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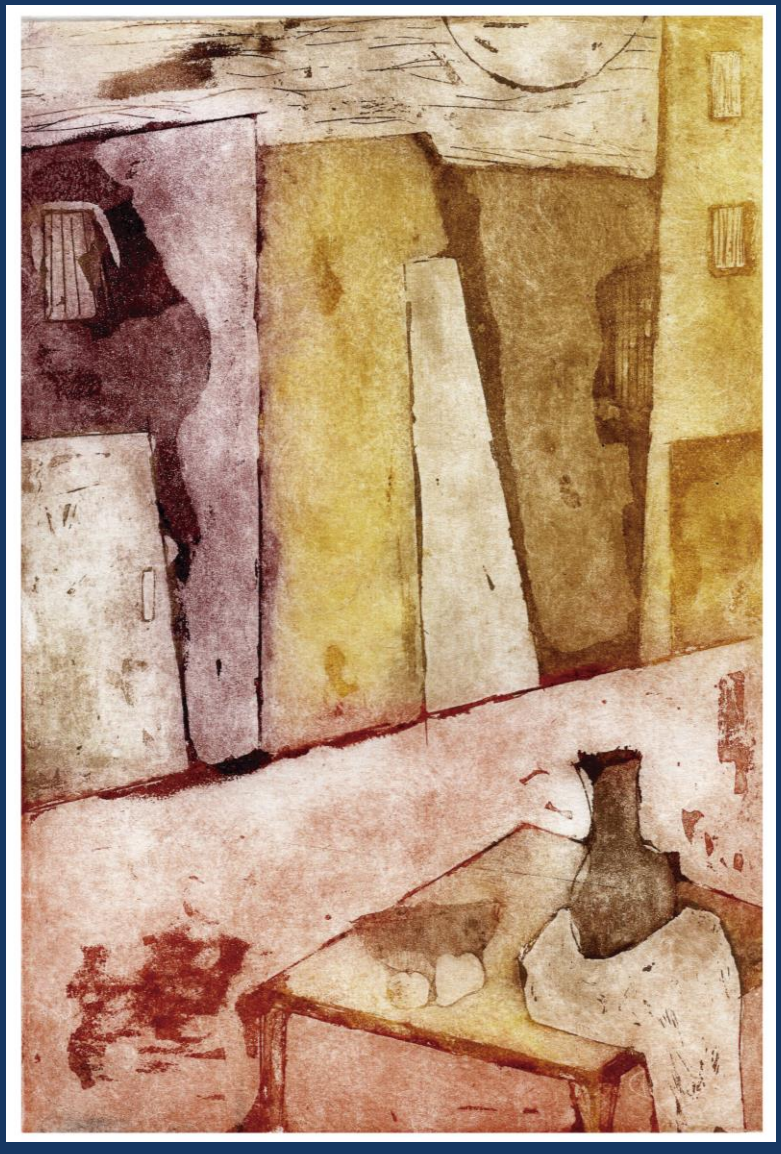

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Atardecer en la tarde
Antonella Ricagni

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IX Reunión Anual de la
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(NANOMED-ar)

VI Reunión Científica Regional de la Asociación Argentina
de Ciencia y Tecnología de Animales de Laboratorio
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con la participación de
The Histochemical Society

13 - 16 de noviembre de 2019
Hotel 13 de Julio - Mar del Plata

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we assayed individual serum to study 28 serodiscordant cases from Argentina and Mexico with borderline serology. These displayed a wide reactivity based on the number of positive peptides and quantification of signal. We observed almost complete lack of correlation between the quantitative values obtained in commercial ELISA (Wiener v4.0) and those obtained by the array. Hence, there is much room for improvement of current serological diagnosis. Based on the reactivity against 6 known antigens, we have separated these sera in three groups: one group of 17 sera reactive against several antigens; a second group of 8 sera reactive with fewer antigens and a group of 3 sera that were negative against most known antigens assayed. Using this information, we will shortlist novel antigens from the high-content screening to improve existing diagnostic kits. In this presentation we will revisit the concept of serodiscordance in the light of all new data arising from this screen.

