Article/Artigo

Molecular characterization of *Trypanosoma cruzi* Mexican strains and their behavior in the mouse experimental model

Caracterização molecular de cepas mexicanas de *Trypanosoma cruzi* e seu comportamento no modelo experimental do camundongo

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ABSTRACT

Introduction: For a long time, the importance of Chagas disease in Mexico, where many regarded it as an exotic malady, was questioned. Considering the great genetic diversity among isolates of *Trypanosoma cruzi,* the importance of this biological characterization, and the paucity of information on the clinical and biological aspects of Chagas disease in Mexico, this study aimed to identify the molecular and biological characterization of *Trypanosoma cruzi* isolates from different endemic areas of this country, especially of the State of Jalisco. **Methods**: Eight Mexican *Trypanosoma cruzi* strains were biologically and genetically characterized (PCR specific for *Trypanosoma cruzi*, multiplex-PCR, amplification of space no transcript of the genes of the mini-exon, amplification of polymorphic regions of the mini-exon, classification by amplification of intergenic regions of the spliced leader genes, RAPD - (random amplified polymorphic DNA). **Results**: Two profiles of parasitaemia were observed, patent (peak parasitaemia of 4.6×10^6 to 10^7 parasites/mL) and subpatent. In addition, all isolates were able to infect 100% of the animals. The isolates mainly displayed tropism for striated (cardiac and skeletal) muscle. PCR amplification of the mini-exon gene classified the eight strains as TcI. The RAPD technique revealed intraspecies variation among isolates, distinguishing strains isolated from humans and triatomines and according to geographic origin. **Conclusions**: The Mexican *T. cruzi* strains are myotrophic and belong to group TcI.

Keywords: *Trypanosoma cruzi.* Mexico. Biological characterization. Genetic characterization.

RESUMO

Introdução: Durante muito tempo, foi questionada a importância da doença de Chagas no México onde muitos a consideravam um padecimento exótico. Considerando a grande diversidade genética existente, entre os isolados de *Trypanosoma cruzi*, a importância da caracterização biológica desses e o escasso número de informações sobre os aspectos clínicos e biológicos da doença de Chagas no México, o objetivo deste trabalho foi realizar a caracterização biológica e molecular de isolados de *Trypanosoma cruzi* originários de diferentes áreas endêmicas deste país, principalmente do Estado de Jalisco. **Métodos**: Oito cepas mexicanas de *Trypanosoma cruzi* foram caracterizadas biologicamente e geneticamente (PCR específica para *Trypanosoma cruzi*, PCR-multiplex, amplificação do espaço não transcrito dos genes do mini-exon, amplificação das regiões polimórficas do gene do mini-exon, classificação pela amplificação de regiões intergênicas dos genes do *spliced leader*, RAPD - *random amplified polymorphic DNA*). **Resultados**: Foram observados dois tipos de parasitemia: patente com picos máximos de parasitemia entre $4{,}6\times10^6$ e 10^7 parasitas/mL e subpatente. Além disso, todos os isolados foram capazes de infectar 100% dos animais. Observou-se tropismo predominante pelo músculo estriado (cardíaco e esquelético). As técnicas de PCR do gene do mini-éxon classificaram as oito cepas como TcI e a técnica de RAPD mostrou variação intra-especifica das mesmas, separando as cepas isoladas de humanos daquelas de triatomíneos e por origem geográfica. **Conclusões**: As cepas mexicanas de *Trypanosoma cruzi* são miotrópicas e correspondem ao TcI.

Palavras-chaves: *Trypanosoma cruzi.* México. Caracterização biológica. Caracterização genética.

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Received in 29/09/2010

Accepted in 13/06/2011

INTRODUCTION

Although, in the past, Chagas disease was considered to be restricted to the American continent where it is found in 18 countries, it has become a worldwide disease due to intense people migration. This disease is a public health problem because it causes increased morbidity and mortality in South America, Central America, and Mexico, where it is considered to be a neglected disease. According to data from the World Health Organization, approximately 8 million people are infected with *Trypanosoma cruzi* on the American continent[1-3].

In Mexico, the importance of Chagas disease has been neglected for many years. The disease was seen as an exotic ailment and did not receive adequate government attention. The prevalence of Chagas disease in the country ranges from 1.6% to 5.8%, with positivity rates of 0.4% to 19% in different states, 0.17% to 17% in blood banks[4-6]. and from 5% to 21.5% in rural areas[6]. In the 1970s, the largest number of acute Chagas disease cases in the country was recorded in the State of Jalisco[7,8]. However, since this time only isolated acute cases have been diagnosed, a fact suggesting a large number of unreported sick people. The prevalence of Chagas disease in this state ranges from 1.8% to 17% in the general population and 21.6% in the rural population[6,9].

