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Conferences

C1 ***Mitochondrial dysfunction and oxidative stress in chagasic disease.***
Nisha Garg (PhD). Department of Microbiology & Immunology and Pathology, University of Texas Medical Branch, USA.

C2 ***Visceral Leishmaniasis in Paraguay.***
Andrés Canese (PhD). Programa Nacional de Control de las Leishmaniasis, Ministerio de Salud Pública y Bienestar Social, Asunción, Paraguay.

C3 ***Analysis of poly [dT-dG].[dC-dA] signals in T.cruzi.***
Beatriz Garat (PhD). Laboratorio de Interacciones Moleculares, Fac. de Ciencias, UdelaROU, Montevideo, Uruguay.

C4 ***Galectins in Trypanosoma cruzi infection: angels or devils?***
Adriana Gruppi (PhD). Fac. de Ciencias Químicas, Universidad Nacional de Córdoba (UNC), Argentina.

C5 ***Use of DNA and viral vectors to induce protective immunity against protozoan parasites.***
Mauricio Rodrígues (PhD). UNIFESP-EPM, San Pablo, Brasil.

C6 ***An antisense RNA silencing system regulates surface antigen expression in the intestinal parasite Giardia lamblia.***
Hugo Luján (PhD). Cátedra de Bioquímica y Biología Molecular, Fac. de Ciencias Médicas, UNC, Argentina.

C7 ***Hormones and Parasites: a world of possibilities to survive and reproduce.***
Marta Romano (PhD) . CINVESTAV-IPN, Mexico DF.

Symposium: Congenital Chagas´ Disease

Chairperson: Edgardo Moretti (PhD). Fac. de Ciencias Médicas, UNC y Servicio Nacional de Chagas, Argentina.

Symposium Workshop:

S1 *Congenital transmission of Trypanosoma cruzi in Argentina: determinants of a sustained trend.*
Ricardo Gürtler (PhD). Lab. de Eco-Epidemiología, Facultad de Ciencias Exactas y Naturales (FCEN), Universidad Nacional de Buenos Aires (UBA), Argentina.

S2 *Laboratory diagnosis and immunological aspects of congenital Chagas' disease.*
Beatriz Basso (PhD). Cátedra de Pediatría, Hospital Universitario de Maternidad y Neonatología, Fac. de Ciencias Médicas, UNC, Argentina.

S3 *Trophoblast-Trypanosoma cruzi interaction: role of the placental alkaline phosphatase in human placenta infection.*
María José Sartori (PhD). Instituto de Biología Celular, Fac. de Ciencias Médicas, UNC, Argentina.

Symposium Conference:

S4 *Congenital Chagas' Disease: Does evidence allow transference?*
Pedro Moya (PhD). Cátedra de Clínica Pediátrica y Neonatológica, Fac. de Ciencias Médicas, UNC, Argentina.

Workshops

W1 Advances in the treatment of Chagas´ Disease

Chairperson: Dr. Patricia Paglini (PhD). Cátedra de Química Biológica, Fac. de Ciencias Médicas, UNC, Argentina.

W1.1 *Advances in the antiparasitical treatment of Chagas´ disease.*
Rafael Gallerano (PhD). Cátedra de Medicina I, Fac. de Ciencias Médicas, UNC, Argentina.

W1.2 *Chagas' disease treatment with tricyclic drugs.*
Rivarola Héctor W. (PhD). Cátedra de Física Biomédica, Fac. de Ciencias Médicas, UNC, Argentina.

W1.3 *Squalene synthase and farnesyl pyrophosphate synthase as molecular targets for chemotherapy of Chagas' disease.*
Juan Bautista Rodríguez (PhD). Departamento de Química Orgánica, FCEN, UBA, Argentina.

W1.4 *Follow-up protocol of treated patients in latent phase. Preliminary results.*
Gustavo Barbieri (PhD). Centro de Chagas y Patología Regional "Dr. Humberto Lugones", Instituto de Biomedicina de la Universidad Católica de Santiago del Estero (UCSE), Argentina.

W2 Biochemistry and Molecular Biology I

Chairperson: Antonio Uttaro (PhD). Fac. de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario (UNR), Argentina.

W2.1 *Biosynthesis of polyunsaturated fatty acids in Trypanosomatids.*
Antonio Uttaro (PhD). Fac. de Ciencias Bioquímicas y Farmacéuticas, UNR, Argentina.

W2.2 *Lipid signals participate in Trypanosoma cruzi metacyclogenesis.*
Jorge Florin-Christensen (PhD). Departamento de Microbiología, Parasitología e Inmunología, Laboratorio de Bioquímica y Biología Celular, Fac. de Medicina, UBA, Argentina.

W2.3 *L-Proline, D-glucose and the intracellular diferentiation process in Trypanosoma cruzi.*
Ariel Silber (PhD). Laboratório de Bioquímica de Parasitas, Departamento de Bioquímica, Universidade de São Paulo, Brasil.

W2.4 *Energy metabolism in Trypanosomes: the putative role of the ATP regeneration systems by phosphotransferases.*
Claudio Pereira (PhD). Laboratorio de Biología Molecular de Trypanosoma cruzi (LBMTC), Instituto de Investigaciones Médicas Alfredo Lanari (UBA-CONICET), Argentina.

W3 Vectors of parasitic diseases

Chairperson: Ricardo Gürtler (PhD). Laboratorio de Eco-Epidemiología, FCEN, UBA, CONICET, Argentina.

W3.1 *Spatiotemporal analysis of Chagas' disease vector Triatoma infestans reinfestation in rural northwestern Argentina*.
María Carla Cecere (PhD). Laboratorio de Eco-Epidemiología, FCEN, UBA, CONICET, Argentina.

W3.2 *Leishmaniasis in Argentina: vectors and epidemic outbreaks.*
Daniel Salomón (PhD). Centro Nacional de Diagnóstico e Investigación en Endemoepidemias -CeNDIE-, ANLIS, Ministerio de Salud, Argentina.

W3.3 *Ticks (Acari: Argasidae, Ixodidae) found in humans in Argentina. Review of their role as vectors.*
A. Guglielmone (PhD). Laboratorio de Sanidad Animal, Estación Experimental Agropecuaria Rafaela, INTA, Rafaela, Argentina.

W3.4 *Geometric morphometry of wings: a new tool for the study of spatial structure of triatominea populations.*
Judith Schachter Broide (PhD). Laboratorio de Eco-Epidemiología, FCEN, UBA, CONICET, Argentina.

