2007/LH36	*Panstrongylus geniculatus*	Caracas	Urban	TcI
34.	MRAT/VE/2007/LH30	*Rattus rattus*	Caracas	Urban	TcI
35.	MRAT/VE/2007/LH34	*Rattus rattus*	Caracas	Urban	TcI
36.	MHOM/VE/2007/LH37	*Homo sapiens*	Caracas	Urban	TcI
37.	MHOM/VE/2007/LH42	*Homo sapiens*	Caracas	Urban	TcI
38.	MHOM/VE/2007/LH46	*Homo sapiens*	Caracas	Urban	TcI
39.	MHOM/VE/2007/LH47	*Homo sapiens*	Caracas	Urban	TcI
40.	MHOM/VE/2007/LH48	*Homo sapiens*	Caracas	Urban	TcI
41.	MHOM/VE/2007/LH49	*Homo sapiens*	Caracas	Urban	TcI
42.	MHOM/VE/2007/LH51	*Homo sapiens*	Caracas	Urban	TcI
43.	MHOM/VE/2007/LH60	*Homo sapiens*	Caracas	Urban	TcI
44.	MDID/VE/2007/LH38	*Didelphis marsupialis*	Cojedes	Rural	TcI
45.	MDID/VE/2007/LH44	*Didelphis marsupialis*	Cojedes	Rural	TcI
46.	MDID/VE/2007/LH45	*Didelphis marsupialis*	Cojedes	Rural	TcIII
47.	MCAN/VE/2007/LH11	*Canis familiaris*	Cojedes	Rural	TcI
48.	MCAN/VE/2007/LH50	*Canis familiaris*	Cojedes	Rural	TcI
49.	MHOM/VE/2007/LH29	*Homo sapiens*	Guárico	Rural	TcI

[a]Reference strains; [b]Possible DTU identified by two of the three markers employed.

tors feces; subsequently, samples were cultured for at maximum two passages to avoid parasite culture selection.

DNA extraction

The DNA extractions from *T. cruzi* cultures was carried out with a mixture of phenol-chloroform-isoamilic alcohol, sodium acetate and ethanol precipitation. DNA concentration and purity were determined by spectrophotometry at 260 and 280 nm (UV/Visible GeneQuant pro RNA/DNA Calculator, Amershan)[18].

Molecular characterization of the T. cruzi isolates

The molecular characterization of *T. cruzi* was carried out using the molecular markers previously described[7]: (i) intergenic region of the non-transcribed mini-exon gene using the primers: TC 5'-CCCCCCTCCCA GGCCACACTG-3', TC1 5'-GTGTCCGCCACCTCCTT CGGGCC-3' and TC2 5'CCTGCAGGCACACGTGTGT GTG-3'; (ii) D7 divergent domain of the 24Sα rDNA employing the primers: D71, 5'-AAGGTGCGTCGACA GTGTGG-3' and D72 5'-TTTTCAGAATGGCCGAA CAGT-3' and (iii) size-variable domain of the 18S rDNA using the primers: V1, 5'-CAAGCGGCTGGGTGGTTA TTCCA-3' and V2, 5'-TTGAGGGAAGGCATGACACA TGT-3'.

For all molecular markers, the amplification reactions included Taq polymerase amplification buffer (100 mM Tris-HCl, pH 8.3), 0.2 mM dNTPs solution, 1.5 mM $MgCl_2$ solution, 1 U of GoTaq® Flexi DNA Polymerase (Promega, Madison, USA), 0.5 µM of each primer, 10 µl of DNA template and water till a 25 µl final total volume. Amplification cycles were performed according to Brisse *et al*[7], using a BIORAD Cycler (Bio-Rad Laboratories, Philadelphia, USA). The PCR products for each reaction were analyzed by electrophoresis on a 2% agarose gel and visualized by ethidium bromide staining.

