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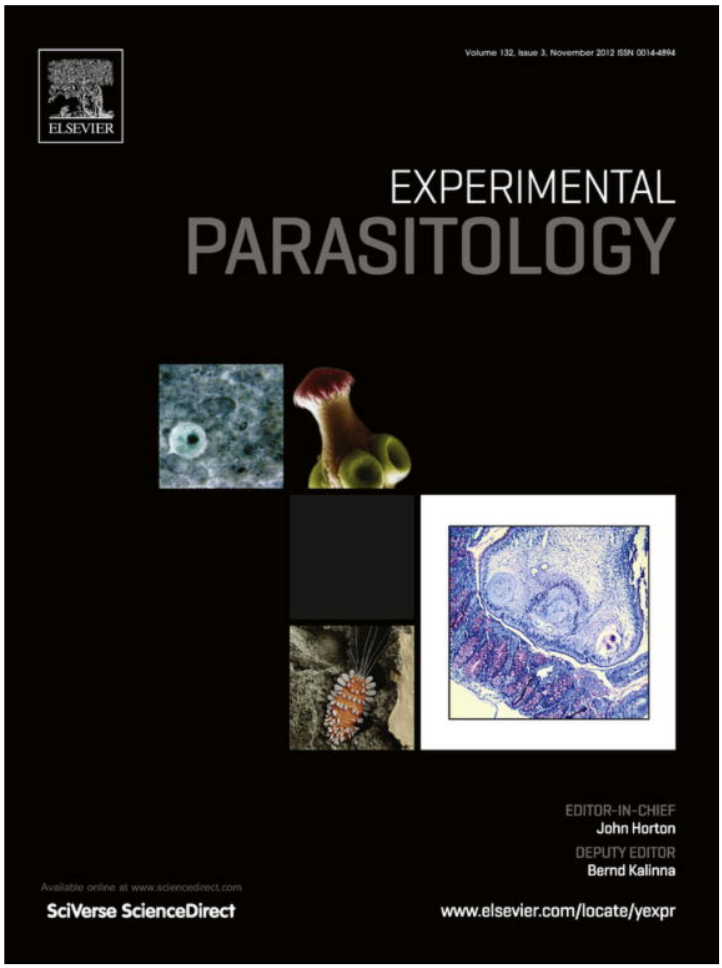


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# *Trypanosoma cruzi* III from armadillos (*Dasypus novemcinctus novemcinctus*) from Northeastern Venezuela and its biological behavior in murine model. Risk of emergency of Chagas' disease

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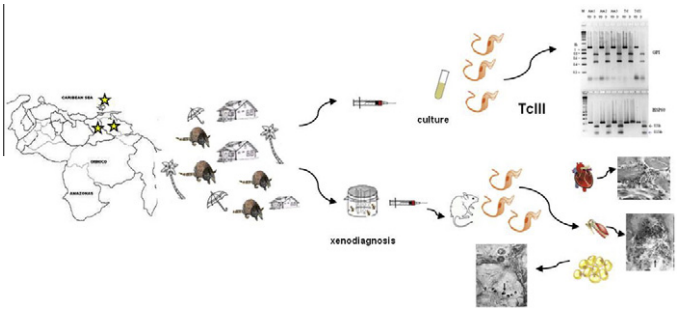
## HIGHLIGHTS

- First report of Trypanosoma cruzi from armadillos - Dasypus novemcintus novemcinctus - from Northeastern Venezuelan states.
- First report of DTU TcIII by PCR–RFLP in the same states.
- The isolates were biologically heterogeneous.
- Risk of emergency of Chagas' disease in Island Margarita (Nueva Esparta State) major touristic area.

## GRAPHICAL ABSTRACT

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## ABSTRACT

*Trypanosoma cruzi*, etiological agent of Chagas' disease, was isolated from armadillos (*Dasypus novemcinctus novemcinctus*) captured in rural communities Northeastern Venezuela from Nueva Esparta State (no endemic for Chagas' disease), Monagas and Anzoátegui States (endemics). The isolates, genetically typed by PCR–RFLP as belonging to the TcIII DTU, have demonstrated in murine model heterogenic parasitemia, mortality and histotropism with marked parasitism in cardiac, skeletal, and smooth myocytes that showed correlation with lymphobasophilic inflammatory infiltrates. Our finding of *T. cruzi* infected armadillos in Isla Margarita (Nueva Esparta State), together with reports of triatomine vectors in this region, the accentuated synanthropy of armadillos, intense economic activity, migration due to tourism and the lack of environmental education programs all of them represent risks that could cause the emergence of Chagas' disease in this area. This is the first report of the TcIII DTU in Northeastern Venezuela, thus widening the geographic distribution of this DTU.