W4 Transmission of Trypanosoma cruzi: Geography, lineages, reservoirs and vectors.

Chairperson: Miguel Angel Basombrío (PhD). Universidad Nacional de Salta (UNS). Argentina.

W4.1 *Spatial component in population ecology of Triatoma infestans analyzed at different geographic scales.*
David Gorla (PhD). CRILAR, Anillaco, La Rioja, Argentina.

W4.2 *Clonal diversity of Trypanosoma cruzi and lineage-host associations in a rural area of the province of Chaco.*
Patricio Diosque. Instituto de Patología Experimental, Facultad de Ciencias de la Salud, UNS, Argentina.

W4.3 *Spatial distribution of lineages of Trypanosoma cruzi in triatomines and domestic animals in a rural area of Santiago del Estero.*
Marta Victoria Cardinal (PhD). Laboratorio de Eco-Epidemiología, FCEN, UBA, Argentina.

W4.4 *Distribution of triatomines in the province of Corrientes, Argentina.*
Elena B. Oscherov (PhD). Cátedra de Artrópodos, Fac. de Ciencias Exactas y Naturales y Agrimensura, Universidad Nacional del Nordeste, Corrientes, Argentina.

W5 Bioquimica y Biología Molecular 2

Chairperson: Sergio Angel (PhD). INTECH, Chascomús, Argentina.

W5.1 *Detoxification of hydroperoxide in trypanosomatids. Cloning and expression of Trypanosoma cruzi thioredoxin.*
Sergio Guerrero (PhD). Centro de Investigaciones sobre Endemias Nacionales (CIEN), Cátedra de Parasitología, Fac. de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Argentina.

W5.2 *Identification and analysis of intraspecific variation of secreted and membrane bound proteins from Echinococcus granulosus.*
Mara Rosenzvit (PhD). Instituto Nacional de Enfermedades Infecciosas, ANLIS "Dr. Carlos G. Malbrán", Argentina.

W5.3 *Characterization of the long-chain E-Isoprenyl Diphosphate Synthase from Trypanosoma cruzi.*
Esteban Bontempi (PhD). Instituto Nacional de Parasitología Dr. Fatala Chabén, Argentina.

W5.4 *Preliminar map of the Trans-splicing/Polyadenylation complex of Trypanosoma cruzi and its application for rational drug design.*
Martín Vazquez (PhD). INGEBI-FBMC, Facultad de Ciencias Exactas y Naturales, UBA, Argentina.

W6 Immune response in parasitic diseases

Chaiperson: Miriam Postan (PhD). Instituto Nacional de Parasitología Dr. Mario Fatala Chabén, Buenos Aires, Argentina.

W6.1 *Dendritic cells are able to segregate microbial and helminth antigens to different compartments, and simultaneously induce microbe-specific Th1 response and helminth-specific Th2 response.*
Laura Cervi (PhD). Department of Pathobiology, University of Pennsylvania, Philadelphia, US.

W6.2 *Chagas' disease and immunosupression: Molecular typing of T. cruzi populations directly from blood and tissue lesions of patients with reactivation.*
Alejandro Schijman (PhD). Laboratorio de Biología molecular de la Enfermedad de Chagas, INGEBI, Buenos Aires, Argentina.

W6.3 *Strategies for the identification of targets of cellular immune responses in chronic Chagas' disease.*
Susana Laucella (PhD). Instituto Nacional de Parasitología Dr. Mario Fatala Chabén, Buenos Aires, Argentina.

W6.4 *The lung as an organ of parasite destruction in trichinellosis.*
María Laura Verzoletti. Instituto de Inmunología Humoral, Catedra de Inmunología, Fac. de Farmacia y Bioquímica, UBA, Argentina.

Posters

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P01.

MONOCLONAL ANTIBODY ANTI-*Trypanosoma evansi* DISTINGUISHES ANTIGENS COMING FROM *Trypanosoma cruzi*, *Babesia equi* AND *Babesia caballi*

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Trypanosoma evansi, *T.cruzi*, *Babesia equi* and *B.caballi* coexist in horses in the sub-tropical area of Argentina. The objective of this research was to study the cross antibody-antigen reaction between these protozoan parasites and evaluate the specificity of the monoclonal antibody 2-4F6 (Mab) against *T.evansi*.

Using an indirect immunosorbent assay (ELISA) test, *T.evansi* antigens had positive reactions with *T.cruzi* and *B.equi* immune sera; *T.cruzi* was positive with *T.evansi* and *B.equi*. This antigen only cross-reacted with *T.evansi* serum and surprisingly not with *B.caballi*. However *B.caballi* gave positive with *B.equi* and *T.evansi* sera. The Mab against *T.evansi* highly reacted with their homologous antigen but not with any of the heterologous antigens.

In an ELISA inhibition test, double concentrations of *T.evansi* antigens from 4 to 512 µg/ml of proteins, blocked between 84% and 92% the Mab activity, while the heterologous antigens employed at the same concentration did not produce inhibition. The antigen recognised by the Mab was not detected in the mentioned heterologous antigens using a double antibody sandwich ELISA technique for antigens detection.

The results in three different tests showed that the Mab 2-4F6 *T.evansi* did not react with *T.cruzi*, *B.equi* and *B.*caballi and could be useful to design a specific diagnostic test for *T.evansi* to be used in areas which in horses, these protozoan parasites coexist.

P02.

AUTOMATIC DETECTION OF *Trypanosoma cruzi* IN BLOOD IN LOW CONCENTRATIONS. COMPARISONS WITH VISUAL METHOD

Alanís E[1], Romero G[2], Alvarez L[1], Martinez C[1], Basombrío MA[3]
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In this work the performance of an opto-mechanic system designed for automatic *T. cruzi* detection in blood samples at low concentrations is analyzed. The method lies on laser interferometry and consists on detecting the parasite through its motility. The results are compared with the current visual method, taking into account: a) time to detect the first parasite, b) time needed to count 100 parasites and c) quantity of parasites detected in 100 fields. To test the repeatability and reliability of the overall system, various samples with low degree of parasitemia and a sample without parasites are analysed in order to dismiss false determinations with the automatic method. A good correlation within both methods is obtained regarding concetration. Nevertheless, at low concentrations, the automatic method detects the first parasite in 37 seconds instead 96 seconds. Furthermore, the automatic system yielded more positive determinations, in concentrations of 0,2 parasites in 100 fields, (4 positive determinations against 2, in 5 experiments). The sensibility of the method allows the use of low magnification (approximately 200X) and a field vision area twice as larger than the visual one. The time needed to analyze each field is approximately 2 seconds, so it is possible to scan a larger sample area in shorter time than with the visual method. These advantages, added to lack of fatigue, are the principal goal of the proposed system. Consequently, this system could become an important tool in clinical laboratory. In some aspects this method can compete advantageously with the Hemoculture methods.

