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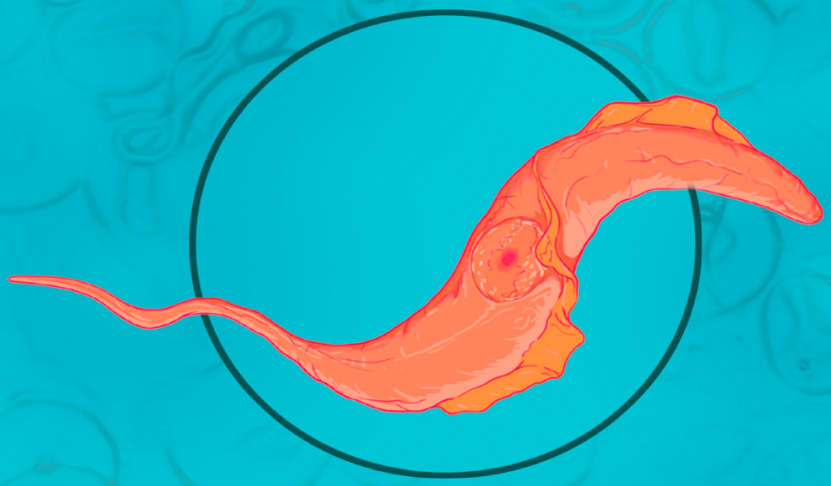

IX Curso Internacional de Tripanosomátidos

Simposio de Biología Molecular de la Enfermedad de Chagas

UNIVERSIDAD DE ANTIOQUIA
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PRESENTATION

IX SYMPOSIUM ON MOLECULAR BIOLOGY OF CHAGAS DISEASE
IX SIMPOSIO DE BIOLOGÍA MOLECULAR DE LA ENFERMEDAD DE CHAGAS

The Symposium on Molecular Biology of Chagas disease and the international course on the molecular biology of Trypanosomatid is an initiative started in 2012 by several Latin American groups from five countries (Argentina, Chile, Colombia, Venezuela, and Uruguay). The main goal of this initiative was to build a network aiming to understand the problem of Chagas disease and others trypanosomiasis from different aspects. In this context, one of the main objectives of the network is to contribute to the formation of advanced human resources. In this context, we have been consistently organizing courses that have contributed to the formation of graduate students, and young researchers and promote the interaction of group leaders from institutes of South America working on Trypanosomatid that causes relevant human diseases. These courses constitute the appropriate environment to discuss current topics in molecular biology and pathogenesis of Chagas disease and other neglected diseases, searching for common interests, giving the opportunity to promote collaborations among groups from different countries of South America, and increasing the chances for emerging innovation in this field.

In 2022, the IX version of this symposium and course will be performed at Universidad de Antioquia, Medellín, Colombia. This year, the emphasis will be focused on the genome, transcriptome, proteome, metabolome, and functional genomics, as well as on relevant aspects of the cell biology of Trypanosomatids, all involved in host-pathogen interactions. In addition, the course will provide practical training, including genomic edition using the CRISPR-Cas9 system, molecular and cellular biology methods, and bioinformatic analysis of genomes and transcriptomes.

I would like to highlight the participation of professors from different countries (Argentina, Brazil, Chile, Spain, Mexico, Colombia, Uruguay, and Venezuela). Moreover, we will have students from different countries, who were supported mainly by UNU-BIOLAC. This year, we want to thank to Grupo La Rabida for supporting most professors, and other companies such as INNOVALAB, ISLA, NOVAVENTA, Post-graduate Biology Program, CODI, and Corporación de Patologías Tropicales for the help during all the organization.

Finally, this meeting is an excellent opportunity to create the Latin American Network in Molecular Biology of Chagas Disease.

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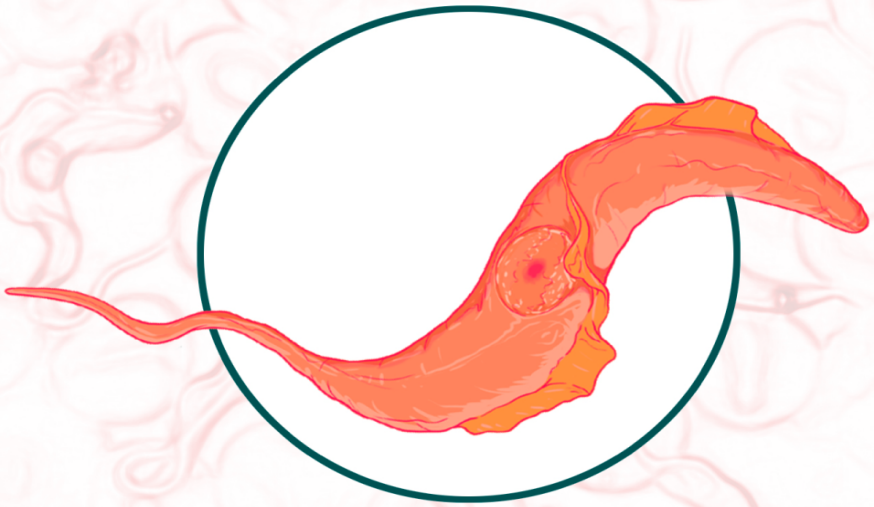

ABSTRACTS OF MAIN LECTURES
IX SYMPOSIUM ON MOLECULAR BIOLOGY OF CHAGAS DISEASE

RESÚMENES CONFERENCIAS MAGISTRALES
IX SIMPOSIO DE BIOLOGÍA MOLECULAR DE LA ENFERMEDAD DE CHAGAS

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Diagnostic of Chagas Disease in Mexico

Diagnóstico de la Enfermedad de Chagas en México

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There are 8 million people infected with the *Trypanosoma cruzi* parasite worldwide, and over 10,000 die every year. Chagas disease is found mainly in the Latin American region, although it has been reported in 17 European countries and in the Western Pacific region (due to migration). Urbanization processes in Latin America during the 20th Century and migration have changed the epidemiology of the disease, turning it into a global risk. The Health Office Administration in Mexico (in Spanish: Secretaría de Salud) proposed to control the vectorial transmission through risk stratification, housing development, chemical vector control, and elimination of connatal and transmission through transfusion of Chagas. The National Institute of Epidemiological Reference (in Spanish: INDRE) processed 33,277 samples from 2001 to 2017; 15,878 (48 %) were positive for Chagas disease. In 2018, the states with the highest rate of incidences were Yucatan (0.8), Quintana Roo (0.8) and Nayarit (0.7). The most crucial vectors identified in some states of the country were: *Triatoma dimidiata*, followed by *T. mexicana* and *T. gerstaeckeri*; TcI is the biotype with which they are mainly infected. There are few studies about the lineage of TcII-TcVII in Mexico, and in these studies appear the presence of several *T. cruzi* lineages on feces from several vectorial species (*T. dimidiata, T. longipennis* y *Meccus pallidipennis*) across Mexico. In Nuevo León, the only current identified vector was *T. gerstaeckeri*; in 2014 the seroprevalence of *T. cruzi* antibodies was 1.93 % (52/2,688); in the electrocardiogram study, 22.85 % (8/35) of infected individuals presented abnormalities in the ECG. These relevant findings in the northeaster region of Mexico place Chagas disease as a serious danger for public health. As for the diagnosis in Mexico, INDRE mentions conventional techniques like parasitological, antibodies detection like immunofluorescence, hemagglutination (HIA) and immunosorbent essay linked to enzymes (ELISA). For pregnant women in blood samples, the molecular diagnosis by polymerase chain reaction (PCR), was the confirmatory method in cases with patients with megaoesophagus, whom has doubtful serology, and bank bloods, while this technique is expensive to be used as a routine test, it can be very helpful in those cases where the infection of the parasites is recent, and the antibodies presence is yet to be detectable. For discrete typification of units (DTU) identification of *T. cruzi* in biological samples, real-time multiplex PCT (MTq-PCR) is the most useful: as well as the intergenetic region (SL-IR), 18S, 18S-AND ribosomal; cytochrome oxidase II (COII); 24Sα, 24Sα-ADN ribosomal; MTq multiplex TaqMan. Entomological work consolidation, such as vectorial control, improvement in housing development, screening of pregnant women living in a risk zone, and universal screening of blood and organ donors, will make possible the elimination of congenital and transfusion transmission.

Keywords: Chagas, diagnosis, prevalence, Mexico, Nuevo Leon

Palabras clave: Chagas, diagnóstico, prevalencia, México, Nuevo León

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Tc323, a *T. cruzi* novel protein with diagnostic potential

Tc323, una nueva proteína de *T. cruzi* con potencial diagnóstico

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Chagas illness, a potentially life-threatening disease, is an infection caused by the hemoflagellate parasite *Trypanosoma cruzi*. Since nowadays none of the available diagnostic methods disclose ∼100% specificity and sensitivity during chronic phase of Chagas disease (CCD), the WHO advises the use of at least two of distinct serological tests for the reliable diagnosis of CCD. From this perspective, we evaluated the diagnostic utility of a hypothetical protein *T. cruzi*, called Tc323, which is only present in this parasite. This protein was identified as the target of a single-chain variable fragment recombinant antibody (scFv 6B6) isolated from a phage display library constructed from B cells of chronic Chagas heart disease patients. Phylogenetic analysis showed that Tc323 is highly conserved throughout evolution in all *T. cruzi* lineage but it lacks orthologous in other kinetoplastid parasites. Due to experimental limitations to produce the full-length protein as recombinant, structural predictions using RaptorX server allowed us to foretell six structural domains (D1-D6) which were cloned into pRSET-A vector and expressed in *E. coli* BL21 Rosetta (DE3) cells. In addition, B cell epitopes predictors mapped that almost all the protein is immunogenic, while the majority of antigenic sequences are within D3 and D6 domains. After optimization of direct ELISA by checkerboard titration, preliminary results showed that recombinant His-tag D3 and D6 domains were recognized by antibodies present in plasma from individuals with CCD but not from those coming from patients with *Leishmania* and non-infected donors. Our finding allowed the identification of two recombinant domains containing Tc323 as a promising immunodiagnosis candidates for chronic Chagas disease in human.

Keywords: chronic Chagas disease, *T. cruzi*, serologic diagnostic, hypothetical proteins

Palabras clave: enfermedad de Chagas crónica, *T. cruzi,* diagnóstico serológico, proteínas hipotéticas

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Point-of-care molecular diagnosis of congenital Chagas disease: from the laboratory to the Maternity

Diagnóstico Molecular de Chagas congénito en puntos de atención: Del laboratorio a la maternidad

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Mother-fetal transmission of *T. cruzi*, causing **congenital Chagas disease (cCD)**, represents one of the main global scenarios of CD, involved in urbanization of CD in endemic and non-endemic countries. Enhancing access to early cCD diagnosis should be a priority at national and regional levels because its prompt treatment achieves a high cure rate, precluding evolution to chronic CD. However, current algorithms involve low sensitive parasitological assays, making necessary serological confirmation after nine months of life. Mainly due to

economical constrains, a high proportion of infants, especially from rural areas is lost to follow-up. Molecular methods for early diagnosis in neonates have been tested to bypass this loss. A TaqMan real-time PCR kit has achieved higher sensitivity than micromethod starting from 1 mL of blood. Studies in infants born to seropositive mothers observed that the first month of life is the best opportunity to perform PCR, when the parasitic load is at its peak and potential false positive results that might arise from contamination with maternal *T. cruzi* DNA are minimised. Loop mediated isothermal amplification (LAMP), is an alternative molecular approach, suitable to resource-limited laboratories, because it does not require a thermocycler, but only a thermoblock or water bath. Furthermore, product visualisation can be done by the naked eye or fluorescence. A prototype kit based on the parasite satellite DNA, that contains dried reagents on the inside of the microtube caps (Eiken Chemical Co, Japan) has been evaluated in archival panels of cCD samples. Aiming at implementing early diagnosis in minimally equipped laboratories associated with Maternities in endemic countries, this *T. cruzi*-LAMP kit was coupled to different rapid DNA extraction methods and supports: 1. An automated DNA extraction device re-purposed from a 3D printer (PrintrLab extraction device, AI Biosciences Inc., College Station, Texas, USA) that uses a Multi Sample DNA extraction kit based on magnetic beads. It has been optimized for 100-200 μL of blood anticoagulated with EDTA and takes less than three hours to yield a result. A recent pilot study in Yacuiba and Villa Montes, Bolivia, showed promising findings with higher sensitivity than the micromethod and high agreement with PCR. 2. An ultra-rapid DNA extraction method (PURE, Procedure for Ultra Rapid Extraction-Eiken Chemical Co, Japan) that uses only 30 μL of starting blood anticoagulated with heparin and the DNA is obtained in around 10-15 minutes. This approach has been analytically validated in blood samples containing serial dilutions of cultured parasites of different discrete typing units and is under prospective evaluation in a multicentre POC study. The more recent challenge is to test dried blood spots using Flinders Technology Associates (FTA®) cards to expand LAMP diagnosis for babies born in domiciles or rural areas without a laboratory suitable for LAMP procedures. These procedures are being actually transferred to laboratories linked to Maternities in endemic sites of Argentina, Bolivia and Paraguay.

Financial Support: ChagasLAMP Project (ref.: G2020-203) from the Global Health Innovative Technology Fund and Small grant program of PAHO/WHO/TDR (LEG ID39002).

Keywords: *Trypanosoma cruzi*, congenital Chagas disease, molecular diagnosis, polymerase chain reaction, loop mediated isothermal amplification, Real time-PCR, LAMP

Palabras clave: *Trypanosoma cruzi*, Chagas congénito, diagnóstico molecular, Reacción en cadena de la polimerasa, amplificación isotérmica mediada por asas, PCR en tiempo real, LAMP

Host-*Trypanosoma cruzi* interactions: role of placenta-specific micro-RNAs during congenital transmission

Interaccción hospedero-*Trypanosoma cruzi*: rol de micro-ARNs placenta-específicos en la transmisión congénita

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Congenital Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, is responsible for 22.5% of

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new cases yearly. However, placental transmission occurs only in 5% of infected mothers, and it has been proposed that the epithelial turnover of the trophoblast is considered a local placental defense against the parasite. Thus, *Trypanosoma cruzi* induces cellular proliferation, differentiation, and apoptotic cell death in the trophoblast, which are regulated by small non-coding RNAs such as microRNAs. On the other hand, *ex vivo* infection of human placental explants induces a specific microRNA profile that includes microRNA related to trophoblast differentiation and apoptotic cell death, such as miR-512-3p miR-515-5p codified at chromosome 19 microRNA cluster (C19MC). Here we determined the expression of miR-512-3p and miR-515-5p validated target genes, specifically human glial cells missing 1 transcription factor and cellular FLICE-like inhibitory protein. In addition, we evaluated the expression of the main trophoblast differentiation marker human chorionic gonadotrophin and caspase 8 during *ex vivo* infection of human placental explants. Moreover, we analyzed how inhibition or overexpression of both microRNAs affects parasite infection. We conclude that the *Trypanosoma cruzi*-induced trophoblast epithelial turnover, particularly the trophoblast differentiation, is at least partially mediated by the placenta-specific miR-512-3p and miR-515-5p and that both miRNAs mediate placental susceptibility to *ex vivo* infection of human placental explants. Knowledge about the role of parasite-modulated microRNAs in the placenta might allow, in the future, their use as biomarkers, prognostic and therapeutic tools for congenital Chagas disease.

Financial Support: ERANET-LAC grant (REF ERANet17/HLH-0142; to UK) and grants from the Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT, Chile), with the REF# 1190341, 1220105 (to UK), 1210159 (to JM), and 11220310 (to ChC).

Keywords: Chagas Disease, micro-RNAs, congenital transmission, placenta

Palabras clave: enfermedad de Chagas, micro-ARNs, transmisión congénita, placenta

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What are the pathways in the DNA damage response: growth arrest, dormancy or death?

¿Cuáles son las vías de respuesta al daño del ADN: detención del crecimiento, latencia o muerte?