In Mexico, genetic characterization by mini-exon analysis of isolates obtained from triatomines, domestic and wild reservoirs, and human cases with cardiac manifestations revealed the presence of *T. cruzi* group I[9-11]. However, there have been few reports of *T. cruzi* II in this country[11].

In 1999, six lineages of *T. cruzi* were established: TcI and TcII with five subgroups denominate *Trypanosoma cruzi* IIa to IIe[12-13]. Recently, an updated nomenclature for the *T. cruzi* was established in a consensus, namely, TcI, TcII (TcIIb), TcIII (TcIIc), TcIV (TcIIa), TcV (TcIId), and TcVI (TcIIe)[14].

Experimental studies show that *T. cruzi* strains cause peculiar tissue lesions as a result of specific and predominant tropism for different types of mammalian cells, such as macrophages, cardiac and skeletal muscle cells, and neurons[15-16].

Studies investigating Mexican *T. cruzi* strains have shown differences in the biological behavior of strains obtained from different hosts and different endemic regions of the country, as well as differences in the resistance to drugs used for the treatment of experimental infection (benznidazole and nifurtimox). In humans, the predominant clinical manifestation of Chagas disease is cardiomyopathy, whereas digestive abnormalities are observed at a low frequency as demonstrated recently[9, 17-20].

In view of the wide genetic diversity among *T. cruzi* isolates, the importance of biological characterization of these strains, and the scarcity of information about clinical and pathological aspects of Chagas disease in México, the aim of the present study was to characterize biologically and molecularly *T. cruzi* isolates originating from different endemic areas in México, especially from the State of Jalisco.

METHODS

The following eight *T. cruzi* strains were studied: three strains isolated from *Triatoma pallidipennis* (CGH2, CGH4, and CGH6 strains), two from *Triatoma longipennis* (CGH1 and CGH3 strains), one from *Triatoma picturata* (KR1 strain), one by xenodiagnosis from a patient in the acute phase of disease (NINOA strain)[21], and one isolated from a patient with the chronic cardiac form (INC-5 strain)[22] (**Table 1**).

A total of 240 non-isogenic male adult (6-8 weeks) mice were used. The animals were kept under suitable temperature, humidity, and availability of water and food *ad libitum*.

Parasitological study

Groups of 30 mice were inoculated intraperitoneally with 3×10^4 blood trypomastigotes with CGH3, CGH4, CGH6, INC-5, and NINOA strains and CGH1, CGH2, and KR1 strains with 3×10^4 culture trypomastigotes obtained from Triatomine Artificial Urine medium.

Blood trypomastigotes were identified and quantified on alternate days for 50 days. Strains presenting patent parasitaemia were quantified using the method of Brener, and those presenting subpatent parasitaemia were monitored using the microhematocrit method.

Histopathology

For histopathological analysis, 10 animals of each group were euthanized by cervical dislocation on the day of parasitaemia peak and the remaining animals at 120 days after infection. The following organs and/or tissues were collected: heart, skeletal (quadriceps) muscle, lung, and gastroesophageal, gastroduodenal and ileocecal junctions. The specimens were fixed in buffered 10% formalin, embedded in paraffin, and cut into 3- to 4-μm-thick sections with a microtome. The sections were stained with hematoxylin and eosin.

We investigated the presence of amastigote nests in the whole tissue area and the inflammatory processes were semiquantitatively classified into absent (-), mild (+), moderate (++), and severe (+++), as previously described[23-26].

DNA obtained from parasites

Blood cultures were performed in liver infusion tryptose (LIT) medium using blood of mice inoculated with the different isolates. Positive blood cultures were maintained in an incubator at 28°C in LIT medium supplemented with 5% fetal bovine serum. For parasite isolation, the 5-mL culture was duplicated with LIT medium every week to obtain exponential growth of the parasite. One week later, a volume of 40mL was completed, and the parasites were washed three times with sterile saline, resuspended in 2mL, and divided into two centrifuge tubes. The parasite suspension was centrifuged at 3,500rpm for 10min at 4°C and decanted. The sediments were stored at -20°C until the time of DNA extraction. DNA was extracted using the phenol-chloroform-isoamyl alcohol method according to Macedo et al.[27].

