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PV075 - ***TRYPANOSOMA CRUZI* I GENOTYPE AMONG STOCKS FROM CHRONIC CHAGASIC PATIENTS UNDER AMBULATORY CARE AT THE EVANDRO CHAGAS NATIONAL INSTITUTE OF INFECTIOUS DISEASES (FIOCRUZ, BRA)**

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Trypanosoma cruzi is the etiologic agent of the Chagas disease in humans, mainly in Latin America, but in the last years became an emerging global health problem. This parasite presents high genetic diversity and can cause different clinical manifestations. In this study, nine trypanosome stocks obtained by hemoculture from chronic chagasic patients were characterized by different approaches. The patients were under ambulatory care at the Evandro Chagas National Institute of Infectious Diseases (Fiocruz), and proceeded from five **Brazilian** States (PE, PB, BA, MG, RS). Materials and Methods. For trypanosome species identification, the isolates were analyzed by classical parasitological approaches and a specific PCR assay. For lineage determination, they were compared by their amplicons of the mini-exon non-transcribed spacer and isoenzymatic patterns. Aiming genotype confirmation, one stock was also analyzed by sequencing of a fragment from TcSC5D gene. Results. All isolates were pure *T. cruzi* cultures, presenting 330 bp products derived from kDNA minicircles. They easily grew in axenic cultures, displaying typical *T. cruzi* stages with large kinetoplast; one stock showed metacyclics only in experimentally infected *Triatoma infestans*. TcI genotype was found in one asymptomatic patient from the State of Paraíba. Six patients were infected with TcII lineage, three individuals presenting clinical symptoms (two with cardiac alterations, and one with megaesophagous), the others being asymptomatic. Two patients infected with TcVI had the disease indeterminate form. Discussion. In Brazil TcII is the main agent of severe chronic infections, whereas TcI is less frequent and usually causes mild chronic disease, unlike that occur in other Latin American countries. Genotyping of *T. cruzi* isolates from patients followed in medical centers is important regarding possible correlations between the parasite lineage and host responses to therapeutic drugs, besides disease prognoses. **Supported by:**FIOCRUZ, UFF, CAPES **Keywords:**Trypanosoma cruzi genotyping; chronic chagas disease; tci

PV076 - **EVIDENCE OF UTR-ASSOCIATED NCRNAS IN *LEISHMANIA***

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It is well established that non-coding RNAs (ncRNAs) regulate a diversified number of cell processes. ncRNAs with independent expression rising from untranslated regions (UTRs) of protein coding genes (so called UTR-associated RNAs, uaRNAs) is a phenomenon conserved in eukaryotes. The discovery of short and unusually AT-rich transcripts in Leishmania major led to a study of putative ncRNAs in the parasite. Three of these odd transcripts were expressed ectopically in L. major in the search for a phenotype, and one of them, named ODD3 that led to a marked phenotype was further investigated. Transcriptomic data in association with RT-PCR and 5'RACE assays suggested that ODD3 is a polyadenylated ncRNA arises from the 3'UTR of one of the copies of the ribosomal protein S16 gene (LmjF.26.0890), a duplicated gene found in tandem on chromosome 26. We analyzed the ODD3 and S16 transcript levels in L. major promastigotes using RT-qPCR. Interestingly, LmjF.26.0890 transcript level does not accompany ODD3 levels; in the stationary phase, LmjF.26.0890 transcript is significantly lower than ODD3 itself. In opposition, LmjF.26.0880 and its 3'UTR are present at equal levels, both higher than LmjF.26.0890 throughout development. We explored the potential of ODD3 as cis or trans-acting element controlling the expression of S16 and other genes. A mutant to overexpress integrated ODD3 into the ribosomal locus was engineered to answer this question. In addition, we generated ODD3 RNA with 4×S1m aptamer tag for the isolation of ODD3 binding proteins. We obtained a list of putative ODD3 binding proteins, 38 proteins in procyclic form and 40 proteins in metacyclic promastigotes. Interestingly, several duplicated ribosomal protein genes in the Leishmania major genome depicted a similar pattern of short transcripts arising from their 3'UTR as shown by RNA-Seq analysis. Therefore, our study indicates that uaRNAs derived from protein coding genes might be a common finding in Leishmania. **Supported by:**FAPESP **Keywords:**Non-coding rna; 3'utr-derived rnas; leishmania

TRYPANOSOMA CRUZI I GENOTYPE AMONG ISOLATES FROM CHRONIC CHAGASIC PATIENTS FOLLOWED AT THE EVANDRO CHAGAS NATIONAL INSTITUTE OF INFECTIOUS DISEASES (FIOCRUZ, BRAZIL)