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## 1. Introduction

*Trypanosoma cruzi* (Kinetoplastida, Trypanosomatidae) is the etiological agent of American trypanosomiasis or Chagas' disease, a zoonosis found in particular landscapes that contain niches harboring wild mammal reservoirs and haematophagous insect vectors (Hemiptera, Reduviidae, Triatominae), which transmit the parasite, generally by fecal contamination, to other mammals.

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These transmission dynamics "sylvatic cycles" are among the least studied in spite of the risk they represent to human health and their wide distribution in Neartic and Neotropical regions (from Salt Lake City, 41° N in the United States to 56° S in the argentine Patagonia as well as some Caribbean islands). In these cycle the parasites circulate among more than 200 mammal species from seven orders and some 140 triatomine species in 15 genera (Dias, 2000; Galvão et al., 2003).

The most ancient reservoirs involved in this cycle are species of Eutheria (Cingulata, Dasypodidae: armadillo; Rodentia, Echimyidae) and Metatheria (Didelphimorphia, Didelphidae: opossum) (Coura, 2010; Teixeira et al., 2006). These synanthropic mammals play an important epidemiological role in Chagas' disease as they move from their natural ecosystems towards those inhabited by humans, producing in consequence the so called "peridomestic" and "domestic cycles". Both the opossum *Didelphis* spp. and the armadillo *Dasypus* spp. show a geographical distribution overlapping that of Chagas' disease (Dias, 2000).

In Venezuela, *T. cruzi* circulates among 22 vector and 13 reservoir genera, among which the widely distributed *Didelphis marsupialis* and *Dasypus novemcinctus novemcinctus* are the most epidemiologically important, resulting in a *T. cruzi* distribution of approximately 80% within the country (Carcavallo et al., 1999; Pifano, 1960). In spite of this, there have been few studies on the eco-epidemiology of the infection-disease, notably in regions such as the Amazon (Feliciangeli et al., 2004) and Nueva Esparta State (Ministerio de Salud, Venezuela, 2007; Pifano, 1961). This last is made up of three islands in which national and international tourism has increased greatly in recent decades.

The pioneering research of Chagas' (1909) and Vianna (1911) had, at an early stage, already demonstrated the histopathological and clinical modalities produced by *T. cruzi* in humans. Zingales et al. (2009) emphasized the intricate heterogeneity of the population structure of the taxon and reviewed the different protocols used to define the biological, biochemical and genetic characteristics of the strains. These authors recommended the use of discrete typing units (DTUs) with a novel nomenclature, TcI–TcVI, in order to better understand the intraspecific organization of the parasite.

The isolates we used in this study were typified following the technique described by Westenberger et al. (2005), which allows the rapid, easy and reliable identification of the isolates belonging to the TcI and TcIII DTUs (Lewis et al., 2009; Zingales et al., 2009).

With this in mind, we must emphasize that the biological properties of the strains under study, especially their histotropism, virulence and pathogenicity, are determining factors for Chagas' disease (Sánchez-Guillén et al., 2006; Coura, 2007).

We report the presence of *T. cruzi* in armadillos and characterize, both parasitologically and molecularly, isolates obtained from these animals captured in three states in Northeast Venezuela: Anzoátegui and Monagas, both endemic for Chagas' disease Nueva Esparta state, not endemic for Chagas' disease, although the presence of these vectors has also been reported (Carcavallo et al., 1999; Morocoima et al., 2010; Pifano, 1961).

## 2. Materials and methods

### 2.1. Study areas

Armadillos were captured from the following áreas: (1) Nueva Esparta State; semiarid areas with a mean annual temperature of 29ºC and temperate, cold, semiarid, mountainous areas (350 m a s l), mean annual temperature 20 °C; specifically from the village of Fuentidueño (N 11° Ó 59"; W 63° 56́ 59"), Díaz Municipality, Margarita Island; (2) Anzoátegui State; from the village of Atapire, mean annual temperature 29 °C, (N 8º 26′ 18″; W 64º 22′ 11″), Francisco de Miranda Municipality, and (3) Monagas State; Aguasay village, mean annual temperature 25 °C, (N 9° 25′ 40″; W 63° 43′ 47″), Aguasay Municipality.

