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Research Articles

Ecopathogenic complexes of American trypanosomiasis in endemic areas of Venezuela: Diagnosis and variability of *Trypanosoma cruzi*

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ABSTRACT

Background & objectives: Trypanosoma cruzi, the causative agent of American trypanosomiasis, has been reported in 180 mammalian species and 154 triatomines species of Neotropic. This is a clonal parasite with variable biological behaviour, associated with the genetics of the parasite and its hosts. To know the eco-pathogenic complex of this zoonosis, it was proposed to characterize *T. cruzi* isolates obtained from triatomines and domestic, peridomestic and wild mammals of the eastern and central-western regions of Venezuela.

Methods: The positivity to *T. cruzi* was established and the isolates were genetically characterized by PCR amplification of the mini-exon gene, the DNA coding for 24Sα and 18S rRNA, and polymorphic sequences-RFLPs. The sampling sites were georeferenced using the MapSource Software and ArcGis 9.3 programs to generate distribution maps of the isolates.

Results: Of the 460 hosts (205 triatomines and 255 mammals), 49% were positive for the parasite. On the other hand, 38 isolates obtained from the triatomines and 23 isolates obtained from mammals were evaluated. The TcI genotype predominated in most of the isolates; however, in those obtained from triatomines the presence of the TcIII genotype in single infections and TcI + TcIII or TcI + TcIV in mixed infections was also evidenced.

Interpretation & conclusion: There is a possibility that the triatomines act as biological syringes for these genotypes associated exclusively to them. The heterogeneity in *T. cruzi* isolates demonstrated the complexity of parasitosis in these regions, presenting its control and prevention as a challenge.

Key words *Trypanosoma cruzi*; infection kinetics; genotype; heterogeneity; hosts

INTRODUCTION

Trypanosoma cruzi (Eucarya, Kinetoplastea, Tripanosomatidae), the causative agent of the American trypanosomosis (AT) or Chagas disease (Chd), is a clonal hemoflagellate, with genetically defined subpopulations, that constitute a biocoenosis, geographically framed in the existence of wild and domestic mammals of up to 10 orders that act as hosts, and strict hematophagous insects, the triatomines, which act as biological vectors. *T. cruzi* is grouped into Discrete Typing Unit (DTUs) which reflect the diversity associated with domestic and wild domestic transmission cycles in a process of parasite selection as it passes through different species of triatomines and mammals including human[1–2].

The AT or Chd as a zoonosis had been considered, until recently exclusive of the Neotropics, affecting 7 million people, mostly circumscribed to the rural habitat[3]. Frequently, it is reported in the United States, Canada, Europe, Japan and Australia, due to the progressive increase of human migrations. In all cases, the use of land, agricultural practices and the indiscriminate handling of animals are linked, affecting environmentally on small and large scale. With the domiciliation of the triatomines and the synanthropic behavior of mammals, the passage of enzootia to the zoonosis has been favored, which increases the exposure to the parasite, as evidenced by the presence of *T. cruzi* in several species of wild hosts, pets

and synanthropic or hunting fauna[4–5].

There are few research studies which overlap the diagnosis of *T. cruzi*, its genotypic variability and the presence of elements of the natural history of parasitism in its niches. In the present work the study of elements of the ecopathogenic complex were proposed. The study of peri-domiciliary and domiciliary endemic areas to the TA or Chd in states of the west, center and the eastern regions of Venezuela, allowed to know the potential geographical expansion of the zoonosis, by analysis of the presence of vectors, reservoirs, distribution of genotypes, performing potential ecological niche models and providing data for monitoring and epidemiological control[6–8].

MATERIAL & METHODS

Study areas

The study was conducted in communities in the central-western region of Venezuela, (Cojedes, Portuguesa, Miranda states and Capital District) and eastern states (Anzoátegui and Sucre states) (Table 1) whose inclusion criteria were the previous records of the presence of the parasite, presence of infected mammals including human and/or presence of *T. cruzi* positive triatomines[6, 8–10]. We reviewed domestic, peridomestic and wild ecotopes as a continuum of peri domiciliary area for the search of mammals and triatomines presence. The study areas were georeferenced through a global positioning system Garmim III GPS (Garmin International, Olathe, KS) using the programs Map Source Software and ArcGis 9.3 (Environmental Systems Research Institute, Redlands, CA, USA) and its biome described according to the life tables of Venezuela[11] (Table 1).

Sampling of triatomines and mammals

A non-probabilistic sampling was carried out, in representative areas with no less than 20 host records (mammals and/or triatomines) in which the presence of *T. cruzi* had been detected in vectors, reservoirs and/or a human population, with zero as presence or absence value[10, 12–13].

Triatomines

Triatomines were collected manually during visits in the day and evening in human dwellings (D = domestic), up to 20 m in diameter from the outermost part of the dwelling (PD = peridomestic) and in an area towards a wild corridor (WC), beyond 30 m from the PD and to the nearby fields (1000 m) that surrounded the localities. The vegetation, furnishings, bird nests, caves, poultry houses and stables were reviewed using 1 hour/man/dwelling, with three operators for three sampling days per region.

Table 1. Geographic regions, localities and coordinates of the study areas.

