

Molecular and biological characterization of *Trypanosoma cruzi* strains isolated from children from Jequitinhonha Valley, State of Minas Gerais, Brazil

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ABSTRACT

Introduction: The biological diversity of *Trypanosoma cruzi* strains plays an important role in the clinical and epidemiological features of Chagas disease. **Methods:** Eight *T. cruzi* strains isolated from children living in a Chagas disease vector-controlled area of Jequitinhonha Valley, State of Minas Gerais, Brazil, were genetically and biologically characterized. **Results:** The characterizations demonstrated that all of the strains belonged to *T. cruzi* II, and showed high infectivity and a variable mean maximum peak of parasitemia. Six strains displayed low parasitemia, and two displayed moderate parasitemia. Later peaks of parasitemia and a predominance of intermediate and large trypomastigotes in all *T. cruzi* strains were observed. The mean pre-patent period was relatively short (4.2 ± 0.25 to 13.7 ± 3.08 days), whereas the patent period ranged from 3.3 ± 1.08 to 34.5 ± 3.52 days. Mortality was observed only in animals infected with strain 806 (62.5%). Histopathological analysis of the heart showed that strains 501 and 806 caused inflammation, but fibrosis was observed only in animals infected with strain 806. **Conclusions:** The results indicate the presence of an association between the biological behavior in mice and the genetic characteristics of the parasites. The study also confirmed general data from Brazil where *T. cruzi* II lineage is the most prevalent in the domiciliary cycle and generally has low virulence, with some strains capable of inducing inflammatory processes and fibrosis.

Keywords: *Trypanosoma cruzi* strains. Jequitinhonha Valley. Molecular characterization. Biological characterization.

INTRODUCTION

Chagas disease is caused by the protozoan parasite *Trypanosoma cruzi* and is an important tropical disease that affects 10 million people worldwide, mostly in Latin America where it is endemic. It is estimated that over 10,000 people die each year due to the clinical manifestations of Chagas disease, which mainly affects the heart and the gastrointestinal tract^{1,2}.

It is well established that *T. cruzi* is a complex taxon that exhibits great genetic variability. *Trypanosoma cruzi* is distributed into six (I to VI) discrete taxonomic units (DTUs)³ that show significant differences with respect to their ecological and geographic distributions⁴. The clonal nature

of *Trypanosoma cruzi*⁵ predicts a linkage between its genetic variability and biological properties, such as morphology, virulence, pathogenesis, mortality, and drug susceptibility, as demonstrated under experimental conditions⁶⁻⁹.

Furthermore, geographical variations in the prevalence of the different clinical manifestations of Chagas disease have also been observed¹, and these variations were associated with the genetic heterogeneity of *T. cruzi* populations¹⁰. Some authors have demonstrated that different *T. cruzi* strains cause peculiar tissue lesions due to their specific and predominant tropisms in different mammalian cells, such as macrophages, cardiac and skeletal muscle cells, and neurons^{11,12}.

Considering the influence of the genetic characteristics of *T. cruzi* populations on their biology, epidemiology^{4,8,13}, and therapeutic susceptibility/resistance *in vitro*⁷ and *in vivo*⁹, the goal of this study was to characterize the genetics, biology, and morphology of *T. cruzi* samples isolated from children living in a vector-controlled area endemic for Chagas disease in Jequitinhonha Valley, State of Minas Gerais, Brazil¹⁴. This study may aid further studies concerning the clinical and therapeutic management of patients within the region studied.

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Received 19 April 2013

Accepted 18 July 2013

METHODS

Patients and *Trypanosoma cruzi* strains

Eight children were identified through a serological inquiry of Chagas disease in Berilo and José Gonçalves de Minas, Jequitinhonha Valley, State of Minas Gerais (MG), Brazil, from a total of 1,412 samples analyzed¹⁴. Five children (8-16 years old) were from Berilo, and three (11-16 years old) were from José Gonçalves de Minas. All of the children presented with the indeterminate clinical form of the disease¹⁴. These municipalities are very close to each other (12km apart). One *T. cruzi* strain was isolated from each child, and a total of eight *T. cruzi* strains were isolated by hemoculture¹⁵. The strains were identified as 1661, 501, 2405, 817, 795, 829, 806, and 855.

Molecular typing

Epimastigote forms obtained from the liver infusion tryptose (LIT) cultures were washed with phosphate-buffered solution (PBS) by centrifugation at 4°C and 3,500rpm. The parasite pellets were stored at -70°C. For molecular typing, polymorphisms of isoenzyme profiles and the triplice assay¹⁶ that explore the combined analysis of 24Sα ribosomal DNA (rDNA) gene, the mini-exon intergenic regions (ITR) gene, and the cytochrome oxidase subunit II (COII) gene polymorphism of *T. cruzi* were employed.