Acknowledgements: to Alejandro Uncos and to Federico Ramos for their technical collaboration. This work was supported by Howard Hughes Medical Institute and the Consejo de Investigación UNSa.

P03.

SEARCH OF ACANTHAMOEBA SPP IN LIQUID OF CONTACT LENSES

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Free life parasites of sort *Acanthamoeba* which are pathogenic for the man exist. The objetive of this work was to investigate the presence of these parasites in the solution where a male patient of 14 years old's contact lenses were conserved. The patient who went to the "Hospital Provincial del Centenario, Servicio de Oftalmología" was affected by epithelial corneal ulcer in his right eye. The liquid was centrifuged at 1500 rpm during 5 minutes and immediately a direct microscopic observation was made at 100x and 400x increases. Smears were also made to stain with Tricromic methods; in both cases could observe ameboideas forms compatible with the morphology of trophozoites of *Acanthamoeba spp*. Germs like *Klebsiella pneumoniae* were observed in the bacterial culture and so the treatment consisted in antiinflamatories, vasodilators and Ofloxacina. The patient imparied his ophthalmological condition and a conjuntival injection l and bilateral queratitis were diagnosed. He was treated with trimetropima + Polimicina b + dexametasona + hexamidina. In patients with ophthalmological pathologies who do not respond to the therapies with extensive spectrum microbial drugs and have the conditions that facilitate the entrance of the amoebas (contact lenses, inadequeate hygiene of them,swimmers, poor personal hygiene,etc), it is necessary to orientate the diagnosis to the search, in the appropiate material, of the Acanthamoeba spp. to prevent a possible queratitis or a primary amebic meningoencephalitis amebic.

P04.

PILOT TRIAL FOR NEWBORN SCREENING OF CONGENITAL CHAGAS: PRELIMINARY RESULTS

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Congenital Chagas (CCh) is a parasitosis associated with serious alterations and high rate of mortality, whose vertical transmission is 4-10%. It constitutes a serious public health problem, however, its early detection and treatment ensure more than 90% of the therapeutic success. Diagnosis is performed through the search of the *Trypanosoma cruzi* using the micro-Strout method in the blood of those newborns (NB) whose mothers presented reactive serology during pregnancy, and posterior serological follow-up at 6-8 months of age in those NB with negative micro-Strout. Nevertheless, since on many occasions the mother is not studied, the measure of IgG antibodies (Ab) in blood specimens of the NB collected on filter paper allows for the presumptive diagnosis of CCh, which will later ask for confirmation in accordance with what was previously mentioned. The objective of this paper is to present the preliminary results obtained in a pilot trial of Newborn Screening (NS) for CCh. For that aim, specimens of whole blood collected on filter paper belonging to 3,386 NB coming from 5 Hospitals were analyzed. IgG Ab anti-*T. cruzi* were measured in those samples using the kit Chagatest ELISA SD recombinante (Neonatal) from Wiener Lab, which was pre-viously validated. 128 NB (3.8%) presented reactive serology, being this value an estimate index of the percentage of mothers with Chagas. In one of the 5 Hospitals, which attends a population mostly living on farms, that index was of 12.3%. So far, none of the 128 NB has been notified with positive micro-Strout and are now waiting to have the follow-up serology tests. Although the method used is not specific for CCh detection, it makes up for the lack of compliance with control methods for Chagas during pregnancy, thus initiating the sequence of diagnostic actions both on the neonate and on the mother.

P05.

SIGNS OF CURE AND THERAPEUTIC RESÍSTANSE IN CHAGAS' DISEASE CHRONIC PATIENTS, IN A THREE YEARS FOLLOW-UP

Lacunza D[1], Sánchez Negrete O[1], Mora MC[1], Segura MA[1], Uncos A[1], Ramos F[1], Garayzabal MI[2], Del Castillo N[2], Basombrío MA[1].
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Benznidazole treatment (5 mg/kg/day, 60 days) was performed in 17 Chagas' disease chronic patients ranging from 19 to 41 years old (Mean: 25.82 ± 6,65 years). All patients belonged to Kurchnir's Group 0, except one of them who belonged to Group 1. The treatment was monitored using Polymerase Chain Reaction (PCR) with 121-122 primers; Hemoculture (HE); ELISA and Indirect Hemo Aglutination (IHA), using commercial kits. These tests were carried out before treatment (BT), and every 6 months after treatment (AT). Eight out of 17 (47.05%), and 5/17 (29.41%) patients, were positive for PCR and HE respectively BT; turning into 9/17 (52.94%) and 0/17 respectively, about 37.9 ± 6,34 months AT. Eight out of 17 patients (47.05%), displaying a high titers (Mean: 1/992) for IHA BT, showed a significant reduction at 38 months AT; while 9/17 (52.94%) patients displaying lower titers BT (Mean: 1/519), kept or increased them AT. Four out 8 patients (50%) showing a reduction in the IHA titers AT, had a positive PCR reaction AT. Five out of 9 (55%) patients who did not reduce the titers, had a positive PCR reaction AT. ELISA tests were positive in all analyzed samples. All the serological tests were performed by a unique operator employing the same kit lot. Infections with *Trypanosoma cruzi*, relatively less virulent strains could explain the early reduction in serological titers, although the PCR results suggest a therapeutic resistance in half or them. May be in these cases PCR tests detect parasite persistence that would not be inducing an antibody response, as was suggested in a group of PCR positive-seronegantive patients by others authors (Salomone OA *et al*. *Trypanosoma cruzi* in persons without serologic evidence of disease, Argentina. Emerging Infectious Diseases, vol. 9, N. 12, december, 2003).
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P06.

