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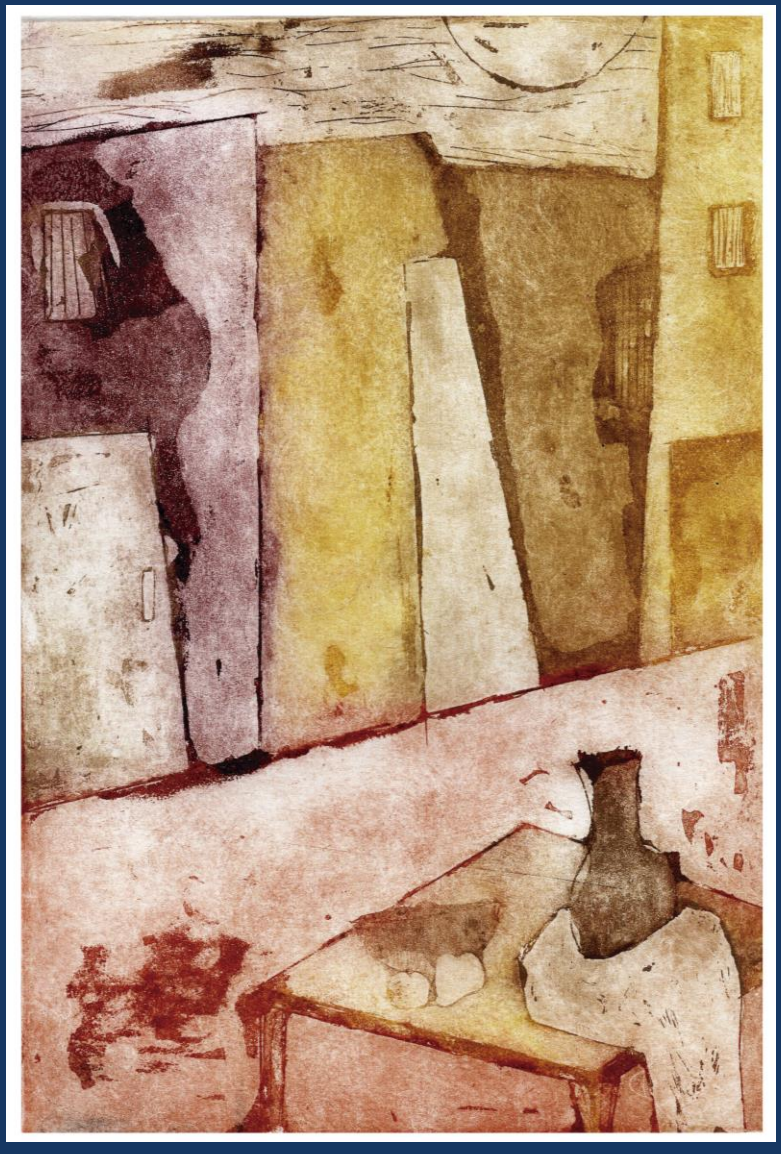

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MIEMBROS EMÉRITOS

La Tapa (*Ver* pág. 4)
Atardecer en la tarde
Antonella Ricagni

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IX Reunión Anual de la
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VI Reunión Científica Regional de la Asociación Argentina
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con la participación de
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13 - 16 de noviembre de 2019
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located in the "Gran Chaco", a highly endemic region for Chagas disease. The PrintrLab was programmed to purify DNA from whole blood-EDTA samples and to provide the incubation step for the T. cruzi-LAMP reaction. The process took about 2.5 hours to yield a result, while manual DNA extraction and subsequent qPCR normally take more than 6. Performance of the "PrintrLab-LAMP" duo was tested with blood-EDTA samples artificially contaminated with 0, 1, 2, 5, 10 and 100 parasites eq/mL and a sensitivity around 2 pararasites eq/mL was achieved. Finally, 70 clinical samples from infants born to seropositive mothers were evaluated and all the micromethod positive ones, 6 samples in total, were detected by the "PrintrLab-LAMP" approach. In conclusion, the "PrintrLab-LAMP" device showed a good sensitivity, the protocol was faster than other molecular techniques and it could be successfully used in a minimally equipped laboratory.

0764 - DEVELOPMENT OF QUANTITATIVE REAL-TIME POLYMERASE CHAIN REACTION COUPLED WITH HIGH-RESOLUTION MELTING (HRM-QPCR) ANALYSIS FOR THE DIAGNOSIS OF TRYPANOSOMA EVANSI IN CANIS LUPUS FAMILIARIS