Polymerase chain reaction and random amplified polymorphic DNA

PCR specific for *T. cruzi* was performed according to Wincker et al.[28] using the following primers that amplify a fragment of 330 bp: 121 (5'-AAA TAA TGT ACG GG(G/T) GAG ATG CAT GA-3') and 122 (5'-GGT TCG ATT GGG GTT GGT GTA ATA TA-3').

For the differential diagnosis of *T. cruzi* and *T. rangeli*, multiplex PCR was performed as described by Souto et al.[29] using primers that amplify a sequence of the D7 divergent domain of the 24Sα gene: D72 (5'-TTT TCA GAA TGG CCG AAC AGT-3'), D75 (5'-GCA GAT CTT GGT TGG CGT AG-3'), and RG3 (5'-GGC CAA AGG GTA AGG CTC-3').

Two techniques were used for the strains genotypic classification. The first technique consisted of amplification of the mini-exon gene as described by Souto et al.[29] using the following set of primers: TC (5'-CCCCCCTCC CAG CCG ACA CTG-3'), TC1 (5'-GTG TCC CCG ACC TCC TTC GGG CC-3'), and TC2 (5'-CCT GCA GGC ACA CGT GTG TGT G-3'). For the second technique, the intergenic regions of the spliced leader genes were amplified in two reactions as described by Burgos et al.[30] In the first reaction, SL-IRac, the UTCC (5'-CGT ACC AAT ATA GTA CAG AAA CTG-3') and

TABLE 1 - Mean features of *Trypanosoma cruzi* Mexican strain.

Characteristic	Strain							
	CGH1	CGH2	CGH3	CGH4	CGH6	KR1	NINOA	INC5
Biological procedence	*T. longipennis*	*T. pallidipennis*	*T. longipennis*	*T. pallidipennis*	*T. palillipennis*	*T. picturata*	human acute phase	human chronic phase
Geographical procedence (state)	Jalisco	Jalisco	Jalisco	Jalisco	Jalisco	Jalisco	Oaxaca	Guanajuato
Parasitaemia peak (DAI)	ND	ND	31	29	27	ND	27 & 33	27
Maximum peak	ND	ND	10^7	4.6×10^6	8×10^6	ND	7.5×10^6	6.1×10^6
Parasitaemia type	sub-patent	sub-patent	patent	patent	patent	sub-patent	patent	patent
Mortality of mice infected (%)	6.6	3.3	3.3	16.6	6.6	10.0	20.0	53.3
Taxonomical group*	TcI	TcI	TcI	TcI	TcI	TcI	TcI	TcI

T: *Triatoma;* **DAI:** days after infection; **ND:** not detected; **TcI:** *Tripanosoma cruzi* I; *Taxonomy was established by Zingales et al.

TCac (5'-CTC CCC AGT GTG GCC TGG G-3') primers were used, in which the 150-bp band corresponds to TcI, the 200-bp band to TcII (a, c), and the 157-bp band to TcII (b, d, e). The second PCR, SL-IR I, confirms TcI identified in the previous reaction when a 475bp is amplified. The UTCC (5'-CGT ACC AAT ATA GTA CAG AAA CTG-3') and TC2 (5'-CCT GCA GGC ACA CGT GTG TG-3') primers were used in the second reaction.

The random amplified polymorphic DNA (RAPD) technique was performed to determine intraspecies variations between the Mexican *T. cruzi* strains as described by D´Avila et al.[31] For this purpose, 1ng DNA was mixed with 1.0U Taq DNA polymerase (Promega, Madison, WI, USA), 125µM dNTPs (Sigma-Aldrich, St. Louis, MO, USA), 1mM $MgCl_2$, and 1.0µL 10× buffer in a final volume of 10µL. Five separate reactions using 6.4 pmol of the following primers were carried out: M13-40-F (5'-GTT TTC CCA GTC ACG AC-3'), λgt11-F (5'-GAC TCC TGG AGC CCG-3'), L-15996 (5'-CTC CAC CAT TAG CAC CCA AAG C-3'), λ31F (5'-GAA GGC ACA TGG CTG AAT ATC-3'), and λ183R (5'-GCG AAA TAC GGG CAG ACA-3'). Amplification was carried out in a PE 9600 thermocycler under the following conditions: denaturation at 95°C for 5 min, annealing at 30°C for 2 min, and extension at 72°C for 1 min, followed by 33 cycles of denaturation at 95°C for 30 s, annealing at 40°C, and extension at 72°C for 1 min and a final step at 72°C for 5 min.