### 2.2. Characteristics of the armadillos. Parasitological tests for the detection of kinetoplastid flagellates

The armadillos were examined to estimate their age, sex and weight (Table 1). Kinetoplastid flagellates were determined using the following protocol: blood samples obtained from the tail veins of each animal were examined as wet smears (400×) and thin smears stained with Giemsa solution (1000×) in order to determine the presence, type of movement and morphotypes of the parasites (Barretto, 1979), as well as to quantify the parasitemia (Brener, 1962). Xenodiagnosis with ten IV-instars nymphs of *R. prolixus*, reared in our insectarium, was then done. Three weeks later, stools obtained by dissecting the intestines of the triatomines were diluted in sterile isotonic solution 0.85% (w/v) and examined as described above, in order to determine the polymorphic stages of the parasites.

Aliquots (0.3 ml) of blood obtained aseptically by cardiac puncture from each armadillo, under anesthetic with 0.2 ml/kg of Ketaset (Ketamine-HCl, Fort Dodge Lab USA), were cultured in blood agar medium (Difco, Thomas Scientific, N Jersey, USA) in a 5-fluorocytocin and gentamycin solution and monitored weekly for flagellates by means of an inverted microscope (400×) for up to two months. Sub-passages of positive cultures were then further cultured in Roswell Park Memorial Institute (RPMI) medium at 28 °C with a maximum of 5 monthly passages in order to avoid alterations in their virulence. Isolates were maintained in liquid nitrogen until analysis.

### 2.3. Biological characterization of T.cruzi infections. Experimental infections. Histoparasitological study

Fecal material from infected nymphs from each xenodiagnosis was inoculated ip. (200 metacyclic trypomastigotes/g body weight) into clean male NMRI mice (12 g mean weight), born at our animal rearing facilities (Tropical Zoology and Ecology Institute), with five mice inoculated per batch. Mice were then monitored (400×) to assess the parasitemia and pleomorphism of the flagellates three days after inoculation and thereafter three times a week until their death or the onset of the chronic phase of the infection. The mortality rate of the mice was recorded daily.

The animal with the highest parasitemia in each batch was then selected for histopathological analysis. Mice were sacrificed

**Table 1**
Characteristics of the armadillos (*Dasypus novemcinctus novemcinctus*) captured from three villages in Northeastern Venezuela and results of the parasitological tests for the detection of kinetoplastid flagellates infection.

| Site of capture | Age; sex; mean weight | Blood′ smears | Xenodiagnosis | Hemoculture |
|---|---|---|---|---|
| Fuentidueño | Juvenils; l M, 2 F; 1.7k | 0/3 | 0/3 | 0/3 |
| (Margarita Island, Nueva Esparta state) | Adults; l M, 1 F; 3k | ½ | 1/2 | 1/2 |
| Atapire (Anzoátegui state) | Adult; 1 M; 3.5k | 0/1 | 0/1 | 1/1 |
| Aguasay (Monagas state) | Adults; 2 M; 3k | 0/2 | 1/2 | 1/2 |
| Total | 8 | 1/8 | 2/8 | 3/8 |

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to obtain samples from heart, skeletal muscle, diaphragm, skin, stomach, duodenum, colon, liver, spleen, pancreas, lungs, kidneys, brain, cerebellum, sternum, bone marrow and adipose tissue. Samples were fixed in formalin (10%), embedded in paraplast, cut into 3μ sections, stained with hematoxylin-eosin and examined microscopically (1000×). Parasite tissue invasion observed for each of the examined organs was then quantified (Morocoima et al., 2006) as follows. The number of pseudocysts/50 fields (400×) was organized into the following intervals: 0; 1–10; 11–20; 21–30; 31–40. Each interval was then coded as ––; +; ++; +++; ++++, corresponding to absent, scarce, moderate, abundant and intense, respectively. The lymphobasophylic inflammatory infiltrates were also classified as: x = absent; xx = scarce and focal; xxx = scarce and disperse; xxxx = abundant and disperse. Histological sections of the organs and tissues showing parasites and inflammatory infiltrations were then photographed at 1000× using a digital camera Genius G-Shot P510.

### 2.4. Molecular typing of T. cruzi isolates

The parasites grown in Roswell Park Memorial Institute (RPMI) medium were typified genotypically by the Polymerase Chain Reaction – Restriction Fragment Length Polymorphism (PCR–RFLP) analysis described by Westenberger et al. (2005) by amplifying region coding for the glucose phosphate isomerase gene (GPI), followed by digestion with the *Hha*I restriction enzyme. *Hha*I generates band profiles identical for the TcI and TcIII DTUs, but different to TcII, TcIV, TcV and TcVI.