EVALUATION OF PCR METHODS TO DETECT AND CHARACTERISE FLAGELATE-PROTOZOANS IN FECAL SPECIMENS FROM TRIATOMINES

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PCR methods were evaluated in order to 1) improve the sensitivity and specificity of detection of *T.cruzi* DNA in fecal samples of triatomines, which were negative by direct microscopical observation and II) to discriminate between *T. cruzi* and other flagelates in faeces from *T .infestans* (Ti), *T. guasayana* (T.g) and *T. garciabesi* (T.gb) with positive MO findings, collected in October 2002, Amamá, Sgo del Estero. I) MO negative samples: A) DNA from 32 faecal samples of *T.i* was isolated with DNAzol (Invitrogen, USA) or boiled during 10 minutes after incubation with Chelex-100 (Sigma, USA). A test of PCR inhibitors was carried out spiking 10 pg of a cloned internal standard to each DNA lysate. B) We compared the PCR concordance between 121-122 (amplicon 330 bp) and 34-67 (amplicon 120 bp) based PCR procedures in 57 DNAzol lysates; C) 93 *T.i* samples lysed using DNAzol were tested by PCR, and 41 of them, with negative PCR findings, were re-tested by a Hot Start approach using a Taq polymerase bound to Antibody (Taq Platinum, Invitrogen, USA). RESULTS: A) An inhibition of 98% and 20% was detected from lysates prepared by boiling or DNAzol, respectively. B) PCR concordance between 121-122 and 34-67 was 100% (3 + /57). C) PCR detected 3 positive cases out of 93, whereas the Hot start-PCR allowed detection of 5 new cases out of the 41 tested. Moreover, 22 samples of T.g and 19 of Tgb, prepared with DNAzol. were studied. None of them was positive for *T.cruzi* DNA. II) MO positive specimens: 43 T.i, 2 *T.g* y one *T.gb* samples were analysed. All T.i were PCR positive confirming their infection with *T. cruzi*. Tg and Tgb samples were PCR negative. In 21 T.i, the lineage was characterised directly from the lysates using miniexon spacer-PCR and D7 rDNA PCR. 19 were *T.cruzi* II,1 was *T.cruzi* I and the other one was a mixed *T.cruzi* I and II infection. Tg and Tgb samples were negative by the miniexon-PCR assay. However, they gave rise to a 255 bp amplicon when amplified by D7 rDNA-PCR. This product had the same molecular weight than one obtained from a Blastochrithidia strain. *T.cruzi* I generated a 270 bp band, *T.cruzi* II a 300 bp amplicon, showing the usefullness of this approach to identify among flagelated protozoans in triatomine vectors.

P07.

ALTERATIONS OF THE HEMOGRAM IN PEDIATRIC PATIENTS WITH ACUTE CHAGAS DISEASE IN SANTIAGO DEL ESTERO, DIAGNOSED BETWEEN 1972 AND 1986

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Introduction: The modifications in the pediatric patient's hemogra present particularities that facilitate certain characteristics in the acute Chagas Disease. These modifications are accentuated in the pediatric group until 2 years of age, in the light as well as in the moderate and mot serious forms of the illness, with myocarditis and meningoencefalitis. **Objectives:** To determine the modifications of the hemogram in children with acute Chagas Diseases up to 2 years of age. **M and M:** Population studied were children that visited the "Centro de Chagas", during years 1972-1986 with: a) characteristic symptoms of acute Chagas Disease, b) confirmation of parasitical tests for T Cruzi (Fresh drop, Strout and/or microhematocrite). Also, hemograms the day of the diagnose. Design of the study: quantification, retrospective, observational and traverse. Statistical treatment: the summarize of measures of central tendency and dispersion. **Results:** 140 patients were included, out of 529 patients with acute disease during the mentioned period. The analyzed variables presented the following results: a) age (in years): X 0,82+DS 0,55 (min. 0,10-máx. 2,0); rural area 91%; Hemoglobin (gms%): X 9,31+1,42 (7,0-11,2), Hematocrito (%): X 29,4+3,64 (21,0-35,0), White Cells (x ml3): X 16.660+8.799 (10.000-50.000) and Linfocitosis (%): X 75,4+8,61 (59,0-90,0). **Conclusions:** 30% of the children presented high parasitism, with marked alterations of the hemograma, with anemia moderate to marked, leukocytosis with values of up to 60.000 white cells per mm3, accompanied by symptoms and specific signs of illness of acute Chagas.

P08.

HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF UMBILICAL CORDS FROM MOTHERS INFECTED BY *Trypanosoma cruzi* IN THE CITY OF SALTA

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We studied with histopathological (HP) and immunohistochemical (IHQ) methods, previously diagnosed with parasitological methods and Polimerase-Chain Reaction (PCR), umbilical cords from new borns with Congenital Chagas Disease. Our objectives were to determine the susceptibility of this organ to *T. cruzi* and to verify the diagnostic value of amastigote detection in the umbilical cords, for the early diagnosis of fetal infection. We worked with 18 umbilical cords recruited from July 1997 to July 2002 at the Maternidad Provincial of Salta. We selected for this study the middle and distal section of each cord, and embedding the tissue in paraffin and staning with Hematoxilyn-Eosin and Immunoperoxidase Techniques. We analized six section of each cord. The intensity and localization of inflammatory process and the presence of amastigotes were recorded. For IHQ we used primary antibodies from rabbit and we applied DAKO LAB Kit systems(labelled streptavidin phosphatase and peroxidase). Amastigotes were detected by HP and confirmed by IHQ in 7 cords (39%). Six of them had moderate or slight inflammatory infiltrates and in one no inflammation was detected. Most nests displayed few parasites. The IHQ method confirmed HP without detecting new infected cords. In conclusion, amastigotes are frecuently found in cords from infected newborns, but this finding is not as sensitive as other methods that detect congenital infection.
Supported by CIUSa, Univ. Nac. de Salta.

P09.
DI DETECTION OF *Trypanosoma cruzi* DNA IN BLOOD FROM CHAGASIC SERONEGATIVE PERSONS

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Current diagnosis of Chronic Chagas Disease relies on serologic detection of specific immunoglobulin G against *Trypanosoma cruzi*. Several authors have informed the presence of parasites in blood from seronegative patients detected by amplification of an specific sequence from the *T.cruzi* genome by polymerase chain reaction (PCR). The goal of the present work was to determine the prevalence of parasitemia by PCR and clinical characteristics of seronegative persons with high epidemiologic risk of Chronic Chagas Disease. We studied 194 persons belonging to two different areas. 110 patients with epidemiologic Chagas disease records were recruited from an urban cardiology clinic, and 84 persons were from a highly disease-endemic area. All patients completed an epidemiologic and clinical questionnaire and had physical examinations (ECG, echocardiogram). IFA, HIA and ELISA were performed to detect chronic infection. We search for DNA of *T.cruzi* in blood by amplification of 220 bp specific nuclear fragment. 80 persons (41%) were negative for serologic test and 12 persons (15%) were positive for PCR amplification. Three patients with negative serologic findings and positive PCR showed clinical signs and symptoms that suggested Chagas Cardiomyopathy. We observed, that is prevalent the presence of parasitemia in patients with high risk of Chronic Chagas Disease with negative conventional serologic test. These results suggest that special care should be taken in diagnosis, therapy and blood transfusion that involve persons with high risk of Chronic Chagas Disease.