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Trypanosoma cruzi is the etiological agent of Chagas disease, a neglected illness that affects millions of people. Thereby, understanding how this pathogen survives stressful conditions can provide insights into its biology and discovery of new drugs. Exposing *T. cruzi* to agents that cause DNA lesions is a stressful condition that can compromise its survival. In this context, a network of pathways called the DNA damage response (DDR) coordinates the repair of lesions to maintain the integrity of the genome. If the damage is not repaired properly, DDR proteins can initiate a signaling cascade for arrested the cell, dormancy or programmed cell death. Of the factors that cause DNA damage, UV and gamma radiation generates lesions that can block or arrest RNA polymerase or DNA polymerase. Such disruption can have a highly deleterious effect on *T. cruzi*, as it transcribes several genes into a single polycistronic pre-mRNA. In this parasite, UV radiation causes immediate apoptosis-like cell death dependent on ATR signaling, a DDR key protein kinase. Interestingly, *T. cruzi* does not repair nuclear UV lesions up to 24h. However, it does not demonstrate increased sensitivity to UV radiation until

this time, suggesting that UV lesions per se are not directly involved in directing cell death. Thus, it is likely that other factors may modulate this signaling process. Blocking RNA polymerase can give rise to R-loops, a structure that can form when RNA hybridizes with DNA, generating a DNA/RNA hybrid and displaced single-stranded DNA. Although R-loops perform physiological functions, they are also associated with genomic instability and could be important to signal to the death. Unlike what happens with UV-induced lesions, lesions caused by gamma radiation cause double strand breaks (DSBs) which block the replication process. This type of injury leads to cell cycle arrest but is not capable of leading to signaled cell death. One of the main markers of the presence of DSBs is the phosphorylated gamma histone 2A (pH2Ax), and the analysis of this modified histone shows that after gamma irradiation there is a large increase in their presence, which may be associated with cell cycle arrest due to the DDR and the action of the ATM protein. Interestingly, even after cell growth resumes, it is possible to verify that pH2Ax levels do not return to pre-irradiation levels. We are investigating whether the maintenance of phosphorylated histone may be related to the presence of dormant cells that increase after gamma irradiation. With these data, our hypothesis is that depending on the triggered DDR we can have different processes occurring in the parasite. Inhibition of transcription can lead to the formation of R-loops that will activate the ATR protein that may signal cell death. On the other hand, the DSBs will activate the ATM protein which may lead, at first, to the arrest of the cell cycle that will allow the repair of the lesions. However, if the injuries are not properly repaired, the signal will not be turned off and may lead the cell to dormancy.

Financial Support: CAPES, CNPq.

Keywords: *Trypanosoma cruzi*, DNA damage, pathways

Palabras clave: *Trypanosoma cruzi*, daño del ADN, vías de respuesta

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Drug target validation against *Trypanosoma cruzi* through Metabolic Control Analysis and Metabolic Modeling

Validación de blancos terapéuticos contra *Trypanosoma cruzi* mediante el análisis del control y modelado metabólicos

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In the validation of therapeutic targets on the intermediary metabolism of protozoan parasites (and in general of pathological cells to determine the essentiality of a protein) the gene expression silencing method is commonly applied. The results of these analyses generally conclude that ALL silenced metabolic enzymes are essential for parasite survival or infectivity. Therefore, it is necessary to apply additional strategies to identify those enzymes that, in addition to being essential, when inhibited at a lower percentage than those obtained by genetic or pharmacological methods, have negative effects on the parameters of antiparasiticidal activity being sought. In the study of the mechanisms that regulate and control the metabolic pathways of a cell, Metabolic Control Analysis (MCA) and kinetic modeling (a Systems Biology approach that consists of building computational models of cell metabolism) are strategies that allow us to quantify the degree of control that an enzyme has over the fluxes of the metabolic pathway to which it belongs. By means of these strategies it is possible to identify those enzymes that have the greatest control of the pathway, which from the metabolic point of view are the sites with the greatest therapeutic potential. These strategies were applied to trypanothione-dependent

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antioxidant metabolism and cysteine metabolism in the *Trypanosoma cruzi* parasite. *In silico, in vitro* and *in vivo* results indicate that mild/moderate inhibition of enzymes/transporters that have the greatest degree of control of a metabolic pathway have a greater negative effect on the parasite than inhibition of an enzyme with little control. Therefore, MCA and computational modeling of metabolic pathways are strategies to help prioritize sites for therapeutic intervention. These approaches can direct basic research for drug design or drug repositioning against validated therapeutic targets.

Financial support: CONACyT 282663

Keywords: *Trypanosoma cruzi*, trypanothione, antioxidant system, metabolic modeling, COPASI, metabolic control analysis

Palabras clave: *Trypanosoma cruzi*, tripanotión, sistema antioxidante, modelado metabólico, COPASI, análisis del control metabólico

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The role of ESCRT in endocytosis and secretion of extracellular vesicles by *Trypanosoma cruzi*

El papel del ESCRT en la endocitosis y la secreción de vesículas extracelulares por *Trypanosoma cruzi*

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The ESCRT machinery (Endosomal Sorting Complex Required for Transport), consists of four multi-subunit complexes, ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III, that play a role in the transport of ubiquitinated cargoes to intraluminal vesicles that bud from multivesicular bodies derived of endosomal compartments. These multivesicular bodies later fuse with lysosomes for degradation of the endocytosed cargoes or with the plasma membrane secreting intraluminal vesicles as a heterogeneous mixture of exosomes. Because Trypanosomatids, which are protozoan parasites that secrete extracellular vesicles and depends on endocytosis, here we analyzed the role of ESCRT complex in *Trypanosoma cruzi*, the agent of Chagas disease. Parasites expressing the Cas9 and T7 RNA polymerase were transfected with DNA fragments coding for two sgRNA that pair the VPS23 gene, a member of the ESCRT-I, together with DNA donor fragments to replace the entire gene by hygromycin and blasticidin resistance markers. Only one of the VPS23 alleles were replaced by one copy of the resistance markers, suggesting that VPS23 is essential for *T. cruzi*. Similar results were obtained for *Leishmania mexicana*. These partial knockouts significantly lower the receptor mediated endocytosis of transferrin, but not the fluid phase BSA-uptake in *T. cruzi*. In addition, they reduced the secretion of extracellular vesicles by epimastigotes. The partial knockout did not affect epimastigote proliferation and metacyclogenesis, and the resulting trypomastigotes were able to infect cells as the parental cell lines. We are currently analyzing whether the secretion of extracellular vesicles is also changed in trypomastigotes released from infected mammalian cells and the capacity of these parasites to promote infection. These results demonstrated the essential role of TcVPS23 by modulating endocytic and secretory activities in *T. cruzi*, and might be useful to understand the role of extracellular vesicles, know to play an important role in the parasite virulence.

Keywords: *Trypanosoma cruzi*, TcVPS23, endocytosis, secretion, vesicles

Palabras clave: *Trypanosoma cruzi*, TcVPS23, endocitosis, secreción, vesículas

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Epigenetics, phenotypic changes, and gene expression in *Trypanosoma cruzi*: a puzzle to be solved

Epigenética, cambios fenotípicos y expresión génica en *Trypanosoma cruzi*: un rompecabezas por resolver

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Genomic organization and gene expression regulation in trypanosomes are remarkable because protein-coding genes are organized into codirectional gene clusters with unrelated functions. Moreover, there is no dedicated promoter for each gene, resulting in polycistronic gene transcription, with posttranscriptional control playing a major role. Nonetheless, these parasites harbor epigenetic modifications at critical regulatory genome features that dynamically change among parasite stages. Here, we will discuss the impact of chromatin changes in a scenario commanded by posttranscriptional control exploring the parasite *Trypanosoma cruzi* and its differentiation program using epigenomic (FAIRE-seq, MNase-seq, Chip-seq and Hi-C data) and transcriptomic (mature and nascent RNA-seq) approaches. The integration of FAIRE and MNase-seq data, two complementary epigenomic approaches, enabled us to identify differences in *T. cruzi* genome compartments, putative transcriptional start regions, and virulence factors. In addition, we also detected a developmental chromatin regulation at tRNA loci (tDNA), which seems to be linked to the translation regulatory mechanism required for parasite differentiation. We are currently mapping the exact moment where the tDNA loci close during metacyclogenesis and its association with tRNAs expression. tDNA locus is also enriched in the histone H2B variant (H2B.V). This latter is also mainly located at dSSSrs and between the core and disruptive genome compartments. Strikingly, a positive correlation was observed between active chromatin and steady-state and nascent transcription levels. Taken together, our results indicate that chromatin changes reflect the unusual gene expression regulation of trypanosomes and the differences among parasite developmental stages, even in the context of a lack of canonical transcriptional control of protein-coding genes.

Supported by: FAPESP, Serrapilheira and CAPES.

Keywords: *Trypanosoma cruzi*, epigenetic, phenotypic changes, gene expression

Palabras clave: *Trypanosoma cruzi*, epigenética, cambios fenotípicos, expresión génica

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Uncovering kinetoplastid-specific components of a super-interactome supporting mRNA export in trypanosomes

Descubrimiento de componentes específicos de cinetoplástidos de un súper interactoma que apoya la exportación de ARNm en tripanosomas

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The nucleocytoplasmic RNA export is an essential pathway for gene expression regulation in eukaryotic cells, but it is still poorly understood in protozoan parasites. In trypanosomatids, transcription is polycistronic and all mRNAs are processed by *trans*-splicing, with export mediated by noncanonical mechanisms. Few orthologs of proteins involved in mRNA export in higher eukaryotes are detectable in trypanosome genomes. We previously described two conserved components of the mRNA export pathway in *T. cruzi*: orthologs of Sub2, a component of the TREX complex, and eIF4AIII (previously Hel45), a core component of the exon junction complex (EJC). Then, we searched for protein interactors of both proteins using cryomilling and mass spectrometry. Significant overlap between TcSub2 and TceIF4AIII-interacting protein cohorts suggests that both proteins associate with similar machinery. The analyses of the TcSub2 and eIF4AIII interactomes identified several interactions with conserved core components of the EJC and uncovered proteins specific to trypanosomatids, named as TcFOP, TcAPI5, TcNTF2L and TcHYP. Additional immunoisolations of the kinetoplastid-specific proteins both validated and extended the superinteractome, showing the presence of proteins involved in the processing, export, quality control and translation of mRNA. Through immunoprecipitation assays coupled with mass spectrometry, we uncover the high connectivity between multiple aspects of mRNA metabolism and kinetoplastid-specific components that create a unique amalgam to support trypanosome mRNA maturation.

Keywords: mRNA export, proteomics, gene expression, trypanosomes

Palabras clave: exportación del ARNm, proteómica, expresión génica, tripanosomas

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Alba-domain protein family of *Trypanosoma cruzi*: An approach to its functions

Aproximación a la función de la familia de proteínas Alba en *Trypanosoma cruzi*

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Trypanosoma cruzi is a parasite characterized by a high genetic diversity and a complex biological cycle that

alternates between two different hosts in which it is subjected to multiple cellular stresses such as thermal or nutritional ones. Therefore, it requires a suitable and fine gene regulation control. In trypanosomatids, gene regulation occurs mainly at the post-transcriptional level. The expression of mRNAs is attained via regulation of their processing, transport, stability, and translation, mediated by the recognition of *cis* elements by RNA-binding proteins (RBPs). Alba-domain proteins are a conserved DNA/RNA-binding proteins family found in Archaea and Eukarya domains. This superfamily of proteins has been split into three major branches that include Archaea proteins and two eukaryotic-specific branches: the Rpp25/Mdp2 group and the Rpp20/Pop7 group. In *Leishmania infantum* and *Trypanosoma brucei* the Alba-proteins of Rpp25/Mdp2 group are involved in translation repression and in the regulation of gene expression during protozoan development. In *T. cruzi*, there is evidence that TcAlba30 protein may aggregate into cytoplasmic foci in parasites submitted to nutritional stress. This protein interacts with β-amastin390 mRNA and its overexpression resulted in the decrease of amastin mRNA levels, suggesting that this protein regulates the β-amastin390 expression. However, the role of other proteins of Rpp25/Mdp2 group and the proteins of Rpp20/Pop7 group identified in the genome of *T. cruzi* are still poorly understood. Therefore, the aim of the present work was to characterize the Alba protein family of *T. cruzi* and its RNA-binding capacity as an approach to uncover its functionality. *In silico* analyzes revealed that the genome of *T. cruzi* strains Brazil and Dm28c (TcI), Y (TcII), CL Brener Esmeraldo-like and TCC (TcVI) has four Alba genes, two for Rpp20/Pop7 group (TcAlba1 and TcAlba2) and two for Rpp25/Mdp2 group (TcAlba30 and TcAlba40). These genes code for highly conserved proteins in *T. cruzi*, but also among other Trypanosomatidae family members. The four proteins have physicochemical and structural characteristics compatible with RBPs. Particularly, the TcAlba30 and TcAlba40 proteins bind *in silico* to two RNA motifs: the Musashi binding element (MBE) and the Sxl binding site (SBS). *In vitro* interaction RNA-Protein assays using a representative of each Alba-family group (TcAlba2 and TcAlba40) showed that TcAlba40 protein, but not the TcAlba2, directly interacted with the 5' UTR region of the *kLYT1* transcript, where a MBE element is found. Likewise, transcription and translation expression of each protein were analyzed in the three *T. cruzi* stages, and the results showed that TcAlba2 and TcAlba40 mRNA are expressed in all stages. Nevertheless, expression of both genes is higher in the trypomastigote form compared to the epimastigote and amastigote forms, being approximately 2-fold for TcAlba2 and 3-fold for TcAlba40. Also, TcAlba proteins family were detected in all three stages, suggesting a continuous expression throughout *T. cruzi*'s life cycle. TcAlba1/2 were constitutively expressed, whereas TcAlba30/40 were mainly expressed in the parasite's replicative stages. Our results suggesting that TcAlba30/40 proteins from Rpp25/Mdp2 group are RBPs and them function may be related to biological processes associated with stages of development of *T. cruzi*.

Keywords: Alba-domain proteins, RNA motifs, RNA-binding proteins, *T. cruzi*

Palabras clave: proteínas Alba, motivos ARN, proteínas de unión a ARN, *T. cruzi*

Transcriptional changes during *Trypanosoma cruzi* metacyclogenesis

Cambios transcripcionales durante la metaciclogénesis de *Trypanosoma cruzi*

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During its life cycle, *Trypanosoma cruzi* undergoes different modifications and one of them is the change from epimastigotes to metacyclic trypomastigotes, known as metacyclogenesis. This differentiation stage is

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essential because the parasite acquires the form to infect humans and develop the disease. In this work, the transcriptome of metacyclic trypomastigotes and epimastigotes was analyzed to identify differentially expressed genes that may be involved in metacyclogenesis. Briefly, *in vitro* induction of metacyclogenesis was performed to obtain metacyclic trypomastigotes from a culture of epimastigotes. RNA-seq in triplicate from metacyclic trypomastigotes and epimastigotes was performed using Illumina/NovaSeq PE150. We implemented a genome reference-based approach using the Dm28c strain to assemble the transcriptome and differential gene expression analysis was done using DESEq2. Gene ontology analysis was performed using Tritrypdb. According to the RNA-seq results, we identified 17,120 total genes. 513 genes were differentially expressed in metacyclic trypomastigotes, 221 were up-regulated and 292 down-regulated. The analysis showed that these genes are related to relevant biological processes in metacyclogenesis. Within these processes, we found that most of the genes associated with infectivity and gene expression regulation were up-regulated in metacyclic trypomastigotes. Instead, the genes involved in cell division, DNA replication, differentiation, cytoskeleton, and metabolism, were mainly down-regulated. The results obtained in this work generate new knowledge about the biology of *T. cruzi*, applied to the understanding of differentiation and parasite-host interaction. In the long term, these genes could be used as potential therapeutic targets in the design of new drugs for Chagas disease.

Keywords: *T. cruzi*, metacyclogenesis, differentiation, transcriptome, RNA-Seq

Palabras clave: *T. cruzi*, metaciclogénesis, diferenciación, transcriptoma, ARN-Seq

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Replication origins in *Trypanosoma cruzi*: genome location and possible epigenetic regulation

Orígenes de replicación en *Trypanosoma cruzi*: ubicación en el genoma y posible regulación epigenética

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DNA replication must be closely controlled to ensure reliable maintenance of the genome in daughter cells. On the other hand, it is a process that can result in mutations. The balance between a careful process and an error-prone one results in higher or lower genetic variability. In *Trypanosoma cruzi*, the etiological agent of Chagas disease, DNA replication is tightly regulated since it is activated or deactivated during its life cycle. Moreover, DNA replication might contribute for the genetic variability, contributing for the success of the infection in the mammalian host. In a previously work, using MFA-seq methodology, we have been showed the presence of DNA replication origins within genes of the DGF-1 family, functional and pseudogenes predicted to encode surface proteins that might contribute to cellular invasion and/or escape from immunity. The presence of origins inside genes is a peculiarity of *T. cruzi* and made us hypothesize that it could contribute for replicative stress in these regions due the frontal collision between transcription and replication machineries (since firing of origins implies in forks emerging in both directions inside the genes and thus one of them will frontal collide with transcription). These replicative stresses might result in increased single-stranded DNA gaps and DNA double strand break (DSB) formation. Then, we also hypothesized that these replicative stresses could be the cause of homologous recombination favored in subtelomeric regions that are enriched by DGF-1 and also trans-sialidase genes. Here, using a single-molecule technique (nanopore-based sequencing followed by D-Nascent analysis) we were able to

detect replication origins fired in every single molecule and therefore we could detect facultative origins and compare them with constitutive origins (fired in many cells of a population) detected by MFA-seq. Using this approach, we could detect origins in other multigenic families besides DGF-1, strongly suggesting that in *T. cruzi* multigenic families contribute with DNA replication and that the presence of origins in these regions might indeed contribute for the genetic variability of these families. In addition, we co-related the presence of origins with the chromatin organization and our data point to a possible epigenetic control of replication firing, evidencing an involvement of chromatin organization with DNA replication in this parasite.