Isoenzyme profiles: the parasite pellets were submitted to enzymatic extraction¹⁷. Six enzymatic systems were used: glucose-6-phosphate isomerase (GPI, E.C. 5.3.1.9), glucose-6-phosphate dehydrogenase (G6PD, E.C. 1.1.1.44), isocitrate dehydrogenase (IDH, E.C. 1.1.1.42), glutamate dehydrogenase nicotinamide adenine dinucleotide (NAD⁺) (GDH1, E.C.1.4.1.2), glutamate dehydrogenase nicotinamide adenine dinucleotide phosphate (NADP⁺) (GDH2, E.C.1.4.1.4), and malic enzyme (ME, E.C.1.1.1.40). The analyses¹⁸ were performed in a horizontal system (Helena, Beaumont, TX, USA) using an acetate-cellulose membrane (Helena, Beaumont, TX, USA). The isoenzyme profile was compared with the reference *T. cruzi* stocks from different DTUs, including P209 c11 (*T. cruzi* I), MAS c11 (*T. cruzi* II), CM17 (*T. cruzi* III), CAN III c11 (*T. cruzi* IV), Bug 2148 c11 (*T. cruzi* V), and TULA c12 (*T. cruzi* VI).

Polymorphism of the 24Sα rDNA gene: for the 24Sα rDNA analysis, 3ng of DNA from each strain was subjected to polymerase chain reaction (PCR) in a total volume of 12.5μL containing 10mM Tris-HCl (pH 9.0), 50mM KCl, 0.1% Triton X-100 (Buffer B, Promega, Madison, WI, USA), 3.5mM MgCl₂ (Promega), 200μM of each 2'-deoxynucleoside 5'-triphosphate (dNTP), 0.25μM of each primer (D71-5'-AAGGTGCGTCGACAGTGTGG-3' and D-72 5'-TTTTTCAGAAATGGCCGAACAGT-3'), and 0.625U of *Taq* DNA polymerase (Phoneutria, Belo Horizonte, MG, Brazil). The amplification program consisted of an initial denaturation step at 94°C for 1min, followed by 30 cycles that included denaturation at 94°C for 30sec, annealing at 60°C for 30sec, and extension for 30sec at 72°C. After 30 cycles, a final extension was performed at 72°C for 10min¹⁹. Then, 5μL of the PCR

product was analyzed on a 6% polyacrylamide gel and visualized by silver staining. Deoxyribonucleic acid (DNA) from *T. cruzi* populations belonging to the *T. cruzi* I (Col1.7G2, 110bp), *T. cruzi* II (JG, 125bp), and *T. cruzi* V (SO3 c15, 110/125bp) DTUs were used as reference strains for the 24Sα rDNA profiles.

Mini-exon intergenic regions (ITR) gene: molecular typing of the ITR spliced leader was performed²⁰ using the primers TcIII (5'-CTCCCCAGTGTGGCCTGGG 3') and UTCC (5'-CGTACCAATATAGTACAGAACTG-3'). This PCR strategy targeting the SL-ITR gene was devised to distinguish populations belonging to *T. cruzi* III and IV (amplicons of approximately 200bp) from populations of *T. cruzi* I, II, V, and VI, which present fragments of approximately 150bp. The reactions were performed in a total volume of 15μL containing 20mM Tris-HCl (pH 8.4), 50mM KCl, 3mM MgCl₂, 250μM of each dNTP, 3μM of each primer, 0.5U Platinum *Taq* DNA polymerase (Invitrogen, Carlsbad, CA), and 1μL of *T. cruzi* DNA covered with mineral oil. The PCR amplification cycles consisted of an initial denaturation of 3min at 94°C, annealing at 68°C for 1min, extension at 72°C for 1min and denaturation at 94°C. Every three cycles, the annealing temperature was decreased by 2 degrees to 66, 64, 62, and 60°C. At the final temperature, the number of cycles was increased to 35 and was followed by a final extension step at 72°C for 10min. The PCR products were analyzed by electrophoresis on a 2% agarose gel stained with ethidium bromide. DNA obtained from strains and clones that yielded approximately 200bp (231, *T. cruzi* III) and 150bp (CL Brener, *T. cruzi* VI) fragments were used as reference amplicons for the SL-ITR gene.