P10.
SIMPLIFIED INDIRECT IMMUNOFLUORESCENCE REACTION FOR THE DIAGNOSIS OF CHAGAS'DISEASE

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The indirect immunofluorescence test (IIF) is one of de most sensitive and specific used by the conventional serology for the diagnosis of Chagas'disease. The IIF, considered as a reference technique, is expensive and consumes a considerable amount of time for its execution. With the purpose of making a faster and less expensive execution, in this work we compared the results obtained in serum with a battery of diagnostic reactions, including the IIF and the results of this modified in: a) incubation and washing times, and b) the conservation of the diluted conjugate.
Ag: epimastigotes of *T cruzi,* strain Tulahuen, 50 parasites/field. Serums obtained with venous puncture. IIF, HAI and AD-2ME were made and in some serum ELISA. Quantified with cut off title 1/32, they were considered as S(+) and S(-). Diluter: SSB pH: 7,2-7,4. Antiserum: anti total human Ig antibodies, of rabbit, marked with fluorescein isotiocianato (I. Pasteur®) diluted in Blue of Evans. Aliquot with sodium azida they were conserved at -20ºC and 4ºC. The technique was carried out twice, A and B, for the modification of the times: n=328 serums, 180 S(+) 148 S(-). A: habitual technique. B: in the 2 steps of the reaction the incubation was of 15' and a single wash was made with SSB and one with distilled water of 1' each one. For the modification of the one conjugated the reaction was carried out for quadruplicate. The 1st stage without modifying. In the 2nd stage recently prepared marked antiserum was used in A and B, n=180 serums, 84 S(+) 96 S (-). In C and D: antiserum conserved at -20ºC and 4ºC during 30, 60 and 110 days, n=60 serums, 29 S(+) 31 S(-). We took like reference the test with recently prepared antiserum. The statistic agreement found (I.Concordance, J. Jouden, Kappa) with the two modifications tried, allow us to reach the conclusion that time is reduced 60%, and the using of the diluted marked antiserum with fluorescein in later determinations simplify the IIF reaction as regards its execution time and allows to economize an expensive reagent, whitout significatives modifications with the reference test.

P11.
PRECOCITY OF SEROLOGICAL TESTS IN THE ACUTE CHAGAS' DISEASE

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Introduction: The *T. cruzi* infection generates humoral and cellular immune response. In the acute phase of the illness, IgM antibodies are liberated during the first week, later IgG antibodies. This is what allows controlling the acute infection that evolves then to the chronicity. **Objective:** To demonstrate the precocity of the serology during the acute phase of Chagas Disease. **M and M:** Population studied were patient that entered to the "Centro de Chagas" during the years 1972-1976, with: a) characteristic symptoms of acute Chagas Disease, b) parasitological tests positive for *T Cruzi*, c) titrated serological tests of Immunofluorescence (TIF) and Hemoaglutination (HAI). Study design: quantitative, retrospective, observational and traversal. Statistical treatment: summarize of measures of central tendency and dispersion. **Results:** Of 529 original patients, only 144 patients completed the inclusion criterions, coming the great majority of rural areas, with age average: 5 (0, 1-15) years. The time of evolution from beginning of the symptoms of the acute period, presented average: 12 (3-33) days. The results of HAI (1:32) presented average: 12 (4-128) titers. The TIF presented average: 64 (16-512) titers. **Conclusions:** The test that use membrane Ag and of the lash TIF resulted of more precocity in becoming positive; showing 65,2% positive at day 0 of infection; for the HAI that uses Ag of the cytoplasm was of 24,8%.

P12.
BLOOD RHEOLOGY STUDY IN EXPERIMENTALLY *Trypanosoma cruzi* (*Tc*)-INFECTED RATS

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Previously, we showed increased plasma viscosity (η_p) and red blood cells (RBC) volume with blood fluidity diminution in *Tc*-infected rats (Medicina 60 (5/2): 790, 2000). In the present study we study the possible causes of such changes. Male "l" rats were infected at weaning with 10^6 Tulahuén strain tripomastigotes by the subcutaneous route. The amount of blood parasites (ps), plasma protein fractions (α_1, β, γ globulins and albumin) by electrophoresis and RBC shapes by electronic microscopy using Bessis classification, were measured at 7 (GA) and 14 (GB) days post-infection (p.i.). GA showed higher ps: 65.3 $\pm$ 28.5 (n=20) vs. GB: 2.4 $\pm$ 1.1 (n=19) x $\pm$ d.s, $p< 0.001$. α_1, β-globulins and albumin fractions were lower in GA and GB vs. control group (GC), $p< 0.05$). γ-globulins were higher in GB vs. GA and GC (GA: 0,8$\pm$ 0.23; GB: 0,95 $\pm$ 0.15; GC (n=18): 0,8 $\pm$ 0.19 (g/dl) respectively, $p<0.05$) and were related with the η_p increases (r: 0,67, $p<0.05$). BRC stomatocyte I shape transformation (GA: 27,77 $\pm$ 6,41; GB: 29.57 $\pm$ 4,.34 % respectively) and echinocyte (GA: 22,4 $\pm$ 8,76; GB: 34,51 $\pm$ 1,11%, $p<0.05$) were found. γ-globulin fraction and non-discocytes BRC increases could explain blood hiperviscosity in the acute stage of *Tc* infection in rats.

P13.