The amplified PCR products were fractionated by polyacrylamide gel electrophoresis after silver staining.

The bands profile obtained by RAPD was analyzed by evaluating the proportion of bands shared among strains. Dendrograms were constructed using the Gel Compar II® software package, version 5.0.

Statistical analysis

The Shapiro-Wilk test was used to determine whether the data presented a normal distribution, and the Levene's test was used to assess the homogeneity of variances.

Two samples were compared using the Mann-Whitney test (independent samples) and the Wilcoxon test (dependent samples). For three or more samples, multiple comparison tests were performed, including the Kruskal-Wallis test followed by Dunn's test (for independent samples) and Friedman's test followed by a nonparametric least significant difference test. Spearman's correlation coefficient was used to analyze the correlation between two variables. For parasitaemia analysis, the area under the curve was calculated by the method of Bezout (trapezoidal rule) to obtain a parameter of overall parasitaemia in relation to days of infection. Cumulative mortality as a function of time was analyzed using Kaplan-Meier survival curves. Statistical analysis was performed with the *statsoft Statistica* 8.0 software, and the level of significance was set at $p<0.05$ for all tests.

Ethical considerations

The study was approved by the Ethics Committee on Animal Experimentation of UFTM (protocol 153).

RESULTS

Parasitaemia

The eight *T. cruzi* isolates studied infected 100% of the inoculated mice. Five (62.5%) strains presented patent parasitaemia as determined by the method of Brener, including three isolates from triatomines (CGH3, CGH4, and CGH6) and the two strains isolated from humans (INC-5 and NINOA). The three strains presenting subpatent parasitaemia (37.5%) using the microhematocrit method were all isolated from triatomines (CGH1, CGH2, and KR1). All animals with patent and subpatent parasitaemia were observed for a period of 50 days (**Figure 1**).

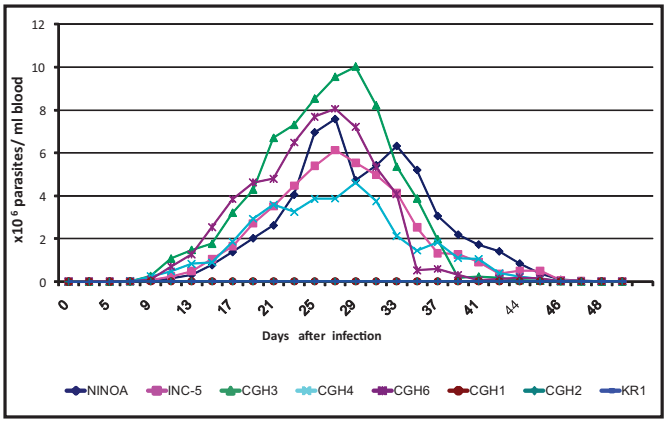

FIGURE 1 - Parasitemia curves by Mexican *Trypanosoma cruzi* strains, intraperitoneally inoculated (3x10⁴ parasites) using the method of Brener on non-isogenic mice and followed for 50 days.

Parasitaemia peak was observed at 27 days in the isolates CGH6 and INC-5, at 29 days in the isolate CGH4, and at 33 days in the isolate CGH3. In contrast, the isolate NINOA presented two peaks at 27 and 33 days after infection. The CGH3 strain showed significantly higher parasitaemia than INC-5 and CGH4 strains when the area under the curve was evaluated ($p=0.032$) and when compared with the CGH4 strain based on peak parasitaemia ($p=0.01$) (**Figure 1**).

Pre-patent period and survival

The pre-patent period was similar in all strains, presenting variations observed between days 2 and 11 after infection. No significant difference in the pre-patent or patent periods duration was observed between strains ($p=0.21$).

The highest mortality rate was observed for mice inoculated with strain INC-5 (53.3%), followed by those inoculated with strains NINOA (20%) and CGH4 (16.6%). Low mortality rates ranging from 3.3% to 10% ($p<0.05$) were observed for all other strains.

Histopathology

Analysis of tissue parasitism in mice inoculated with the different Mexican strains showed the presence of amastigote nests in all tissues of infected animals in both the acute and chronic phases. The highest percentage of infected tissues was observed in animals inoculated with the strain INC-5 during the two phases (**Figure 2**). In addition, skeletal muscle was the only tissue in which parasites were detected in 100% of the animals during the two phases, except for strain NINOA that was observed in 92.8% of the animals during the chronic phase.