Eastern region			
		Coordinates	
State	Communities	N	W
Anzoátegui	Altos de Guanta	10º 13’ 30.4”	64º 32’ 8.8”
	Angostura	10° 4’ 60”	64° 34’ 60”
	Bergantín	10° 1’ 7.6”	64° 21’ 45.8”
	Cambural	9° 51’ 00.7”	64° 50’ 00.1”
	El Enial	10° 7’ 32.6”	64° 37’ 6.5”
	La Ceiba	8° 50’ 60”	63° 46’ 60”
	Los Olivos	9º 47’ 44.2”	63° 13’ 55.3”
	Los Ranchos	10°11’ 08.0”	64° 37’ 05.2”
	Naricual	10° 4’ 41.5”	64° 37’ 11.8”
	San José de las Margaritas del Llano	9° 28’ 52.9”	64° 37’ 23.7”
Sucre	Altos de Sucre	10º 13’ 4.67”	64º 32’ 59.4”
	El Maco	10º 11’ 60”	63º 49’ 60”
	La Sabana	10° 13’ 4.9”	64°25’15.9”
	San Pedro	10° 13’ 6.9”	64°25’17.9”
	Yagaracual	10º 18’ 27.0”	64º 20’ 29.8”
Central-western region			
Capital District	Caricuao	10º 25’ 48.5”	66º 58’ 35.3”
	Cotiza	10º 31’ 3.2”	66º 54’ 31.3”
	El Marques	10º 29’ 47.1”	66º 48’ 45.6”
	La Pastora	10º 30’ 44.9”	66º 55’ 11.5”
	La Vega	10º 27’ 37.3”	66º 56’ 31.9”
	Valle Coche	10º 28’ 37.8”	66º 53” 45.3”
Miranda	Araira	10º 27’ 27.0”	66º 29’ 44.2”
	Alto Prado	10º 26’ 22.4”	66º 53’ 35.0”
	Baruta	10º 22’ 43.5”	66º 51’ 7.8”
	Chacao	10º 29’ 37.9”	66º 51’ 23.7”
	Colinas Bello monte	10º 28’ 53.8”	66º 52’ 36.0”
	Cupira	10º 9’ 38.4”	65º 41’ 54.7”
	El Cafetal	10º 28’ 10.7”	66º 49’ 43.1”
	El Hatillo	10º 25’ 25.3”	66º 49’ 31.1”
	Hoyo de la Puerta	10º 22’ 29.0”	66º 53’ 8.2”
	Terrazas Club Hípico	10° 26´53.2”	66° 52´26.9”
	Ocumare del Tuy	10º 8’ 19.55”	66º 52’ 26.9”
	Parque Caiza	10° 28’ 12,5”	66° 45 0.26”
	Petare	10º 28’ 13.6”	66º 47’ 57.3”
	Santa Teresa del Tuy	10º 16’ 15.0”	66º 42’ 14.83”
Cojedes	La Escopeta	9º 65’ 9.89”	68º 58’ 8.9”
	Las Rosas	9º 76’ 31”	68º 62’ 62”
	Nuevo Mundo	9° 53’ 39.8”	68° 33’ 15.8”
Portuguesa	Jabillal	9° 42’ 39”	69° 18’ 36”
	Las Panelas	8° 58’30”	69° 58’ 00.8”
	Araure	9° 34’ 31.6”	69° 13’ 51.0”

The samplings were carried out twice a year in each region, taking care to cover periods of rain and drought[14–15].

An active search for triatomines was achieved by training the inhabitants for the recognition of triatomines and their safe collection, when they approached homes attracted by light. The triatomines were registered accord-

ing to place of collection, time and sex, geo-referenced and maintained for review and identification according to Lent and Wygodzinsky[16].

Mammals

In the D, PD and WC, mammals were captured, using "National" traps (Tomahawk livetraps Co. Tomahawk, Wisconsin, USA, Mod.201 -40.6x12.7x12.7 cm; Mod 204-50.8x17.9x17.9 cm) and traps "Sherman" (Tomahawk livetraps Co; Mod 101 -25.4x7.6x7.6 cm) separated each by 10 m and placed between 6 pm and 6 am. The traps were baited with universal bait. Mammal sampling was carried out twice a year in each region at a rate of 3 days per visit to the house and 4 hours/man/night effort for each day of the capture, in rain and drought period". Mammals were identified according to Sánchez and Lew[17]. The captured mammals were sedated with intramuscular Ketamine® at a rate of 10 mg/kg for blood sampling by cardiac puncture and sterile blood culture on NNN diphasic blood agar (Novy, Nicolle and McNeal) to search for trypanosomatids, by interdiary review of cultures, for up to six months after inoculum. Additionally, a xenodiagnosis was performed, with 10 to 20 individuals of *R. prolixus* (triatomines) of III or IV nymphal stage, proven healthy and reared in the laboratory to obtain flagellates compatible with blood *Trypanosoma* morphotypes[18].