Purification of PCR mini-exon product

The amplification product of the intergenic region of the *T. cruzi* mini-exon was purified from agarose gels with the commercial Wizard® SV Gel kit and PCR Clean-Up System (Promega, Madison, USA), according to the manufacturer's protocol.

DNA sequencing

Sequencing of DNA fragments was performed at the Sequencing Departmentof the National Center for Microbiology, Instituto de Salud Carlos III, Madrid, Spain, using the 373 Asystem, Model 377 (Applied Biosystem); samples to be sequenced were submitted to Big Dye Terminator Cycle Sequencing ready reaction kit protocol (ABI-PRISM, PE Biosystems, Life Technologies, NY, USA).

Phylogenetic analysis

The multiple alignments were performed using the ClustalW application and BioEdit Sequence Alignment Editor, version 7.0.5.3[19]. Phylogenetic and molecular evolutionary analyses were conducted by MEGA programme, version 4.1. The evolutionary history was inferred using the Neighbor-Joining method[20–21].

RESULTS

Molecular characterization of T. cruzi isolates

The characterization of *T. cruzi* isolates through the amplification of the intergenic region of mini-exon gene, rDNA 24Sα and 18S rDNA, yielded 42 samples, out of 44 (95.5%), showing a TcI DTU profile, demonstrated by amplification of specific bands of 350, 110 and 175 bp, respectively. This amplification pattern was similar to the ones exhibited by the TcI reference strains (EP, EP6c and Dm28c) included in the experiments. Amplicons of 300, 110 and 165 bp were only observed in the TcV reference isolates, corresponding to the mini-exon, rDNA 24Sα and 18S rDNA genes, respectively. Surprisingly, the mini-exon intergenic region was not amplified in two *T. cruzi* isolates; *Canis familiaris* LH8 isolate from a peridomestic rural ecotope of Altos de Guanta village, Anzoátegui state, and *Didelphis marsupialis* LH45 isolate from a peridomestic rural ecotope of Cojedes state. These two isolates showed rDNA 24Sα PCR amplicons of 125 and 110 bp, corresponding to the TcIV and TcIII lineage respectively. In addition, the LH8 isolate had a 175 bp-18S rDNA amplification product, characteristic of TcI-DTU, instead of the 155 bp of TcIV-; so LH8 isolate was identified as "possible TcIV", considering it showed two out of three markers employed. The LH45 isolate showed a possible TcIII pattern (165 bp band) using this marker (Table 1).

DNA sequencing

The multiple alignments of sequences showed high similarity among the nucleotide sequences of the isolates characterized as TcI. These TcI isolates were very different from TcV reference strains, showing an important nucleotide variation. Although, almost all isolates from Anzoátegui, Caracas, Cojedes and Guárico were identified as DTU TcI populations, the sequences of the mini-exon marker in these isolates show some nucleotide variability. A length of 322 bp was obtained in all isolates; the genetic variability amongst TcI isolates was of 15.5%.

There was nucleotide divergence in 50 positions, corresponding to single nucleotide polymorphisms (SNP) with 15 transitions (4.7%), 17 transversions (5.3%) and 23 insertion-deletions (7.1%). There were 272 constant positions. The TcI isolates obtained from vectors and mammalian hosts in Caracas were a group intrinsically homogeneous, showing differences with the sequences of the rural isolates from Anzoátegui, Cojedes and Guárico (Table 2).