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INTRODUCTION

Trypanosoma cruzi is the etiologic agent of the Chagas disease in humans, mainly in Latin America, including Brazil. However, the international migration, generally of asymptomatic patients to non-endemic countries of the North America, western Pacific region and Europe, has spread the Chagas disease by non-vectorial routes. Nowadays, the chagasic infection is considered an emerging global health problem. *Trypanosoma cruzi* presents high genetic diversity with the recognition of at least six lineages (TcI-VI). This parasite can cause different clinical manifestations, including regional variations. In this study, nine trypanosome stocks obtained by hemoculture from chronic chagasic patients who were under ambulatory care at the Evandro Chagas National Institute of Infectious Diseases (INI, Fiocruz) were characterized by different approaches. The patients proceeded from five Brazilian States (Pernambuco, Paraíba, Bahia, Minas Gerais and Rio Grande do Sul).

PATIENTS, MATERIALS AND METHODS

The data on the patients who donated blood for trypanosome hemocultures are presented on Table. All trypanosome isolates used in this work were deposited in the Trypanosomatid Collection at Oswaldo Cruz Institute, receiving a code-number (CT-IOC). For trypanosome identification, the isolates were firstly analyzed by classical parasitological approaches (morphology, biometry, growth in LIT or NNN+LIT medium, and differentiation to typical metacyclics), besides a PCR assay using the primers 121/122 and an isoenzyme analysis at the MDH *locus*. For lineage determination, they were compared by the amplicons of the mini-exon non-transcribed spacer using a multiplex PCR assay (primers Tc1/Tc2/Tc3/Tr/ME) and electrophoretic patterns of isoenzymes at three *loci* (GPI, PGM and ME). For genotype confirmation of one stock, it was also analyzed by sequencing of a fragment of the TcSC5D gene from *T. cruzi* CL-Brener genome. *Trypanosoma rangeli* stocks were included in some studies for comparative purposes.

This research was approved by Fiocruz Ethical Committee # 0050.0.009.000-05.

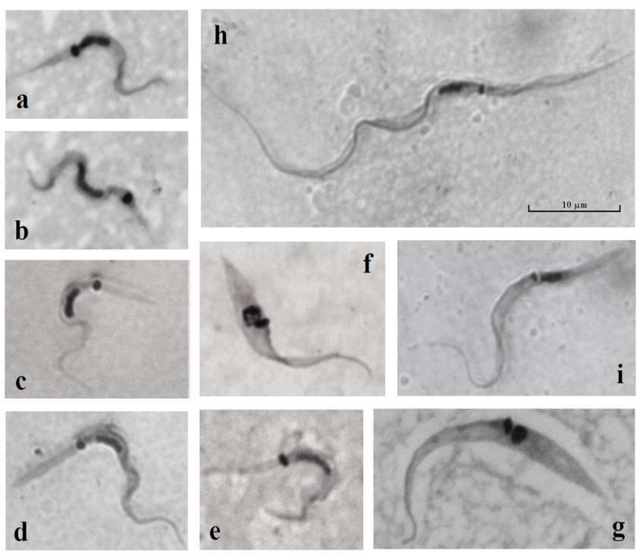

Figure 1 - Representative forms from axenic cultures of *Trypanosoma cruzi* and *T. rangeli* isolates from Chagas disease patients followed at INI (Fiocruz). *Trypanosoma cruzi* stages: metacyclic trypomastigotes (a-e) and epimastigotes (f, g). *T. rangeli* stages: trypomastigote (h) and epimastigotes (i) (CT-IOC 535). Compare the differences in size between the trypomastigotes of *T. cruzi* and *T. rangeli*, as well as the dimensions of their kinetoplasts. *T. cruzi* lineages are represented, as follows: TcI: (a, b) (CT-IOC 541); TcII: (c, d, f) (CT-IOC 543, 544); TcVI: (e, g) (CT-IOC 539, 540). Giemsa-stained smears under optical microscopy (×1,000). All images are at the same magnification.