Parasitological diagnosis

The feces and digestive tract of collected triatomines were checked, in sterile isotonic solution (0.85%) under a microscope (400X) for the search of flagellates compatible with *T. cruzi* morphotypes. This procedure was also performed on experimental xenodiagnosis triatomines used on mammals, from 7 days post intake and for up to 6 months. The hemolymph and salivary glands were examined under a microscope to search for *Trypanosoma rangeli*[19]. The metacyclic trypomastigotes of the fecal samples/ salivary glands of triatomines were inoculated via i.p., at a rate of 200 metacyclics/g of weight in male *Mus musculus* NMRI of 10 g. The recovery of the isolates and closure of the cycle was carried out by means of xenodiagnosis on the animals with the highest parasitaemia, for subsequent extraction of 0.5 mL of blood for NNN culture[18].

Molecular diagnosis and variability of T. cruzi isolates

Stool samples of triatomines or material of NNN culture were subjected to DNA extraction by Wizard Genomic® kit (Promega). The purity was calculated by the $A_{260/280nm}$ ratio and the integrity was corroborated by electrophoresis in 0.8% agarose gel at 80V, in TAE buffer (0.04 M Trisma Acetic acid, 0.001 M EDTA) stained with Ethidium Bromide and visualized in Gel Doc 1000 (Bio-Rad). The molecular diagnosis of *T. cruzi* was made by amplification of the kDNA sequence, based on the polymerase chain reaction (PCR)[20]. The genetic variability of

Table 2. Genotypic characterization of *Trypanosoma cruzi* isolates from triatomines and mammals of the eastern and central-western regions of Venezuela.

Triatomines isolates							
Eastern region							
Quantity	Molecular markers						DTU
	ME (bp)	24S/D7 (bp)	18S / (bp)	HSP60 *(EcoRV)* (bp)	GPI *(HhaI)* (bp)	H1 *(AatII)* (bp)	
13	200	110	175	462	817+447	364+122	TCI (76%)
3	150	110	165	314+148	817+447	364+122	TCIII (18%)
1	150+200	110	155+175	462	817+447	364+122	TCIII+TCI=TCV (6%)
Central-western region							
23	200	110	175	462	817+447	364+122	TCI (96%)
1	200+250	110+125	155+175	462	817+447	364+122	TCIII+TCI=TCV (4%)
Mammals isolates							
Eastern Region							
Quantity	Molecular markers						DTU
	ME (bp)	24S (bp)	18S / (bp)	HSP60 *(EcoRV)* (bp)	GPI *(HhaI)* (bp)	H1 *(AatII)* (bp)	
12	200	110	175	462	817+447	364+122	TCI (100%)
Central-western region							
10	200	110	175	462	817+447	364+122	TCI (100%)

ME= intergenic region of the miniexon; 24S= divergent domain of the 24Sα fraction of rDNA; 18S= size-variable domain of the 18S fraction of rDNA; PCR-RFLP= PCR / Restriction Fragment Length Polymorphism of Heat Shock Protein 60 (HSP60), Glucose-6-phosphate isomerase (GPI) and Histone 1 (H1); Discreet Typing Units (DTU), known as TCI, TCIII; TCV in accord to Zingales 2; bp= pairs of bases.

T. cruzi isolates was studied by using molecular markers: (i) intergenic region of the miniexon, (ii) D7 divergent domain of the 24Sα rDNA and (iii) size-variable domain of the 18S rDNA. PCR-RFLP of HSP60, GPI and H1 was performed[21–22]. The products were evidenced by electrophoresis in agarose gels (Agarose Ultra pure Sigma®) at 2% using TAE (40 mM Tris Acetate Buffer, 0.5 M EDTA pH 8.0) under running conditions of 80-100V. The DNA bands stained with ethidium bromide were visualized with UV using the Gel Doc 1000 system (BioRad). The size of the products of the PCRs was estimated by comparing the band pattern of the molecular size marker 100 bp (Promega®) or 100 bp Hypperladder IV (Axygen®) (Table 2).

Modelling of the actual and potential niche of T. cruzi isolated from mammals and triatomines

The *T. cruzi* distribution map was generated with georeferenced records of parasite presence in mammals and triatomines, associated with 19 climatic variables and a spatial resolution of a 1 Km^2 pixel in Ecuador, resulting from the interpolation of mean temperature data, monthly precipitation and altitude data climatic (interpolation of average monthly climatic data from meteorological stations over 30-50 years - WorldClim project)[23].

Using the MAXENT software, a run of ten models was performed to generate a model of average values using 1000 iterations, a convergence threshold of 1.0 x 10-5, with 75% of the records for the calibration of the model and 25% for its evaluation (Maximum Entropy model). The output of the MAXENT model was converted by DIVA GIS software, resulting in the map with a cut-off threshold of the 10th percentile. The percentage contribution of each variable and the Jackknife test allowed to know the importance of each variable, alone or as a whole, on the potential distribution of the parasite in their hosts[24–25].

Ethics statement

All applicable international, national, and/or institutional guidelines were followed. The study was approved by the Ethics Committee of the Instituto de Biomedicina de la Universidad de Carabobo, under the number CBIIB (UC) 2012-2, which endorsed the Project 2007001442 Science Mission-MPPCTI, which has been supporting part of the research carried out.