In order to differentiate between TcI and TcIII, we amplified the intergenic region of the heat shock protein 60 gene (HSP60) and then the PCR products with the *Eco*RV restriction enzyme. This enzyme only digests amplified fragments from theTcIII DTU and does not recognize restriction sites for the TcI DTU as demonstrated by Westenberger et al. (2005).

This technique allowed the rapid, easy and reliable identification of the isolates belonging to the TcI and TcIII DTUs (Lewis et al., 2009; Zingales et al., 2009). International reference strains W250 cl10B (TcI), Esmeraldo (TcII), MA26X (TcIII) and CAN III (TcIV) obtained from the Criobank at the London School of Hygiene and Tropical Medicine were used for comparison.

### 2.5. Ethical guidelines

All experiments involving animals were conducted according to the current Bioethical Laws as laid down by the Venezuelan Science and Technology Ministry, and were approved by the Ethics Committee and the Committee for Animal Care of the National Fund for Science and Technology (FONACIT, Caracas, Venezuela).

## 3. Results

### 3.1. Parasitological characterization of T. cruzi

Of the eight nine-banded armadillos (*Dasypus n. novemcinctus*) Linnaeus, 1758 (Cingulata, Dasypodidae) captured, flagellates were observed in 6 adults; none of the juveniles were infected (Table 1). Stumpy trypomastigotes were observed from the wet and thin Giemsa stained smears of blood in one animal and xenodiagnosis and the hemocultures showed polymorphic states that included metacyclic trypomastigotes in the other five. Of these armadillos, one from each of the regions under study was randomly chosen for analysis. The parasitemia of the armadillo when its blood was directly examined was 0.1 trypomastigotes $\times 10^5$/ml blood.

The parasitemic peaks (of both slender and broad trypomastigotes) in mice inoculated with the M/DAS/VE/2007/JBC, M/DAS/VE/2007/RSC and M/DAS/VE/2007/CAJ isolates obtained from armadillos captured in Fuentidueño, Aguasay and Atapire were 7.0, 0.7 and 0.4 trypomastigotes $\times 10^5$/ml blood (data not shown) producing mortalities of 100%, 40% and 20%, respectively. The M/DAS/VE/2007/JBC isolate from Margarita Island (Nueva Esparta state) showed the most virulent behavior as it was the only one found in the wet blood smears of the armadillos, as well as producing the highest parasitemia levels in the experimental mice causing 100% mortality.

The histoparasitological study showed pseudocysts with parasites in 13 out of the 16 organs and tissues examined. Similar levels of histotropism were also observed with a marked predilection for the myocytes of cardiac, skeletal and smooth tissues, where an elevated number of intracellular parasites (31–40 pseudocysts/50 fields/400×) were detected. There were also abundant and dispersed inflammatory infiltrates although differences in the specific muscle tissues invaded were found. Parasitism in the pancreas,

**Table 2**
Levels of tissue parasitism and inflammatory infiltrates produced in NMRI mice experimentally infected with *Trypanosoma cruzi* isolated from armadillos (*Dasypus novemcinctus novemcintus*) from villages in Northeastern Venezuela.

| Organs and tissues | Isolates | | | | | |
|---|---|---|---|---|---|---|
| | M/DAS/VE/2007/JBC | | M/DAS/VE/2007/CAJ | | M/DAS/VE/2007/RSC | |
| Heart | ++ | xx | ++++ | xxxx | ++++ | xxx |
| Skeletal muscle | ++ | xxx | ++++ | xxxx | ++++ | xxxx |
| Diaphragm | ++++ | xxxx | +++ | xxx | ++ | xx |
| Skin | ++ | xx | – | xx | + | xx |
| Stomach (smooth muscle) | ++++ | xxxx | +++ | xx | +++ | xx |
| Duodenum(smooth muscle) | + | x | ++ | xx | + | xx |
| Colon (smooth muscle) | – | x | – | x | + | xx |
| Pancreas | – | x | – | x | ++ | x |
| Liver | + | xx | ++ | xx | + | xx |
| Spleen | – | x | – | x | – | x |
| Sternum | + (Cdb.) | x | + (Cdb.) | x | + (Cdt.) | x |
| | – (B.marrow) | – | + (B.marrow) | – | + (B.marrow) | – |
| Lungs | – | x | – | x | ++++ | xx |
| Kidney | – | x | – | x | – | x |
| Brain | – | x | – | x | – | x |
| Cerebellum | + (molec.s.) | x | – | x | – | x |
| Adipose tissue | – | x | – | x | ++ | x |

+ = tisue parasitism; x = inflamatory infiltrates; Cdb. = chondroblasts; Cdt. = chondrocytes; B. marrow = bone marrow; molec. s. = molecular stratum. Isolates: M/DAS/VE/2007/JBC (Isla Margarita; Nueva Esparta state; M/DAS/VE/2007/CAJ (Anzoátegui state); M/DAS/VE/2007/RSC-Monagas state). (H/E.).