Keywords: Replication, replication origins, *Trypanosoma cruzi*, epigenetic

Palabras clave: Replicación, orígenes de replicación, *Trypanosoma cruzi*, epigenética

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Dynamics of the *Trypanosoma cruzi* genome

Dinámica del genoma de *Trypanosoma cruzi*

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The *Trypanosoma cruzi* parasite is able to invade almost any cell type, circulate freely in the blood or extracellular matrix, pass through the digestive tract of its insect vector and survive after being eliminated in the feces. It is also capable of infecting many mammals, including humans. This versatility of invasion and infection gives this parasite a virtually unique characteristic when compared to other pathogens. To cope with these characteristics requires a great adaptive capacity, and a fine regulation of gene expression, to allow adaptation to such different environments. This seems to go in the opposite direction of what is usually known about these parasitic protozoa, that they do not present the classical eukaryotic gene regulation mechanisms, and that their tendency is to transcribe most of the open reading frames, proceeding later to a post-transcriptional regulation. The surface envelope of *T. cruzi*, which is in a certain way its "letter of introduction" to the different cell types and/or immune systems they face, may constitute one of the explanations for the broad spectrum of infective capacity of these parasites. This surface is mainly constituted by three protein families encoded by hundreds of genes: mucin-like proteins (MUC), transialidases (TS) and mucin-associated proteins (MASP). They are GPI-anchored, with MUC and TS having been extensively studied, while MASP were discovered more recently, when the genome sequence of *T. cruzi* was first determined. An additional feature is that their genes are in the same regions of the genome. Thus, the *T. cruzi* genome consists of a core compartment (CC) with a high degree of synteny with the rest of the trypanosomatids (*Leishmania* spp., African trypanosomes), and a disruptive compartment (DC) where synteny with these species is lost. There are disruptive chromosomes (DC>90%), core chromosomes (CC>90%) and mixed chromosomes. Analyzing the steady state levels of mRNA we see that CC presents high and medium levels of expression -independently of the stage- and DC presents low levels of expression, also in all stages. On the other hand, the analysis of differentially expressed genes shows that in the CC they are distributed similarly in the different stages, while the CR shows highly significant differential expression in the trypomastigote stage, indicating that it is a genome specialized in the host-pathogen interaction. These different expression characteristics between compartments are due to epigenetic mechanisms and chromatin organization: chromosomal interactions, chromatin condensation,

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nucleosome positioning and methylation patterns, which seem to be determinant in the properties of each compartment.

Keywords: *Trypanosoma cruzi*, genomics, transcriptomcs, epigenetics

Palabras clave: *Trypanosoma cruzi*, genoma, transcriptoma, epigenética

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Nuclearly encoded mitochondrial genes and their role in the adaptation to mechanical transmission in African trypanosomes

Rol de los genes mitocondriales codificados en el núcleo en la adaptación de los tripanosomas africanos a la vida anaeróbica

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African trypanosomiasis comprises a group of diseases caused by various species of Trypanosoma of the Salivaria group, which affect both humans (sleeping sickness) and domestic and wild animals (Nagana). *Trypanosoma vivax*, the main cause of Nagana, is naturally vectored by the tsetse fly, whose distribution is restricted to the sub-Saharan zone of the African continent. In the proboscis of these insects, *T. vivax* undergoes a stage of its life cycle (epimastigote) of rapid cell division. During this replicative phase, trypanosomes need to carry out oxidative phosphorylation, due to the lack of nutrients in this environment. In contrast, in the stage of the life cycle that occurs in mammalian blood (rich in glucose), glycolysis is the almost exclusive source of energy supply. Thus, the respiratory chain plays a key role exclusively during passage through the insect vector. *T. vixax* was introduced to the American continent several centuries ago, where due to the absence of its natural vector (the tsetse fly), these parasites are transmitted by several species of hematophagous flies (Tabanidae and Stomoxys). These flies function as exclusively mechanical vectors (similar to an "infected needle"), so the parasite does not go through the replicative epimastigote phase that depends on mitochondrial respiration. This absence of respiratory requirement led us to propose that the mitochondrial genome of *T. vivax*, which encodes some protein subunits of the respiratory chain, should have undergone changes in response to the new lifestyle based on mechanical transmission. Analysis of the mitochondrial genomes of Venezuelan and one African strain (Y486) allowed us to identify several loss-of-function mutations (reading frame shifts). The mitochondrial editing system of these trypanosomes was drastically reduced in American *T. vivax*, with loss of guide RNAs linked to most of the mitochondrial genes that require such modifications. Expanding the range of American strains, we observed that most likely the incursion into America occurred several times, and in each of these independently occurred degradation of the coding capacity of the mitochondrial genomes. We then analyzed the adaptation to mechanical transmission of mitochondrial genes encoded in the nucleus (about 700 genes) and it is to be expected that many of these genes are changing in response to this drastic "environmental" modification. This same process analyzed in several strains of *T. vivax* that have acquired adaptation to mechanical transmission independently, as well as in *T. evansi*, offers the opportunity to study an adaptive process occurring simultaneously and independently in several lineages. Another affected system is the variable surface protein (VSG) coding system, associated with antigenic variation, since mechanical transmission implies not going through the phase in which parasites perform recombination (epimastigote). This surely affects the capacity to generate variability and has promoted important changes, which is reflected

in the fact that some American strains present a substantial reduction of genomic regions encoding VSG genes.

Keywords: *Trypanosoma vivax*, mechanical transmission, editing

Palabras clave: *Trypanosoma vivax*, transmisión mecánica, editing

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Deciphering the structure of *Trypanosoma cruzi* nuclear multiprotein complexes

Descifrando la estructura de complejos multiproteicos nucleares de *Trypanosoma cruzi*

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Chromatin structure is maintained by a series of multiprotein complexes that regulate DNA transcription, replication and repair. Recently, different components of these complexes have been identified by immunoprecipitation and proteomic analyses. However, given the low sequence identity it is not straightforward to identify some components by orthology methods or to predict the interactions between them. By an artificial intelligence approach using Rosetta-Fold and Alpha-Fold2 we were able to predict the structure of two subcomplexes containing the bromodomain proteins BDF5 and BDF6. The predicted interactions were corroborated by double hybrid assays in yeast and a working pipeline was established that will allow the prediction of larger complexes. BDF5 is essential and would function as a general regulator of transcription, in a complex with three bromodomains and Histone Acetyl Transferase activity. On the other hand, BDF6 is part of a TinTin-like complex, a subcomponent of the NuA4 complex, and is essential for trypomastigote infectivity and the development of intracellular amastigotes.

Keywords: *Trypanosoma cruzi*, multiprotein complexes, BDF5, BDF6

Palabras clave: *Trypanosoma cruzi*, complejos multiproteicos, BDF5, BDF6

Origin and evolution of Eukaryotes Telomeres: the organization *Leishmania* and *Trypanosoma cruzi* telomeres

Origen y Evolución de los Telómeros: organización de los telómeros en *Leishmania* y *Trypanosoma cruzi*

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Very early in the evolution of eukaryotic organisms, linear chromosomes appeared and with them arose the need to protect their ends to avoid being treated as breaks or accidents, and to solve the problem of the culmination of the replication of the delayed chain (lagging strand). The appearance of the telomeric structure (telomeres and associated proteins) served to attack the first problem, while the second was solved with the telomerase enzyme acting in concert with the proteins associated with telomeres. The region between the first autosomal gene and the telomere is called subtelomere, which is characterized by being genetically unstable, allowing homologous and heterologous interchromosomal recombination without compromising the synteny of the rest of the chromosome. In some organisms such as *Trypanosoma brucei, Plasmodium falciparum*, and *Saccaromyces cereviseae* these regions play a crucial role in surface antigenic variation or selective gene silencing. In our studies we have found a very particular telomeric structure in *Leishmania major* which evokes a possible telomerase-independent mechanism, and a complex subtelomere formed by sequence blocks. Whereas in *Trypanosoma cruzi* there may be coding genes (and their pseudogenes) very close to the telomere mostly belonging to multigene families such as RHS, DGF.1, Transialidases and abundant non-LTR retrotransposons. Our experiments have demonstrated the ability of these subtelomeres to make non-homologous interchromosomal recombination and this fact added to the great diversity present in these subtelomeres has led us to postulate that these subtelomeres are sites for the generation of genetic variability to confer an adaptive advantage to these parasites. In this lecture we will review the telomeric structure and a comparative analysis between the telomeres of Trypanosomatids and the rest of eukaryotic organisms.

Keywords: telomeres, evolution, parasites, genome dynamics, *Trypanosoma cruzi, Leishmania*

Palabras clave: telómeros, evolución, parásitos, dinámica genómica *Trypanosoma cruzi, Leishmania*

Signaling and adaptive mechanisms in trypanosomatids: a possible Achilles heel in the fight against trypanosomiasis

Señalización y mecanismos adaptativos en tripanosomátidos: un posible talón de Aquiles en la lucha contra la tripanosomiasis

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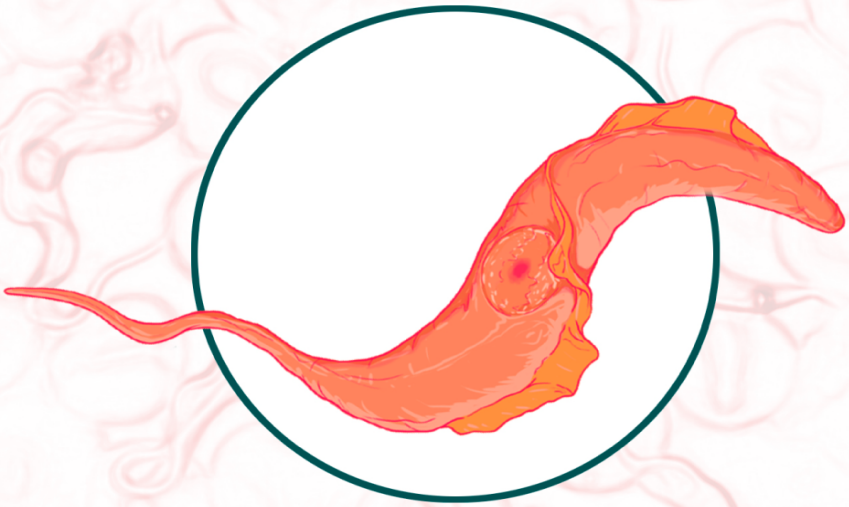
Trypanosoma cruzi is the etiological agent of Chagas disease or American trypanosomiasis, a neglected tropical condition historically endemic to Latin America that, due to human migration, has spread beyond its traditional boundaries. It is now estimated that 6 to 8 million people suffer from this disease worldwide. *T. cruzi* is a protozoan parasite that presents a complex life cycle, during which it must cope with sudden changes in the environment to which it must respond immediately to survive. Cyclic nucleotide phosphodiesterases have been implicated in the proliferation, differentiation and osmotic regulation of trypanosomatids. In some trypanosomatid species they have been validated as molecular targets for the development of new therapeutic agents. It has also been shown that many inhibitors of mammalian PDEs lack an inhibitory effect on trypanosomatid enzymes, indicating that there are differences in the substrate specificity of parasite and host enzymes, foreshadowing the possibility of identifying compounds that selectively inhibit the parasite enzyme. The limited possibilities to edit *T. cruzi* genome have made it difficult to carry out knockout and knockdown

experiments, and drug target validation has been limited to biochemical evaluations. Recently, the development of CRISPR/Cas9 as a tool for genomic edition brought a new perspective to the study of *Trypanosoma cruzi*. According to the most often applied protocols, epimastigotes are co-transfected with a single plasmid bearing both the gene for Cas9-GFP expression and a sequence to be translated into a single guide RNA (sgRNA), jointly with a lineal donor DNA encompassing a selection marker flanked by sequences homologous to the target gene. Here, we tested an alternative approach for the generation of Phosphodiesterase (PDE) knockout parasites. We obtained epimastigotes from Tul II strain stably expressing Cas9-GFP in the nucleus in all parasite stages, with no detrimental effects on epimastigote growth or differentiation nor on trypomastigote infection capability. These Cas9-GFP epimastigotes were co-transfected with the sgRNA + DNA donor pair, according to the intended gene target. sgRNA were obtained by *in vitro* transcription using a template DNA bearing the specific + scaffold sequence under a T7 promoter. To obtain the donor DNA we designed a pre-donor formed by a sequence including several restriction enzyme recognition sites flanked by 30-bp arms homologous to the sequence adjacent sgRNA annealing target. This pre-donor allowed to easily generate a variety of donor DNAs by cloning alternative selection markers. DNA extracts (boiling-preps) from 4-day post-transfection cultures were evaluated by PCR using mixed primer pairs: while one of the primers annealed to the target gene, the second primer annealed to a sequence in the donor DNA, allowing assessment of its correct insertion in the gene of interest. Advantages of this take on CRISPR/Cas9 edition include its versatility for choosing and switching between alternative selection markers and a quick and affordable generation of the components of the system and analysis of the transfected cultures, while possibly facilitating complementation assays on the KO lines. Finally, our results indicate that loss of PDEs (even partial) is incompatible with normal parasite function/parasite viability.

Keywords: *Trypanosoma cruzi*, Chagas disease, signal transduction, phosphodiesterases, CRISPR, drug discovery

Palabras clave: *Trypanosoma cruzi*, enfermedad de Chagas, transducción de señales, fosfodiesterasas, CRISPR, descubrimiento de drogas



ABSTRACTS POSTERS
IX SYMPOSIUM ON MOLECULAR BIOLOGY OF CHAGAS DISEASE

RESÚMENES CARTELES
IX SIMPOSIO DE BIOLOGÍA MOLECULAR DE LA ENFERMEDAD DE CHAGAS

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Understanding nuclear architecture of *Trypanosoma cruzi*: Topological Associated Domains (TADs) and the role of tDNAs in TAD boundaries

Entendiendo la arquitectura nuclear de *Trypanosoma cruzi*: Dominios Topológicamente Asociados (TADs) y el papel de los tDNAs en los límites de TADs

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The tridimensional chromatin organization is being recently studied through chromatin conformation capture (3C) techniques. Topologically associated domains (TADs), revealed by 3C-based approaches, are sub-megabase divisions of chromosomes that are physically related by higher contact with each other than with their neighbors. The boundaries of TADs are marked by a decrease in DNA-DNA interaction, higher chromatin accessibility, and enrichment of DNA motifs acting as binding sites for regulatory proteins. Understanding these 3D genome organization aspects is highly relevant for gene expression and regulation. Concerned about the study of chromatin packing in *Trypanosoma cruzi*, our group encountered genes of the core compartment (conserved genes) in open chromatin regions while disruptive/both genes (multigenic families) in closed chromatin sites. We also showed that dynamic nucleosomes are more prevalent in the replicative than non-replicative forms, which possibly justifies the lower level of global transcription in the non-replicative stages. In the present work, we aim to comprehend to which extent these behaviors can be ruled by the formation of TADs, by the positioning of genomic regulatory features on its boundaries, and how it impacts gene expression since regulation mainly occurs at a post-transcriptional level. We are using high-throughput chromosome conformation capture (HiC) data of *T. cruzi* Brazil A4. Through the HiCExplorer pipeline, we generated Hi-C contact matrices of 20 to 2 Kb and compared TADs formation across resolutions. Results revealed that the lesser the matrix resolution, the lesser the TAD amount. We classified and ranked the chromosomes according to CORE, DISRUPTIVE, and BOTH genomic compartments, and we developed bash scripts to determine the percentage of genes from each compartment per TADs. Of these, 51% account for mixed TADs (containing genes from more than one compartment), and 49% account for pure TADs (genes of only one compartment). Among the pure ranked TADs, those with only CORE genes ranged from 4 to 178 Kb in length, and those with only DISRUPTIVE or only BOTH ranged from 4 to 28 Kb in length. This data suggests that DISRUPTIVE/BOTH genes are more packed than CORE genes or that genomic regions composed only by DISRUPTIVE/BOTH genes are shorter in length than CORE regions so that smaller TADs are a consequence. Next, to gain insights into genomic features at the TAD borders, we calculated the distance between the middle of TAD boundaries and their surrounding genes. We found 70% (47/68) of tDNAs at the TAD boundaries, what may reflect a strategy to facilitate its accessibility due to being essential for translation. We integrated RNA-seq and FAIRE-seq available data, and its coverage throughout the *T. cruzi* genome reinforced higher transcription levels, and also open chromatin to TADs harboring tDNA genes. In summary, our data show important aspects of the tridimensional organization of the *T. cruzi* genome and reveal tDNAs preference to occupy TAD boundaries.