REINFECTIONS IN THE CHRONIC PHASE OF EXPERIMENTAL CHAGAS' DISEASE DID NOT AGGRAVATE THE MIOCARDIOPATHY INDUCE BY *Trypanosoma cruzi*

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On the basis of the clinical features of Chagas' disease, the patients could suffer a the digestive form of the disease (10%) or the cardiac one (30%). The variability of the symptoms range from a mild electrocardiographic alteration to sudden death and it may be due to many interrelated factors, two of them are the parasite strain and the reinfections. We have previously demonstrated that reinfections induce severe cardiac damages in the acute and indeterminate phase of the infection. In the present work, we analyse, in albino Swiss mice infected with *T. cruzi* Tulahuen (Tul) strain (n=70) and SGO-Z12 isolate (n=70) (SGO), the effect of reinfections carried out in at 150 days post infection (dpi). We studied parasitaemia, survival, electrocardiographic abnormalities, heart histopathology and affinity and density of ß adrenergic receptors'. The maximum parasite number was 1,19 ± 0,84 p/ml (160 dpi) for Tul group while there were not parasites in blood in SGO mice. The survival rate in reinfected and Tul infected groups, 320 dpi, was similar (43-50%) while in the group infected with SGO-Z12 isolate it was higher (64%) ($p<0.01$) the % of mice that showed at least one kind of electrocardiographic alteration was similar in all groups (150, 185, 240, 320) (52-71%). The ß -adrenergic receptors showed in the chronic stage an affinity (Kd, nM) of 11,21 ± 0,26 and density (Bmax, fmol/mg. Prot) of 53,33 ± 0,71 for Tul and for SGO a affinity of 7,32 ± 0,19 and a density 184,02 ± 2,10. These values were significantly different in the groups reinfected ($p<0.01$). The heart histopathology in all groups showed the typical alterations of the chagasic cardiomiopahty. These data showed that the reinfected process in the early chronic phase of experimental Chagas' disease developed an immune response that did not modify the natural history of Chagas' disease.

P14.

"*IN VIVO*" STUDY ON THE INFECTIVITY OF 2 ISOLATES FROM DIFFERENT LINEAGES OF *Trypanosoma cruzi*

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This investigation analyzed parameters of virulence, immunogenicity and pathogenicity of two isolates of different lineages of *T. cruzi* from the Chaco province. Forty BALB/C mice were inoculated with 2 x 10^4 sanguineous trypomastygotes of the I (n=20) and IId ((n =20) *T. cruzi* lineages. Microstrout was performed in both groups two times weekly for 60 days after inoculation (post inoculation, PI). Serological samples were taken on days 17, 30 and 60 PI. 10 animals of each group were autopsied on days 20 and 60 PI, samples were taken from the heart, muscle, urinary bladder and colon for histological analysis. The level of infection of the organs was characterized as null, mild, moderate and severe then the number of amastygote nests/mm^2 was calculated. Several of the mice inoculated with the *T.cruzi* IId produced positive microstrouts between days 5 and 20 PI, reaching a maximum of infected individuals (50%, 10/20) on day 12 PI. On the other hand, only 5% (1/20) of the mice inoculated with *T.cruzi* I tested positive on day 12 PI. IgG anti- *T.cruzi* was detected in all the animals on day 60PI, but the ELISA positivity was greater on day 30 PI for the group inoculated with *T. cruzi* I. On day 17 PI all the animals were negative for the ELISA test. In mice inoculated with *T.cruzi* I, a significantly higher level of inflammation was detected in the muscle, urinary bladder and colon ($p<0.001$). No differences in the heart were observed in either group. The number of amastygote nests, however, was significantly higher in the heart, urinary bladder and muscle ($p<0.001$) of animals inoculated with *T.cruzi* I on day 20 PI. These preliminary data suggest that in the early stage of infection, the T. *cruzi* I isolate provokes more tissue parasitemia, less blood parasitemia and more inflammation than the *T. cruzi* IId isolate.

P15.

TRYPANOCIDAL ACTIVITY OF THE DRUG TAK-187 IN A MURINE MODEL OF ACUTE INFECTION BY *Trypanosoma cruzi*

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The trypanocidal activity of an experimental triazole, TAK 187, which inhibits the enzyme C 14a demethylase of *T. cruzi*, was evaluated in experimentally infected animals. Sixty Swiss mice were infected by ip route with 10^3 Tulahuen strain trypomastigotes and distributed in 3 experimental groups, which received treatment starting at day 12 p.i. Bzl group (n=20): treated with benznidazole, 200 mg/Kg/day during 30 days; group TAK (n=20): treated with TAK 187, 20 mg/Kg/day, every other day during 60 days; control group, vehicle alone. Every animal was examined parasitologically by fresh blood mounts 2-3 times a week during 45 days, by hemoculture on day 91 and by blood PCR on day 112 p.i. All animals surviving were sacrificed on day 198 p.i. Serum samples were drawn for ELISA and tissue samples of urinary bladder, heart, liver and skeletal muscle were fixed in 10% formalin for histological studies. Results: TAK 187 indued a large reduction of the parasite load of infected animals, comparable to the effect of BZL: 70% (14/20) of controls presented positive hemoculture by day 91 p.i., whereas 100% were negative in BZL and TAK groups. by day 91 ($p<0.001$). *T. cruzi* kinetoplastic DNA was detected in 93% (12/13) of controls, 33% (6/19) of BZL group and 78% (13/18) of TAK group. Both TAK and BZL groups displayed lower antibody levels than controls ($p<0.001$) and milder histopathological alterations in liver, skeletal muscle and heart. TAK 187 displayed a stronger protective effect than BZL against heart lesions. BZL is the only drug being now in use in Argentina. These results suggest that TAK 187 produce a therapeutic effect similar to that of BZL, but using 10- fold lower doses.

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P16.

USE OF MOLECULAR AND SEROLOGICAL TECHNIQUES TO EVALUATE ETIOLOGICAL TREATMENT IN EXPERIMENTAL INFECTION BY *Trypanosoma cruzi*

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The usefulness of molecular and serological techniques was assessed to monitor cure after specific chemotherapy with Benznidazole (BZL), in experimentally infected animals. Forty Swiss mice were infected by intraperitoneally route with 500 Tulahuen strain trypomastigotes and distributed in 4 experimental groups: acute (n=10) and chronic (n=10) treated with BZL, acute (n=10) and chronic (n=10) control untreated animals. BZL was administrated 200 mg/Kg/day during 30 days, starting at day 15 p.i. in the acute group and 150 p.i. day. in the chronic group. PCR samples were taken at 13 p.i. day from 10 of the acute group, and at 105 and 148 p.i. day from all chronic animals. Post-treatment (Pos-T) samples were taken from both groups at 35 and 127 Post-T day. Antibody levels were analyzed by ELISA in every animal at 7, 35, 127 and 210 Pos-T day. Results: Statically significant differences in antibody levels were detected by ELISA only between treated and untreated acute animals ($p=0.0286$). Both acute and chronic groups displayed lower parasite load when compared with the initial. PCR reaction was negative in all acute treated mice (5/5) analyzed at 127 Pos-T day, while all the control group mice displayed a positive PCR reaction. Eight out of 9 chronic treated mice (88%) became PCR negative at 127 Pos-T day while only 2 out of 9 control mice (22%) displayed a negative reaction ($p=0.0152$). These results suggest that PCR could be useful to evaluate the treatment efficacy in the chronic phase of Chagas disease.