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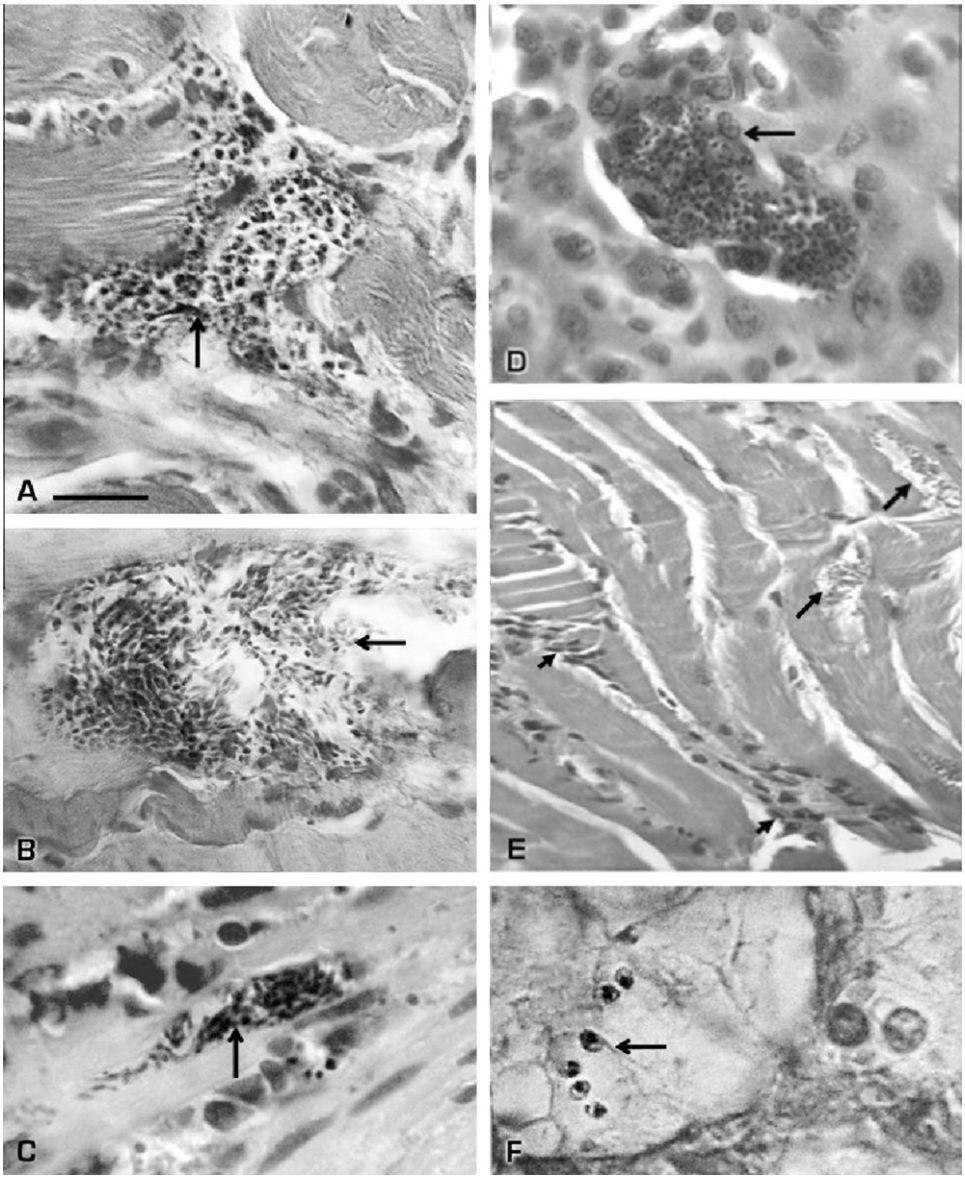