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Keywords: chromosome conformation capture, topological associated domains, tDNAs, *Trypanosoma cruzi*

Palabras clave: captura de la conformación de la cromatina, dominios topológicamente asociados, tDNAs, *Trypanosoma cruzi*

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Combining AlphaFold2-multimer, interactomic datasets and yeast two-hybrid to survey divergent trypanosomatid complexes

Combinando AlphaFold2-multimer, datos interactómicos y doble híbridos de levadura para estudiar complejos divergentes en tripanosomátidos

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Little is known about acetylation-regulated signaling pathways in early branching organisms such as trypanosomatids. In part, this is because, during bioinformatic analysis of the proteins involved, serious limitations arise when confronted with their highly divergent sequences. Moreover, they would not act in isolation, but in complexes that assemble to fulfill one or multiple functions, increasing the complexity of the system. Recently, in an effort to increase knowledge in this area, several interactomic data sets of *Trypanosoma brucei* acetyl-lysine readers, writers and erasers were published. On the other hand, molecular biologists have received "new eyes" with the public release of AlphaFold2 and AlphaFold2-multimer, which allowed them to reveal with a high degree of confidence the shape of proteins and their potential mode of interaction. These tertiary and quaternary structure predictions should be used as a tool with high predictive value when inferring protein function. In this work, we show different examples that combine logical correlations between experiments, interactomic datasets and in silico predictions of AlphaFold2-multimer with the aim of validating the models predicted by the latter. Among the cases analyzed are the complexes formed by the distant homologs of the *T. cruzi* MRG (MORF4 Related Gene) and BET (Bromo and Extra-Terminal) proteins, all proteins involved in acetylation signaling pathways described in other eukaryotes. Our results led us to identify a structurally conserved complex that is orthologous to the human and yeast TINTIN complex, a more divergent complex formed by the MRG domain of BDF5 (Bromodomain Factor 5) and an unexpected complex formed by the bromodomain factors BDF4 and BDF1, which together reconstruct a protein with two bromodomains and an Extra-Terminal domain; an architecture typically found in human and yeast BET proteins.

Keywords: protein complexes, AlphaFold2, interaction prediction, bromodomains, acetylations

Palabras clave: complejos proteicos, AlphaFold2, predicción de interacción, bromodominios, acetilaciones

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Glucokinase overexpression affects glucose uptake rate and growth of *Trypanosoma cruzi* epimastigotes (EP pLEW13/pTcINDEX-TcGlcK)

La sobreexpresión de la glucoquinasa afecta la velocidad de consumo de la glucosa y el crecimiento de epimastigotes de *Trypanosoma cruzi* (EP pLEW13/pTcINDEX-TcGlcK)

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Trypanosoma cruzi, the etiological agent of Chagas disease, contains two enzymes that phosphorylate glucose,

hexokinase (HK) and glucokinase (GlcK), both located in the glycosome. These enzymes present significant differences in the Km for glucose (45 μM for HK and 1000 μM for GlcK), HK has 20-fold higher affinity for glucose than GlcK. Furthermore, HK is more abundant than GlcK in glycosomes, being the concentration of HK 9-fold higher than GlcK. The two enzymes differ in the selectivity for the anomers of the D-Glucose, GlcK has a moderate preference for the β anomer, whereas HK has for the α-anomer. Based on these differences, it has been suggested that the main function of GlcK is not glycolytic. In this work, recombinant lines of *T. cruzi* epimastigotes overexpressing GlcK in the presence of tetracycline (EP pLEW13/pTcINDEX-TcGlcK) were obtained. Induction of GlcK overexpression in recombinant epimastigotes affects the growth, with a 2.5-fold longer generation time of the induced parasites compared to the uninduced culture, which was 0.953 days. However, the glucose consumption rate of epimastigotes with induced overexpression of GlcK was approximately 2-fold higher than the uninduced epimastigotes (12.3 and 5.64 nmoles glucose. hour^{-1}. 1 x 10^6 cells^{-1}, respectively). We also assessed whether the GlcK overexpression phenotype was dependent on the source of energy and carbon present in the culture medium. When recombinant epimastigotes were grown in LIT medium with low glucose ($\approx$ 2 mM) and in LIT (low glucose) supplemented with L-proline (10 mM) or galactose (22.2 mM), in the presence (Tet$^+$) or absence (Tet$^-$) of tetracycline, the generation time (days) was always approximately 2-fold longer compared to the uninduced epimastigotes (Tet$^-$). In the case of the cultures in medium with low glucose, the generation time values were for Tet$^+$ 1.44 and for Tet$^-$ 0.781. In the same medium supplemented with L-proline, Tet$^+$ 1.63 and Tet$^-$ 0.972, or galactose, Tet$^+$ 1.85 and Tet$^-$ 0.763. These results indicated that overexpression of GlcK in glycosomes affects the growth of the parasite, regardless of the energy and carbon source present in the culture medium. A possible explanation for this phenotype is that the overexpression of GlcK affects the glycolytic flux, diverting a greater amount of β-D-glucose-6-phosphate to the pentose phosphate pathway, generating an imbalance of the ATP/ADP ratio in glycosomes with the subsequent collapse of the glycolysis.

Keywords: glucokinase, glycosome, overexpression, glucose-6-phosphate metabolism, pTcINDEX, *Trypanosoma cruzi*

Palabras clave: glucoquinasa, glicosoma, sobreexpresión, metabolismo de la glucosa-6-fosfato, pTcINDEX y *Trypanosoma cruzi*

Efficacy and selectivity of new triazole analogues against *Trypanosoma cruzi*

Eficacia y selectividad de nuevos análogos de triazol frente a *Trypanosoma cruzi*

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Although centuries old, Chagas disease (CD) remains neglected and affects 6-7 million individuals worldwide. Today, CD is globalized due to the population flow between different continents, making it a worldwide public health problem. CD is often asymptomatic in the acute phase and characterized by patent parasitemia. Chronic Chagas' cardiomyopathy is the main clinical manifestation of the disease, with high rates of sudden death. The clinical treatment of CD, based on 2 nitroderivatives: Benznidazole (Bz) and Nifurtimox (Nif),

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has important limitations and serious adverse effects. Clinical trials have reported the therapeutic failure of promising candidates and demonstrated that Bz is unable to prevent the evolution of chronic chagasic cardiomyopathy, making the search for new effective and safe drugs urgent. The triazole nucleus is considered a privileged structure in medicinal chemistry and has broad biological activity, including activity against *Trypanosoma cruzi*. In this study, new triazole derivatives were evaluated for efficacy and selectivity against *T. cruzi*. Computational analyses and phenotypic screening assays were employed in the identification of triazole analogues effective against intracellular trypomastigotes and amastigotes forms of *T. cruzi*. Analyses of physicochemical properties revealed that the new analogues do not violate Lipinski's rule and fulfill the main criteria for druglikeness. The new triazole derivatives showed low toxicity in VERO cells (CC_{50} > 200 μM), even after 72h of treatment. Notably, 3 triazole analogues with efficacy against intracellular amastigotes similar to Bz, but potentially active against trypomastigotes. Promising candidates were also evaluated for their pharmacokinetic profile. ADMET predictions (absorption, distribution, metabolism, excretion and toxicity) indicate that the compounds are permeable to the blood-brain barrier, human intestinal epithelium and Caco-2 cells. The triazole analogues have predicted mitochondrial localization, are cytochrome P450 inhibitors, and have no predicted genotoxicity and mutagenicity. These preliminary data stimulate the analysis of more predictive biomodels of translational efficacy, aiming at the identification of new drugs with the possibility to advance the pipeline of preclinical and clinical trials for Chagas disease therapy.

Keywords: *Trypanosoma cruzi*, chemotherapy, triazole, Chagas disease

Palabras clave: *Trypanosoma cruzi*, quimioterapia, triazol, enfermedad de Chagas

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Biological activity of 5-(1-aryl-1*H*-pyrazol-4-yl)-1,3,4-thiadiazol-2-amine derivatives against *Trypanosoma cruzi*

Actividad biológica de los derivados de 5-(1-aril-1*H*-pirazol-4-il)-1,3,4-tiadiazol-2-amina contra *Trypanosoma cruzi*

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Chagas disease (CD), caused by the protozoan *Trypanosoma cruzi*, is a neglected disease with a high morbidity and mortality rate. CD affects 6-7 million individuals worldwide and is now a global public health problem. Clinical treatment, based on nitroderivatives introduced more than 50 years ago, has important limitations. Benznidazole (Bz) and nifurtimox (Nif) are partially active in the acute phase and have low efficacy in the chronic phase of the disease, in addition to having serious adverse effects responsible for treatment discontinuation. Bz, the reference drug, is not able to prevent the evolution of chronic chagasic cardiomyopathy, an important clinical manifestation of CD. In this study, the compound 5-amino-1-aryl-4-(4,5-dihydro-1*H*-imidazol-2-yl)-1*H*-pyrazole, previously identified by our group as active against *T. cruzi*, was optimized by the addition of the pharmacophore group thiadiazole, based on a search in the ChEMBL database. A total of 24 new compounds, series 1 (**1a-l**) and 2 (**2a-l**), were synthesized and the derivatives evaluated for efficacy and selectivity against *T.*

cruzi. Computational tools, employed to evaluate the physicochemical properties of the new analogues, revealed that the pyrazole-thiadiazole derivatives do not violate Lipinski rules and have predicted good oral availability. Phenotypic screening results demonstrated good trypanocidal activity of 2 pyrazole-thiadiazole analogues with IC_{50} values near 15 μM for intracellular amastigotes and selectivity index (SI) >30. The derivatives of series 1 and 2 were not toxic in mammalian cells (CC_{50} >500 μM). These preliminary results highlight 2 pyrazole-thiadiazole analogues as promising candidates with the possibility of advancing preclinical *in vitro* assays. Predictive biomodels of translational efficacy, 3D culture model and *in vitro* parasitism recrudescence, will be evaluated. In-silico approaches will be employed for molecular target identification and the mechanism of action of promising compounds will be investigated. Thus, this study will contribute with new knowledge in the area of Chagas disease chemotherapy.

Keywords: *Trypanosoma cruzi*, quimioterapia, híbridos de pirazol-tiadiazol, enfermedad de Chagas

Palabras clave: *Trypanosoma cruzi*, chemotherapy, pyrazole-thiadiazole hybrids, Chagas disease

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Effect of pyrazol-benzoimidazole derivatives against *Trypanosoma cruzi*

Efecto de los derivados de pirazol-benzoimidazol contra *Trypanosoma cruzi*

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Chagas disease (CD), endemic in 21 countries in Latin America, is a neglected tropical disease (NTD), whose infectious agent is the protozoan *Trypanosoma cruzi*. Currently globalized, CD is a worldwide public health problem. This silent disease can progress to digestive (megaesophagus and megacolon), neurological, and cardiac manifestations. Clinical treatment remains based on 2 nitroderivatives, benznidazole (Bz) and nifurtimox (Nif), which have important limitations in efficacy and have severe side effects. The therapeutic failure of posaconazole, ravuconazole and fexinidazole in clinical trials highlight the urgency in identifying new safe and effective drugs in different stages of the disease. Thus, this study aims to evaluate the trypanocidal activity of pyrazole-benzimidazole hybrids (**1a-l**) in terms of their effect on intracellular trypomastigotes and amastigotes of *T. cruzi*. Computational approaches were initially employed aiming to evaluate the physicochemical and pharmacokinetic properties of the pyrazole-benzimidazole analogues. The Datawarrior software and the SwissADME program, available online, were used based on Lipinski, Ghose, Veber and Egan rules. Also, we evaluated the electrostatic and lipophilic potential of the pyrazole-benzimidazole analogues using the JMol and Molinspiration programs, respectively. Phenotypic screening was performed using *T. cruzi* Dm28c genetically engineered for luciferase expression and cytotoxicity assay performed by ATP quantification using CellTiter Glo. *In silico* analyses revealed that the pyrazole-benzimidazole derivatives show predicted good oral bioavailability and pharmacokinetics. The series of compounds consist of small molecules with low molecular weight (PM) 339.195, number of hydrogen donor atoms (HBD) equal to 1 and hydrogen acceptors (HBA) between 3-4, number of rotatable bonds between 2-3, polar surface area (TPSA) $\leq$ 46.5, and lipophilicity coefficient (cLogP) in the range of 2.07 to 3.83, fulfilling the requirements for druglikeness. Based on the lipophilic and molecular electrostatic potential, the analogues have predicted good interaction with the target, most notably compounds **1c**, **1d** and **1(f-k)**

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which have regions of high lipophilicity. The Boiled-egg plot revealed that the compounds have good absorption by the intestinal epithelium and cross the blood-brain barrier, suggesting good solubility and permeability. The analyzed compounds showed low cytotoxicity in Vero cells, reaching CC_{50} values $\geq$ 355 μM. Phenotypic screening revealed that the tested pyrazol-benzimidazole analogues are active against *T. cruzi*, but reach IC_{50} values higher than Bz, the reference drug. New pyrazole-benzimidazole analogues are being synthesized aiming to improve their efficacy against *T. cruzi*.

Keywords: *Trypanosoma cruzi*, chemotherapy, pyrazole-benzimidazole hybrids, Chagas disease

Palabras clave: *Trypanosoma cruzi*, quimioterapia, híbridos de pirazol-benzoimidazol, enfermedad de Chagas

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Relationship between chemical composition, botanical origin and antiparasitic activity of Costarican propolis against *Trypanosoma cruzi*

Determinación de la capacidad antiparasitaria contra *Trypanosoma cruzi* de propóleos costarricenses y su relación con su composición química y su origen botánico

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Chagas disease affects millions of people throughout Latin America; more than 300,000 new cases and 12,000 deaths are reported yearly. Due the high incidence, social and economic impact, the search for efficient, effective, and safe treatments for this disease has become a priority in the region. Based on previously published studies, it has been determined that some propolis extracts with polyisoprenylated benzophenones (BPI), such as nemorosone, have important trypanocidal activity. Furthermore, BPIs with activity against *Trypanosoma cruzi* can be found in floral resins of species of the genus *Clusia* in the southern region of Costa Rica. Therefore, this study proposes to evaluate *in vitro* and *in vivo* the antiparasitic effect of propolis containing BPI from the Southern Region of Costa Rica in the search for novel molecules that could be used as a potential treatment for chronic experimental trypanosomiasis. We hypothesize, that propolis is a better source for a wide range of concentrated BPIs than the original botanical source. For this study, 20 samples of propolis from the southern region of Costa Rica were taken from 10 randomly selected apiaries. The samples will be purified using medium pressure chromatographic techniques (MPLC) and the chemical composition will be determined by HPTLC (high-performance thin layer chromatography). At the *in vitro* level, we will work with the 3T3 cell line to evaluate the cytotoxicity of the compounds. The cytotoxicity and selectivity will be tested in the *T. cruzi* clone Dm28c. We also propose to validate the safety and efficacy of the selected compounds *in vivo* in C57B6/J mice.

Keywords: *Trypanosoma cruzi*, propolis, nemorosone, floral resins

Palabras clave: *Trypanosoma cruzi*, propóleo, nemorosona, resinas florales

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Activity of purified alkaloids from the plant of the genus *Hamelia* on *Trypanosoma cruzi in vitro* models of Chagas disease

Actividad de alcaloides purificados de la planta del género *Hamelia* sobre *Trypanosoma cruzi* en modelos *in vitro* de la enfermedad de Chagas

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In 2010, the World Health Organization (WHO) estimated that in Costa Rica there are approximately 7,000 people infected with *Trypanosoma cruzi*. These infections continue to increase year after year and in 2020, twenty new patients with Chagas disease were registered. Currently, benzimidazole and nifurtimox are the only drugs that exist to treat Chagas disease, however, these are not effective in chronic disease and are poorly tolerated by patients. Because of this, there is a need to find new drugs with antichagasic activity that cause fewer adverse effects in the patient and are more effective against chronic infection. For this purpose, secondary metabolites of plants are a valuable source for the search of compounds to reduce the severity of experimental and eventually clinical trypanosomiasis. Among these plants are the species of the genus *Hamelia*, which have attracted attention because they present secondary metabolites with antimicrobial, analgesic, anti-inflammatory and antiparasitic properties. Preliminary results have shown activity of tetracyclic oxindole alkaloids from *H. patens*, of which pteropodine and isorinchofylline were the most effective against intracellular amastigotes. In addition, it was determined that the absence of the E ring and the R conformations at C7 and C20 in an oxindole alkaloid provide specificity for the intracellular form of the parasite and high potency. Due to these particularities, the objective of this research is to analyze novel structures of tetracyclic oxindole alkaloids isolated from *H. xerocarpa* which are hypothesized to have high potency and high selectivity compared to human cell lines. The potency of the alkaloids will be evaluated in two life stages of *T. cruzi* strains Dm28c and ALF, the latter being isolated from *Triatoma dimidiata* in Heredia, Costa Rica. Cytotoxicity will also be evaluated in NIH/3T3 and VeroE6 cell lines, with the aim of identifying an active alkaloid compound with a higher selectivity index towards intracellular amastigotes.