The least infectious strains during the acute phase were CGH4, CGH3, and CGH2. The CGH1 strain was the least infectious strain during the chronic phase. However, the strains CGH3, CGH4, CGH2, and KR1 were more infectious during the chronic phase compared with the acute phase.

The largest number of amastigote nests in the atria and ventricles was observed in animals inoculated with strain INC-5, irrespective of the phase of infection. In the atria, this number was significantly higher during the acute phase when compared with isolates CGH2, CGH3, CGH4, and NINOA, and during the chronic phase when compared with strains CGH2, CGH3, and CGH4. However, in

the ventricles, the INC-5 strain in the amastigote nests was significantly higher during the acute phase when compared with strains CGH1, CGH2, CGH3, CGH4, and KR1, and during the chronic phase when compared with strains CGH1, CGH2, CGH3, and CGH4 ($p<0.001$).

Mice inoculated with strain NINOA presented a larger number of amastigote nests in skeletal muscle during the acute phase when compared with strains CGH2, CGH3, CGH4, and KR1, and during the chronic phase when compared with strains CGH1 and CGH4 ($p<0.01$).

A significant difference in the number of amastigote nests at the gastroesophageal junction was observed between strains NINOA and CGH6 ($p=0.022$). There were no significant differences in the number of amastigote nests in the lung or at the gastroesophageal and ileocecal junctions between animals inoculated with the strains studied.

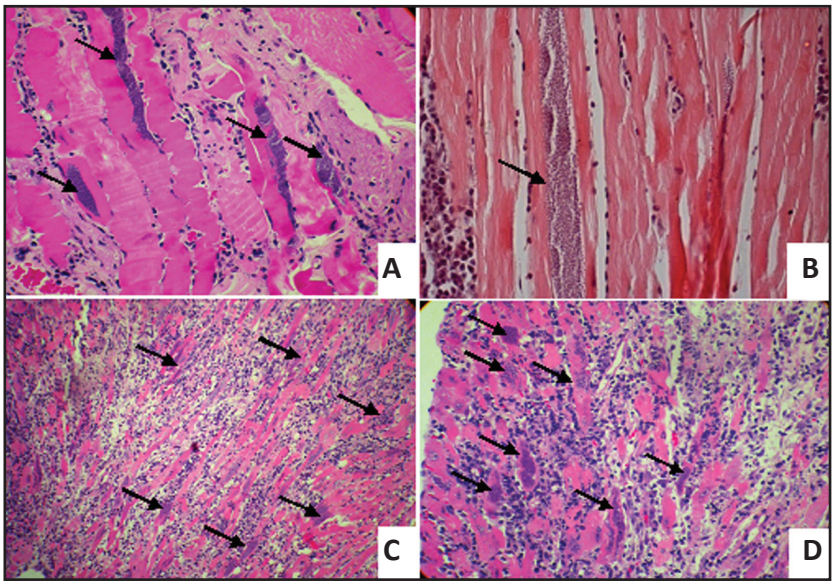

FIGURE 2 - Representative histopathological sections of skeletal muscle and heart of mice infected with *Trypanosoma cruzi*, showing the inflammatory infiltrates and amastigote nests (arrow). Histopathological sections were stained with hematoxylin and eosin (200x magnification).

A: skeletal muscle infected with NINOA-*T. cruzi* strain; **B:** skeletal muscle infected with KR1-*T. cruzi* strain; **C:** ventricle infected with INC-5-*T. cruzi* strain; **D:** ventricle infected with NINOA-*T. cruzi* strain.

Tissue inflammatory process

Strain INC-5 induced the most intense inflammatory process in the largest number of tissues when compared with the other strains during the two phases of the disease.

During the acute phase, strains INC-5 and CGH1 induced moderate to intense inflammatory processes in the atria and the isolates CGH2, CGH6, and NINOA induced moderate inflammation. Moderate to intense inflammatory processes were observed in the skeletal muscle of animals inoculated with the strains CGH2, CGH3, CGH4, NINOA, and INC-5. In the ventricle, the strain INC-5 induced moderate to severe inflammation. Mild to moderate inflammation was observed in the lungs of all mice inoculated with the strains **(Table 2)**.