Phylogenetic analysis

The phylogenetic tree, corresponding to the parasite

Table 2. Variable positions in mini-exon sequences of the TcI isolates studied

Isolate	Locality	Variable position																					
		39	76	78	81	97	100	122	127	198	289	290	291	292	293	294	295	296	301	302	303	304
D m28 c	Guárico	G	A	G	C	A	C	A	A	C	G	T	–	–	–	–	–	–	T	–	G	C
EP	Guárico	–	G	G	G	G	C	G	A	C	A	C	A	C	A	C	A	C	T	G	G	C
EP6c	Guárico	–	A	G	G	G	C	G	A	A	A	C	A	C	A	C	A	C	T	G	G	C
LH1	Anzoátegui	G	A	G	C	G	G	A	A	C	G	T	–	–	–	–	–	–	T	–	–	G
LH4	Anzoátegui	G	A	G	C	G	G	A	A	C	T	–	–	–	–	–	–	–	T	G	G	C
LH5	Anzoátegui	G	A	G	C	G	G	A	A	C	G	T	–	–	–	–	–	–	G	T	G	–
LH6	Anzoátegui	G	A	G	C	G	G	A	A	C	T	–	–	–	–	–	–	–	G	T	G	G
LH12	Anzoátegui	G	A	G	C	G	G	A	A	C	T	–	–	–	–	–	–	–	G	T	G	G
LH13	Anzoátegui	G	A	G	C	G	G	A	A	C	T	–	–	–	–	–	–	–	G	T	G	G
LH19	Anzoátegui	G	A	G	C	G	G	A	A	C	G	T	–	–	–	–	–	–	T	T	G	–
LH20	Anzoátegui	G	A	G	C	G	G	A	A	C	G	T	–	–	–	–	–	–	T	–	–	G
LH23	Anzoátegui	G	A	G	C	G	G	A	A	C	G	T	–	–	–	–	–	–	T	–	–	G
LH26	Anzoátegui	G	A	G	C	G	G	A	A	C	G	T	–	–	–	–	–	–	T	–	–	G
LH2	Anzoátegui	G	A	G	C	G	G	A	A	C	G	T	–	–	–	–	–	–	T	–	G	–
LH3	Anzoátegui	G	A	G	C	G	G	A	A	C	G	T	–	–	–	–	–	–	T	T	G	–
LH10	Anzoátegui	G	A	G	C	G	G	A	A	C	T	–	–	–	–	–	–	–	G	T	G	G
LH18	Anzoátegui	G	A	G	C	G	G	A	A	C	G	–	–	–	–	–	–	–	G	T	G	G
LH21	Anzoátegui	G	A	G	C	G	G	A	A	C	G	T	–	–	–	–	–	–	T	–	–	G
LH22	Anzoátegui	G	A	G	C	G	G	A	A	C	G	T	–	–	–	–	–	–	T	–	–	G
LH25	Anzoátegui	G	A	G	C	G	G	A	A	C	G	T	–	–	–	–	–	–	T	T	G	–
LH27	Anzoátegui	G	A	G	C	G	G	A	A	C	G	T	–	–	–	–	–	–	T	–	–	G
LH28	Anzoátegui	G	A	G	C	G	G	A	A	C	G	–	–	–	–	–	–	–	G	T	G	–
LH7	Anzoátegui	G	A	G	C	G	G	A	A	C	G	–	–	–	–	–	–	–	T	T	G	G
LH9	Anzoátegui	G	A	G	C	G	G	A	A	C	T	–	–	–	–	–	–	–	G	T	G	G
LH24	Anzoátegui	G	A	G	C	G	G	A	A	C	G	T	–	–	–	–	–	–	T	T	G	–
LH14	Anzoátegui	G	A	G	C	G	G	A	A	C	G	–	–	–	–	–	–	–	G	T	G	–
LH33	Anzoátegui	G	A	G	C	G	G	A	A	C	G	T	–	–	–	–	–	–	T	–	–	G
LH43	Anzoátegui	G	A	G	C	G	G	A	A	C	G	T	–	–	–	–	–	–	G	T	G	–
LH35	Caracas	G	G	A	C	A	C	A	C	A	G	T	–	–	–	–	–	–	T	–	–	G
LH36	Caracas	G	G	A	C	A	C	A	C	A	G	T	–	–	–	–	–	–	T	–	–	G
LH30	Caracas	G	G	A	C	A	C	A	C	A	G	T	–	–	–	–	–	–	T	–	–	G
LH34	Caracas	G	G	A	C	A	C	A	C	A	G	T	–	–	–	–	–	–	T	–	–	G
LH37	Caracas	G	G	A	C	A	C	A	C	A	G	T	–	–	–	–	–	–	T	–	–	G
LH42	Caracas	G	G	A	C	A	C	A	C	A	G	T	–	–	–	–	–	–	T	–	–	G
LH46	Caracas	G	G	A	C	A	C	A	C	A	G	T	–	–	–	–	–	–	T	–	–	G
LH47	Caracas	G	G	A	C	A	C	A	C	A	G	T	–	–	–	–	–	–	T	–	–	G
LH48	Caracas	G	G	A	C	A	C	A	C	A	G	T	–	–	–	–	–	–	T	–	–	G
LH49	Caracas	G	G	A	C	A	C	A	C	A	G	T	–	–	–	–	–	–	T	–	–	G
LH51	Caracas	G	G	A	C	A	C	A	C	A	G	T	–	–	–	–	–	–	T	–	–	G
LH60	Caracas	G	G	A	C	A	C	A	C	A	G	T	–	–	–	–	–	–	T	–	–	G
LH38	Cojedes	G	A	G	C	A	G	A	A	C	T	–	–	–	–	–	–	–	G	T	G	C
LH44	Cojedes	–	G	G	G	G	C	G	A	A	A	C	A	C	A	C	A	C	T	G	T	G
LH11	Cojedes	G	A	G	C	G	G	A	A	C	G	T	–	–	–	–	–	–	G	T	G	G
LH50	Cojedes	–	G	G	G	G	C	G	A	A	A	C	A	C	A	C	A	C	T	G	T	G
LH29	Guárico	–	G	G	G	G	G	G	A	C	A	C	A	C	A	C	A	C	T	G	G	C