RESULTS

Triatomines

We obtained 205 triatomines of which 133 (65%) were collected in the eastern region and 72 (35%) in the western region. The species in order of predominance in the eastern region were *T. maculata*, *P. geniculates, R. prolixus*, *R. picitipes*, *Ps. arthuri, E. cuspidatus*, observing greater variability in terms of triatomine species sampled in relation to the central-western region in which only three species were collected, which in order of predominance were *P. geniculatus*, *R. prolixus* and *T. maculata*.

The prevalence of positive triatomines to *T. cruzi*, parasites in faeces and PCR (presence of a 330 bp band for the amplification of kDNA), was 62% (82/133 triatomines) discriminated by species of higher prevalence of infection such as *T. maculata*, *P. geniculatus*, *R. prolixus*, *Ps. arthuri*, *R. pictipes* and *E. cuspidatus* for the eastern region. The prevalence of positive triatomines to *T. cruzi* was 71% (51/72 triatomines), discriminated by species with greater prevalence of the parasite such as *P. geniculatus* and *R. prolixus,* for the central-western region. Of the totality of positive triatomines for *T. cruzi*, 28.4% was used for its culture and genotypic characterization.

Mammals

255 specimens of 16 species of the Venezuelan mastofauna were obtained, of which 83 (32%) were found in the eastern region and 172 (67%) in the central-western region. The species for the eastern region were varied, with domestic, synanthropic and wildlife exemplars, representing 32% of the studied mammals. The species in decreasing predominance were: *Canis familiaris, Didelphis marsupialis, Equus asinus, Rattus rattus, Bos taurus, Equus asinus x Equus caballus, Equus caballus, Riphidomys couesi, Cerdocyon thous, Cuniculus paca, Dasypus novencinctus, Desmodus rotundus, Odocoileus margaritae, Odocoileus virginianus, Sus scrofa* and *Tamandua tetradactyla*. The species of mammals collected in the central-eastern region, were essentially domestic or synanthropic, representing 30% of the mammals studied with a predominance in decreasing order for *C. familiaris, D. marsupialis, Equus asinus, R. rattus and Equus aisnus x Equus caballus*.

The positivity of these mammals to *T. cruzi,* eliminate established by parasitological (hemoculture/xenodiagnostic) and/or molecular methods (amplification of kDNA) was 43.4% (36/83 mammals) in the eastern region, discriminated from higher to lower prevalence eliminate of infection: *D. marsupialis, C. familiaris, Equus asinus, Bos taurus, R. rattus, Cerdocyon thous, Odocoileus margaritae* and *Tamandua tetradactyla.* The *T. cruzi* positivity of mammals was 30% (51/172 mammals) in the central-western region, discriminated from higher to lower prevalence : *Canis familiaris, D. marsupialis,*

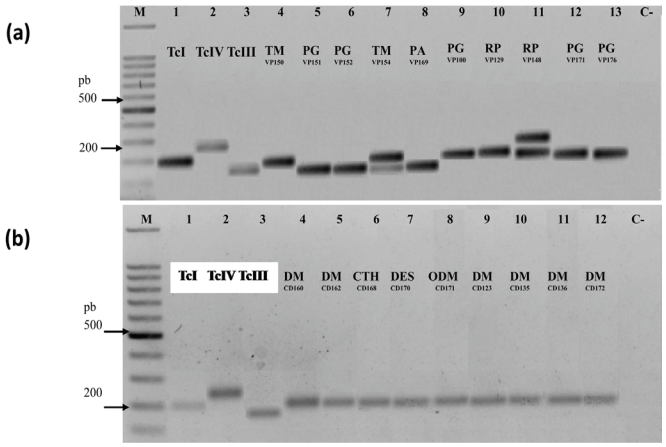

Fig. 1: Amplification of the non-transcribed spacer of the mini-exon gene of *Trypanosoma cruzi* isolates from triatomes and mammals of the Eastern and Central-Western regions/Venezuela. Agarose gel electrophoresis (2%). TcI: 200 base pairs (bp) band, TcIV: 250 bp band; TcIII: 150 bp band. (a) Triatomines: (M) Molecular Size marker-100 bp (Promega); (1) + Control TcI (EP strain, from a patient in acute stage of Chagas disease); (2) + Control TcIV (MA286XC Strain, from *Dasypus novemcintus* in Paraguay); (3) + Control TcIII (Cachi1 Strain, from *D. novemcintus* Anzoátegui-Venezuela); (4-8) Isolates from *Triatoma maculata* (TM); *Panstrongylus geniculatus* (PG); *Psammolestes arthuri* (PA) from Eastern region; (9-13) Isolates from TM; PG; *Rhodnius prolixus* (RP) from Central-Western region. (b) Mammals: (4-8) Isolates from *Didelphis marsupialis* (DM), *Cerdocyon thous* (CTH); *Odocoileus margaritae* (ODM) from Eastern region; (9-12) Isolates from DM from Central-Western region. C – negative control.