Polymorphism of the COII gene: for molecular characterization, parasite DNA was extracted²¹, and the mitochondrial COII gene was amplified using the primers Tcmit-10 (5'-CCATATATTGTTGCATTATT-3') and Tcmit-21 (5'-TTGTAATAGGAGTCATGTTT-3') to produce a 375bp fragment from *T. cruzi* maxicircle DNA²². Each PCR was performed in a final volume of 15μL containing 10mM Tris-HCl (pH 8.4), 50mM KCl, 0.1% Triton X-100, 1.5mM MgCl₂ (Buffer IB, Phoneutria, MG, Brazil), 1.0U *Taq* DNA Polymerase (Promega), 250μM of each dNTP, 0.3μM of each primer, and 3ng of DNA template. The amplification program consisted of a denaturation step of 5min at 95°C followed by 40 cycles of a 45sec denaturation step at 95°C, annealing for 45 sec at 48°C, and primer extension for 1min at 72°C. After PCR amplification, the amplicons were digested with *AluI* (Promega) for 16h according to the manufacturer's instructions. The digested products were analyzed on 6% polyacrylamide gels and visualized by silver staining. COII restriction fragment length polymorphism DNA samples from *T. cruzi* populations belonging to *T. cruzi* I (Col1.7G2, mitochondrial haplotype A), *T. cruzi* II (JG, mitochondrial haplotype C), and *T. cruzi* VI (CL Brener, which shares the mitochondrial haplotype B with all *T. cruzi* DTUs) were used as references.

Biological characterization

Animals and experimental *Trypanosoma cruzi* infection: Female swiss mice (28-30 days old) obtained from the CCA (Centro de Ciência Animal) of the Universidade Federal de Ouro Preto (UFOP), MG, Brazil, were used in this study.

The study was approved by the Ethics Committee in Animal Experimentation of UFOP, MG, Brazil (Process 2009/10). The animals were maintained according to the guidelines of the *Colégio Brasileiro de Experimentação Animal* (COBEA).

Metacyclic trypomastigotes obtained from LIT cultures were intraperitoneally inoculated into the mice. For each *T. cruzi* strain, 8 mice were infected with 1.0×10^4 blood trypomastigotes from previously infected animals. The following parameters were evaluated:

Infectivity and mortality: infectivity (%INF), defined as the percentage of mice that presented a positive fresh blood examination (FBE) and/or hemoculture (Hm); and mortality (%MOR), which was registered daily after inoculation and expressed as the cumulative percentage of death throughout the infection period.

Fresh blood examination: five microliters of blood collected from the tail vein were examined on alternate days, and parasitemia was evaluated²³. The examination began four days after inoculation and continued for five consecutive days after negativation. The results were expressed as the percentage of mice with positive FBE (%+FBE). The following parameters were also assessed: pre-patent period (PPP), patent period (PP), maximum peak of parasitemia (MPP), and day of maximum peak of parasitemia (DMPP).

Morphology of blood trypomastigotes: parasite morphology was evaluated daily by microscopic examination of fresh blood obtained from the mouse tail vein during the acute phase of infection. The percentages of slender, intermediate, and large parasites were recorded²⁴.

Hemoculture: thirty days after infection, the animals that had negative FBE results were subjected to hemoculture²⁵. Each tube of culture was examined for the presence of parasites after 30, 60, 90, and 120 days, and the results were expressed as the percentage of mice with a positive Hm (%+Hm).

Histopathology: for each *T. cruzi* strain, three infected mice were euthanized during the chronic phase of the infection (290 days after inoculation). The heart was fixed in 10% buffered formalin (pH 7.2) and embedded in paraffin. Sections (5µm thick) were mounted on glass slides and stained with hematoxylin-eosin. Morphometric studies of inflammation involved the analysis of 15 randomly selected fields (total area, $1.1 \times 10^6 \mu\text{m}^2$) of tissue sections on a single slide per animal. Inflammatory infiltration in the heart was quantified by counting the cellular nuclei. Animals that were not infected showed an average of 200 cellular nuclei in their cardiac muscular tissue. Thus, presence of inflammation was considered when the heart of a mouse presented more than 200 cellular nuclei. Images registered with a 40× objective were analyzed with Leica QWin software (Leica Microsystems, Wetzlar, Germany).

Statistical analysis

The data for the biological parameters (PP, PM, DPMP, and area under the parasitemia curve) were analyzed using the program Prism 5 for Windows, version 5.0. The Kolmogorov-Smirnov test of normality was used for data corresponding to all parameters. Data with normal distributions were evaluated

by analysis of variance (ANOVA) followed by the Newman-Keuls post-test. For the data that were not normally distributed, the non-parametric Mann-Whitney test was employed. The analyses of mortality and infectivity were performed using the chi-square test of homogeneity. Statistical significance was defined as $p < 0.05$.

Ethical considerations

The inclusion of patients in the study and the blood collections were performed after the patients signed a consent form approved by the Ethics Committee for Research in Humans from René Rachou Research Center (CPqRR), of the Oswaldo Cruz Foundation (FIOCRUZ), Belo Horizonte, MG (Process Number 007/02)

RESULTS

Molecular typing

The molecular characterization revealed that all of the *T. cruzi* strains analyzed had genetic profiles indicative of *T. cruzi* II³. **Figures 1A and B** show the typical electrophoretic profiles for GPI and G6PD, respectively.