(–) Denote gaps in these positions. The shading denote specific changes in the sequences of isolates from Caracas.

populations analyzed, showed that the *T. cruzi* isolates from Caracas had more homogeneous sequences than the ones from the other regions (Fig. 1). To highlight this result, dotted lines box, grouping the *T. cruzi* isolates from Caracas was included in Fig. 1, as they were the only isolates from urban environments and were clustered with a high bootstrap. In contrast, the other isolates were interspersed throughout the different clades, regardless of their geographic origin, all were from rural environments and bootstrap values were lower compared to the Caracas isolates clade form. Conserved substitutions in several positions (14) were observed among the isolates from both urban and rural areas, highlighting the nucleotide variability between the Caracas isolates (urban isolates) and the other isolates from Anzoátegui, Cojedes and Guárico regions (rural isolates). Indeed, mini-exon sequences comparisons were made with other *T. cruzi* TcI isolates, obtained in different areas of Venezuela and reported in GenBank. It was observed that most of them were grouped together with the isolates from Cojedes Guárico and Anzoátegui, whereas the Caracas isolates were a separate genetic group (data not shown). Estimates of average evolutionary divergence within groups using the maximum composite likelihood method in MEGA4, showed 0.012 for rural isolates and 0.001 for urban ones, while the divergence between rural isolates and urban ones was 0.039.