0696 - MOLECULAR DETECTION OF TRYPANOSOMA CRUZI IN OCULAR TISSUE FROM PUTATIVE CORNEAL DONORS

Marta STARCENBAUM BOUCHEZ(1) | Elisabeth CITTADINO(1) | Gianfranco ALI SANTORO(2) | Héctor FONTANA(1) | M Susana LEGUIZAMÓN(2) | **Juan BURGOS** (2)

HOSPITAL DE OFTALMOLOGÍA SANTA LUCÍA (1); IIBIO-UNSAM (2)

Infection by Trypanosoma cruzi, the etiological agent of Chagas disease, is endemic of America where 6-8 million subjects are infected (prevalence in Argentina 3.6%). Due to heart and intestines are T. cruzi target organs, seropositive individuals are excluded as donors, whereas for kidney transplant the use of both dead and living seropositive donors to negative recipients is accepted. About cornea transplants, seropositive donors are rejected even for seropositive acceptors nevertheless WHO consensus makes general indications in which infected donors can be accepted, only in extreme cases and subject to the informed consent. This general consideration is applied for safety without having been, to date, proved the parasite presence in the transplanted tissue. Herein we analyzed ocular tissues (20 corneas and corresponding sclera rings, and 7 eye muscles) from ten deceased seropositive donors (6/4 M/F, 30-74 years old) from Argentina that were admitted consecutively at Hospital Santa Lucia in Buenos Aires, Argentina. DNA extraction was carried out by means of QIAgen (DNeasy blood and tissue kit) with a previous incubation with proteinase. DNA integrity was checked by PCR amplification of the 290bp β-actin amplicon. Presence of T. cruzi DNA was analyzed by means of PCR reactions targeted to the variable region of kinetoplastid DNA (kDNA) (primers 121 and 122) and to the nuclear satellite sequence (TCZ1 and TCZ2). Considering tissue samples, 10 % of corneas (2/20), 20 % of sclera rings (4/20), and 14.3 % of eye muscles (1/7) have positive PCR findings. From patient analysis, corneas were T. cruzi positive in 20 % (2/10) of corneas, 40 % (4/10) sclera rings, and 25 % (1/4) eye muscles. Interestingly, the two donors with positive corneas also had sclera positive findings, suggesting higher parasite burden or a special tissue tropism. This is the first report of T. cruzi presence in human cornea that bring light on the use of seropositive patients as donors.

0706 - ANALYSIS OF JOINT VARIATION BETWEEN HUMAN CASES OF TEGUMENTARY LEISHMANIASIS AND SAND FLY ABUNDANCE IN A HYPER–ENDEMIC AREA OF ARGENTINA.

Maria Cristina ALMAZAN (1) | Griselda Noemí COPA(1) | José Fernando GIL(2) | Inés LÓPEZ QUIROGA(3) | Carlos Lorenzo HOYOS(1) | Silvana Pamela CAJAL(1) | Melisa Evangelina DÍAZ FERNÁNDEZ(3) | Julio Rubén NASSER(3) | Alejandro Javier KROLEWIECKI(1) | Rubén Oscar CIMINO(1) | Jorge Diego MARCO(4) | Andrea Paola BARROSO(4)

INSTITUTO DE INVESTIGACIONES DE ENFERMEDADES TROPICALES (1); INSTITUTO DE INVESTIGACIONES EN ENERGIA NO CONVENCIONAL (2); CÁTEDRA DE QUÍMICA BIOLÓGICA. FACULTAD DE CIENCIAS NATURALES. UNIVERSIDAD NACIONAL DE SALTA (3); INSTITUTO DE PATOLOGIA EXPERIMENTAL (4)