Analysis of the 24Sα rDNA gene revealed the presence of the 125bp fragment (rDNA type 1) in all strains, compatible with *T. cruzi* II or *T. cruzi* VI (**Figure 2A**). The analysis of the amplified products of ITS revealed the presence of 150bp fragments that were compatible with *T. cruzi* II or *T. cruzi* VI for all strains (**Figure 2B**). The restriction fragment length polymorphism (RFLP) profiles for the COII gene showed that all of the strains had the 81 and 212bp fragments related to mitochondrial haplotype C that are characteristic of *T. cruzi* II (**Figure 2C**).

Biological characterization

Infectivity and mortality: the percentage of infectivity (%INF) observed in mice inoculated with six *T. cruzi* strains (795, 806, 817, 829, 1661, and 855) isolated from children was 100%, as demonstrated by a positive FBE and Hm for all of these strains (**Table 1**). It was necessary to carry out Hm to confirm infection in three animals. One animal infected with strain 829 was positive by Hm, but the animals infected with strains 501 and 2405 were negative (**Table 1**). The experimental infections with strains 501 and 2405 both had a %INF of 87.5% (**Table 1**). No significant differences were observed between strains with respect to this parameter.

No mortality was observed in the majority of the animals infected with the eight *T. cruzi* strains during the acute and chronic phases of infection; the exception was strain 806, which was associated with a mortality rate of 62.5% (5/8) in the first month of infection (**Table 1**). Regarding this parameter, this strain was significantly different from the other strains studied.

Parasitemia curve: to classify the *Trypanosoma cruzi* strains, an arbitrary criterion was used: low parasitemia (<300,000/0.1mL of blood), moderate parasitemia (300,000-1,000,000/0.1mL of blood), and high parasitemia (>1,000,000/0.1mL of blood). Six strains

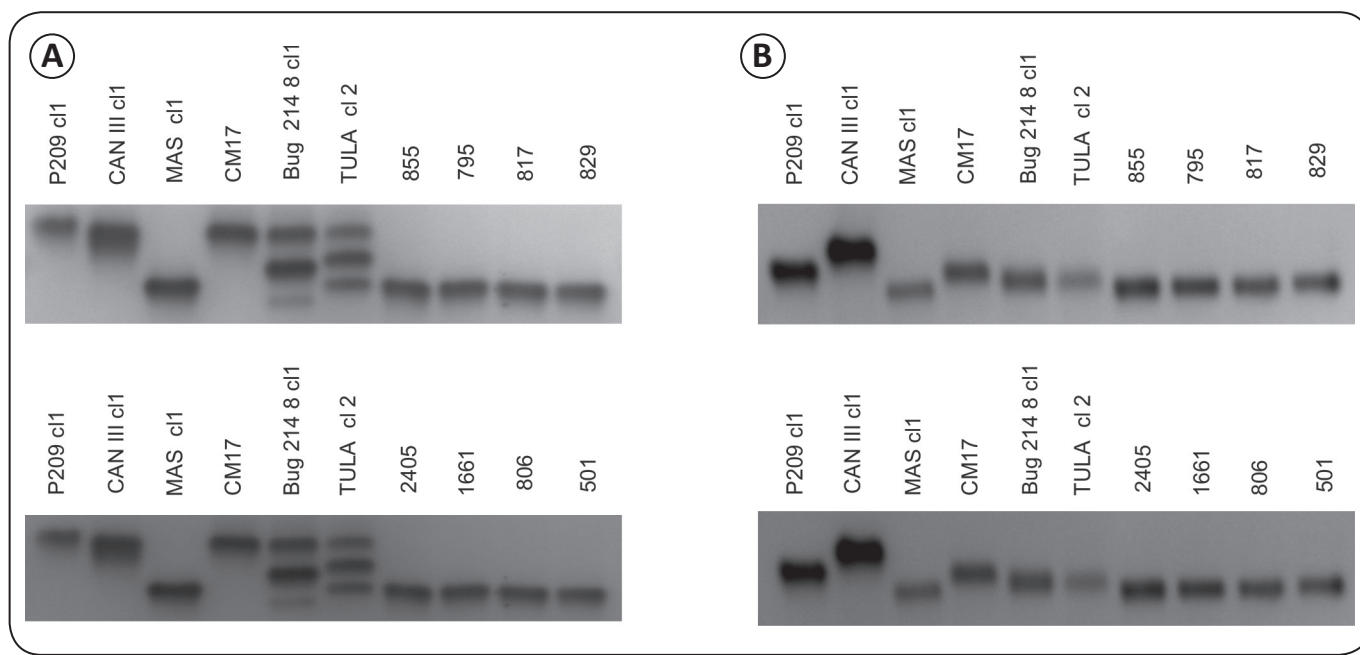


FIGURE 1 - Electrophoretic profiles of the glucose-6-phosphate isomerase (A) and glucose-6-phosphate dehydrogenase (B) enzymes from *Trypanosoma cruzi* strains isolated from children living in Berilo and José Gonçalves de Minas, Jequitinhonha Valley, State of Minas Gerais, Brazil. Reference stocks: P209 cl1 (*T. cruzi* I), MAS cl1 (*T. cruzi* II), CM17 (*T. cruzi* III), CAN III cl1 (*T. cruzi* IV), Bug 2148 cl1 (*T. cruzi* V), and TULA cl2 (*T. cruzi* VI). *Trypanosoma cruzi* strains isolated from children: 855, 795, 817, 829, 2405, 1661, 806, and 501.