P17.

EVALUATION OF THE CELLULAR DAMAGE INDUCED BY THE ANTIGEN ES OF *Toxocara canis* IN ISOLATED HEPATOCYTES, MAINTAINED IN PRIMARY CULTURE

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Toxocara canis is a nematode parasite found in dogs. It is the most important agent of human toxocariosis, causing visceral larva migrans and ocular toxocariosis. The infective larvae produce the *in vitro* excretion-secretion antigen (ES) which is made up of a set of biologically active glycoproteins. Larva migration affects the liver, the lungs, the brain and other organs, as it could be observed *in vivo* studies. The aim of this work was to evaluate the cellular damage caused by the *in vitro* ES antigen in rat hepatocytes. The antigen was obtained through the Savigny technique. The isolation of the hepatic cells was carried out by the method described by Berry *et al*, using Wistar adult rats. The percentage of viable cells was calculated by the tripan blue exclusion technique. Suspensions with a minimum of 85% of entire cells were used. $2x10^6$ hepatocytes were cultured in RPMI-1640 medium with 10% of inactive fetal bovine serum and antibiotics, during 2 hs. at 37ºC in a 5% of CO_2 atmosphere in order to stick the cells to the glass. 10 to 150 µg/ml ES antigen concentrations were assayed together with 2 controls with and without triton, during 4, 18 and 24hs. Cellular damage was estimated by release of cytosolic and/or mitocondrial enzymes (LDH, ALT, AST) to the extracelular medium and the hepatocyte viability was assessed by means of direct fluorescence. The maximum effect was reached with 50 µg / ml ES, 43% colored cells died after 18hs and the liberated enzymes: LDH 57%, ALT 31% and AST 28% expressed regarding the values of the control with triton (100% of each enzyme). The cellular damage would be due to, among other factors, to enzymes present in the composition of the ES antigen, secreted by the parasite larvae, as the serine proteasa.

P18.

EFFECT OF A JUVENILE HORMONE ANALOGUE ON *L.amazonensis* AND *L.braziliensis*

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Leishmaniasis, a disease that is caused by a protozoan parasite (*Leishmania sp.*), is transmitted by means of a dipteran vector (*Phlebotominae,* "sandfly"). Leishmaniasis presents in America three main varieties of clinico-pathological forms: cutaneous disease, mucocutaneous disease and visceral disease. In Argentina cutaneous disease is an endemic parasitosis and its rate of primocutaneous infection and mucous lesion is approximately the 20% of cutaneous primary unresponsiveness. The aim of the present work was to study the biological effects of methoprene (Metho), a juvenile hormone analogue, on *L.(V)braziliensis* (L.b) and *L.(L)amazonensis*(L.a). Previously, 200 µM of Metho caused growth inhibition (95%) of *L.mexicana mexicana*-promastigotes (Stoka,1996), and 150 µM of Metho caused cytolytic effects (100%) on bloodstream trypomastigotes (Esteva *et al*., 2002). In this way promastigotes of *L.b* and *L.a (2x10^5/ml)* were incubated in BHT medium+SFB20% with different quantities of Metho. It was observed that the most important inhibitory effects of Metho were in: *L.a* 72 hours (92.2 and 96.6%) and *L.b 48 hours* (93 and 96%) with 250 and 500 µM of Metho respectively. In addition two groups of hamsters (30 days-old) were infected with $1x10^6$ *L.a.* promastigotes (intradermic). One of these groups was treated during 4 weeks with 5mg of Metho/day/animal, by means of a gastric-sound, using sunflower oil as vehicle. The other group was treated under the same conditions but without the addition of Metho. Weekly the diameter of leg-lesion was measured; the result was the same in both groups. **Conclusions** "*In vitro*" experiments showed an important parasiticide effect on *L.b* and *L.a.* In contrast, "*in vivo*" experiments did not show differences between control and treated-groups.

P19.

SEROLOGIC-CLINICAL BEHAVIOUR OF ADULT CHRONIC CHAGASIC ASYMPTOMATIC AT THE BEGINNING, UNTREATED AND TREATED WITH TRYPANOCIDAL DRUGS DURING AN AVERAGE PERIOD OF 20 YEARS

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It is even a controversial point the efficiency of the trypanocidal drugs (benznidazole; nifurtimox) in adult chronic chagasic. In this work was evaluated the behaviour of the conventional serology (CS)and clinical evolution of adult chronic chagasic, asymptomatic at the beginning, untreated and treated with trypanocidal drugs during an average period of 20 years. In the study group (n=114)(17 to 46 year-old) 57 patients received specific antiparasitic treatment and 57 remained untreated. All the patients were performed clinical exams, ECG, X-chest ray and xenodiagnosis (Xd) to the 67% of them. All patien's sero stored during the follow-up were simultaneously analysed by means of CS (DA-2ME; IHA; IIF). Serologic evolution of the treated patients: 33.3% the antibody titers remained constant; 38.6% decreased and 28% (16/57) showed non-reactive the final CS. 7/16 were repeatedly negative. Despite patients were not controlled in equal period, the time of becoming negative after of the treatment was very variable. It was not observed correlation with the age at which the patients received treatment. The serological titers were not decreased in the untreated patients. The geometric mean of IIF-assessed antibody titers, at the beginning and at the final of follow-up were: 90±3,3 - 36±5,2 in the treated and those of non-treated ones 72±3,3 - 93±4,4. Clinical evolution: 3/57 (5.3%) treated patients presented disturbances suggesting CrChM (average age 31 year-old) and 10/57 (17.5%) non-treated (average age: 33 year-old). Alterations more common were: LAFB and frequent VE. The 95% of Xd performed before of the treatment. were (+). All them were (-) after itself, independent of serological and clinical evolution. The treated adults chagasic had lower clinical evolution that the untreated. It was observed decrease and/or disappearence of anti-*T. cruzi* antibody in the 67% of who were treated, while in the untreated controls were not observed diminution of the serologic titers during the follow-up.