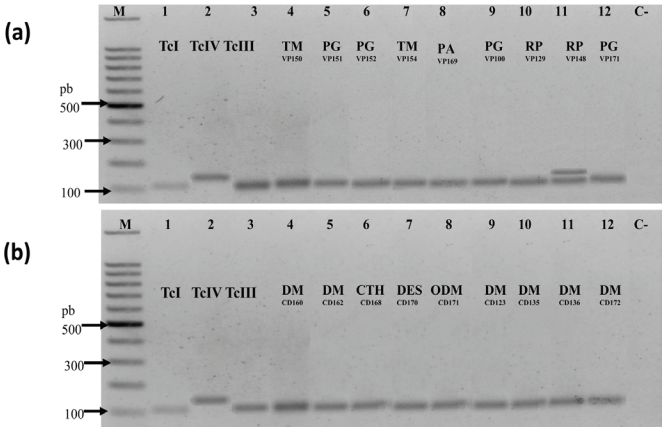

Fig. 2: Amplification of 24Sα fraction of rDNA of *Trypanosoma cruzi* isolates from triatomes and mammals of the Eastern and Central-Western regions/Venezuela. Agarose gel electrophoresis (2%). TcI/TcIII: 110 base pairs (bp) band; TcIV: 125 bp band. (a) Triatomines: (M) Molecular Size marker 100 bp (Promega); (1) + Control TcI (EP strain, from a patient in acute stage of Chagas disease); (2) + Control TcIV (MA286XC Strain, from *Dasypus novemcintus* in Paraguay); (3) + Control TcIII (Cachi1 Strain, from *D. novemcintus* Anzoátegui-Venezuela; (4–8) Isolates from *Triatoma maculata* (TM); *Panstrongylus geniculatus* (PG); *Psammolestes arthuri* (PA) from Eastern region; (9–13) Isolates from TM; PG; *Rhodnius prolixus* (RP) from Central-Western region.

(b) Mammals: (4-7) Isolates from *Didelphis marsupialis* (DM), *Cerdocyon thous* (CTH); *Odocoileus margaritae* (ODM); Desmodus rotundus (DES) from Eastern region; (9–12) Isolates from DM from Central-Western region. C – negative control.

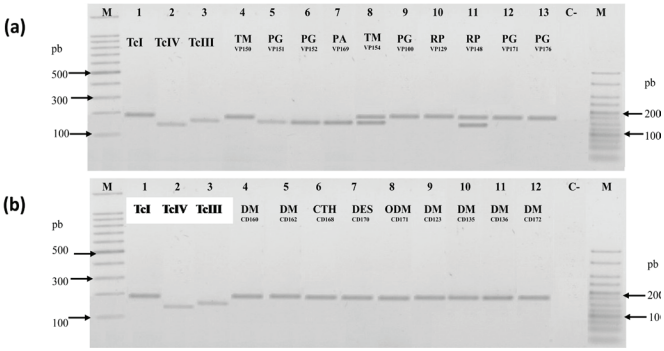

Fig. 3: Amplification of 18S fraction of rDNA of *Trypanosoma cruzi* isolates from triatomes and mammals of the Eastern and Central-Western regions/Venezuela. Agarose gel electrophoresis (2%). TcI: 175 base pairs (bp) band; TcIII: 165 bp band; TcIV: 155 bp band.(a) Triatomines:Molecular Size marker 100 bp (Promega); (1) + Control TcI (EP strain, from a patient in acute stage of Chagas disease); (2) + Control TcIV (MA286XC Strain, from *Dasypus novemcintus* in Paraguay); (3) + Control TcIII (Cachi1 Strain, from *D. novemcintus* Anzoátegui-Venezuela; (4-8) Isolates from *Triatoma maculata* (TM); *Panstrongylus geniculatus* elimintae TM (PG); *Psammolestes arthuri* (PA) from Eastern region; (9–13) Isolates from TM; PG; *Rhodnius prolixus* (RP) from Central-Western region. Control; (14) Molecular Size marker Hyperladder V (Novagen).

(b) Mammals: (4-7) Isolates from *Didelphis marsupialis* (DM), *Cerdocyon thous* (CTH); *Odocoileus margaritae* (ODM) ; Desmosus rotundus (DES) from Eastern region; (8-12) Isolates from DM from Central-Western region. C – negative control.

Equus asinus, Equus aisnus x Equus caballus, R. rattus.

Study of genetic variability

The genetic variability pattern of *T. cruzi* subpopulations observed by the ME, 24Sα, 18S markers and RFLPs, for 38 triatomine isolates, revealed that for the eastern region, 72% (10/14) amplified for TcI and 21% (3/14) for TcIII (2 isolates obtained from *P. geniculates* and 1 from *Ps. arthuri*). The remaining 7% (1/14, obtained from *T. maculata*) showed a mixed infection TcI/ TcIII and RFLP pattern compatible with TcV. Of the 24 *T. cruzi* isolates from triatomines from the central-western region, 96% (23/24) amplified for TcI and 4% (1/24) revealed a mixed infection TcI/TcIV in one specimen of *R. prolixus* enlightening genetic heterogeneity in these triatomine isolates (Table 2 & Fig. 1–4).