(829, 795, 855, 817, 501, and 2405) showed low parasitemia, and two strains (806 and 1661) demonstrated moderate parasitemia (**Figure 3** and **Table 1**).

Samples 806 and 1661 showed moderate parasitemia and presented higher numbers of parasites in the peripheral blood ($p \leq 0.05$) than the other strains, but no significant differences were observed between them (**Figure 3** and **Table 1**). Significant differences were also detected between the areas under the parasitemia curves of strains 795 and 829. No differences were detected when the other pairs of strains were compared.

Strains 806 and 1661 were more virulent (higher parasitemia) than the other strains and exhibited the lowest mean PPP (6.0 days for strain 806 and 4.2 ± 0.25 days for strain 1,661, Table 1). These strains showed an earlier mean DMPP, which was 15.75 ± 2.15 days for the 806 strain and 17.7 ± 0.79 days for the 1,661 strain (**Table 1**).

Strain 829 exhibited the lowest number of parasites in the peripheral blood during the PP (5.500 trypomastigotes/0.1mL of blood), a longer PPP (13.7 ± 3.08), and a shorter PP (3.3 ± 1.08) compared with the other strains (**Table 1**). This strain was also the least virulent (**Figure 3**).

In general, the mean PPPs were relatively short and oscillated between 4.2 ± 0.25 and 13.7 ± 3.08 days, whereas the PP was between 3.3 ± 1.08 and 34.5 ± 3.52 days. The MPP values oscillated between 5.5 ± 1.45 and $840.9 \pm 364.43 \times 10^3$ trypomastigotes/0.1mL of blood. A DMPP was also observed, with values between 15.75 ± 2.15 and 26.25 ± 4.86 days. The biological parameters of all of the tested strains of *T. cruzi* are shown in **Table 1**.

Morphological analysis of blood trypomastigotes revealed a predominance of intermediate and large forms for all strains. Strain 806 exhibited the highest percentage of slender trypomastigotes, which disappeared as the infection progressed.

Histopathology: histopathological analyses of the hearts of mice infected with the *T. cruzi* strains showed mononuclear infiltrate cells during the chronic phase of the infection in the animals infected with strains 501 and 806 (**Figure 4**), and fibrosis was detected only in animals infected with strain 806 (data not shown). Inflammation and fibrosis were absent during the chronic phase of the infection in the animals infected with the other strains.

DISCUSSION

Variations in the clinical forms of Chagas disease and in the susceptibility/resistance of *T. cruzi* to specific treatment have been observed during evaluations of patients from distinct endemic regions^{1,26}. These differences may be attributed to *T. cruzi* and host genetic diversity¹⁰ in addition to environmental conditions, which were the same for all experimental groups in this study and were consequently not considered in our analyses²⁷.

These observations have prompted several research groups to isolate and characterize different samples of the parasite from distinct hosts and vectors species from domestic, peridomestic, and sylvatic environments²⁸⁻³¹. Differences in *T. cruzi* strains or clones related to biological behavior in vertebrate and invertebrate hosts, resistance to drugs, induction of immune response, and geographical distribution were also demonstrated^{4,8,9,11,25,32}.