**Fig. 1.** Histological parasitism in NMRI mice experimentally infected with isolates of *Trypanosoma cruzi* from armadillos (*Dasypus novemcinctus novemcinctus*) from villages in Northeastern Venezuela. (A) Heart (isolate CAJ); (B) skeletal muscle (isolate JBC); (C) colon (smooth muscle) (isolate RSC); (D) liver (isolate JBC); (E) skeletal muscle (isolate JBC); (F) adipose tissue (isolate RSC). (H–E; A/E. 400×; F. 1000×; isolate CAJ = M/DAS/VE/2007/CAJ-Anzoátegui State; isolate JBC = M/DAS/VE/2007/JBC-Isla Margarita-Nueva Esparta State; isolate RSC = M/DAS/VE/2007/RSC-Monagas State. (arrows: seudocysts of *T. cruzi* with amastigote and flagellate stages; arrow heads: inflammatory infiltrations; scale bar = 15 m).

liver, and lungs was scarce and the spleen, kidney and brain were not invaded by any of the isolates under study (Table 2; Fig. 1).

These results taken together allowed us to characterize the infecting parasite as *Trypanosoma (Schizotrypanum) cruzi* Chagas, 1909 according to Barretto (1979).

### *3.2. PCR-RFLP of isolates from armadillos (Dasypus novemcinctus novemcinctus) from villages in Northeastern Venezuela*

The GPI fragment after digestion with the *Hha*I enzyme (Fig. 2, top panel), for the isolates M/DAS/VE/2007/JBC, M/DAS/VE/2007/CAJ and M/DAS/VE/2007/RSC, revealed similar patterns for TcI and TcIII genotypes. In a second step, in order to identify the genotype of the *T. cruzi* isolates, the amplification products of the intergenic region of the HSP60 gene were digested with the *Eco*RV enzyme, which only generates restriction products with TcIII DTU, as shown by the *T. cruzi* isolates from armadillos, in the same way as with the reference strain MA26X, representative of TcIII DTU, thus confirming the TcIII DTU of the parasites (Fig. 2, bottom panel).

## 4. Discussion and conclusions

It was Chagas (1909) who first identified a natural *T. cruzi* infection in *D. n. novemcinctus*, thus demonstrating its importance as a reservoir. Since then, this species has been shown to have high infection indices with patent parasitemia in both wild and peridomestic environments, as it co-habits in caves (a natural focus for trypanosomiasis) with *T. maculata, Rhodnius prolixus* and *Panstrogylus geniculatus*, this last very attracted to the light in human inhabited regions (Barretto, 1979).

*D. n. novemcinctus* has also been reported as a reservoir for *T. cruzi* in the United States and Mexico (Barr et al., 1991; Packchanian, 1942; Salazar-Schettino et al., 1997; Yaeger, 1988); Costa

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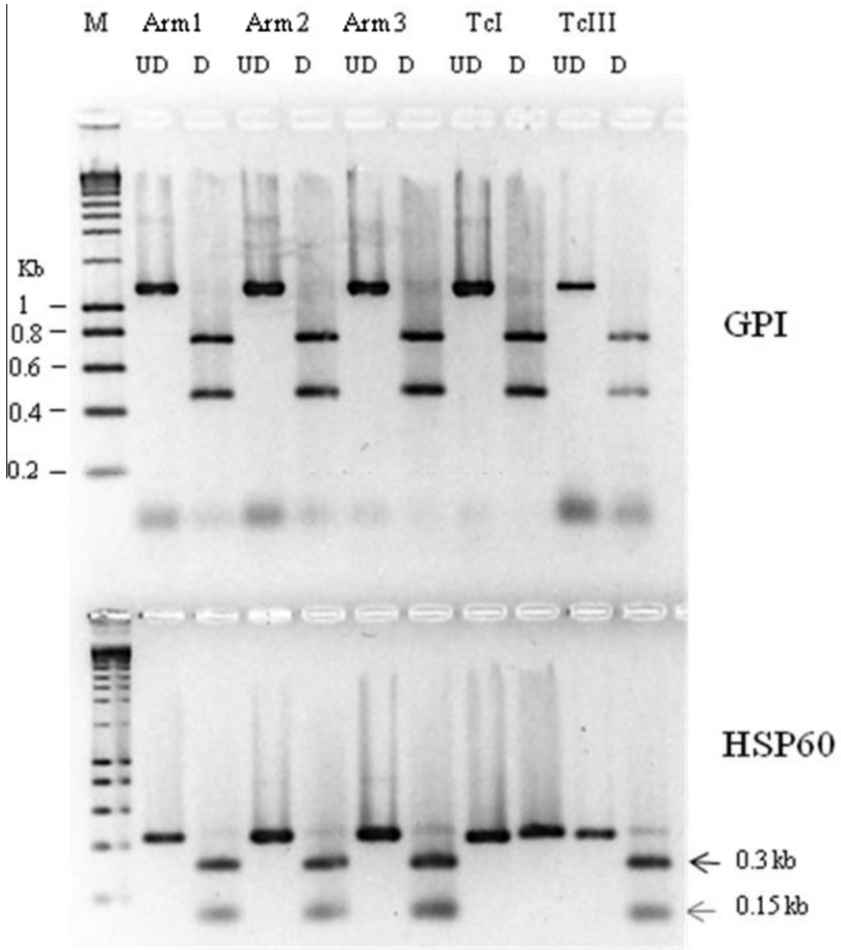