Leishmaniases are a group of diseases caused by Leishmania parasites that are transmitted by sand fly female bite. In Argentina, the north of Salta province is a hyper-endemic area of Tegumentary Leishmaniasis (TL), being Oran department one of the most affected zones. To achieve deeper knowledge about the disease transmission in that region, we studied the joint variation of TL cases and sand fly abundance in two periurban sites of Oran. Sand fly captures were executed with CDC traps placed at the neighborhoods El Cedral (EC) (one night/sampling) and Taranto (TA) (three nights/sampling) across a year. Species identification of female sandflies was made by observation of spermatheca and cibarium. Also, the clinical information of patients diagnosed at Instituto de Investigaciones de Enfermedades Tropicales (IIET) since 1989 to 2018 was analyzed to determine the monthly mean of TL cases and the time of evolution of lesions. A total of 102 female sandflies were caught in EC neighborhood, while 1,434 in TA. The most abundant species was Nyssomyia neivai. The months with the highest proportion of gravid females were December and February for EC and TA neighborhoods, respectively ($p<0.05$). Regarding patient information, the male: female ratio was 6:1 with a median age of 32 years old. The time of evolution determined was one month. It was seen that the peak of patient cases took place in March for EC and in May for TA neighborhoods, namely three months later. This lag between gravidness period (high risk of infection) and peaks of TL cases may be explained due to the time of evolution (one month), plus an incubation period that seems to last two months. Considering the sex ratio and the productive age of patients, the transmission could have been mainly sylvatic during work activities. The analysis of joint variation allowed reaching a better characterization of disease transmission which is fundamental for designing and implementing prevention and control measures.

0732 - FIELD IMPLEMENTATION OF A 3D PRINTER BASED DNA EXTRACTION METHOD COUPLED TO LAMP FOR CONGENITAL CHAGAS DISEASE DIAGNOSIS

Diana Patricia WEHRENDT (1) | Season WONG(2) | Lizeth ROJAS PANOZO(3) | Silvia RIVERA NINA(3) | Lilian PINTO(3) | Marcelo ABRIL(4) | Daniel LOZANO(3) | Albert PICADO(5) | Joaquim GASCON(6) | Faustino TORRICO(3) | Julio ALONSO PADILLA(6) | Alejandro SCHIJMAN(1)

INGEBI-CONICET (1); AI BIOSCIENCES (2); CEADES (3); FUNDACIÓN MUNDO SANO (4); FIND (5); IS-GLOBAL (6)

Congenital Chagas disease entails the transmission of Trypanosoma cruzi infection from a mother to her child. With currently available chemotherapies, the cure rate for infected children is almost 100 % if administered early upon infection. It is therefore of great relevance to diagnose newborns on time. However, the algorithm to detect congenital T. cruzi infection involves the performance of microhematocrite or micromethod at delivery or during the first months of life and a confirmatory serology at 10 months of age. In highly endemic areas where people live far away from reference centers, many infants never go back to confirm the diagnosis and receive treatment if infected. The challenge is then to implement sensitive and rapid diagnostic techniques that can be performed in minimally equipped laboratories. At present there is a prototype loop isothermal amplification molecular test available (T. cruzi-LAMP kit, Eiken, Japan), with similar sensitivity to that of real time PCR (qPCR), but easier to use. Nonetheless, highly purified DNA is needed and obtaining it is time consuming and requires equipment unavailable in endemic regions. Thus, our aim was to couple the T. cruzi-LAMP kit to a recently developed DNA extraction device based on a low cost 3D printer (named PrintrLab), and to test its use in a hospital

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

Arturo MUÑOZ | Raul H LUCERO | Bettina L BRUSÉS | Laura B FORMICHELLI | Alejandro G SCHIJMAN

INSTITUTO DE MEDICINA REGIONAL, UNIVERSIDAD NACIONAL DEL NORDESTE

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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UNIVERSIDADE FEDERAL DO PARANÁ (1); IMPAM (UBA-CONICET) (2)

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).