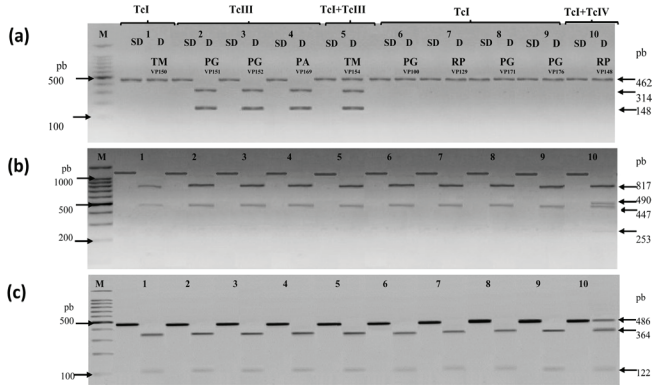

Fig. 4: PCR-RFLP genotyping profiles of *Trypanosoma cruzi* isolates obtained from triatominos of the Eastern and Central-western regions/Venezuela. 2% agarose gel electrophoresis in TAE 1X. Each pair of lines corresponds to undigested PCR products (UD), followed by digested products (D) for HSP60/EcoRV (A), GPI/HhaI (B), H1/AatII (C). From left to right: (M) Molecular Size marker 100 bp (Promega 100); (1–5) isolates from *Triatoma maculata* (TM), *Panstrongylus geniculatus* (PG), *Psammolestes arthuri* (PA) from Eastern region; (6–10) isolates from PG and *Rhodnius prolixus* (RP) from Central-Western region.

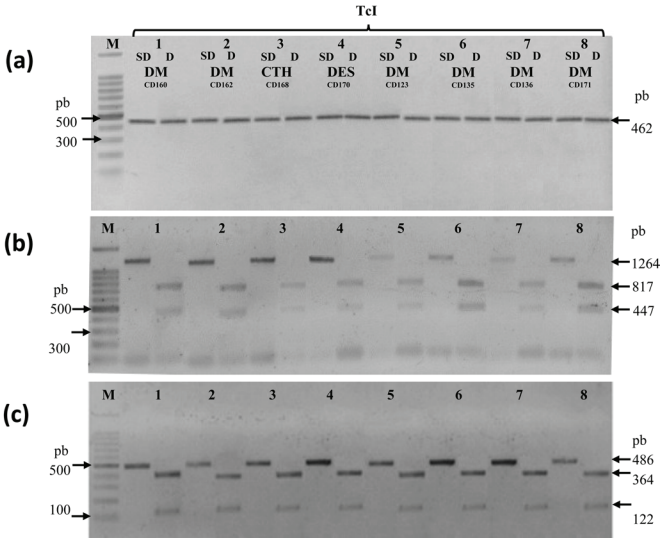

Fig. 5: PCR-RFLP genotyping profiles of *Trypanosoma cruzi* isolates obtained from mammals of the Eastern and Central-western regions/Venezuela. 2% agarose gel electrophoresis in TAE 1X. Each pair of lines corresponds to undigested PCR products (UD), followed by digested products (D) for HSP60/EcoRV (A), GPI/HhaI (B), H1/AatII (C). From left to right: (M) Molecular Size marker 100 bp (Promega 100); (1-4) isolates from *Didelphis marsupialis* (DM), *Cerdocyon thous* (CTH); *Desmodus rotundus* (DES) from Eastern region; (5-10) isolates from DM from Central-western region.

Analysis using ME, 24Sα, 18S markers and RFLPs from the 23 *T. cruzi* isolates obtained from mammals in the eastern and central-western regions of Venezuela,

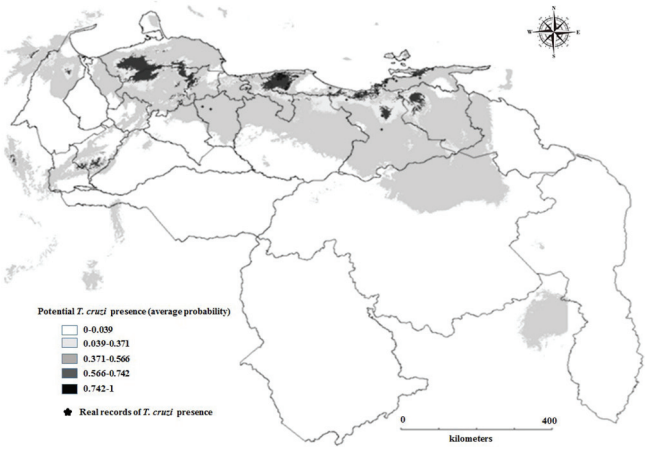

Fig. 6: Model of potential distribution of *T. cruzi* showing the probability of occurrence based on data of presence of *T. cruzi* in mammals/triatomines of the eastern and central-western regions of Venezuela. The intense black colors indicate higher probability of occurrence; gray indicate medium probability; pale gray indicate low probability. The black dots indicate real records of the presence of the parasite determined in the study areas.

confirmed homogeneity with respect to the DTU of the parasite, all exclusively TcI (Table 2 & Fig. 1– 3, 5).