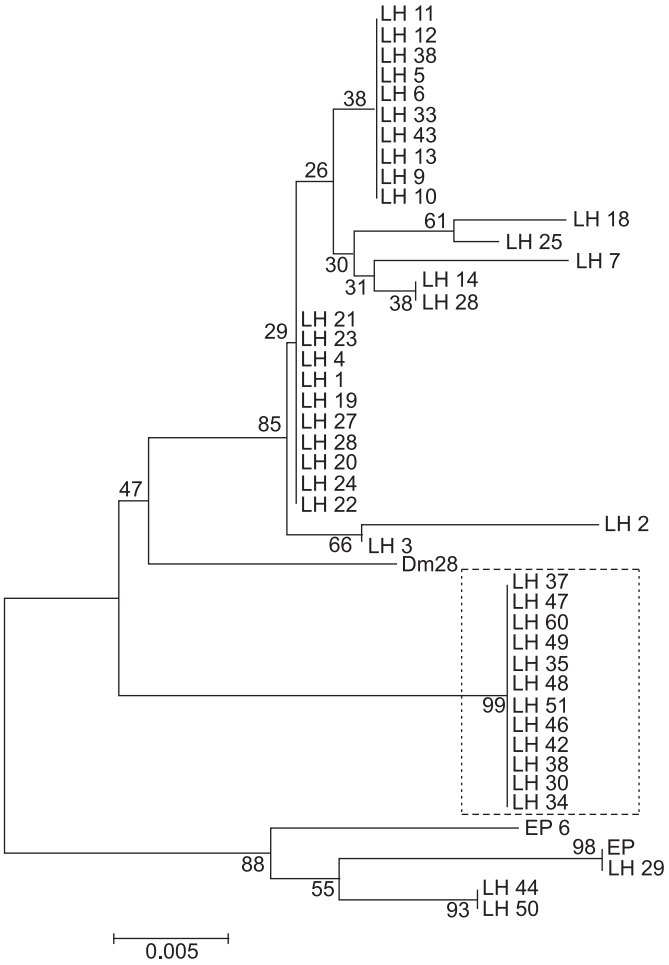

Fig. 1: Evolutionary relationships of Venezuelan *Trypanosoma cruzi* TcI isolates.The evolutionary history was inferred using the Neighbor-Joining method[20]. The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches. Phylogenetic analyses were conducted in MEGA 4[21]. In the dotted lines box, the isolates of *T. cruzi* from Caracas are grouped.

DISCUSSION

T. cruzi is a hemoflagellate of clonal structure with occasional genetic recombination, composed of several subpopulations circulating in vectors, reservoirs and humans from domestic and/or sylvatic cycles. These stable clones are of great significance in terms of adaptive evolution to new environments, as new vectors or reservoirs including humans and its distribution would influence the clinical course of human disease and some epidemiological associations for different regions[22].

In Venezuela, recent studies suggested the re-emergence of Chagas disease, with important epidemiological changes that favored the presence of the disease not only in endemic regions with rural conditions housing, but also in urban areas with diverse human dwellings[3-4]. Thus, urban areas include housing zinc roof, concrete walls, high class buildings, that permit new habitats for wildlife and synanthropic vectors, such as *Panstrongylus geniculatus or Triatoma maculata* in sympatry with the primary vector, *Rhodnius prolixus,* which represent risk factors for transmission of the disease[3–4, 14, 23–24].These epidemiological changes could be associated with genetic variability in isolates of *T. cruzi*, which has not been deeply studied in Venezuela.

In the present study, the characterization of the *T. cruzi* isolates from vectors and reservoirs collected in Anzoátegui, Caracas and Cojedes, showed high frequency of TcI-DTU (95.5%), in agreement with other reports of this frequent DTU in at least 17 states of Venezuela, and occasional occurrence of TcIII and TcIV genotypes[15-17, 25].

TcI has been considered as a homogeneous group; however, some recent studies have reported variations in the intergenic region of the mini-exon sequence in TcI isolates derived from vectors, reservoirs and humans, and collected in different countries as Bolivia, Mexico, Brazil, Colombia, and Argentina, being identified as DTU variants[7, 9–10, 26–27].

In this study, we found evidence of TcI heterogene-

ity by mini-exon DNA sequencing, as was also suggested in studies of *T. cruzi* isolates from human and vectors from western region or urban Venezuelan capital (Caracas), characterized as TcI but without association with one particular clinical manifestation of Chagas disease[14, 17]. In addition, genetic variability within *T. cruzi* I strains from orally and non-orally transmitted human cases were also reported in Venezuela[25].

