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# XXXIII

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**PV075 - TRYPANOSOMA CRUZI 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 4xS1m 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