Potential geographic distribution of the elements of the zoonoses

The predictive model generated by Diva GIS and MAXENT with the geolocalized positives mammals and triatomines in the present study, produced a map of potential distribution of *T. cruzi* with occurrence, based on *T. cruzi* presence data in mammals and/or triatomines associated with preponderating bioclimatic characteristics. The highlighted areas indicated that there was a 0.556-1 probability of finding the *T. cruzi* parasite in mammals and/or insects, in focal areas of the coastal mountain range such as Sucre, Monagas and Anzoátegui states (of the Eastern region) and Miranda, Aragua, Lara, Falcón, and Zulia (from the central-western region). In an exceptional way, the model predicted with the same probability the presence of *T. cruzi* in mammals and/or insects, in focal areas of Merida State.

The variables of those regions that would seem to have contributed more in percentage for the potential establishment of the zoonosis were: precipitation of the driest month (23.8%), precipitation of the coldest quarter (23.7%) and average temperature of the warmest quarter (10%). The six variables of minor contribution were: Annual Precipitation (7.9%); Isothermality (7 %); Seasonality of the temperature (5.8%), Precipitation of the wettest quarter (5.4%), Precipitation of the wettest month (4%);

Altitude (3.6%), Average daytime temperature range (Temperature, Maximum-Temperature Minimum, 2.9%) and Average temperature of the wettest quarter (2.7%). The bioclimatic variable that by itself predicted the potential establishment of a niche of the zoonosis with the highest contribution values in data distribution (Jackknife AUC test) was the annual precipitation, which gave more useful information when analyzed in isolation. An area under the curve (AUC) of 0.94, gave effectiveness to the model of potential *T. cruzi* distribution in the study regions (Fig. 6).

DISCUSSION

The AT or Chd, is a complex parasitosis that manifests from an enzootia to an anthroponosis, depending on the trophic network in which it is circumscribed and in which several species of triatomines and mammals can participate, including the human. Recently, it has been considered a re-emerging parasitic disease conditioned by anthropogenic changes in wild and urban habitats[3, 26–28].

The TA in Venezuela has traditionally been associated with the foot of the Andean mountain, the Central-Eastern mountain range and the elevations of the central-western plains, areas of greater distribution of human settlements[29]. Recent studies have revealed an increase in incidence in coastal areas and other regions of eastern and southern Venezuela, such as Anzoátegui, Nueva Esparta and Amazonia[9, 30–31].

The vectors in the transmission cycle

In Venezuela, 60% of the confirmed cases of Chd have been reported in Anzoátegui, Aragua, Barinas, Carabobo, Cojedes, Portuguesa, Trujillo and Yaracuy, the majority of these being associated with the presence of *R. prolixus* domiciliary primary vector[6, 8, 10, 30]. This scenario has changed in the central-western region of Venezuela, due to the occasional visit or colonization in human habitation by *T. maculata* and *P. geniculatus*, both attracted by artificial light from nearby wild niches, or by the decrease of mammals, their source of food, due to human anthropized habitats or subsistence hunting activities[30, 32–34].

The results presented here, although they are a specific entry, revealed diversity of the triatominofauna, with a preponderant presence of *P. geniculatus*, followed by *T. maculata* and *R. prolixus* species with relevant epidemiological roles in Venezuela and with the potential of domiciliation, evidenced in the eventual existence of nymphal stages in human settlements. Other species found, would be acting as occasional vectors such as *Ps. arthuri*, *E. cuspidatus* and *R. pictipes*, all subject of mandatory epidemiological surveillance in the domestic, peridomestic and wild transmission cycle[6, 13, 15, 35–39].

In particular, *Ps. arthuri* reported in Anzoátegui, Cojedes and Portuguesa with natural infection by *T. cruzi*, could be considered a cryptic vector, increasing in peridomestic ecotopes, specifically in bird nests, less than five meters from human habitation and in sympatry with other triatomines and some mammals. These potential vectors could, at any time migrate to homes and be in contact with humans and peridomestic or domestic animals, constituting a risk factor[39].

The genotype of *T. cruzi* that appeared mostly in *P. geniculatus*, *T. maculata* and *R prolixus* was TcI, originally associated with wild cycles, however, it has recently been syndicated to domestic transmission cycles that go from Central America to the Amazon basin (north of South America)[2]. The species *E. cuspidatus*, associated mainly with domestic and peridomestic ecotopes, has been found infected with TcI in the present work. The reports of infection in the literature only refer to *E. mucronatus*, species of more recent speciation, infected with TcI. Thus, this would be a new finding of consideration in the distribution of this *T. cruzi* genotype[13, 38].

R. pictipes, registered in association with wild cycles for *T. cruzi* and *T. rangeli* in palms, bromeliads, caves of mammals and dry trees, was found in peridomestic ecotopes, with 100% positivity for TcI, indicating its high degree of adaptation to anthropized environments as cited in literature[38]. In the present work, TcIII was found in unique infections in *P. geniculatus* and *Ps. arthuri*, The distribution of TcIII, has been increasing in the country, acquiring epidemiological importance, insofar as it has been considered a bioindicator of the existence of biological corridors between the cycles of wild and peridomestic transmission and particularly associated with the armadillos as reservoirs[2, 6, 29, 39–40].