During the chronic phase, the strain INC-5 induced an intense inflammatory process in the atrium. Moderate to severe inflammation in this tissue was induced by isolate NINOA and mild to moderate inflammation by the isolates CGH1, CGH2, CGH4, CGH6, and KR1. The most severe inflammatory process in the ventricle was induced by the strain INC-5. In the skeletal muscle, moderate to severe inflammation was induced by the isolates CGH2, NINOA, and INC-5, whereas the other isolates induced mild to moderate inflammation. In the lung, all strains induced a mild to moderate inflammatory process **(Table 2)**.

Correlation between tissue parasitism and inflammation

The correlation between tissue parasitism and inflammation was established for the different strains during the two phases of the disease. During the acute phase, a strong correlation ($0.81 \geq r < 1$) between

TABLE 2 - Comparison of the inflammatory process in the different tissues during the acute and chronic phases of infection.

	Strain							
Tissue	**CGH1**	**CGH2**	**CGH3**	**CGH4**	**CGH6**	**KR1**	**NINOA**	**INC-5**
Acute phase								
atrium	++/+++	++	+	+	++	+	++	++/+++
ventricle	+	+	+	+	+	+	+	++/+++
skeletal muscle	+/++	++/+++	++/+++	++/+++	+	+/++	++/+++	++/+++
lung	+/++	+/++	+/++	+/++	+/++	+/++	+/++	+/++
gastroesophageal junction	+	+	+	+	+	+	+	+
gastroduodenal junction	+	+	+	+	+	+	+	+
ileocecal junction	+	+	+	+	+	+	+	+
Chronic phase								
atrium	++	++	+	+/++	+/++	+/++	++/+++	+++
ventricle	+	+	+	+	+	+	+	+++
skeletal muscle	+/++	++/+++	+/++	+/++	+/++	+/++	++/+++	++/+++
lung	+/++	+/++	+/++	+/++	+/++	+/++	+/++	+/++
gastroesophageal junction	+	+	+	+	+	+	+	+
gastroduodenal junction	+	+	+	+	+	+	+	+
ileocecal junction	+	+	+	+	+	+	+	+

+: mild; ++: moderate; +++: severe.

parasitism and inflammation at the ileocecal and gastroesophageal junctions was observed for strains CGH1 and INC-5, respectively. This correlation was moderate ($0.51 \geq r < 0.8$) for strains CGH2 (atrium and gastroesophageal junction), CGH3 (gastroesophageal junction), and KR1 (lung, gastroesophageal and ileocecal junction), and weak ($0.01 \geq r < 0.5$) for strain CGH4 in the lung.

During the chronic phase, a strong correlation ($0.81 \geq r < 1$) was observed for strains CGH2 (gastroesophageal junction), CGH3 (gastroesophageal and ileocecal junction), and KR1 (ileocecal junction), and a moderate correlation ($0.51 \geq r < 0.8$) for strains CGH3 (gastroesophageal junction), KR1 (gastroesophageal and ileocecal junction), and NINOA (ventricle and ileocecal junction).

No correlation between parasitism and inflammation in the tissue studied was found for strain CGH6 during any of the phases of infection. A strong correlation between inflammation and parasitism during the acute phase was observed for strains CGH1 and INC-5, whereas during the chronic phase, no correlation was found in any of the organs studied. Intestinal tissue was the only tissue in which a correlation between the inflammatory process and the number of amastigote nests was observed in most of the Mexican *T. cruzi* strains during both phases of infection.

Polymerase chain reaction and random amplified polymorphic DNA results

PCR specific for Trypanosoma cruzi: PCR with the 121-122 primers was performed to confirm the identity of the *T. cruzi* isolates. The 330-bp fragment specific for *T. cruzi* was verified in all strains.

PCR using primers D72, D75, and RG3: This PCR technique allowed us to rule out the presence of *T. rangeli* among the isolates and confirmed the presence of *T. cruzi*.

PCR of the mini-exon gene: amplification of conserved regions of the mini-exon gene permitted the classification of all Mexican strains studied as *Trypanosoma cruzi* I (350-bp amplified fragment). An amplified fragment of 300 bp was detected for the TcII sample as a control **(Figure 3A).**

PCR of the intergenic regions of the spliced leader gene: This technique confirmed that the Mexican *T. cruzi* isolates belong to the TcI group (475-bp product; see **Figure 3B**).

