Molecular characterization of kinetoplast of three Venezuelan isolates of *Trypanosoma vivax.*
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Trypanosomes are characterized by the presence of a Kinetoplast that contains mitochondrial DNA organized into a complex network of interlocked minicircles and maxicircles. Maxicircles contain typical mitochondrial genes most of which are translatable only after RNA editing, whereas minicircles encode small guide RNAs required for decrypting the maxicircle transcripts. In this study we amplified by PCR fragments of mini and maxicircles from three Venezuelan isolates of *Trypanosoma vivax.* We obtained partial sequences of minicircles containing the highly conserved origin of replication and established the presence of ATPase subunit 6 and NADH dehydrogenase subunit 8.

Key words: *Trypanosoma vivax*, kinetoplast, kDNA, maxicircle, minicircle.

Kinetoplast is a structure characteristic of trypanosomes that contain mitochondrial DNA organized into a complex network of interlocked minicircles and maxicircles. Minicircles encode small RNA guides required for decrypting the maxicircle transcripts in order to generate functional open reading frames in the process known as RNA editing. Maxicircles contain typical mitochondrial genes most of which are translatable only after a post transcriptional RNA modification. Expression of these genes is necessary when parasites develop a life cycle within the insect vector.

*Trypanosoma vivax* is one of the most important hemoparasites in Latin America due to the broad range of animals that infects causing significant losses in cattle. Epidemiologic transmission of *T. vivax* is cyclical in Africa, where parasite undergoes biological changes inside the tse-tse fly, prior to transmission, and undergoes mechanical transmission when parasite is carried by other hematophagous flies that infect other vertebrate hosts in South America.

The only report of kinetoplast DNA of *Trypanosoma vivax* (Borst et al 1985) revealed the presence of a very large and homogeneous maxicircle by electron microscopic and restriction digests. On the other hand, *T. vivax* minicircles appeared to be heterogeneous and this species was shown to have the smallest minicircle of all the Kinetoplastids that have been studied.

Nevertheless, there is no report with reference to molecular analysis of this organelle yet. Due to the above, in this study we isolated kDNA from three geographical Venezuelan isolates of different degree of virulence of *Trypanosoma vivax,* named Liem (Li) highly virulent, Apure (Ap) with an intermediate virulence and Parmana (Par) with the lowest virulence.

We carried out a PCR test with specific maxicircle and minicircle primers that were designed based on *T. brucei* and *T. evansi* sequences. PCR products that were chosen were cloned into a pGEM-T vector, sequenced and analyzed by bioinformatic tools. We obtained a partial sequence of minicircles that shows homology with others trypanosomatids species, also we noted that the sequence contain a highly conserved sequence that correspond to minicircle origin of replication. Also and in agreement with Borst’s reports of *T. vivax* minicircles, the three isolates exhibited heterogeneous minicircle sequences. Besides this we found two complete sequences of mitochondrial genes that corresponding to subunit 6 of ATPase and subunit 8 of NADH dehydrogenase.

This study represents the first report of any molecular analysis of *Trypanosoma vivax* kDNA that have been reported to date.

Reference
Borst P., Fase-Fowler F., Weijers P.J. Kinetoplast DNA from *Trypanosoma vivax* and *T. congolense*. Molecular and Biochemical Parasitology, 15 (2), pp. 129-142 (1985)

Acknowledgements:
Misión Ciencia 2007001425, IVIC 305 and DID-USB