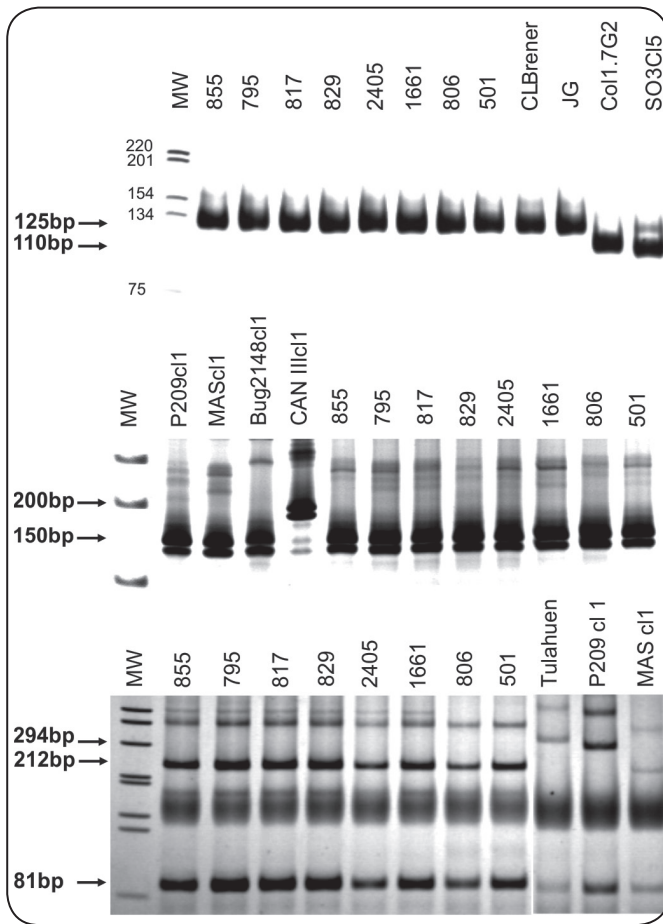


FIGURE 2 - Genetic characterization of *Trypanosoma cruzi* strains (855, 795, 817, 829, 2405, 1661, 806, and 501) isolated from children living in Berilo and José Gonçalves de Minas, Jequitinhonha Valley, State of Minas Gerais, Brazil. A: Analysis of the D7 amplified region of the ribosomal DNA (rDNA) 24Sα gene, a 1kb marker (Invitrogen, USA). Reference stocks: CL Brener (*T. cruzi* VI) and JG (*T. cruzi* II) strains (125bp, rDNA 1), the Col1.7G2 clone (*T. cruzi* I) (110bp, rDNA 2), and the SO3 cl5 clone (*T. cruzi* V) (110/125bp, rDNA 1/2); B: Molecular typing of the mini-exon intergenic regions (ITR) gene of *Trypanosoma cruzi*, a 100bp marker. Reference stocks: P209 cl1 (*T. cruzi* I), MAS cl1 (*T. cruzi* II), Bug 2148 cl1 (*T. cruzi* V; 150bp), and CAN III cl1 (*T. cruzi* III; 200bp); C: restriction fragment length polymorphism (RFLP) profiles of the mitochondrial COII gene of *T. cruzi*, a 25bp marker. Reference stocks: (A) P209 cl1, strains with 264 and 81bp restriction fragments are classified as *T. cruzi* I (mitochondrial A haplotype); (B) Tulahuen, strains with 294 and 81bp restriction fragments can be classified as *T. cruzi* III, *T. cruzi* IV, *T. cruzi* V, or *T. cruzi* VI (mitochondrial B haplotype); (C) MAS cl1, strains with 212 and 81bp restriction fragments are classified as *T. cruzi* II (mitochondrial C haplotype).

Because parasite genetics and biology may be associated with the clinical aspects of the disease^{33,34}, the goal of the present work was to determine the characteristics of *T. cruzi* strains isolated from children in the Jequitinhonha Valley, MG, Brazil, an endemic area where the clinical aspects of Chagas disease have not yet been well characterized. Additionally, the correlation between a parasite's genotype and biology was explored.

The molecular typing revealed that all of the strains studied were *T. cruzi* II, similar to other endemic, central regions of Brazil^{16,35-38} and other countries of the South Cone in Latin America³⁹⁻⁴¹. Other researchers have studied samples of *T. cruzi*

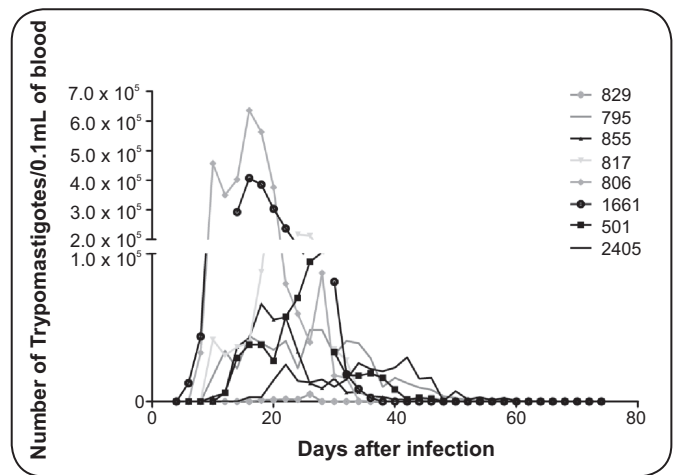


FIGURE 3 - Parasitemia curves (daily average) observed in Swiss mice inoculated with 1.0×10^4 blood trypomastigotes of the *Trypanosoma cruzi* strains (829, 795, 855, 817, 806, 1661, 501, and 2405) isolated from children in the Berilo and José Gonçalves de Minas municipalities, Jequitinhonha Valley, State of Minas Gerais, Brazil.