Random amplified polymorphic DNA

The primer λ183R presented the higher ability to classify the Mexican *T. cruzi* isolates. The strains could be divided into two polymorphic branches, with a similarity coefficient of 28.4%. The first branch comprised the six strains isolated from triatomines (CGH3, CGH6, CGH4, CGH1, CGH2, and KR1). The second arm included the two strains of human origin (NINOA and INC-5), which presented a similarity coefficient of 50%. Two interesting facts should be mentioned: First, the strain KR1 isolated from *T. picturata* was separated from strains CGH3, CGH6, CGH4, CGH1, and CGH2 isolated from *T. palillipennis* or *T. longipennis*, with a coefficient of similarity of 45.2%; and second, the strains presenting patent parasitaemia were separated from those presenting subpatent parasitaemia**.**

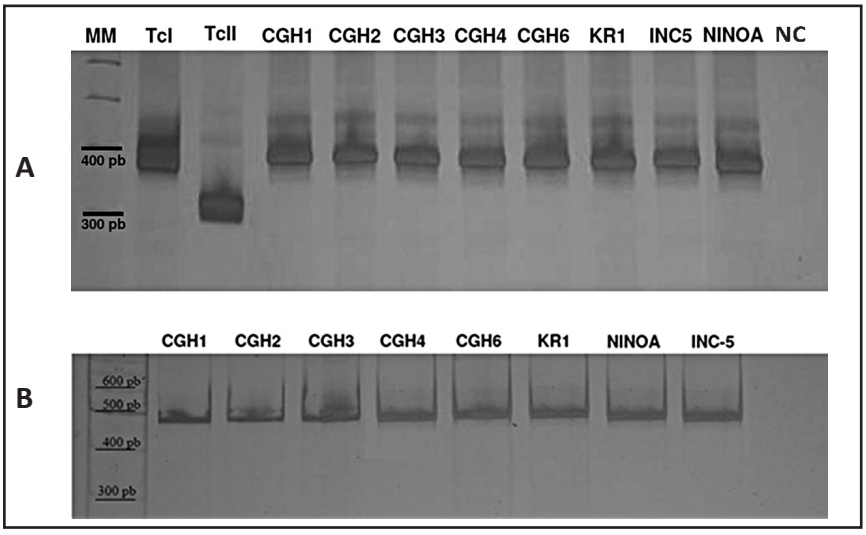

FIGURE 3 - Polyacrylamide gels 6% silver stained. A) Analyzed using primers Tc, Tc1, and Tc2. All samples from Mexican *Trypanosoma cruzi* strains, amplified fragments of 350 bpb, were specific for TCI; (B) by using primers TCAC and UTCC.

Molecular marker (MM); TCI, MUT *T. cruzi*-I strain (350 bp); TcII, HEL *T. cruzi*-II strain (300 bp); NC: negative control.

DISCUSSION

Mexico is a tropical country where tropical diseases, such as Chagas disease, are common. Control programs for Chagas disease and its vector have only recently been implemented in this country, whereas such programs have been already established in South American countries for several years[3,32]. The high rates of infection of triatomines observed in the State of Jalisco suggest an increased exposure of people living in this region to *T. cruzi*[33-35]. This fact has raised our interest to biologically and genetically characterize the strains circulating in this state.

The biological and genetic characterization of *T. cruzi* strains isolated in endemic areas of Mexico is important for the understanding of the epidemiology and biology of the parasite in these regions. Studies of this parasite strains conducted since 1950 have shown that *T. cruzi* can be found in different vectors and reservoirs and that parasites isolated from different regions of the same country behave differently when inoculated into experimental models[36-37].

The strains studied here showed a high degree of infectivity, with 100% of the mice inoculated being infected. However, there are no studies conducted in Mexico demonstrating the infectivity profile of different *T. cruzi* isolates in mice.

In the present study, five of the eight strains presented patent parasitaemia (CGH3, CGH4, CGH6, NINOA, and INC-5). This parasitaemia is the most frequent profile reported in experimental studies[18,38-39]. Three strains (CGH1, CGH2, and KR1) presented subpatent parasitaemia, and there are no reports of *T. cruzi* strains from Mexico showing this behavior in experimental models. The mortality of mice inoculated with the strains of this study ranged from 3.3% to 53.3%. In this respect, studies conducted in Mexico and, specifically in the state of Jalisco, have shown that strains originating from this state are able to kill up to 100% of experimentally infected animals[17-18,40].