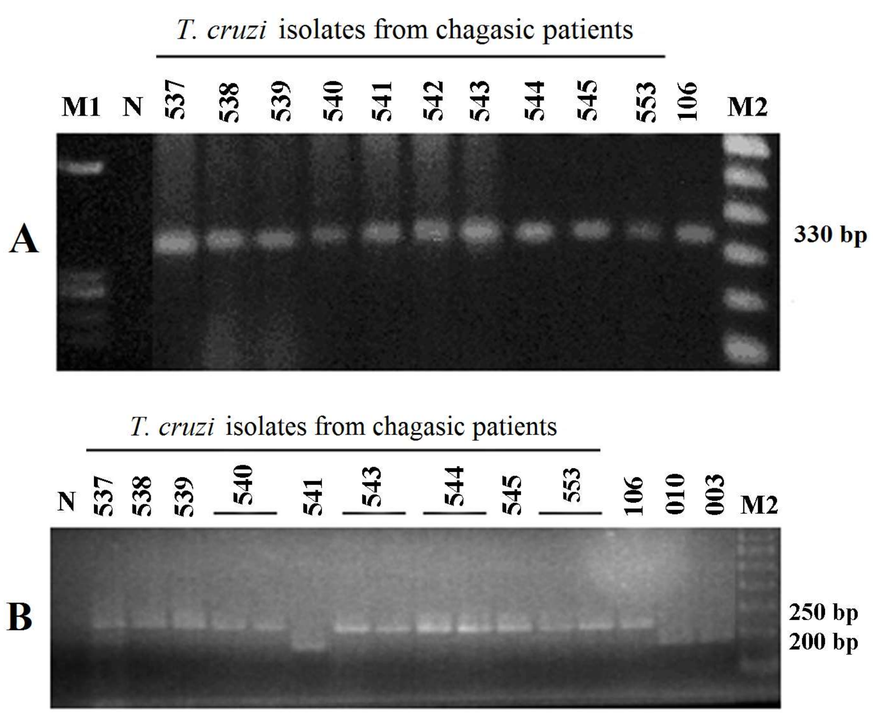

Figure 2 – Polymerase chain reaction products of *Trypanosoma cruzi* isolates from Chagas disease patients followed at the INI (Fiocruz), and reference strains. A: typical *T. cruzi* amplicons (330 bp) derived from kDNA minicircle using the primers 121/122. B: amplicons from the mini-exon non-transcribed spacer using a PCR multiplex assay with the primers Tc1/Tc2/Tc3/Tr/ME; 200 and 250 bp bands were from TcI and TcII major DTUs, respectively. The numbers at the top of the gel indicate the code-number of each stock at the Trypanosomatid Collection (CT-IOC). *Trypanosoma cruzi* reference strains: CT-IOC 106 (Y), 010 (Dm28c) and 003 (F). (N) negative control. (M1) ϕX174 DNA Hae digest. (M2) 100 bp ladder.

Presented at the XXXII Annual Meeting of the Brazilian Society of Protozoology (Caxambu, MG, novermber 2016).

RESULTS

All isolates under study were pure *T. cruzi* cultures (Fig. 1a-f), presenting 330 bp products derived from kDNA minicircles (Fig. 2A), and MDH, PGM and ME patterns distinct from those of *T. rangeli* (Figs. 3, 4). The isolates usually grew well in axenic cultures, displaying typical *T. cruzi* stages with large kinetoplast, being distinguishable from *T. rangeli* (Fig. 1h-i). One stock showed metacyclics only in experimentally infected *Triatoma infestans*. Three *T. cruzi* lineages (TcI, II and VI) were identified in the isolates from the patients (Figs. 2B, 4). TcI genotype was found in one asymptomatic patient from the State of Paraíba. Six patients were infected with TcII lineage, three individuals presenting clinical symptoms (two with cardiac alterations, and one with megaesophagus), the others being asymptomatic. Two patients infected with TcVI had indeterminate form. Results are summarized on Table.

Table - Data on the chronic Chagas disease patients followed at INI (Fiocruz) who donate blood for parasite isolation, and on the isolates of *Trypanosoma cruzi* obtained from them, including their code-number in the Trypanosomatid Collection (CT-IOC), molecular and biochemical characterization, besides their lineage assignment.