**Fig. 2.** PCR–RFLP of isolates from armadillos (*Dasypus novemcinctus novemcinctus*) from villages in Northeastern Venezuela. Top panel: amplification of the coding region for the GPI gene and digestion products obtained with the *Hha*I enzyme; Bottom panel: amplification of the intergenic region of the HSP60 gene and digestion products obtained with the *Eco*RV enzyme. (UD = un digested, D = digested. Arrows indicate the digestion products of 300 and 150 bp obtained with HSP60 and *Eco*RV for the three *T. cruzi* isolates from armadillos Arm 1, Arm 2, Arm 3 and with the TcIII reference strain MA26X. (Arm 1 = Isolate M/DAS/VE/2007/JBC-Isla Margarita-Nueva Esparta State; Arm 2 = Isolate M/DAS/VE/2007/CAJ-Anzoátegui State; Arm 3 = Isolate M/DAS/VE/2007/RSC-Monagas State. TcI = WA250 cl10B; M = Molecular marker Hyperladder I, Bioline).

Rica (Zeledón et al., 1975); Colombia (Saravia et al., 1987); Brazil (Barretto, 1979; Coura, 2007; Deane, 1961; Gaunt and Miles, 2000; Miles et al., 1981); Paraguay and Bolivia (Llewellyn et al., 2009; Marcili et al., 2009; Yeo et al., 2005). The few studies done in Venezuela (Pifano, 1960; Tonn et al., 1982) have merely indicated the presence of the parasite in this animal, without characterizing it either parasitologically or molecularly.

The relationship between the *T. cruzi* DTUs and its principal natural host was proposed by Yeo et al. (2005) and Llewellyn et al. (2009), who observed that the majority of isolates from *D. novemcinctus* from several Latin American regions belonged to the TcIIc (TcIII) DTU, thus re-enforcing their hypothesis of a possible ancient evolutive association between this DTU and armadillo species. TcIIc (TcIII) stocks have been isolated from marsupials, rodents, carnivores and triatomines from different natural ecosystems in Venezuela (Cordillera Oriental to the West of the country), Colombia, Bolivia, Brazil and Paraguay (Araújo et al., 2011; Freitas et al., 2006; Llewelyn et al., 2009; Marcili et al., 2009; Brisse et al., 2003). In addition, this isolate has also been reported, although rarely, from humans and domestic reservoirs and triatomines in Brazil, Paraguay and Argentina (Abolis et al., 2011; Brisse et al., 2003; Câmara et al., 2010; Freitas et al., 2006; Marcili et al., 2009; Martins et al. (2006); Monteiro et al., 2010). Thus, previous studies have shown that TcIII DTU has a distribution extending at least from Western Venezuela to the Argentine Chaco. Here, we show for the first time, the presence of the TcIII DTU in Northeastern Venezuela, thus increasing its geographical distribution and ratifying its close association with *D. novemcinctus*. We therefore suggest that this Venezuelan region should be considered a reservoir of this DTU and the state of Nueva Esparta, at present considered as a non-endemic zone, at risk from Chagas' disease.