Some theories, indicate that the parasite-vector association is much older than that of the parasite-mammal, since the insects were primarily predators with secondary incorporation of the hematophagy[41], favoring their action as true "biological syringes" that spread more than one parasite genotype, this could explain the genetic variability that is revealed in mixed infections with the TcI + TcIV and TcI + TcIII, genotypes, observed in *T. maculata* and *R. prolixus* respectively.

The mammal in the transmission cycle

In the present work several species of mammals were studied, acting as potential reservoirs for *T. cruzi*, all infected with TcI, with the ability to interact with triatomine vectors mentioned above and in areas where an infected human population has been registered, being able to form

ecopathogenic complexes[1, 6, 26, 37, 42–43]. In this study *D. marsupialis* was the species, with the highest prevalence, 50% exclusively TcI, that has been considered in some ecosystems a primary synanthropic reservoir, with wide geographic distribution, omnivorous habits, and eclectic niches, such as the corridors between wild and domestic environments. It is characterized by high rates of infection and can eliminate infectious forms in the urine and the content of their anal glands, eventually contaminating food and home equipments, which constitutes a high risk[27, 44–45].

Other wild mammals, components of the Venezuelan hunting fauna, were positive, such as *T. tetradactyla*, *O. margaritae*, *D. novencintus*, *P. tajacu*, *C. paca*, *C. thous*, which have been reported as infected with *T. cruzi* in the country and in other areas of America. These species could act as reservoirs and dispersers, especially when participate in the human food chain; it is necessary to establish their population dynamics within the transmission cycle[1, 33]. It should be noted that the infection in chiroptera specimens such as *D. rotundus* and in foxes such as *C. thous* is the first record, being that the results obtained here constitute a contribution to the data reservoirs in Venezuela.

The positivity to *T. cruzi* of animals used for livestock such as equidae, is also a finding of great epidemiological importance since in critical situations in agricultural practice, humans concentrate the population of these animals in areas, for saving food, water and labour for management, leading to overcrowding. This aggregation would facilitate the attraction of vectors, forcing a bottleneck, and creating a greater risk of transmissibility, in artificially favoured transmission cycles. The possibility of transmission of *T. cruzi* in humans involved in livestock management, their families, or in a livestock-marketing route, by handling raw meat and/or consuming undercooked meat with circulating blood stages of the parasite it is something that could not be ruled out[46–47].

In the exposed results, the positivity to *T. cruzi* in dogs constitutes an important contribution to the few studies about the role of the dog in the transmission cycle, in Venezuela. The dog would be considered in surveillance programs, for its role as a sentinel of the AT in areas with re-emergence, post chemical vector control or post human treatment. In non-endemic areas, it could act as an indicator of the emergence of the zoonosis[30, 42, 46, 48–50]. In our study, the isolates of *T. cruzi* obtained from *D. marsupialis*, cinegentic mastofauna and synanthropic and domestic fauna, revealed long periods of infection with continuous parasitic load, low parasitemia values and variable mortality rate in a murine model. This would guarantee continuous transmissibility, independent of the geographical area (data not shown).

State of art

T. cruzi is a complex of heterogeneous subpopulations, over artificial and natural stress as the uncontrolled extension of the cities towards forests and savannas, deforestation, migration of the hosts (including humans) from wild/ rural landscapes to suburban and urban habitats, leading to selection of parasite subpopulations.

In our study, this heterogeneity was reflected in the higher prevalence of TcI, followed by the TcIII genotype prevalence and a little representation of mixed genotypes TcI + TcIII and TcI + TcIV (TcV?). The presence of the TcI genotype in the three ecotopes would indicate its transmission among the hosts independent of the ecotope, breaking the classical conception of associating a genotype with a specific transmission cycle[2].

Likewise, the eclectic range of mammals and triatomines species associated with TcI (*E. cuspidatus*, *R. pictipes*, *O. margaritae*, *D. rotundus*, *P. tajacu* and *C. thous*) would explain why the ample TcI dispersion in the country, conditioned by hosts niches that favour host-parasite encounter and determine the host role in the transmission cycles[6, 26, 37, 43, 50].

The parasite-host relationship is part of a larger system, represented by communities and ecosystems in which all the elements of the association are present, especially when its distribution coincides with major human settlement distributions in Venezuela. The phenomenon of emerging, neglected or re-merging diseases would be understood since the complex paradigm. These maladies involve ecological and evolutionary mechanisms that participate in the passage from wild to domestic environments and vice versa[51].

The studies of parasite reservoirs are important for control and epidemiological surveillance. The risk would imply the presence of these hosts in peridomestic and domestic ecotopes, associated with hunting and consumption of wild animals. Likewise, know the *T. cruzi* prevalence in cinegetic wild fauna, favours the early detection of a wide range of zoonosis. However, with a punctual entry is difficult to define exactly the role of some mammal species; longitudinal studies are more representative to clarify this eco-pathogenic complex[1, 26].

Research on TA in Venezuela has been focused on the clinical and epidemiological behaviour; occasionally some researchers have analysed the biological tendencies of the parasitosis, but not as a whole. This study contributes to discern to some aspects of the real or potential eco-pathogenic complex and provides elements for the modelling of *T. cruzi* distribution.

Conflict of interest: None

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