Data on the patients					*T. cruzi* isolates		Molecular and biochemical analyses			Genotypes
Names	Ages	Genres	Origins	Clinical forms	Stocks	CT-IOC	kDNA*	Mini-exon*	Zymodemes	Lineages
L.F.S.	49	M	PE	Indeterm.	LFS49	537/542§	330	250	Z2	TcII
J.M.C.	55	M	MG	Digestive (1)	JMC55	538	330	250	Z2	TcII
M.C.J.B.	51	F	BA	Indeterm.	MCJB51	539	330	250	ZB	TcVI
L.T.A.	47	F	RS	Indeterm.	LTA47	540	330	250	ZB	TcVI
J.N.S.	65	M	PB	Indeterm.	JNS65	541	330	200	Z1	TcI**
J.M.M.	51	M	BA	Indeterm.	JMM51	543	330	250	Z2	TcII
J.J.C.	66	M	PE	Cardiac (2)	JJC66	544	330	250	Z2	TcII
M.N.A.G.	44	F	MG	Cardiac (2)	MNAG44	545	330	250	Z2	TcII
C.S.	53	M	MG	Indeterm.	CS53	553	330	250	Z2	TcII

Genres: M= male; F= female. Origins (Brazilian States): PE (Pernambuco), MG (Minas Gerais), BA (Bahia), RS (Rio Grande do Sul), PB (Paraíba). Indeterm.: Indeterminate form. (1) Megaesophagous. (2) Cardiac form, stage A. (*) data in base pairs: bp. (**): Genotype confirmed by sequencing and BLAST analysis. (§) CT-IOC 542 is a subculture of the isolate 537.

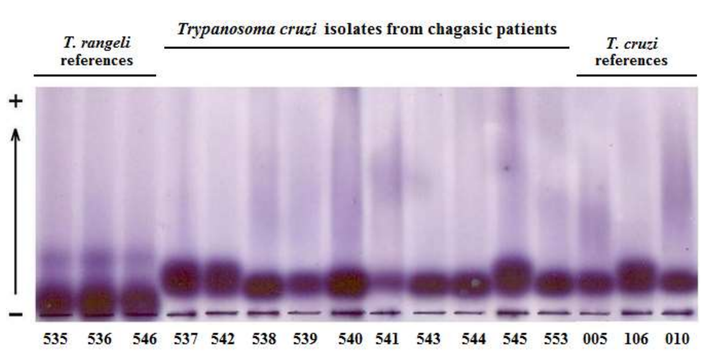

Figure 3 - Isoenzyme patterns at malate dehydrogenase *locus* (MDH) displayed by the *Trypanosoma cruzi* isolates from chagasic patients followed at the INI (Fiocruz), and reference strains. The numbers at the bottom are the codes of each stock at the Trypanosomatid Collection (CT-IOC). *Trypanosoma rangeli* references were also from chagasic patients: CT-IOC 535 and 536/546. *T. cruzi* references: CT-IOC 005 (CL Brener), 106 (Y) and 010 (Dm28c). *Trypanosoma cruzi* and *T. rangeli* can be distinguished at this *locus*.

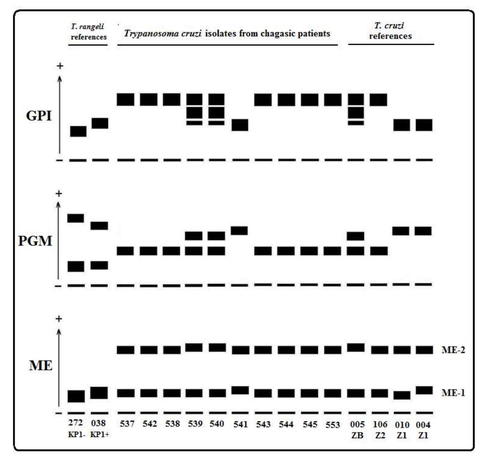

Figure 4 - Diagrammatic representation of the electrophoretic patterns of glucose phosphate isomerase (GPI), phosphoglucomutase (PGM) and malic enzyme (ME) displayed by *Trypanosoma cruzi* isolates from chagasic patients followed at the INI (Fiocruz), and reference strains. At the bottom are the code-numbers of each stock in the Trypanosomatid Collection (CT-IOC). *T. rangeli* KP1(+) and KP1(–) references strains: CT-IOC 272 (SC-61) and 038 (H14), respectively. *T. cruzi* references: CT-IOC 005 (CL Brener), 106 (Y), 010 (Dm28c) and 004 (Colombiana). Zymodemes found in the isolates from the chagasic patients, as follows: Z1 (CT-IOC 541), Z2 (CT-IOC 537/542, 538, 543, 544, 545, 553) and ZB (CT-IOC 539, 540).

DISCUSSION

The main *T. cruzi* genotypes found in Brazilian chronic patients were indentified in this study, including TcI, which is less frequent and usually causes asymptomatic disease, unlike that occurs in other American countries. It is emphasized the importance of *T. cruzi* genotyping accounting for possible correlations between the parasite lineage and the patient' response to therapeutic treatment, or disease clinical manifestations.