Keywords: Chagas disease, *Trypanosoma cruzi*, *Hamelia*, alkaloids

Palabras clave: enfermedad de Chagas, *Trypanosoma cruzi*, *Hamelia*, alcaloides

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Identification of innate immune or biochemical factors in triatomines with lytic capacity against *Trypanosoma rangeli* and *Trypanosoma cruzi*

Identificación de factores inmunes o bioquímicos innatos en triatominos con capacidad lítica contra *Trypanosoma rangeli* y *Trypanosoma cruzi*

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Chagas disease vectors are modulators of trypanosomatid transmission. Their immune system comprises humoral and cellular responses. In LIPT, we detected lytic factors in foregut, hemolymph and salivary glands of *Rhodnius prolixus* against *T. rangeli* and *T. cruzi* genotypes. Comparative proteomic analyses between *R. prolixus* and *R. colombiensis* (vector without trypanolytic activity) have shown that these insects may have different mechanisms of innate immune activity. We set out to evaluate the differential effector mechanisms involved in the modulation of trypanosome transmission at the level of salivary glands and hemolymph of *R. prolixus* and *R. colombiensis*. Hemolymph was collected by cutting a leg of the insect and centrifuged at 14,000 rpm to collect the cell-free supernatant, 20 pairs of salivary glands were extracted and protein identification was performed by LC-MS/MS. Spectra were analyzed using MASCOT with a score less than $p<0.05$. Scaffold 4.0 validated proteins with an identity equal to or greater than 90%, with four peptides and three replicates. Proteins involved in immune response were filtered by functional association in the GO database. Relative quantification of proteins was performed by a label-free method using the normalized spectral abundance factor (NSAF). At the hemolymph level, we evaluated oxidative activity by measuring superoxide enzyme. We identified in hemolymph a greater number and diversity of proteins in *R. prolixus* compared to *R. colombiensis* with a total of 111 and 93 protein identifications, respectively, some with immune activity such as antimicrobial peptides, profenol oxidase and lipocalins were found. In salivary glands we also found a greater number and diversity of proteins in *R. prolixus* compared to *R. colombiensis* with a total of 1143 and 873 respectively, there was a greater number of nitrophorins, triabins and lipocalins in *R. prolixus*. Nitrophorins in *R. prolixus* presented higher relative abundance according to NSAF data, so additional RT-PCR analysis of their expression was performed. Nitrophorins NP1-NP4 and NP7 were detected in *R. prolixus*, however, in *R. colombiensis* only nitrophorins NP1 and NP2 were detected. These results are interesting considering that nitrophorins are expressed in saliva and act as immunity factors releasing nitric oxide (NO), NO reacts with other free radicals such as superoxide generating peroxynitrite and all these molecules have antibacterial and antiparasitic properties. On the other hand, the superoxide measurement assay reveals that there is greater activity of this radical in *R. prolixus* compared to *R. colombiensis*. We highlight in our results the action of these free radicals, such as superoxide and NO, which are part of the immunity of triatomines against trypanosomatids and the overexpression of nitrophorins and the presence of greater superoxide activity in *R. prolixus*, suggesting that these mechanisms could be involved in trypanolytic activity and complementing the action of innate immunity of other mechanisms such as the prophenoloxidase system, activity of antimicrobial peptides or the action of hemocytes.

Keywords: innate immunity, *R. prolixus*, lytic factor, nitrophorins, nitric oxide

Palabras clave: inmunidad innata, *R. prolixus*, *R. colombiensis*, factor lítico, nitroforinas, óxido nítrico

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Assembly of the genome of a Colombian strain of *Trypanosoma cruzi I* (TcI) from the department of Tolima as a tool for the analysis of genetic diversity and evolution

Ensamblaje del genoma de una cepa colombiana de *Trypanosoma cruzi* I (TcI) empleando lecturas largas procedente del departamento del Tolima como herramienta para el análisis de variabilidad genética y evolución

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Trypanosoma cruzi, the causative agent of Chagas disease, is a digenic parasite that affects about 7 million people and on average 14,000 die per year. *T. cruzi* has a wide genetic diversity and is divided into six DTUs; (TcI - TcVI), TcI is the most abundant DTU in Colombia and despite the advances in the study of *T. cruzi* haplotypes in the country, there is a great lack of knowledge about the genomics of this parasite since its genes are found in dispersed groups of tandem repeats. Most of the assemblies of *T. cruzi* I have been performed with 454, Sanger and Illumina technologies, but these assemblies are highly fragmented due to the complexity of the genome. According to the aforementioned, the present work aims to provide high quality genome assembly and gene annotation of a Colombian strain of *T. cruzi* I to analyze genetic variability within and between species of the family Trypanosomatidae. A *T. cruzi* strain characterized as TcI isolated from *Didelphis marsupialis* from the municipality of Coyaima-Tolima was used. High quality DNA was obtained using Gentra Puregene Kit and sequencing was performed using Pacific Bioscience (PacBio) HiFi technology. Haploid assemblies of 120 and 313 contigs were obtained with Hifiasm and Flye, respectively. The strain has a diploid genome of approximately 90 Mb (∼40 Mb haploid), and about 14,000 genes. Using different tools (BUSCO, Canu, Hifiasm total and NGSEP ploidy 1 and 2) we report for the first time an assembly in which the information of the two copies of each chromosome is separated. Mapping of the raw PacBio reads to different TcI and TcII reference assemblies was performed. Variant calling was done with NGSEPlinux_4.1.0 software where a low number of variants of the aligned reads to the assemblies made from the same reads is reported. Based on SNP analysis, a dendogram was performed with TcI genomes isolated from Colombia, where 2 different groups were observed, at one end were positioned all parasite strains isolated from a patient with HIV and cardiomyopathy, at the other end were positioned all parasite strains isolated from an acute Chagasic patient infected by oral transmission and our assembly. Paralogous genes in the *T. cruzi* genome (Flye assembly) and orthologous genes present in 5 different species of the family Trypanosomatidae including our *T. cruzi* assembly were determined using OrthoFinder. It is concluded that new sequencing technologies help to improve the quality of the *T. cruzi* genome and to understand the complexity of this parasite, the importance of using long read technologies in genomes with high complexity such as *T. cruzi* is demonstrated, to understand the variability within the DTU TcI and this work proposes this strain as a reference genome for *T. cruzi* in Colombia.

Keywords: *Trypanosoma cruzi*, mixed infections, 454 technologies, Sanger, Illumina, Pacific Bioscience (PacBio), reference genome

Palabras clave: *Trypanosoma cruzi*, infecciones mixtas, tecnologías 454, Sanger, Illumina, Pacific Bioscience (PacBio), genoma de referencia

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Obtaining the mitochondrial genome (kDNA) of a strain of *Trypanosoma cruzi* from endemic areas of Chagas disease in the department of Tolima (Colombia) for genomic and evolutionary studies

Obtención del genoma mitocondrial (kDNA) de una cepa de *Trypanosoma cruzi* procedente de zonas endémicas de la enfermedad de Chagas en el departamento de Tolima (Colombia) para estudios genómicos y evolutivos

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Chagas disease is caused by the protozoan parasite *Trypanosoma cruzi*, class Kinetoplastea, family Trypanosomatidae. In Colombia it is estimated that there are between 700,000 and 1,200,000 inhabitants infected with *T. cruzi* and about 8,000,000 people at risk of acquiring the infection. Kinetoplastids are characterized by a single mitochondrion with an intricate organization, consisting of a network of dozens of maxicircles and thousands of concatenated minicircles representing up to 20-25% of the total amount of DNA per cell. Maxicircles contain the genes involved in the mitochondrial electron transport chain and their study may help to understand the phylogenetic and evolutionary relationships of this group of parasites and adaptive processes. The aim of the present work was to identify the genomic characteristics of the maxicircles and minicircles (kDNA) of *T. cruzi* circulating in Colombia, by using sequencing technologies based on long reads, to obtain complete genomes that encompass all complex regions that present a repetitive naturalness and low percentages of Guanine/Citokine, difficult to appreciate through studies with short reads. A strain from a rural area of the municipality of Coyaima-Tolima isolated from the natural reservoir *Didelphis marsupialis* was used, which was sequenced by PacBio Hifi 100x technology. As for the maxicircles, 10 molecules were assembled de novo, with a size range between 21,950-47,166 bp, whose heterogeneity in their final size is mainly due to the length of the divergent region which presents sizes ranging from 6,400 bp to 32,000 bp. Through bioinformatics tools, a final molecule with a size of 47,166 bp was obtained and its circular nature was verified by bioinformatics methods. Its annotation revealed a total of 2 ribosomal genes and 18 structural genes that showed perfect synteny with mitochondrial genomes of other trypanosomatids previously reported. However, partial and complete deletions in the ND5 and RPS12 genes were found in some assembled molecules, suggesting possible heteroplasmy phenomena within the sequenced strain. Additionally, it was possible to obtain the complete divergent region, finding that its size was being underestimated. For this reason, a deeper analysis of this region, which has been the least studied of this genome, was performed, distinguishing specific characteristics such as its repetitive nature. A representative repertoire of sequences was obtained for the minicircles, which presented genomic and size heterogeneity, finding different numbers of conserved regions (Conserved Sequence Blocks-CSB) and hypervariables. In the present work, the first report of mitochondrial genomes of a strain isolated from southern Tolima was carried out. The usefulness of sequencing based on long reads for the analysis of complex genomes, such as the kinetoplast (kDNA), which presents low percentages of Guanine/Citokine and very repetitive regions, was demonstrated, thus contributing to the study of unexplored regions such as the divergent region, laying the foundations for future studies that can evaluate the biological implications that this region has in molecular processes in trypanosomatids.

Keywords: *Trypanosoma cruzi*, kDNA, maxicircles, minicircles, divergent region

Palabras clave: *Trypanosoma cruzi*, kDNA, maxicírculos, minicírculos, región divergente

Analysis of *Trypanosoma cruzi* metacyclogenesis in different Discrete Typing Units (DTUs)

Análisis de la metaciclogénesis de *Trypanosoma cruzi* en diferentes Unidades Discretas de Tipificacion (DTUs)

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Trypanosoma cruzi has a wide genetic variability which has favored the division of this species into subgroups that can be identified by common molecular, genetic, biochemical and immunological markers. Currently, it is classified into six discrete typing units of TcI-TcVI and TcBat. These have differential characteristics related to their prevalence, geographic distribution, hosts, and vectors. Also, biological characteristics such as virulence and replication rates. Metacyclogenesis is a very important process in the life cycle of *T. cruzi* since, at this stage, non-infectious epimastigotes (EP) become infectious metacyclic trypomastigotes (MT). Therefore, the aim of this study was to determine whether differences in the time required to reach the peak of metacyclogenesis in cultures of different DTUs. *T. cruzi* strains TcI (DA/MG), TcII (Y), TcIII (845), TcIV (85) and TcVI (Tulahuen) were cultured with a concentration of 1×10^8 EP/mL and three biological replicates in liver infusion medium (LIT) supplemented with 10% fetal bovine serum at 26 °C, conducive to nutritional stress conditions that give rise to the process of metacyclogenesis. They were quantified by Neubauer chamber for 12 days and by Giemsa staining where 300 parasite morphologies were classified into EP and MT. Results were tabulated in Excel and analysis of variance was performed by R software to determine statistical significance. The starting point of metacyclogenesis for DA was found at day 3, MG (4), TcII (6), TcIII and TcIV (3), and TcVI (4). For strains belonging to the DTU TcI only statistically significant differences were found from day 5. However, the number of MT was higher in MG than in DA (52'000.000 MT/9'000.000 MT). On the other hand, the strain that presented a lower number of MT was TcIV. The data presented a normal distribution and in the two-way ANOVA analysis statistically significant differences were found with a P value <0.0001 for the number of metacyclic trypomastigotes as a function of strain/day. The *T. cruzi* strain that presented a higher efficiency in the metacyclogenesis process was the MG strain, which shows that there are differences not only between DTUs but additionally between strains of the same DTUs, which could be related to circulation and transmission of the same.

Keywords: *Trypanosoma cruzi*, metacyclogenesis, discrete typing units, growth curves

Palabras clave: *Trypanosoma cruzi*, metaciclogénesis, unidades discretas de tipificación, curvas de crecimiento

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Characterization of TcCAL1: a calcium binding protein and its role in the life cycle of *Trypanosoma cruzi*

Caracterización de TcCAL1: una proteína con dominios de unión a calcio y su función en el ciclo de vida de *Trypanosoma cruzi*

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In *Trypanosoma cruzi*, several studies have reported that intracellular calcium (iCa^{2+}) levels increase during metacyclogenesis and parasite adhesion to host cells. Also, channels that generate variations in iCa^{2+} concentration have been studied. However, most of the proteins that interact with this ion, possibly decoding its signals, have not been characterized. TcCAL1 is a 103 amino acid protein with EF-hand type domains for Ca^{2+} binding, with no known function and specific to kinetoplastids. In this work, we studied the role of TcCAL1 in some aspects of the parasite life cycle. By western blot, it was demonstrated that TcCAL1 is more abundantly expressed in the trypomastigote forms, compared to the amastigote and epimastigote stages. Through immunofluorescence microscopy, it was determined that TcCAL1 is localized throughout the cell body of the three stages mentioned. Also, cultures overexpressing TcCAL1 fused to a six-histidine tag (pTREX/TcCAL1x6His) and cultures containing the empty pTREX vector (controls) were obtained. In metacyclogenetic assays, overexpression of TcCAL1x6His significantly decreased the percentages of differentiation from epimastigote to metacyclic trypomastigote forms. When invasion processes were evaluated, overexpression of TcCAL1x6His caused an increase in the adhesion percentages of metacyclic trypomastigotes to the surface of Vero cells, as well as the number of parasites attached per cell. Similarly, the percentages of infected Vero cells and the number of intracellular amastigotes per cell increased in cultures overexpressing TcCAL1x6His. However, parasites overexpressing TcCAL1x6His showed similar epimastigote proliferation rates to controls, as well as differentiation of metacyclic trypomastigotes to axenic amastigotes. On the other hand, a yeast two-hybrid assay was performed expressing TcCAL1 as bait in conjunction with a *T. cruzi* cDNA library. As a result, two TcCAL1-interacting proteins were identified, with characteristic armadillo-like or prefoldin-like domains, respectively. Such interactions were studied by co-immunoprecipitation and mass spectrometry, where a protein with prefoldin domains was also identified. However, no proteins with armadillo domains were detected. Co-localization by immunofluorescence microscopy of epimastigotes expressing a fragment of the armadillo domain protein fused to an HA tag was evaluated using anti-HA and anti-TcCAL1 antibodies. As a result, signal intensity overlap was observed for both antibodies. These results allow us to hypothesize that TcCAL1x6His limits iCa^{2+} concentration in the parasite, negatively affecting metacyclogenesis. Also, we propose that overexpression of TcCAL1x6His promotes the invasiveness of *T. cruzi* to host cells by activating some Ca^{2+}-dependent function. Future studies aim to determine Ca^{2+} binding to TcCAL1 and to quantify iCa^{2+} levels in parasites overexpressing TcCAL1x6His against different components of the extracellular matrix. This study reaffirms the importance of studying uncharacterized proteins exclusive to the parasite to deepen our knowledge of *T. cruzi* biology.

Keywords: *Trypanosoma cruzi*, calcium binding protein, host-cell invasion

Palabras clave: *Trypanosoma cruzi*, proteína de unión a calcio, invasión en células hospedadoras

*Tc*BDF6: an essential protein for the infectivity of *Trypanosoma cruzi*

*Tc*BDF6: una proteína imprescindible para la infectividad de *Trypanosoma cruzi*

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Protein acetylation at lysine residues is a dynamic posttranslational modification that regulates various protein functions. The only known domain capable of recognizing acetylated lysines is the bromodomain (BD), which functions as a scaffold for the assembly of macromolecular complexes. In Trypanosoma cruzi, there are 8 coding sequences with BD: TcBDF1-8. Since the *Tc*BDF6 sequence is the most divergent and cannot be phylogenetically related to mammalian BDs, selective inhibitors with potential trypanocidal activity could be found. The aim of this work was to generate *Tc*BDF6 mutant strains and evaluate their morphology, replication and infectivity, as well as their possible role in DNA repair. For this purpose, the gene coding for TcBDF6 was disrupted in epimastigotes of strain Dm28c using the CRISPR-Cas9 technique and heterozygous (Dm28c BDF6$^{-/+}$) and homozygous (Dm28c BDF6$^{-/-}$) mutant lines were selected by limiting dilution cloning, indicating that this is a non-essential protein for epimastigotes. However, the mutant lines showed differences in epimastigote growth and morphology compared to parasites of the control strain Dm28c ("wild type"). In UV exposure assays, to analyze the implication of this bromodomain in DNA double-strand damage repair mechanisms, it was observed that all the strains studied showed similar UV sensitivity, indicating that TcBDF6 would not be involved in such mechanisms. We observed that, like the control strain, the mutant strains are able to generate metacyclic trypomastigotes (MT) *in vitro*. When Vero cells were infected with TM Dm28cBDF6 $^{-/+}$, there was a delay in amastigotes development and trypomastigote release, which started on day 20 post-infection (in the control strain it occurred at 7 days). The released trypomastigotes showed morphological differences, but infected Vero cells normally, releasing trypomastigotes again at day 7 post-infection. In contrast, even when TM Dm28cBDF6$^{-/-}$ were able to infect Vero cells, they showed a significantly lower infection percentage than the other strains (on average, one amastigotes per cell), being unable to release trypomastigotes post-infection. These results suggest that *Tc*BDF6 would be crucial in the infectivity and intracellular replication of the parasite, and could become an interesting therapeutic target for the treatment of Chagas disease.