Regarding the present work, the TcI genetic variability observed could be associated with geographical distribution, as it was proposed in other studies[26]. The nucleotide variability found in mini-exon gene could suggest that the SNPs identified have simply accumulated during clonal diversification of TcI in geographically isolated populations, or an adaptive parasite response to different environments[27]. Due to the presence of progressive epidemiological changes in Venezuela, it would be interesting to evaluate more isolates and other markers.

The TcI nucleotide sequences of isolates from vectors and reservoirs, collected in rural habitats from Anzoátegui and Cojedes, showed variations in relation to the sequences of *T. cruzi* isolates from urban habitats of Caracas. This finding could show a possible segregation and selection of subpopulations in function of geographic area.

In Anzoátegui counties, northeastern part of Venezuela, the parasite populations circulate in rural and semirural biotope using different vectors, such as *R. prolixus*, *T. maculata* and *P. geniculatus* and more sporadically *P. rufotuberculatus* and *Eratyrus mucronatus*[24, 28]. These vectors feed on a variety of reservoirs, which may contribute to genetic recombination events, resulting in appearance of new hybrids or genotypes of the parasite, which be infective to domiciled reservoirs, including *C. familiaris*.

The presence of a *C. familiaris* isolate from Anzoátegui, LH8, with a possible TcIV pattern, that did not have all the characteristic markers, could reflect one mixed infection with several DTUs, probably due to the epidemiological role of dogs, as sentinel of *T. cruzi* infections, and the probability of infection in different areas, where owners move with them.

The *T. cruzi* TcI isolates from Caracas showed sequences more homogeneous. Perhaps, in the Caracas region, human induced environmental changes through deforestation and uncontrolled urbanization, which may have altered the original ecological niche, where the parasite circulated in a zoonotic cycle, with a variety of vectors and wild reservoirs, similar to those observed in Anzoátegui and Cojedes regions. In response to the environmental changes, certain vector species could have been adapted to the human habitat, specially attracted by home light and blood sources in the new urban scenario. In the new areas, the extinction of certain species of vectors and reservoirs favors the dominance of a single vector, *P. geniculatus,* and few reservoirs such as *Rattus rattus*, *D. marsupials* and *Homo sapiens*[29]. These hosts could have acted as biological filters that, over the years, select parasites subpopulations in a closed zoonotic cycle, genetically distinct from the TcI DTU from rural regions. Similar features of genetic variability associated to different epidemiological cycles have been also described in Colombia[9].

The sequence comparisons between the mini-exon markers of the 42 isolates and other eight sequences from Venezuelans *T. cruzi* TcI isolates deposited in the GenBank showed that they did not have a preferential distribution in relation to geographical area, whereas the Caracas isolates described in the present study remained as a separate group.

Although, the isolates from Caracas characterized in this study were few, its biological characterization in murine models showed higher virulence than the Anzoátegui and Cojedes isolates, and a particular tropism for the central nervous system, ocular tissues, genital organs, bone, cartilage, kidney, lung, liver, and pancreas[30]. In this sense, in the area of Caracas, severe symptomatic cases have been observed recently, many of them associated with oral transmission, showing more pronounced symptoms than those associated to fecal contamination through vector transmission[4, 25]. This increased virulence could be a biological expression in response to genetic recombination events and the result of parasite adaptation to environmental changes, new vectors and reservoirs.

It would be important to have more parasites isolated from humans, vectors and reservoirs of different geographical areas to confirm this trend towards a genetic difference between isolates from rural areas and those from urban areas. However, the difficulties found in *T. cruzi* isolation as well as the classification of some rural, urban and hybrid environments are important limitations to carry out this type of investigations.Therefore, it is advisable to perform characterization studies in order to elucidate the relation of genetic variability within the TcI, with the emergent epidemiological pattern that is currently being observed in Chagas disease in Venezuela.

ACKNOWLEDGEMENTS

This investigation received financial support from Proyecto Misión Ciencia N° 2008000911-6, FONACIT, MPPS and Proyecto Estratégico UCV-UC-UDO, FONACIT, MPPS, Nº 2011000470.