Mice inoculated with the subpatent strains (CGH1, CGH2, and KR1) continued to be positive over 1 year of follow-up using the microhematocrit method. Roellig et al.[41] observed that the genotypes of parasites belonging to groups TcI and TcIIe (now TcVI) are characterized by maintaining subpatent parasitaemia during the chronic phase and infecting *Rhodnius prolixus*. According to these authors, maintenance of the sylvatic cycle of *T. cruzi* depends on competitive vectors that find infected hosts. The strains studied here belonged to group TcI. The biological and genetic diversity of *T. cruzi* and its ability to infect different mammalian hosts support the hypothesis that some animal species may maintain parasitaemia for a longer period than others, an ability that renders this host a good reservoir. Differences between potential reservoirs might be due to the species and genetics of the host and parasite genotype, or a combination of both.

In the present study, large amastigote nests were observed in mice muscle tissue with subpatent parasitaemia. This fact suggests that trypomastigotes are susceptible to complement. When the amastigote nest ruptures, undifferentiated amastigote forms with invasive capacity are released as reported by Mortara et al.[42], resulting in the persistence of infection for a long period.

The Mexican isolates used in this study presented tropism for skeletal muscle and heart (**Figure 2**). Tropism for these tissues has been reported for strains found in Mexico and in the State of Jalisco[43].

The importance of lung tropism in *T. cruzi* is unknown, but studies suggest that this is not as strong as other tissue tropisms, such as the tropism for cardiac and skeletal muscle, or that it does not provoke organ alterations[19,44]. Melnikov et al.[19] detected few amastigote nests in the lungs of 32% to 90% of mice infected with Mexican *T. cruzi* isolates. The author found bronchial lesions, edema, and inflammation as well as alveolar hemorrhage depending on the strain. In the present study, the infection rate of this organ ranged from 40% to 100% during the acute phase and from 50% to 73% during the chronic phase, but the number of amastigote nests was low as reported by Melnikov et al.[19]. These results suggest that the Mexican strains may present tropism for muscles found in large vessels and in the peribronchial muscle tissue of the lungs.

The immunogenicity of *T. cruzi* isolates differs among the organs and tissues infected by the parasite. This fact might have influenced the correlation between the number of amastigote nests and inflammation.

Interestingly, limb paresis, especially in the hind limbs, was observed in some of the animals inoculated with strains presenting patent parasitaemia (NINOA and INC-5), and the animals died within a few hours. Histopathological examination of these animals showed intense tissue parasitism in the striated muscle accompanied by large amastigote nests adopting muscle fiber morphology.

Two main genetic groups of *T. cruzi* were described in the past decade: *T. cruzi* I (TcI), which is associated with a sylvatic transmission cycle, and *T. cruzi* II (TcII), which is more frequently found in the domestic environment. This relationship between genotype and *T. cruzi* transmission cycle cannot be applied to countries in Central America or Mexico because genotype TcI is the main group isolated from domestic, peridomestic, and sylvatic transmission cycles in these regions[9-11]. In the present study, different molecular biology techniques demonstrated that the isolates studied belonged to the TcI group.

The RAPD technique is characterized by the random amplification of DNA fragments. Some of the primers used may permit the clustering of strains according to geographic and/or biological origin[45]. In the present study, the λ183R primer was the best primer for classification of the Mexican *T. cruzi* strains. This technique, thus, permitted obtaining clusters of strains according to biological origin (human and triatomine) and parasitaemia produced in an experimental model (non-isogenic mice). Using this technique, Bosseno et al.[46] demonstrated high homogeneity among Mexican *T. cruzi* isolates obtained from different hosts; however, this homogeneity was not correlated with the virulence of the strains. The intraspecies variations observed in the strains studied might be related to differences in biological behavior *in vivo*. Lana et al[47]. observed that clonal genotypes of *T. cruzi* differ significantly in terms of infectivity, demonstrating an association between genotype diversity, tropism, and pathogenicity.

Finally, the present study demonstrated that the Mexican strains are infectious *in vivo*. All strains showed tropism for striated (skeletal and cardiac) muscle. Five of the eight isolates presented patent parasitaemia, whereas the remaining three isolates determined subpatent parasitaemia. In these isolates, parasitaemia was detected up to 1 year after the infection, showing good adaptation to the host used (mouse). This fact may render this host a good reservoir. The present study confirmed the predominance of *T. cruzi* genotype I in Mexico, and the RAPD technique demonstrated intraspecies variability among strains, which were considered to be genetically different.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FINANCIAL SUPPORT

This study was supported by the following Brazilian funding agencies: *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)*, *Fundação de Amparo a Pesquisa de Minas Gerais (FAPEMIG)* and *Conselho Nacional de Pesquisa (CNPq)*.

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