Keywords: *Trypanosoma cruzi*, bromodomain, acetylations, *Tc*BDF6, infectivity

Palabras clave: *Trypanosoma cruzi*, bromodominios, acetilaciones, infección

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Study of High Mobility Group B protein form *Trypanosoma cruzi* through CRISPR/Cas9-edited cell lines

Estudio de la proteína High Mobility Group B de *Trypanosoma cruzi* mediante la obtención de líneas celulares de *Trypanosoma cruzi* editadas genéticamente mediante CRISPR/Cas9

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High Mobility Group B (HMGB) proteins are non-histone chromatin proteins that bind to DNA affecting its degree of compaction and thus participate in the regulation of gene expression and other processes such as recombination, replication and DNA repair. As a strategy for the study of the *Tc*HMGB protein, a *T. cruzi* strain capable of inducible *Tc*HMGB overexpression was obtained from the p*Tc*INDEX-GW vector (Dm28/p*Tc*INDEX-GW-TcHMGB). It was observed that overexpression of *Tc*HMGB in the parasite resulted in changes in the degree of chromatin compaction and in the structure of the nucleus. In addition, replication rate, cell cycle, cell division and cell infectivity were affected *in vitro*, suggesting that protein levels must be regulated to maintain parasite fitness. The next strategy to study the *Tc*HMGB protein was to edit the *T. cruzi* genome and attempt to obtain knock out (KO) mutant strains for *Tc*HMGB and the expressed hemagglutinin (HA) tagged protein using the CRISPR/Cas9 system. For this, plasmids were constructed to express the Cas9 nuclease and a guide RNA that directs the enzyme to the desired cleavage site (pTREX-Cas9/sgRNA-*Tc*HMGB) and donor DNA fragments were designed and obtained by PCR to promote DNA repair by homologous recombination by introducing resistance sequences for transfectant selection. *T. cruzi* Dm28c epimastigotes were cotransfected with the plasmid pTREX-Cas9/sgRNA-*Tc*HMGB and donor DNA corresponding to each editing strategy (KO and tagged) and after 5 weeks, parasites resistant to the antibiotics used in each case were selected. After multiple attempts, tchmgb gene editing was verified by PCR. In addition, we observed a reduction of mRNA and protein levels by qRT-PCR and western blot and immunofluorescence, respectively. Transfected parasites were maintained in axenic cultures of epimastigotes. Interestingly, we observed a loss of stability of the edited (KO) strain. This may be a consequence of the high plasticity of the *T. cruzi* genome that may have allowed a gene rearrangement to restore the tchmgb gene copy. In addition, we determined that the edited strain (KO) grew more slowly than the WT strain, which contributed to the gradual loss of the edited parasites over time. Parasites expressing the tagged protein will be used to investigate the distribution of *Tc*HMGB in the *T. cruzi* genome by chromatin immunoprecipitation and sequencing (ChIP-seq). Finally, given the impossibility of obtaining a stable KO strain, the role of *Tc*HMGB on transcription will be evaluated by RNA-seq using the *T. cruzi* strain capable of overexpressing *Tc*HMGB. These results will allow further study of the role of *Tc*HMGB protein on gene transcription.entina

Keywords: *Trypanosoma cruzi*, CRISPR-Cas, protein *Tc*HMGB

Palabras clave: *Trypanosoma cruzi*, CRISPR-Cas, proteína *Tc*HMGB

Microbiome alterations driven by *Trypanosoma cruzi* infection in two disjunctive mouse models

Alteraciones del microbioma provocadas por la infección de *Trypanosoma cruzi* en dos modelos distintos de ratón

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Alterations caused by *Trypanosoma cruzi* in gut microbiome may play a key role in host-parasite interactions and may be involved in the establishment and progression of infection. Therefore, the aim of this study was to evaluate the impact of *T. cruzi* on gut microbiome from animal models. We implemented a murine model with two mouse strains, BALBc and C57BL/6. A control group (n=5) and an infected group (n=5) with *T. cruzi* (Tulahuen) were followed-up for 16 days. Parasitemia (blood-heart), cytokines (serum) and shotgun metagenomics (feces) were performed. Changes in cytokines, mainly an increase in IFNg, IL-6 and TNF-a were observed. In the microbiome, species such as *Faecalibaculum rodentium, Bifidobacterium pseudolongum, Bacteroides thetaiotaomicron* and *Lactobacillus johnsoni* showed a decrease in abundance while *Akkermansia muciniphila* and *Blautia*, increased. We also observed changes in the abundance of certain viruses. Functional changes showed a decrease in metabolic pathways associated with fatty acids and aromatic amino acid synthesis and pyruvate/lactate fermentation pathways. Assemblies and bins enabled reconstruction of genomes from metagenomes (MAGs). *T. cruzi* produces intrinsic effects on the gut microbiome. These changes may generate a pro-inflammatory response and an alteration of gut microbiome that may be related to establishing/progression of infection. Functional changes indicate a reduction in amino acid synthesis pathways such as tryptophan, likely due to their utilization by this protozoan. As well, a decline of short-chain fatty acid synthesis such as butyrate correlates with loss of anaerobic environment increasing dysbiosis. Data also allowed the reconstruction of good quality MAGs of bacteria such as *Akkermansia muciniphila* and *L. johnsoni* enabling comparative genomic analyses from metagenomic data.

Keywords: metagenomics, *Trypanosoma cruzi*, Chagas disease, mice models

Palabras clave: metagenómica, *Trypanosoma cruzi*, enfermedad de Chagas, modelos murinos

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Replication study of genetic markers associated with Chagas disease in Latin American population

Estudio de replicación de marcadores genéticos asociados a la enfermedad de Chagas en población latinoamericana

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Chagas disease (CD) is an infectious disease caused by the *Trypanosoma cruzi* parasite, which is endemic in 21 Latin American countries and represents a serious public health problem worldwide due to migratory processes. The pathophysiological and molecular mechanisms of CD are not fully elucidated; however, the role of the immune response and the inflammatory component in the development of the disease is recognized. In addition, the fact that about 30 % to 40 % of patients infected with *T. cruzi* develop a cardiac form of the disease named Chronic Chagasic Cardiomyopathy (CCC) or that individuals with the same risk of infection may or may not present it, highlights the relevance of the host genetic component in the disease. Therefore, the identification of genetic markers associated with the risk of developing the pathology is fundamental to establishing prevention, management, and treatment strategies. This study evaluated the gene regions previously associated with *T.*

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cruzi infection and/or development of CCC in four Latin American populations from endemic areas of CD through a case-control study in 3413 individuals classified as seronegative (n=1104), asymptomatic seropositive (n=1328) and with CCC (n=981) from Colombian, Argentinean, Bolivian and Brazilian populations. The analysis was performed with the results obtained from a genome-wide association study (GWAS) carried out by the Ibero-American Network for Genomic Medicine in Chagas Disease, RIMGECH, using the Illumina Global Screening Array microarray and imputed data from the University of Michigan server. Fifty-six gene regions (± 2kb) associated in previous studies with CD were selected, and a meta-analysis was performed with significant SNPs per gene region. Statistical analyses were performed with PLINK software and six SNPs were identified in the TLR4, CXCL9, MASP2, HLA-DPB1, FCN2, and CYP21A2 genes associated with protection or risk for *T. cruzi* infection in the Colombian and Argentine cohorts. Ten SNPs of the ITGAM, CCR5, VDR, TLR2, PTPN22, HLA-A, HLA-G, and CCL19 genes were significantly associated with protection or risk for the development of CCC. Finally, in the populations analyzed, some SNPs previously identified in different studies were replicated, which supports their role in the risk of infection or development of the cardiac phase of the disease.

Keywords: Chagas disease, *Trypanosoma cruzi*, SNPs, GWAS

Palabras clave: enfermedad de Chagas, *Trypanosoma cruzi*, SNPs, GWAS

Molecular characterization of *Trypanosoma* spp. isolates obtained from wild vectors from the municipality of Coyaima, Tolima and its implications in the epidemiology of Chagas disease in Colombia

Caracterización molecular de aislados de *Trypanosoma* spp. obtenidos de vectores silvestres procedentes del municipio de Coyaima, Tolima y sus implicaciones en la epidemiologia de la enfermedad de Chagas en Colombia

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One of the zoonoses that has affected mankind since the last century is Chagas disease (CD), which is highly correlated with socioeconomic deficits and is therefore considered a neglected disease. It is estimated to affect 6 million people and, in the Americas, there are 30,000 new cases each year and 12,000 deaths. Currently, around 70 million people in the Americas live in Chagas-exposed areas and are at risk of contracting the disease. The main mechanism of transmission of this disease is vector-borne and although mortality has decreased significantly, the disease can cause irreversible and chronic consequences in the heart, digestive and nervous systems. The etiological agent of this pathology is the flagellate parasite *Trypanosoma cruzi*, an infective parasite for several vertebrate species, which is transmitted by hematophagous invertebrates. Associated with CD, we also find *T. rangeli*, which in co-infection can modulate the transmission process of *T. cruzi* in the vector. Cases of CD have been reported in the municipality of Coyaima, Tolima since 1988 and despite the application of various public policies to reduce the incidence of this infection, the population is still at risk of infection, therefore the objective of this study was to identify Trypanosomatids from isolates obtained from wild vectors from this municipality. A total of 100 *Rhodnius colombiensis* nymphs were captured from the Totarco Tamarindo village in the municipality of Coyaima, Tolima, from which the salivary glands were extracted

with the aid of entomological forceps and the intestinal contents by abdominal incision. Subsequently, direct examination, xenoculture and hemo-culture were performed in NNN-LIT 10% biphasic medium. DNA extraction was performed using the Phenol Chloroform Alcohol Isoamyl Alcohol (FCAI) methodology and subsequent molecular detection by amplification of the hypervariable region of the minicircles using the S35, S36, KP1L primer set. Based on direct observation we obtained 49 nymphs positive for trypanosomatid forms in intestinal contents and 6 positive nymphs in salivary glands. On the other hand, molecular characterization yielded 41 samples of intestinal contents positive for *T. cruzi* (amplification fragment of 330 bp) and 14 positives for *T. rangeli* (amplification fragments between 300-450 bp). These data show that in the municipality of Coyaima there is still a high index of infected wild vectors, which are considered a potential source of infection for the surrounding communities. In this sense, it is important to promote entomo-parasitic surveillance studies to feed epidemiological information on CD and on the other hand, to work on education and awareness strategies with the community to reduce the incidence of the disease in the municipality.

Keywords: trypanosome, wild reservoirs, Chagas disease, molecular characterization

Palabras clave: tripanosoma, vectores silvestres, enfermedad de Chagas, caracterización molecular

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New *Trypanosoma cruzi* transmission scenarios in the department of Boyacá, Colombia after the interruption of transmission by *Rhodnius prolixus*

Nuevos escenarios de transmisión de *Trypanosoma cruzi* en el departamento de Boyacá, Colombia después de la interrupción de transmisión por *Rhodnius prolixus*

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In Colombia, the Chagas Disease (CD) interruption program has had its most tremendous success in the department of Boyacá with the certification by PAHO-WHO as free of intradomiciliary *Rhodnius prolixus* transmission in 24 municipalities. However, reinfestation by native triatomine species puts the advanced process at risk due to secondary species' reactivation or maintenance of transmission. In this study, we conducted entomological surveillance and spatial distribution of triatomines in 52 municipalities where a sustained insecticide intervention was done to achieve PAHO-WHO certification. Using molecular methods in triatomines, an eco-epidemiological study was conducted in the two areas of the department with the highest reinfestation, to determine the transmission cycles in which the native vector populations participate. Identification of food sources, rate of natural infection, circulating *T. cruzi* genotypes, and serological and molecular in domestic dogs were also analyzed. We observed two distribution clusters, the first one in the Northeastern department area, with a high presence and domiciliation of *Triatoma dimidiata*, and the second in the Southwestern area, with a predominance and domiciliation of *Triatoma venosa*. In the reinfestation by *T. dimidiata* region, high entomological rates were found in homes and peridomiciles, with natural infection of 40%, and predominance of TcI Dom in vectors and humans as the only source of blood intake for this species. Domestic dogs had a seroprevalence of 4.6%, while *T. cruzi* DNA could not be detected by PCR. On the other hand, in the Southwestern area, an infestation was found that was more associated with the peridomicile. The natural infection in the *T. venosa* vector was 13.9 %, and only TcI sylvatic was detected. In this new scenario, we identified four food sources: humans, dogs, rats, and chickens. The seroprevalence in dogs was 46.5%, and 36.4% of infected dogs were positive PCR for *T. cruzi*

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in blood. *T.dimidiata* and *T. venosa* have become the main insect vectors in transmitting *T. cruzi* after the elimination of *R. prolixus*. Molecular analysis was able to identify areas of parasite transmission. *T. dimidiata* is associated with domestic transmission cycles but with the ability to connect both transmission cycles (domestic-wild). In this scenario, dogs play a secondary role in local ecoepidemiology. Meanwhile, *T. venosa* is associated with the peridomicile from where it maintains enzootic transmission, and dogs have an active role as reservoirs. The development of these studies raises intervention priorities for the department to consolidate the CD control program.

Keywords: *Trypanosoma cruzi*, Chagas disease, *Rhodnius prolixus*, transmission

Palabras clave: *Trypanosoma cruzi*, enfermedad de Chagas, *Rhodnius prolixus*, transmisión

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Detection and Molecular Characterization of Trypanosomatids isolated from Wild Reservoirs from endemic areas of Tolima

Detección y Caracterización Molecular de Tripanosomátidos aislados de Reservorios Silvestres procedentes de áreas endemicas del Tolima

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Zoonoses are infectious diseases transmissible from vertebrate animals to humans under natural conditions. This concept implies that the pathogens causing the infection or disease have, in general, a wild animal reservoir, usually asymptomatic, which can transmit the pathogen directly to humans or domestic animals, which in turn can transmit it to humans. Pathogens that generally cause zoonoses can be bacteria, viruses or parasites. The latter can cause fatal diseases such as Chagas disease, responsible for around 12,000 deaths per year, which is transmitted by the hemoflagellate parasite *Trypanosoma cruzi*. Recently, the emergence and re-emergence of this type of zoonosis has been observed, a phenomenon closely related to ecological, climatic and socio-cultural changes that have determined that the animal population shares its habitat with humans with increasing frequency. Thus, it is necessary to carry out constant epidemiological surveillance of wild reservoirs in areas where the urban population closely adjoins the natural habitats of these reservoirs. The identification and characterization of American Trypanosomatidae is possible using conserved regions of the kDNA minicircles, which allow differentiating *T. cruzi* from *T. rangeli*, as well as the presence of lineages within *T. rangeli*. For this reason, the aim is to perform the diagnosis and molecular characterization of trypanosomatids present in wild reservoirs that may present a focus of infection for the nearby urban population. Blood samples from six mammals captured in the department of Tolima (two *Cebus albifrons* monkeys, three opossums *Didelphis marsupialis* and one armadillo *Dasypus* spp.) were used. Using these blood samples, blood cultures were performed in NNN-LIT 10% biphasic medium and DNA extraction was performed using the Genomic DNA Purification Kit from ThermoFisher Scientific. 15 days after the blood cultures were performed, they were reviewed by optical microscopy using an aliquot of the liquid medium and by smears with GIEMSA staining, finding forms similar to *T. cruzi* and *T. rangeli*. Using the DNA purified from the samples, molecular characterization was performed using primer sets S35, S36 and S35, S36, KP1L, and it was found that blood samples from *C. albifrons* and *Dasypus* spp. monkeys were infected with *T. rangeli* (amplification fragment of 765 bp and between 300 - 450 bp) and blood samples from opossums *D. marsupialis* were found to be infected

with *T. cruzi* (amplification fragment of 330 bp). Likewise, samples with *T. rangeli* were found to belong to the KP1 (-) subpopulation or lineage, given the absence of the 165 bp amplification fragment, which corresponds to the amplification of the KP1 minicircle. Thus, the importance of diagnosis and epidemiological surveillance of wild reservoirs to detect possible foci of deadly parasitic diseases, such as Chagas disease, is highlighted.