REFERENCES

1. *Chagas disease (American trypanosomiasis). 2010*. Fact Sheet No. 340. Available from: *http://www.who.int/mediacentre/factsheets/fs340/en/index.html* (Accessed on June 24, 2014).
2. Prata A. Clinical and epidemiological aspects of Chagas disease. *Lancet Infect Dis* 2001; *1:* 92–100.
3. Añez N, Crisante G, Rojas A. Update on Chagas disease in Venezuela – A review. *Mem Inst Oswaldo Cruz* 2004; *99:* 781–7.
4. Alarcón de Noya B, Díaz-Bello Z, Colmenares C, Ruiz-Guevara R, Mauriello L, Zavala-Jaspe R, *et al*. Large urban outbreak of orally acquired acute Chagas disease at a school in Caracas, Venezuela. *J Infect Dis* 2010; *201:* 1308–15.
5. Devera R, Fernandes O, Coura JR. Should *Trypanosoma cruzi* be called "cruzi" complex ? A review of the parasite diversity and the potential of selecting population after *in vitro* culturing and mice infection. *Mem Inst Oswaldo Cruz* 2003; *98:* 1–12.
6. Souto R, Fernandes O, Macedo A, Campbell D, Zingales B. DNA markers define two major phylogenetic lineages of *Trypanosoma cruzi. Mol Biochem Parasitol* 1996; *83:* 141–52.
7. Brisse S, Verhoef J, Tibayrenc M. Characterisation of large and small subunit rRNA and mini-exon genes further supports the distinction of six *Trypanosoma cruzi* lineages. *Int J Parasitol* 2001; *31:* 1218–26.
8. Zingales B, Andrade SG, Briones MR, Campbell DA, Chiari E, Fernandes O, *et al*. A new consensus for *Trypanosoma cruzi* intraspecific nomenclature: Second revision meeting recommends TcI to TcVI. *Mem Inst Oswaldo Cruz* 2009; *104:* 1051–4.
9. Herrera C, Guhl F, Falla A, Fajardo A, Montilla M, Vallejo GA, *et al*. Genetic variability and phylogenetic relationships within *Trypanosoma cruzi* I isolated in Colombia based on mini-exon gene sequences. *J Parasitol Res* 2009. doi:10.1155/2009/897364.
10. Falla A, Herrera C, Fajardo A, Montilla M, Vallejo G A, Guhl F. Haplotype identification within *Trypanosoma cruzi* I in Colombian isolates from several reservoirs, vectors and humans. *Acta Trop* 2009; *110:* 15–21.
11. Ramírez JD, Duque MC, Montilla M, Cucunubá ZM, Guhl F. Multilocus PCR-RFLP profiling in *Trypanosoma cruzi* I highlights an intraspecific genetic variation pattern. *Infect Genet Evol* 2012; *12:* 1743–50.
12. Macedo A, Oliveira R, Pena S. Chagas disease: Role of parasite genetic variation in pathogenesis. *Expert Rev Mol Med* 2002; *4:* 1–16.
13. Higo H, Miura S, Horio M, Mimori T, Hamano S, Agatsuma T, *et al*. Genotypic variation among lineages of *Trypanosoma cruzi* and its geographic aspects. *Parasitol Int* 2004; *53:* 337–44.
14. Añez N, Crisante G, da Silva FM, Rojas A, Carrasco H, Umezawa ES, *et al*. Predominance of lineage I among *Trypanosoma cruzi* isolates from Venezuelan patients with different clinical profiles of acute Chagas disease. *Trop Med Int Health* 2004; *9:* 1319–26.
15. Llewellyn MS, Miles MA, Carrasco HJ, Lewis MD, Yeo M, Vargas J, *et al*. Genome-scale multilocus microsatellite typing of *Trypanosoma cruzi* discrete typing unit I reveals phylogeographic structure and specific genotypes linked to human infection. *PLoS Pathog* 2009; *5*(5): e1000410. doi: 10.1371/journal.ppat.1000410.