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The Trypanosomiasis caused by Trypanosoma evansi affects a wide diversity of mammals being zoonotic potential in man, with a case reported in 2005 in India. This haemoflagellate protozoan can parasitize most domestic mammals, being horses, dogs, and cattle the most affected species. Diagnostic tools for this parasitic infection are scarce, even though this trypanosomiasis can be very lethal if the animals are not treated. This work reports the development of a multi-diagnosis assay based on qPCR coupled to HRM that differentiates infections with diverse species of trypanosomatids and Leishmanias with zoonotic potential in peripheral blood samples from canines. The molecular marker selected was the Internal Transcribed Spacer (ITS1) present in the ribosomal RNA locus. This marker is highly conserved and present size variability among trypanosomes species. The results using as a template gDNA of different trypanosomatid species showed specific amplification with distinctive patterns in Melting Curves for T. evansi, T. cruzi, T. brucei, T. rangeli and different species of Leishmanias. This was confirmed in agarose gels, resulting in single or multiple bands with a size range from 250 to 480 bp. Its clinical validation was carried out on 14 peripheral blood samples from domestic canines from northeastern Argentina. The results showed positivity for infection with T. evansi in 36 % of the samples. Additionally, through this standardized technique, in one sample it could be detected infection with Leishmania infantum with low parasitemia, confirmed by sequencing and subsequent alignment of the ITS1 region with reference sequences. Therefore, molecular diagnosis of animal trypanosomiasis by HRM-qPCR represents a viable tool for wide-scale epidemiological studies, which may be used to report the true prevalence of the infection and allow implementation strategies to control these zoonotic diseases in Argentina, as well as the rest of South America, Africa, and Asia.

0831 - CHARACTERIZATION OF EXTRACELLULAR VESICLES DERIVED FROM THE INTERACTION OF TRYPANOSOMA CRUZI WITH HOST CELLS IN THE MODULATION OF IMMUNE SYSTEM

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The protozoan Trypanosoma cruzi is the etiologic agent of Chagas' disease, initially restricted to the Americas, but has spread throughout the world, reaching millions of individuals. T. cruzi has a complex biological cycle where it needs to evade the immune system and invade host cells to complete the infection. One of the most effective mechanisms in innate immune defense against pathogens is the complement system, which consists of a set of proteins that are activated in cascade and which culminates in the formation of a pore in the membrane of the microorganism, causing its lysis. T. cruzi have developed several mechanisms to escape the complement system and to invade eukaryotic cells, expressing different molecules and releasing extracellular vesicles. Extracellular vesicles (EVs) are small vesicles composed of a lipid bilayer which comprises microvesicles and exosomes, according to their size and biogenesis. Our group have shown the release of EVs during the interaction between the parasite and host cells promotes complement system inhibition and increases the invasion of metacyclic forms of T. cruzi to host cells. Here, our aim was to understand the secretion of EVs by different stages of the parasite and how these EVs could manipulate host immune system to effects the infection. Parasites from CL Brener and Dm28 strains of T. cruzi was differentiate to metacyclic forms (METAs) by a nutrient starvation process and tissue-culture derived trypomastigotes (TCT) was obtained from supernatant of infected VERO cells monolayers. To induce EVs secretion, the different stages from the parasites was exposed to THP1 cells in a relation of 5:1 (parasites:cells) for one hour at 37 °C. Subpopulations of EVs was isolated by differential centrifugation method, with large EVs (LEVs, predominantly microvesicles) obtained from a 11,000 xg centrifugation and small EVs (SEVs, predominantly exosomes) from a subsequent 100,000 xg centrifugation. The two subpopulations of EVs was differentially secreted from the parasites and had different features. Moreover, it was seen that EVs from different strains was capable of inducing a cytokine response in dendritic cells, acting as communicators during the infection and modulating the immune system. The next steps of this work is to understand if different subpopulation of EVs have different functions in the resistance and invasion of T. cruzi and to characterize the role of T. cruzi EVs in modulating the secretion of cytokines and nitric oxide by macrophages.

0868 - LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) AS A DIAGNOSTIC TOOL FOR CUTANEOUS LEISHMANIASIS

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Cutaneous leishmaniasis (CL) is the most common form of leishmaniasis and causes skin lesions, mainly ulcers, on exposed parts of the body, leaving life-long scars and serious disability or stigma. Biopsy is widely used for the diagnosis of CL to obtain specimens for direct diagnoses (smear and culture). Molecular diagnosis is a promising alternative; although it is not well suited for adoption in laboratories with limited resources. Isothermal DNA amplification methods have the advantage of not requiring expensive equipment. The aim of this work was to use a previously reported LAMP assay to detect CL colorimetrically (Mikita et al., 2014, Rivero et al., 2017). LAMP reactions were performed using pan-Leishmania primers based on the 18S-rDNA sequences. Briefly, 5 µl of DNA extracted from cultures or biopsy specimens were subjected to amplification in reaction mixtures containing 40 pmol FIP and BIP primers, 20 pmol LF and LB primers, 5 pmol F3 and B3 primers, 1 µl (8 units) Bst DNA polymerase (New England Biolabs), the reaction buffer (20 mM Tris–HCl, 10 mM KCl, 8 mM $MgSO_4$, 10 mM $(NH4)_2SO_4$, 0.1% Tween-20), and 1.4 mM of each dNTP using a heat block for the amplification cycle. The LAMP assay was set up testing different concentrations of betaine and temperatures. Two approaches were used to confirm the amplification by using electrophoresis in agarose gel and by visual inspection after the addition of the fluorescent dye SYBR® Green (Invitrogen, S7563).