Keywords: trypanosomatids, wild reservoirs, Chagas disease, epidemiological surveillance

Palabras clave: tripanosomátidos, reservorios silvestres, enfermedad de Chagas, vigilancia epidemiológica

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Chagas disease epidemiology in Honduras

Epidemiología de la enfermedad de Chagas en Honduras

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Honduras has been a member of the Initiative of Central American Countries for the prevention and control of Chagas disease (IPCA) since 1997 and was the driving force behind it, which has been formed by all the countries that make up the Central American strip. This in turn is part of 4 other initiatives that were formed in the countries of South America and one where initially only Mexico was part of it. Several achievements have been made, among them the elimination of the urban area of the *T. infestans* vector, which is native to Bolivia and has been the main vector in the countries of the Southern Cone. Chagas disease has been endemic throughout Honduras, except in the Caribbean coastal region. The most vulnerable and affected population are those living in mountainous areas in houses built with natural materials (adobe, bahareque, muddy and thatched roofs). In 1983-1984, an average seroprevalence of 15.6% was found in 12 of the 18 departments of Honduras (Comayagua, Copan, El Paraíso, Francisco Morazán, Intibucá, La Paz, Lempira, Ocotepeque, Olancho, Valle and Yoro). A prevention and control program were initiated in 2003, and by 2010 the main vector transmitting *R. prolixus* had been successfully eliminated. The estimated seroprevalence in children under 15 years of age decreased from 5.0% in 2003-2007 to 0.4% in 2010. In 2012 as part of a reorganization of the health system, the National Chagas Control Program was integrated into a national program for neglected infectious diseases, which focuses on nine diseases. At the same time, the Ministry of Health decentralized management responsibility for the control and surveillance of Chagas disease to the regional level and also promoted the delegation of health service delivery to decentralized entities formed by NGOs, commonwealths and municipalities. Cases of Chagas disease have been detected in a timely manner, generally through serological surveys in the school population, screening in blood banks, studies of chronic cardiac diseases in hospitals and by clinics in health facilities. Although clinical detection is infrequent due in part to the lack of physician training in this area and the variable clinical manifestations of the disease and the short duration of acute symptoms. Screening has continued in those places that were endemic for Chagas disease and where the *T. dimidiata* vector persists in peridomiciliary and jungle areas, where surveys continue to be conducted among schoolchildren and detection of Chagas disease in pregnant women through rapid serological tests. These tests are confirmed in regional laboratories for subsequent vector control and treatment. It is estimated that the national prevalence of Chagas disease in Honduras is 0.59%, and reactive serology rates for *T. cruzi* in blood banks decreased from 1.5% in 2008 to 0.77 in 2017, which is currently maintained.

Keywords: Chagas, epidemiology, cases, age, gender, *Trypanosoma cruzi*

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Palabras clave: Chagas, epidemiología, casos, edad, género, *Trypanosoma cruzi*

Host-pathogen interaction: Role of *Trypanosoma cruzi*-derived exovesicles during *ex vivo* infection of human placental explants.

Interaccción hospedero-patógeno: Rol de exovesículas derivadas de *Trypanosoma cruzi* en la infección *ex vivo* de explantes de placenta humana.

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Chagas disease (CD) is a multisystemic zoonotic infection whose causative agent is the protozoan parasite *Trypanosoma cruzi*. According to WHO, the CD is considered a neglected tropical disease that is endemic in Latin America. However, it has spread beyond its traditional geographical limits because of migration and congenital transmission, constituting a global public health problem. Congenital transmission depends on a complex network of host-pathogen interactions, where the placenta and virulence factors secreted in exovesicles play a fundamental role. Thus, to infect the developing fetus, the parasite must cross the placental barrier composed of a bistratified epithelium (trophoblast), fetal connective tissue (villous stroma) that contains fetal blood vessels, and the basal lamina that supports the different epithelia. To study the possible role of *T. cruzi* trypomastigote exovesicles (Tryp-TcEVs) in the susceptibility to infection and tissue damage caused by the parasite during *ex vivo* infection of human placental explants (HPE) we proposed two specific aims: 1) To determine the effect of Tryp-TcEVs during *ex vivo* infection of HPE by quantification of parasite DNA load; 2) To determine the role of Tryp-TcEVs in *T. cruzi*-induced tissue damage by histopathological and histochemical analysis of HPE. To do this, Tryp-TcEVs were obtained from trypomastigote cultures in MEM medium, followed by two ultra-centrifugations, filtration, and quality control in TEM, in collaboration with Dr. Antonio Osuna (University of Granada, Spain). Parasites were obtained by culture in VERO cells (ATTC ® CCL - 81™). Term placentas were obtained from the Obstetrics and Gynecology Department of the Hospital San José, Santiago de Chile. HPEs of 5 mm^3 were obtained, followed by PBS washing and incubation in RPMI medium for 24 hours in the absence or presence of parasites ($1x10^5$ parasites/mL) and Tryp-TcEVs (5 μg/mL). Then, qPCR was performed to detect *T. cruzi* satellite DNA to evaluate parasite load (182-bp) (TCZ-F (5'-GCTCTCTTGCCCACAMAMGGGGGTGC-3'3') and TCZ-R (5'-CAAGCAGCGGATAGTTCAGG- 3'3')) using the human GADPH gene as housekeeping gene (100-bp) (hGDH-F (5'-TGATGCGTGTGTACAAGCGTTTTTT-3'3') and hGDH-R (5'-ACATGGTATTCACCACCACCCCAC TAT-3'3')). Data were analyzed using the double comparative control method ($\Delta\Delta$Ct). Routine histological processing was performed to assess the tissue damage, and samples were stained with Hematoxylin-Eosin, Masson's Trichrome, Picrosirious red, and Schiff's Periodic Acid. Our analysis shows that HPE incubated in the presence of Tryp-TcEVs (5 μg/mL) and *T. cruzi* trypomastigotes ($1x10^5$ parasites/mL) for 24 hours significantly increased their parasite load compared to those infected only with the parasite. In addition, it was evidenced that Tryp-TcEVs increase the damage caused by the parasite since increased trophoblast detachment, loss of continuity and organization of the basal lamina and villous stroma were observed. Finally, as a conclusion, we can confirm that Tryp-TcEVs contribute to the destruction of the placental barrier caused by the parasite, favoring infection.

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Keywords: Chagas disease, exovesicles, congenital transmission, placenta

Palabras clave: enfermedad de Chagas, exovesículas, transmisión congénita, placenta

Diversity of trypanosomatid parasites of anurans and reptiles, and their hematophagous insects in the Amazon biomes and the Atlantic Forest in municipalities of Brazil

Diversidad de parásitos tripanosomátidos de anuros y reptiles, y de sus insectos hematófagos en los biomas Amazónico y el bosque Atlántico de municipios de Brasil

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The protozoa of the Family Trypanosomatidae (Class: Kinetoplastida) are parasites of vertebrates, invertebrates and plants. The family includes 25 genera (20 monoxenes and 5 heteroxenes, including *Trypanosoma* and *Leishmania*), which can be transmitted by vectors of the orders Diptera (Families Corethrellidae, Culicidae and Psychodidae), Hemiptera (family Reduviidae) and Arhynchobdellida (leeches). In anurans, approximately 60 species of trypanosomatids of the genus *Trypanosoma* are reported, forming an aquatic clade, which are evolutionarily important because they are considered the link between the trypanosomes of aquatic and terrestrial hosts. In reptiles, trypanosomatids of the genus *Leishmania*, subgenera *Sauroleishmania* ("lizard Leishmania") and *Leishmania* (*L. donovani* and *L. tropica*) are reported in Africa, Asia, and Europe. Of the genus *Trypanosoma*, the parasite *T. cruzi* is reported. In the American continent there are records of *L. (S.) henrici* in reptile species introduced from the Old World. The parasite *T. cruzi* was reported in the following reptile species in Chile: *Microlophus atacamensis, Liolaemus* sp. and *Gartthia gaudichaudii*. Phlebotomine sandflies of the subtribe *Sergentomyiina*, genera *Deanemyia* and *Mycropigomyia* are vectors of these parasites in reptiles. In spite of the great diversity of species of these parasites in these vertebrates, and in their vectors, they have been little studied, because they have no importance in human or veterinary medicine. The objective of this work is to know the diversity of trypanosomatids of anurans and reptiles and their insect vectors (dipterans) in the Amazon and Atlantic Forest biomes in Brazil. The work is being carried out in the municipalities of Pacoti and Guaramiranga, Ceará (Atlantic Forest biome), and in Presidente Figueredo and Urucurituba (Amazon biome). The capture methodology applied was time constrained audio-visual search (TAVS). The sampling was performed in the morning and at night. The collected animals were sacrificed following the ethical norms for the use of animals, through anesthesia protocols. Liver, spleen and blood samples were taken (filter paper, and for parasite isolation in NNN/LIT (Novy-MazNeal-Nicolle/Liver infusion Tryptose) biphasic culture medium, supplemented with 10% SFB). Blood smears were also analyzed

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for morphological identification of the parasites. DNA was extracted from the tissues and PCR will be done with specific primers for the Trypanosomatidae family. The amplicons corresponding to Trypanosomatidae will be purified and sequenced using the BigDye Terminator v3.1 kit in an ABI 3730. Hematophagous insects were captured with CDC traps modified with sound boxes, stored in absolute ethanol, identified using dichotomous keys, and DNA was extracted to identify the presence of parasites and the source of blood feeding, with specific markers. Samples were collected from 48 anurans and 23 reptiles (N = 71) of the following species: Anurans: *Pristimantis relictus, P. streldidialis, Leptodactylus mystaceus, Scinax tropicalia, Rhinella dapsilis, Scinax sp., Trachycephalus typhonius, Scinax scihalus, Dendropsophus tapacurensis, Rhinella crucifer, Boana raniceps*. Reptiles: *Anolis* sp., *Polychrus marmoratus, Lachesis mutans, Leposternon polystegum, Apostolepis cearensis, Enyalius broniati, Coleodactylus meridionalis, Enyalius bibronii, Norops fuscoauratus, Copeoglossum nigropunctatum*. Parasite identification in vertebrate blood smears, liver DNA extraction and PCR for parasite detection using oligonucleotides of SSUrRNA and gGAPDH genes are currently underway.

Keywords: Trypanosomatidae, anurans, reptiles, insect vectors, hematophagous

Palabras clave: Trypanosomatidae, anuros, reptiles, insectos vectores, hematófagos

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Clinical and epidemiological aspects of the piroplasmosis and trypanosomiasis in horses from the Northeastern of Colombia

Aspectos clínicos y epidemiológicos de la piroplasmosis y tripanosomiasis en caballos del Nororiente de Colombia

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Piroplasmosis and tripanosomiasis are debilitating diseases of great economic impact on the equine industry of Latin America. Considering the lack of studies in the northeastern part of Colombia, this study aims to determine the clinical and epidemiological features associated with infection of the *Babesia, Theileria*, and *Trypanosoma* genera in horses from this geographical area. A total of 280 horses from Arauca, Meta, and Santander departments were analyzed by molecular methods to detect infection by *Babesia caballi, Theileria equi, Trypanosoma evansi*, and *T. vivax*. Furthermore, clinical and epidemiological analyses were carried out on the data sets. Molecular analysis showed a prevalence of 25.7 % and 3.9 % for *T. equi* and *T. evansi*, respectively, without positive animals for *B. caballi* and *T. vivax*. Higher prevalences of *T. equi* were detected in Santander and Meta, whereas *T. evansi* was detected exclusively in Santander. Clinical analyses showed significant alterations in PCV, red blood cells, mean corpuscular volume, and body condition in positive animals, while epidemiological analyses showed a significant association of tick infestation and lack of insect control with the infection by *T. equi* and *T. evansi*, respectively. Our analysis shows a considerable infection rate by *T. equi* in horses from northeastern Colombia, which affects the clinical and body condition of these animals. Control of ticks and treatment of symptomatic animals should be considered to reduce the economic impact of this industry.

Keywords: hemotropics, ticks, vectors, infection, mammals

Palabras clave: hemotrópicos, garrapatas, vectores, infección, mamíferos

Sequencing of Colombian strains of *Leishmania (Viannia) braziliensis* using Oxford Nanopore technology

Secuenciación de cepas colombianas de *Leishmania (Viannia) braziliensis* mediante tecnología Oxford Nanopore

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Leishmaniasis is a disease caused by the protozoan parasite of the genus *Leishmania* belonging to the family Trypanosomatidae. In Colombia, Leishmaniasis is endemic and represents a major public health problem associated mainly with poverty in rural areas. There are three main forms of the disease, cutaneous (98.3 % of cases), mucocutaneous (1. % of cases) and visceral (0.3 % of cases), whose main etiological agents are species of the subgenus *Leishmania* (*L. mexicana, L. amazonensis*) and the subgenus *Viannia* (*Leishmania braziliensis, L. panamensis* and *L. guyanensis*). Despite the advances in the knowledge of the distribution of *Leishmania* spp. in Colombia, there is a great lack of knowledge about the structural genomics of the species circulating in our country, whose study will contribute to the understanding of the biological characteristics involved in the epidemiology of Leishmaniasis and will help to understand adaptive phenomena of these parasites. According to the above mentioned, this project aims to contribute to the knowledge of the genomics of the species of *L. braziliensis* circulating in Colombia using fourth generation sequencing Oxford Nanopore, for further studies of diversity at the genomic level. To meet this objective, three strains of *L. braziliensis* isolated from three Colombian patients, which are part of the stock of the Group of Infections and Health in the Tropics of the National University of Colombia, were sequenced. DNA extraction was performed with the QIAamp DNA Mini kit using $1x10^8$ parasites/ml per strain and sequencing was performed using the Nanopore MinION technology generating a total of 1.4 Gb of data per strain. Quality assessment of reads was performed with Nanoplot and base calling was performed with Guppy v6.0 using the high precision model. We obtained reads with an N50 of 13Kbp, with lengths from 700bp to 85Kbp. Therefore, each strain was assembled using the NECAT error correction step comparing the NGSEP and Flye assemblers. Quality assessment of the assemblies was performed with QUAST and BUSCO using the Brazilian strain *L. braziliensis* M2904 as a reference. Each strain was assembled into approximately 90 contigs, where the largest contig had a length of 2.3 Mbp, which corresponds to the largest chromosome for the species. Using the Abacas and Mummer tools it was possible to assign the contigs to the chromosomes of *L. braziliensis*. Sixty-five percent of the 35 chromosomes are contained in a contig and the telomeric region was located in 32 chromosomes, of which 16 have telomeres at both ends of the chromosome. This is the first sequencing and assembly project of Colombian *L. braziliensis* parasites using Nanopore technology. Our results showed that it is possible to use ONT reads to reconstruct the 35 chromosomes of these parasites. This is an easy and low-cost alternative to study the diversity of *Leishmania* in our country, to improve our understanding of its evolutionary history and to evaluate the genomic structural complexity of the genus and its incidence in adaptive processes.

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Keywords: *Leishmania braziliensis*, bioinformatics, genomics, nanopore, next generation sequencing

Palabras clave: *Leishmania braziliensis*, bioinformática, genómica, nanoporos, secuenciación de última generacións

Use of the Oxford Nanopore platform to obtain maxicircles (kDNA) of Colombian isolates of *Leishmania (Viannia) braziliensis* for genomic and evolutionary studies

Uso de plataforma Oxford Nanopore para la obtención de maxicirculos (kDNA) de aislados colombianos de *Leishmania (Viannia) braziliensis* para estudios genómicos y evolutivos

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Leishmania (Viannia) braziliensis is one of the main responsible of cutaneous leishmaniasis in Colombia. Its mitochondrial genome consists of maxicircles and minicircles. The maxicircles of all kinetoplastid flagellates are functional analogs of the mitochondrial genome of other eukaryotes. They consist of two distinct parts, referred as the coding region and the divergent region. The divergent region is composed of highly repetitive sequences and are the least explored segment of the mitochondrial genome of trypanosomatids, which makes their sequencing and assembly difficult, and for this reason there is a low report of complete maxicircle sequences available, as has been the case for organisms of the genus *Leishmania*. Therefore, the aim of this work is to show the obtaining of maxicircles from 7 clinical isolates of *L. braziliensis* through sequences from Oxford Nanopore Technology and their comparison with other trypanosomatids. The seven clinical isolates were assembled *de novo* using NGSEP and NECAT software, to confirm the identity of the clinical isolates as *Leishmania braziliensis*, a BLASTn was performed using the 7 assemblies obtained and the kinetoplast of a *L. braziliensis* strain as a reference (Access number: MN904516.1). In total 10 maxicircles were obtained from the *de novo* assembly and were verified by BLAST and%GC using the ribosomal genes 12S rRNA and 9S rRNA of *L. peruviana* since they are phylogenetically closely related (Accession number: BK010881.1). As for the annotation of the assembled maxicircles, the *L. panamensis* maxicircle was used as reference genome (Accession number: MK570510.1). And for the evaluation of nucleotide identity the annotated maxicircle was used in conjunction with other trypanosomatids of medical importance through the MUMmer software. A total of 10 maxicircles were obtained in the 7 assembled isolates with a size range of 24,300 to 31,000 bp and an average size of 27,400 bp. The size of the coding region comprised a total of 14,909 bp and the 10 assemblies had an average variable region size of 12,119 bp. Using bioinformatics tools, a circular maxicircle genome with a size of 27,737 bp was obtained, for the semi-automated annotation process were annotated 17 genes in the conserved region corresponding to the mitochondrial electron transport chain and 2 genes corresponding to ribosomal rRNAs. And finally, the nucleotide identity analysis of this maxicircle showed the following identities in the next order: *L. peruviana* with 99.02%, *L. guyanensis* with 98.03%, *L. panamensis* with 97.65%, *L. infantum* with 84.65%, *L. tarentolae* with 84.38%, *L. donovani* with 84.30%, *L. major* with 83.33%, *L. mexicana* with 83.28%, *T. cruzi* with 78.11% and finally *T. brucei* with 77.30%. The results of this work revealed that mitochondrial genomes can be obtained

by means of ONT long reads with high quality and low cost, which allow the study of genomic variability and parasite biology and adaptive processes.