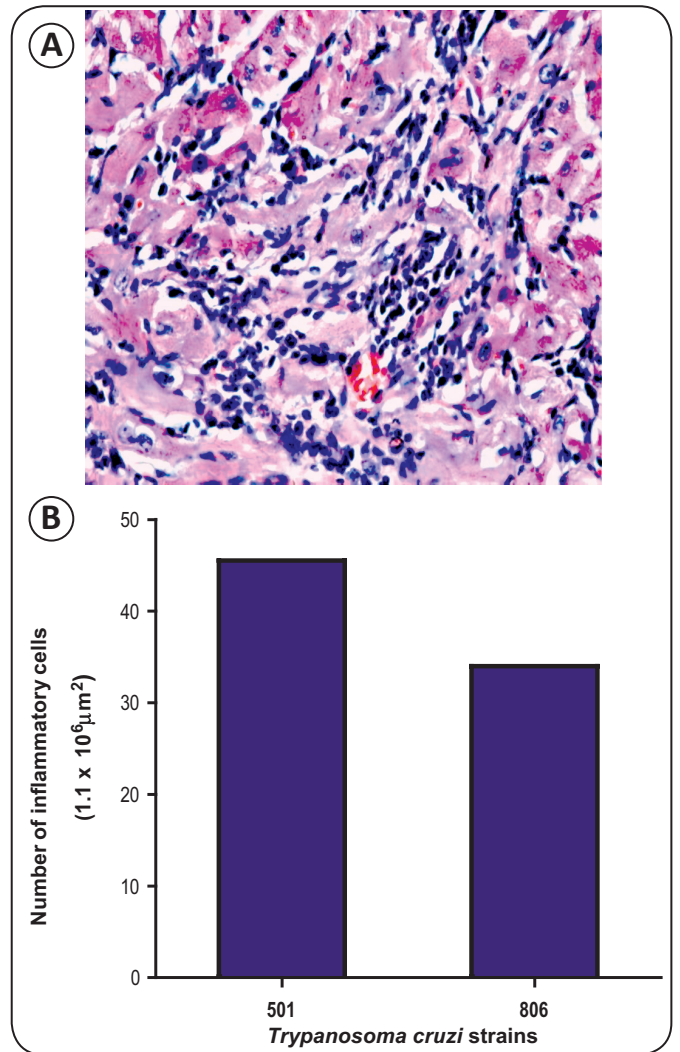


FIGURE 4 - A: Histopathological aspects of the inflammatory infiltrate in the cardiac muscle of animals infected with *Trypanosoma cruzi* strains 501 or 806; B: Quantification of the inflammatory cell number in animals infected with these strains. Data were expressed as mean. Hematoxylin-eosin stain, bar: 50μm.

TABLE 1 - Means of the biological parameters evaluated in Swiss mice inoculated with the *Trypanosoma cruzi* strains isolated from children living in Berilo and José Gonçalves de Minas, Jequitinhonha Valley, State of Minas Gerais, Brazil.

Strains <i>T. cruzi</i>	PPP	PP	MPP x 10 ³	DMPP	% +FBE	% +Hm	% INF	% MOR
829	13.7 ± 3.08	3.3 ± 1.08	5.5 ± 1.45	18 ± 4.05	87.5 (7/8)	100.0 (1/1)	100.0 (8/8)	0.0
795	9 ± 0.65	34.5 ± 3.52	68.6 ± 17.17	21.5 ± 2.44	100.0 (8/8)	NP	100.0 (8/8)	0.0
855	9.7 ± 0.59	21.5 ± 3.75	98.1 ± 31.70	18.2 ± 0.79	100.0 (8/8)	NP	100.0 (8/8)	0.0
817	8 ± 0	26.25 ± 3.14	238.2 ± 84.61	23.5 ± 0.82	100.0 (8/8)	NP	100.0 (8/8)	0.0
806	6 ± 0	20.2 ± 1.79	840.9 ± 364.43	15.75 ± 2.15	100.0 (8/8)	NP	100.0 (8/8)	62.5 (5/8)
1661	4.2 ± 0.25	27.2 ± 1.19	471.7 ± 96.75	17.7 ± 0.79	100.0 (8/8)	NP	100.0 (8/8)	0.0
501	9.5 ± 1.45	25 ± 4.0	122.5 ± 43.94	21 ± 3.52	87.5 (7/8)	0.0 (0/1)	87.5 (7/8)	0.0
2405	13.5 ± 2.02	23.25 ± 5.80	58 ± 16.71	26.25 ± 4.86	87.5 (7/8)	0.0 (0/1)	87.5 (7/8)	0.0

PPP: pre-patent period; PP: patent period; MPP: maximum peak of parasitemia; DMPP: day of maximum peak of parasitemia. Data are expressed as the mean ± standard error, NP: not performed; % +FBE: percentage of mice with a positive fresh blood examination; % +Hm: percentage of mice with a positive hemoculture; % INF: percentage of infectivity (percentage of mice that presented positive FBE and/or Hm); % MOR: percentage of mortality.

isolated from patients residing in Virgem da Lapa, a municipality near the studied region⁴², and they detected the presence of the same genetic group found in our study. However, it is important to note that the author of the Virgem da Lapa⁴² study used an isoenzymatic characterization method that is unable to differentiate *T. cruzi* II from hybrid groups.