16. MorocoimaA, Carrasco HJ, Boadas J, Chique JD, Herrera L, Urdaneta-Morales S. *Trypanosoma cruzi* III from armadillos (*Dasypus novemcinctus novemcinctus*) from northeastern Venezuela and its biological behavior in murine model. Risk of emergency of Chagas' disease. *Exp Parasitol* 2012; *132:* 341–7.
17. Carrasco HJ, Segovia M, Llewellyn MS, Morocoima A, Urdaneta-Morales S, Martínez C, *et al*. Geographical distribution of *Trypanosoma cruzi* genotypes in Venezuela. *PloS Negl Trop Dis* 2012; *6*(6): e1707. doi: 10.1371/journal.pntd.0001707.
18. Sambrook J, Russel D. *Molecular cloning: A laboratory manual*. III edn. New York: Cold Spring Harbor 2001; p. 6.4–6.12.
19. Hall TA. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl Acids Symp Ser* 1999; *41:* 95–8.
20. Saitou N, Nei M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987; *4:* 406–25.
21. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* 2007; *24:* 1596–9.
22. Gaunt M, Yeo M, Frame I, Stothard J, Carrasco H, Taylor MC, *et al*. Mechanism of genetic exchange in American trypanosomes. *Nature* 2003; *421:* 936–9.
23. Morocoima A, Chique J, Zavala-Jaspe R, Díaz-Bello Z, Ferrer E, Urdaneta-Morales S, *et al*. Commercial coconut palm as an ecotope of Chagas disease vectors in northeastern Venezuela. *J Vector Borne Dis* 2010; *47:* 76–84.
24. Morocoima A, Coriano H, Navas C, De Sousa L, Ferrer E, Herrera L. *Panstrongylusrufo tuberculatus* (Hemiptera, Reduviidae, Triatominae) infected with *Trypanosoma cruzi* in the state of Anzoátegui (Venezuela). *Bol Mal Salud Amb* 2012; *52:* 135–8.
25. Segovia M, Carrasco HJ, Martínez CE, Messenger LA, Nessi A, Londoño JC, *et al*. Molecular epidemiologic source tracking of orally transmitted Chagas disease, Venezuela. *Emerg Infect Dis* 2013; *19:* 1098–101.
26. O' Connor O, Bosseno M, Barnabé C, Douzery E, Breniere S. Genetic clustering of *Trypanosoma cruzi* I lineage evidenced by intergenic mini-exon gene sequencing. *Infect Genet Evol* 2007; *7:* 587–93.
27. Tomasini N, Lauthier JJ, Monje Rumi MM, Ragone PG, Alberti D'Amato, AA, Pérez Brandan C, *et al*. Interest and limitations of sliced leader intergenic region sequences for analyzing *Trypanosoma cruzi* I phylogenetic diversity in the Argentinean Chaco. *Infect Genet Evol* 2011; *11:* 300–7.
28. Morocoima A, Chique J, Herrera L, Urdaneta-Morales S. *Eratyrus mucronatus* (Stal, 1859) (Hemiptera, Reduviidae, Triatominae): Primer registro para el estado Anzoátegui (Venezuela). *Bol Mal Salud Amb* 2010; *50:* 307–10.
29. Herrera L, Urdaneta S. Synanthropic rodent reservoirs of *Trypanosoma (Schizotrypanum) cruzi* in the valley of Caracas, Venezuela. *Rev Inst Med Trop Sao Paulo*1997; *39:* 279–82.
30. Morocoima A, Rodríguez M, Herrera L,Urdaneta S. *Trypanosomacruzi*: Experimental parasitism of bone and cartilage. *Parasitol Res* 2006; *99:* 663–8.

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Received: 24 July 2014 *Accepted in revised form:* 10 December 2014