Keywords: *Leishmania (V.) braziliensis*, maxicircle, genomics, bioinformatics, nanopore, next generation sequencing

Palabras clave: *Leishmania (V.) braziliensis*, bioinformática, genómica, nanoporos, secuenciación de última generación

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Changes at the genomic level of clinical isolates of *Leishmania (Viannia) braziliensis* given by *in vitro* maintenance

Cambios a nivel genómico de aislamientos clínicos de *Leishmania (Viannia) braziliensis* dados por el mantenimiento *in vitro*

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Parasites of the genus *Leishmania*, etiological agents of Leishmaniasis, have been extensively studied due to the wide spectrum of clinical manifestations that they generate in humans, the impact of the disease in affected populations and the low availability of treatments. Most of the studies on this parasite, in both promastigote and amastigote stages, have been developed using cultures of the parasite kept *in vitro* by long time. *Leishmania* spp. presents a high genetic plasticity, due to variations at chromosomal level (rearrangements, aneuploidy, etc.) or in specific regions such as genes (copy number variation). This genomic mosaicism in *Leishmania* spp. can act as an essential adaptive mechanism that allows rapid selection of a particular genome structure under specific conditions. This plasticity means that the *in vitro* maintenance of parasite isolates can induce genotypic and eventually phenotypic alterations such as loss of virulence and infectivity. In this sense, this study presents an analysis of the genomic and phenotypic variation *in vitr*o growth of promastigotes and [*in vitro* susceptibility to Amphotericin B (AmB) in promastigotes and amastigotes], resulting from the *in vitro* maintenance of promastigote stages of a clinical isolate of *L. (V). braziliensis*. Here we perform a comparison of the same parasite isolate between few culture passages after parasite isolation from patient (<10, early) vs multiple culture passages (>20, late). Phenotypic characterization data show that the *in vitro* doubling time during the logarithmic phase was 9.2 h (95% CI, 8.283 to 10.31) for the early isolate and 8.3 h (95% CI, 7.769 to 8.887) for the late version. Susceptibility to AmB determined from the IC_{50}, was 0.101 μg/mL (95% CI, 0.09063 to 0.1129) for the early version and 0.057 μg/mL (95% CI, 0.05200 to 0.06357) for the late version with a significant difference between them (F-Test, $p<0.0001$). Regarding genomic analysis, a significant variation in the number of copies of different gene families was observed. Within the genes with known IDs, a loss in 484 gene families and a gain of 291 gene families were observed for late version with respect to early one. When comparing with the reference genome, a certain degree of similarity between losses and gains resulting from *in vitro* selection was observed. To understand the biological processes associated with the expansion/contraction in gene copy number, a gene ontology (GO) enrichment analysis was carried out, showing that gene losses resulting from *in vitro* growth were mainly associated with long-chain fatty acid metabolism, tryptophan catabolism and glycolysis. Gene gains are mainly associated with microtubule movements, vesicular localization and response

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to oxidative stress. The results obtained suggest that the *in vitro* maintenance of *L. (V.) braziliensis* isolates leads to important variations with respect to the natural genotype and phenotype.

Keywords: *Leishmania (V). braziliensis*, genomics, *in vitro* passage, promastigotes

Palabras clave: *Leishmania (V). braziliensis*, genómica, pases *in vitro*, promastigote

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Phenotypic and genotypic characterization of *Leishmania (Viannia) braziliensis* clinical isolates in Colombia

Caracterización fenotípica y genotípica de aislamientos clínicos de *Leishmania (Viannia) braziliensis* en Colombia

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Human leishmaniasis is a disease caused by infection with protozoan parasites of the genus *Leishmania*, presenting tegumentary (TL) or visceral manifestations. In Colombia, TL is predominant and is associated with *Leishmania* species of the subgenus *Viannia* such as *Leishmania (V.) panamensis*, *L. (V.) braziliensis*, and *L. (V.) guyanensis*. As the first line of treatment, pentavalent antimony salts are used and as the second line miltefosine, pentamidine, or amphotericin are prescribed. There is a variable percentage of therapeutic failure in TL for each of these drugs, and it is not well known the role of the parasite genetics regarding the treatment failure. The potential loss of susceptibility to amphotericin B (AmB) is a major issue since it is generally the last treatment option and natural resistance has not been described to AmB in *Leishmania (Viannia)* species. The present work aims to establish whether there is genomic variation between clinical isolates of *L. (V.) braziliensis* with different degrees of AmB susceptibility. For this purpose, phenotypic and genetic features of thirteen clinical isolates of *L. (V.) braziliensis* from the Colombian Orinoco and Amazon regions were analyzed. The isolates were evaluated in early culture stages (<10 passages) and two of them in late culture stages (>20). The strain MHOM/BR/75/M2904 was included as the reference genome. The *in vitro* doubling time of these isolates in their promastigote stage grown in Schneider medium ranged from 8.099h to 17.87h (median=9.352 h, IQR=3.983). Susceptibility to AmB, calculated as the IC_{50}, ranged from 0.016 to 0.207 μg/mL (median= 0.071 μg/mL, IQR= 0.058) in promastigotes and from 0.008 to 0.244 μg/mL (median= 0.017 μg/mL, IQR= 0.012) in amastigotes. The *in vitro* infectivity to human macrophages, calculated as the percentage of infection to U937 derived macrophages, ranged from 26% to 64% (median= 42%, IQR= 15.5). These data correspond with a heterogeneous sample and groups with similar behavior for the evaluated variables were identified - *e.g.*, isolates particularly sensitive or tolerant to AmB. Subsequently, a ploidy analysis was performed observing that the 13 isolates of *L. (V) braziliensis* were found to be predominantly diploid with exception of chromosome 31 which is tetraploid. Some isolates showed chromosome 33 aneuploidy and there it was found that chromosome 34, from all isolates, presents an increase in read depth of a 60Kb region (13 genes) and an LD1 amplification of 140Kb (38 genes) was found in one specific isolate. These observations constitute a preliminary analysis and once the genomic profiling is complete, a correlation between it and the parasite phenotype will be assessed.

Keywords: *Leishmania (V.) braziliensis*, amphotericin B, clinical isolates, doubling time, IC_{50}, infectivity, LD1 amplification, ploidy

Palabras clave: *Leishmania (V.) braziliensis*, anfotericina B, aislamientos clínicos, tiempo de duplicación, IC_{50}, infectividad, amplificación LD1, ploodía

Functional characterization of Lathosterol oxidase (LOS) and Vesicle-associated membrane protein (VAMP) as factors involved in decreased susceptibility to amphotericin B in *Leishmania (Viannia) braziliensis*

Caracterización funcional de la latosterol oxidasa (LOS) y la proteína de membrana asociada a vesículas (VAMP) como factores implicados en la disminución de susceptibilidad a la Anfotericina B en *Leishmania (Viannia) braziliensis*

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Leishmaniasis is an infectious disease caused by species of the genus *Leishmania*. Among its main clinical manifestations, cutaneous leishmaniasis (CL) is the most prevalent in Colombia and is produced by endemic species such as *Leishmania (V.) panamensis*, *L. (V.) braziliensis*, and *L. (V.) guyanensis*. Although leishmaniasis is treatable, treatment failure to second-line therapy, such as amphotericin B (AmB), has been reported in recent years. Therefore, it is essential to understand the parasite molecular mechanisms that may explain differences in susceptibility to currently available drugs. In trypanosomatid parasites, lathosterol oxidase (LOS) and vesicle-associated membrane protein (VAMP) have been described as factors whose negative regulation decreases sensitivity to AmB. In addition, our previous findings evidenced that *L. (V.) braziliensis* clones with induced resistance to Miltefosine and low sensitivity to AmB had lower mRNA levels for these genes compared to the uninduced control. However, the involvement of both genes in increased AmB tolerance in *L. (V.) braziliensis* is still unclear. Hence, we aimed to evaluate the role of LOS and VAMP in increased tolerance to AmB in *L. (V.) braziliensis* by comparing their gene structure among clinical isolates with different susceptibility to AmB, as well as the *in silico* design of a CRISPR-Cas9 system for eventual functional validation. For this purpose, the sequence of LOS and VAMP genes of 13 clinical isolates of *L. braziliensis* with different sensitivity to AmB were identified using genomic data produced by Illumina sequencing and mapped against the *L. braziliensis* MHOM/BR/75/M2904 genome. Multiple alignments of *Lb*LOS and *Lb*VAMP genes among isolates revealed the presence of polymorphisms (Single nucleotide polymorphisms -SNPs-, insertions/deletions) mainly in the 5' and 3' untranslated regions (UTRs) of both genes, most frequently in LOS UTRs. Preliminary analyses have shown non-specific polymorphisms in *Lb*LOS and *Lb*VAMP UTRs of clinical isolates with higher AmB tolerance that can be related to a particular regulation of *Lb*LOS and *Lb*VAMP expression. Therefore, it is crucial to validate changes in the expression of both genes among the different clinical isolates. Finally, a CRISPR-cas9 strategy for LOS and VAMP knockout in *L. braziliensis* M2903 was designed *in silico* using the EuPaGDT and LeishGEdit platforms. From these analyses, two sets of sgRNAs were designed for partial or total excision of LOS and VAMP ORFs, sgRNAs specificity was verified by off-target absence in the *L. braziliensis* MHOM/BR/75/M2903 genome. These studies will allow us to determine whether the gain of AmB tolerance

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is certainly related to *Lb*LOS and *Lb*VAMP depletion, suggesting this mechanism as a factor associated with AmB tolerance in *L. braziliensis*.

Keywords: Leishmaniasis, *Leishmania (V.) braziliensis*, amphotericin B, LOS, VAMP, UTRs, CRISPR-Cas9

Palabras clave: Leishmaniasis, *Leishmania (V.) braziliensis*, anfotericina B, LOS, VAMP, UTRs, CRISPR-Cas9

Understanding the epidemiological features of *Leishmania infantum* in dogs from the Metropolitan Area of Bucaramanga Santander, Colombia

Entendiendo las características epidemiológicas de *Leishmania infantum* en perros del Área Metropolitana de Bucaramanga, Santander, Colombia

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Dogs are the main reservoir of *Leishmania infantum* so their epidemiological surveillance is essential to control visceral leishmaniasis. Considering the paucity of studies of canine leishmaniasis in Colombia, the present study aims to determine the prevalence of *Leishmania* spp., in healthy and symptomatic dogs from the Metropolitan Area of Bucaramanga (MAB), Santander. The symptomatic group, corresponding to 33 dogs with differential diagnoses of canine leishmaniasis that attended clinical centers of this area, whereas the healthy one corresponds to 215 dogs from the MAB, that attend government schedules for rabies vaccination. In both groups, DNA was extracted from the blood samples, whereas a lymph node aspiration was additionally processed in the symptomatic group. CDC traps were used in strategy points for the capture of vector insects. For both mammals and vectors, the molecular diagnosis was performed using a PCR targeting the *Hsp*70 gene of *Leishmania* spp., and the PCR products were sequenced using Sanger methods for species identification. Serological analyzes were performed using immunofluorescence antibody test (IFAT). Molecular analyses showed a positivity rate of 24,2% (8/33) to L. infantum in the symptomatic group, of which 12.1% (4/33), 9% (3/33), and 3% (1/33) came from Bucaramanga, Giron, and Floridablanca, respectively. Higher positivity was detected in lymph node aspirations (6/33) compared with blood samples (2/33) ($P < 0.005$). Clinical signs associated to infection were alopecia, cachexia, lymphadenitis, anemia, and lymphopenia. A total seroprevalence of 24.8% was detected by IFAT, with higher values in symptomatic group (42.4%), followed by asymptomatic one (21%). Respect to the vector, a total of 33 *Lutzomyia* specimens were collected, bellow for two main species (*L. camposi* and *L. dubitans*), without *Leishmania* spp., infection. Our results show an active transmission cycle of *L. infantum* in dogs from MAB, with a higher rate of infection in symptomatic ones, that suggest high pathogenicity, probably associated with genetic variants of the pathogen, as well as ecological conditions in the study area.

Keywords: zoonoses, reservoirs, vectors, trypanosomatids, leishmaniasis.

Palabras clave: zoonosis, reservorios, vectores, tripanosomatidos, leishmaniasis.

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Characterization of molecular clocks that control the progress and coordination of the cell cycle in Apicomplexans

Caracterización de relojes moleculares que controlan el avance y coordinación del ciclo celular en Apicomplejos

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One third of the world's population is chronically infected by *Toxoplasma gondii* (Phylum Apicomplexa). Chronic infections cause morbidity and mortality in humans as well as in livestock, causing important losses in the agro-industrial sector. Cell replication in these parasites is their main mechanism of pathogenesis. Therefore, understanding cell cycle regulation is central to the identification of potential drug targets. However, their study is limited because in cell culture, about 80% of the parasites are in G1. The low frequency of parasites in S and M phases limits the understanding of the events leading to the generation of new parasites. *Toxoplasma* controls the different phases of the cell cycle through soluble factors and physical anchors associated with the centrosome, coordinating mitosis and cytokinesis in time and space. In this project we propose: 1) to obtain synchronizable populations for the study of the different phases of the *T. gondii* cell cycle, and 2) to use these populations to identify factors that control cell cycle progression, focusing on triggers of centrosome duplication and the formation of physical anchors between the centrosome, chromosomes and daughter cells. For this, we propose the generation of "synchronizable" strains through endogenous labeling of cyclins and kinases with fluorescent proteins. Cyclins and kinases were selected with fluctuating expression profiles throughout the cell cycle, with peaks of expression at different times of the cycle. The fusion of these fluctuating factors to fluorescent proteins will allow us to determine the moment of the cell cycle in which the parasites are found and to separate them by cell sorting to obtain synchronized populations. It has been possible to generate a strain that expresses CyclinY (CycY) fused to tdTomato, which has its highest expression peak in G1. In turn, a knock-in analogous to that of CycY will be performed on this strain in which cyclin 1 and Crk 6 and 4 (Cdk related kinase) will be marked with mNeonGreen. The latter proteins have peaks of expression at different times than CycY, making it possible to discern different times in the cell cycle based on fluorescence. These strains are being generated by introducing into the endogenous loci of the genes coding for CycY, Cyc1, Crk4 and Crk6, an insert containing the gene for tdTomato or mNeonGreen and a selection cassette. These populations will be used in RNA-seq assays, and to generate complementary DNA libraries for yeast two-hybrid assays to identify specific centrosomal factor interactors. These tools will allow us to map cell stage-specific gene expression and generate fundamental time-resolved information to understand the molecular mechanisms underlying successful cell division in this parasite.

Keywords: Apicomplexan, cell cycle, cytokinesis, centrosome

Palabras clave: Apicomplejos, ciclo celular, citocinesis, centrosoma

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Enteroparasites of zoonotic interest (*Blastocystis* spp., *Microsporidium* spp. and *Cryptosporidium* spp.) from captive birds, seized and marketed in Mexico City

Búsqueda de enteroparásitos de interés zoonótico (*Blastocystis* spp., *Microsporidium* spp. y *Cryptosporidium* spp.) de aves en cautiverio, incautadas y comercializadas en la Ciudad de México

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Zoonoses are infections or diseases that are transmitted from vertebrate animals to humans, by direct means, fomites or vectors. Among them, we find those caused by parasites such as cryptosporidiosis, trypanosomiasis, blastocystosis, giardiasis and microsporidiosis, among others. The present work shows the partial results of the search for three parasites of clinical importance, in commercialized, seized and captive birds. Two areas of high bird-human contact were sampled: busy markets and zoos in Mexico City. A total of 131 species-specific samples were collected. Within the avian groups with the highest prevalence were psittaciformes, passerines and galliformes. In addition, to find risk factors that can favor bird-human infection. Of these samples, by staining methods (Kynyoun), 12 and 15 presumptively positive samples for *Cryptosporidium* spp. were found for each area, respectively. For *Microsporidium* spp. there is a presumption (Trichrome mod. Weber) of 14 samples in zoos. For the molecular identification of *Cryptosporidium* spp. and *Blastocystis* spp., primers were designed targeting the 18s rRNA gene to perform endpoint PCR. For *Microsporidium* spp., primers already designed by other authors are being used to carry out multiplex/nested PCR, together with the use of restriction enzymes for species identification. It is expected to associate the sequencing results with human genotypes and understand the impact of birds on the ecoepidemiology of these parasites.

Keywords: Parasites, molecular diagnosis, zoonoses

Palabras clave: Parásitos, diagnóstico molecular, zoonosis