As has already been mentioned, since the classic papers of Chagas' (1909) and Vianna (1911) among others, the complexity of the development of this disease in mammals has been amply demonstrated as regards the clinical, parasitological and pathological aspects. More recent studies have shown the strict correlation between the heterogenic pathogenicity of *T. cruzi* strains or its clones, with its behavior in animal models (Andrade, 1985; Andrade et al., 2010; Bice and Zeledón, 1970; Herrera and Urdaneta-Morales, 1992; Herrera et al., 2003, 2004; Lenzi et al., 1996; Lisboa et al., 2007; Melo and Brener, 1978; Moreno et al., 2002; Morocoima et al., 2006; Postan et al., 1983; Sánchez-Guillén et al., 2006; Toledo et al., 2002). These investigations have shown the hugely eclectic nature that *T. cruzi* displays for intracellular invasion, differentiation and multiplication, as well as its capacity for distributing itself via the blood stream to practically all the organs and tissues of the mammal host.

These results have been confirmed by genetic analysis that has indicated a strong linkage between the long-term clonal evolution of the parasite (that produces polyclonal and thus very heterogenic

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strains) and its biological variability. Other approaches suggest the adaption of the clones by natural selection to the diverse cycles of *T. cruzi* by natural selection, whereby different DTUs would be present in different epidemiological pictures (Andrade et al., 2010; Araújo et al., 2011; Lisboa et al., 2007; Rodríguez et al., 2002; Sánchez-Guillén et al., 2006; Teixeira et al., 2006; Toledo et al., 2002).

This leads us to affirm that although genetic studies are vital for clarifying the intraspecific heterogeneity of the taxon (Zingales et al., 2009), they are by themselves of scarce biological importance for the unraveling of the complexity of the infective process (Sánchez-Guillén et al., 2006) and should thus be complemented with parasitological and eco-epidemiological research.

As far as we know, this is the first study that describes the biological behavior of the TcIII DTU. The three stocks, as shown by a murine model, produced: infection in all the animals tested, different levels of acute parasitemia, similar histotropism and inflammatory infiltrate levels and different mortality rates. We wish to emphasize that the parasitemic peaks were in marked contrast with intracellular parasite loads and inflammatory infiltrates (Table 2), thus indicating heterogeneity between isolates with respect to these biological parameters. We would also like to highlight our observations of the intracellular parasitism by *T. cruzi* amastigotes and trypomastigotes in chondroblasts, chondrocytes, adipocytes, cerebellum molecular stratum, and bone marrow and cartilage, which has been scarcely reported (Herrera et al., 2005; Morocoima et al., 2006). These results indicate the eclecticism and paninfectivity of the TcIII DTU, with the systemic invasion of cells of endo-, meso-, and ectodermic origin (Table 2).

Biological heterogeneity has previously been shown for TCI and TCII isolates from different mammal hosts (Lisboa et al., 2007). Our research has shown, however, heterogeneity in TcIII DTU isolates obtained from the same host species.

The differences of the progress of Chagas' disease in humans and laboratory animals has proved to be a point of controversy (Chagas', 1909; Vianna, 1911; Brener, 1965, 1973; Watkins, 1966; Bice and Zeledón, 1970; Melo and Brener, 1978), among others. This was, however, resolved by Postan et al. (1983) who found that mice inoculated with single- cell-isolate clones of *T. cruzi* from the same source developed different infections, thus confirming the genetic heterogeneity of the protozoa expressed by different populations of natural strains.

More recently, considerable heterogeneity in the parameters used to characterize the biological diversity of *T. cruzi* (TcDTUs) evaluated in experimental animals (infectivity, modes of development of the parasitemia and inflammatory processes, blood trypomastigote morphology, tissular tropism, virulencia (histopathological lesions), and mortality rate) has been reported (Andrade et al., 1999; Lana et al., 2000; Toledo et al., 2002; Martins et al., 2006; Sánchez-Guillén et al., 2006; Lisboa et al., 2007).

Although the control of this parasitosis has been qualified as successful in Venezuela (Ache and Matos, 2001), reports of acute infections in under five year olds in several Venezuelan states indicate active transmission (Añez et al., 2004).

The synantropy of many wild mammals with humans and domestic animals, intense national and international tourism, deforestation and urbanization with modification of the structure of wild ecotopes and the domiciliation of wild triatomines are the most important factors for the establishment of infection in humans (Coura, 2010; Miles et al., 1981; Teixeira et al., 2006).

These aspects apply particularly to Nueva Esparta State, which apart from the risks already mentioned is considered to be non-endemic for Chagas' disease (Ministerio de Salud. Venezuela, 2007) in spite of the presence of the primary insect vectors along the coast in Margarita Island (Carcavallo et al., 1999; Pifano, 1961).

The results obtained in this study provide further evidence for the possible emergence of Chagas' disease in this touristic zone considered to be one of the most important in the Caribbean and thus which should be treated as a priority for public health.

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