The biological characterization showed that the majority of the strains of *T. cruzi* isolated from children were infective for Swiss mice, presented low virulence because six of eight strains showed low parasitemia, later peaks of parasitemia, had a predominance of large blood trypomastigotes throughout the acute phase, and showed low mortality rates, characteristics well-matched with *T. cruzi* strains belonging to *T. cruzi* II^{8,11,12}. For strains 806 and 1661, moderate parasitemia was observed, particularly for strain 806, which displayed a mortality rate of 62.5%. However, during five successive blood passages (data not shown) in animals infected with this same strain, no mortality was observed, what confirms the low virulence characteristic of *T. cruzi* II strains. These results corroborate previous studies that demonstrated that the majority of the *T. cruzi* strains isolated from humans living in different states of central and southern Brazil^{11,43-45}, including Virgem da Lapa⁴² and Berilo²⁹ cities of Minas Gerais, presented low virulence and mortality in mice.

The higher parasitemia of strain 806 may be associated with a high percentage of slender blood trypomastigotes early in the infection; this observation is consistent with the results of other studies^{11,28,29,43,46}. The PPP and the DMPP for strains 806 and 1,661, the most virulent of the strains investigated, were significantly different and precocious compared with those of the other strains. The parasitemia was significantly higher compared with that of the other strains. However, strain 829 presented a longer PPP, a later DMPP, a shorter PP, and a lower MPP than the other strains studied. The long period for parasitemia detection by fresh blood examination of mice and the lower and later peak of parasitemia observed in animals infected with this strain are characteristics of low virulence compared with the other strains studied. The DMPP

was later for the majority of the strains, a common characteristic of strains of low virulence^{11,28,29,42}. This observation may be associated with the predominance of large blood trypomastigotes during the acute phase of the infection in mice, as observed in samples from patients in Bahia and Minas Gerais, Brazil^{11,28,29,42,43}.

The presence of large trypomastigote forms is related to a later increase in parasitemia and a later DMPP because this form of the parasite is less infective, slower to invade host cells, and, consequently, slower to undergo cellular division. Thus, its growth is delayed compared with the strains with a predominance of slender trypomastigotes, which circulate for a shorter period of time after inoculation⁴⁷.

Histopathological analysis of the hearts of infected animals demonstrated that strains 501 and 806 were the only strains that resulted in an inflammatory process. Fibrosis was detected only in mice infected with strain 806 and has been observed in several *T. cruzi* strains isolated from Minas Gerais and Bahia, Brazil^{45,48}. The histopathological results observed in this study may be associated with the parasitemia curve or the virulence of the strains because both strains 501 and 806 presented higher parasitemia, similar to strain 1661. We demonstrated a correlation between parasitemia and inflammation in mice, which is in accordance with the results of other authors^{29,42} who have studied cardiotropic *T. cruzi* strains isolated from patients in endemic areas of Minas Gerais, including the Berilo municipality.

As observed in the present study, other authors^{46,47} have demonstrated that strains that multiply slowly present a later peak of parasitemia, are predominantly myotropic, and show a predominance of large blood trypomastigotes during the acute phase of infection. Thus, the present study demonstrated for the first time that *T. cruzi* strains isolated from children living in the Berilo and José Gonçalves de Minas municipalities in Jequitinhonha Valley, MG, which were genetically characterized as *T. cruzi* II with appropriate molecular markers¹⁶, showed similar biological characteristics in mice, as in other experimental studies^{7,8,13,49}.

Finally, the results obtained in this study reinforce the idea that both the genetic and biological characterization of *T. cruzi* strains may be important because these methods are the most appropriate to determine whether there is a correlation between these characteristics and the clinical-pathological manifestations of Chagas disease, a polemic and complex theme in Chagas disease^{33,34}. Therefore, we estimate that this work may contribute to a better understanding of the state of Chagas disease in the Jequitinhonha Valley. This knowledge may help our team provide clinical assistance and etiological treatment for the patients in the studied region, which is considered one of the most important endemic areas of this disease in our country⁵⁰.

ACKNOWLEDGMENTS

We are thankful to the municipal authorities and the local health teams in Berilo and José Gonçalves de Minas for their support in the development of this study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FINANCIAL SUPPORT

We would like to thank *Fundação de Amparo à Pesquisa de Minas Gerais* (FAPEMIG) (*Rede Mineira de Bioterismo/FAPEMIG*), announcement *Programa de Pesquisa para o Sistema Único de Saúde* (PPSUS/05), *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq), *Agência Brasileira da Inovação-Fundo de Infra-Estrutura* (FINEP-CTInfra), *Secretaria de Educação Superior/Ministério da Educação* (SESU/MEC), and *Pró-Reitoria de Extensão/Universidade Federal de Ouro Preto* (PROEX/UFOP) for providing financial support for this study and the author's research fellowship.

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