P20.

ARCHAEABACTERIA: FROM THE NATIVE SALARS TO ARCHAEOSOMES IN THE LAB

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Recent work form another groups had determined that the so-called *archaeosomes* (vesicles formed from polar lipids extracted from archeabacteria) are excellent adyuvants of humoral and cellular immune responses. In this work we will briefly describe the steps conducing to the optimisation of the growth conditions of micro-organisms extracted from the surface of a patagonic saline. First, we could isolate 3 morphological different colonies (from different strata: grey crystals GC, black mud BM and red crystals RC). The half of the gene RNA R16S for each of these colonies was sequenced using two primers for the *Archaea* Domain. The three colonies resulted to be extreme *halophilic archaea*, with high probability to belong to *Halorubrum* genera. In order to prepare the archaeosomes, the total polar lipids were extracted from batches from each type of colony and lipid films were obtained by evaporating the solvent under N_2 stream. The lipid films were suspended in 10 mM Tris pH 7.4; the resulted vesicular suspensions were negatively stained and observed under electronic microscope. The micrographs resulted the confirmation of the obtainment of the first archaeosomes prepared from extreme halophilic archaea grown in native ground; the formed vesicles resulted to be multilamellar, with an average size smaller than 500 nm. Further experiments showed that the three types of archaeosomes were efficiently internalised (phagocytosed) with retention of their aqueous content in macrophages cells. No change of viability of Vero cells and low cytotoxicity on J774 cells was found for 1, 10, 50 and 100 µg archaeolipids vs. 50000 cells. These preliminary results showed that: a) It is possible to prepare *in vitro* structurally stable archaeosomes from material extracted from extreme halophilic archaea grown in native ground. b) The archaeosomes are not cytotoxic *in vitro* and c) they are efficiently phagocytosed by macrophages. Due to their inherent absence of toxicity, archaeolipids are good candidates to be used as vaccine adjuvants in future approaches.

P21.

ACTION OF 8.O.4´ NEOLIGNANS ON *Trypanosoma cruzi*. MORPHOLOGIC ALTERATIONS OF EPIMASTIGOTES AND INHIBITION OF THE METACYCLOGENESIS

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Of the great structural variety of neolignans, the 8.O.4´ type represents a small group whose members have been isolated mainly fromn the family of the Myristicaceae and have been demonstrated to have wide spectrum of biological activities. Eight synthetic racemic structures in their ketone and alcoholic forms and three phenylpropanoids, halves of the active neolignans, were evaluated by their capacity to inhibit the growth of epimastigotes (Y strain) of *Trypanosoma cruzi* in axenic cultures. The results indicated that the dimers (IC_{50} = 23-42 mg/ml) have greater activity than their monomers (IC_{50} 50mg/ml) and ketones (IC_{50} = 23-33 mg/ml) have major activity that the alcohols. Our objective was to deepen the characterization of the activity of three active compounds analyzing the morphologic changes and their action on the metacyclogenesis. The structures of the epimastigotes were evaluated by optical microscopy through Giemsa colorations and electronic microscopy of transmission. Alterations in the nuclear morphology, loss of flagellum and vacualization have been observed. The metacyclogenesis *in vitro* was evaluated on the Dm28 strain by means of TAU/TAU3AAG culture, two concentrations below the IC_{50} of each compound were used. Under these conditions the three compounds showed an inhibiting activity on metacyclogenesis in concentrations 20 folds lower than IC_{50}.

P22.

EFFECT OF *Bidens pilosa* RAW EXTRACS IN *Trypanosoma cruzi* MONOPHASIC CULTURES, TULAHUÉN 0 STRAIN

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Chagas' disease, or american tripanosomaisis, is a pathology which affects million of people in whole America. Since 95 years of its discovery, an efficient chimiotherapy of infected patients is not yet available. In search of natural substances with tripanocid properties, raw extracts hydroetanolic of *Bidens pilosa* were tried. This is autochthonous herbaceous plant widely spread in the litoral region. The extracts were in 5 different concentrations , from 5 to 200 mg/L, in static monophasic cultures of *Trypanosoma cruzi*, Tulahuén 0 strain. The results show a depresor effect of development in 150 mg/L to 200 mg/L concentrations. Celular morphology observations make evident the rise of epimastigotes shapes, in a 20% of the celular population, with a length of the 30% of the corresponding to the epimastigotes share of the reference lot, to the concentrations of the extract already mentioned.

P23.

DEVELOPMENT OF LIQUID DOSAGE FORMS OF BENZIMIDAZOLE ANTI-HELMINTHIC AGENTS

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Albendazole is a benzimidazole derivative with a broad spectrum of activity against human and animal helminthe parasites. Benznidazole, is one of the drugs most frequently used for the treatment of Chagas disease. It is given orally at a dose regimen of 5 to 10 mg/kg. However, both anthelmintic drugs are poorly water-soluble reducing the flexibility for drug formulation and administration. Solid dispersions in water-soluble carriers have attracted considerable interest as a means of improving the dissolution rate, and the bioavailability of drugs. Objectives: The aim of this work was to evaluate the use of solid dispersions and cosolvents to increase the dissolution rate and the bioavailability of these two poorly water-soluble drugs. Experimental: Solid dispersions and physical mixtures were prepared with Albendazole:PEG 6000 at different ratios, by means of solvent and melting methods. The particle size and the phase solubility method were evaluated. Studies of the effect of different cosolvents on the dissolution of Benznidazole were conducted using propylene glycol, transcutol, sorbitol, and PEG 400. Results: The amount of Albendazole released increased with an increasing proportion of PEG 6000. The phase solubility studies showed an increase on the solubility of Albendazole in presence of ethanol at pH 1,2. On the other hand, the use of cosolvents improved the solubility of Benznidazole, allowing us to preformulate an oral and parenteral dosage form. Therefore, the procedures detailed here seems to be effective to increase the aqueous solubility and inducing a better bioavailability of these antiparasite agents.

P24.

CHANGES OF THE β ADRENERGIC SYSTEM IN THE ACUTE PHASE OF THE *Trypanosoma cruzi* MYOCARDIOPATHY

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The chagasic myocardiopathy is considered a cardioneuropathy with the sympathetic and parasympathetic systems affected; alterations in the level and function of proteins involved in cAMP mediated signaling would be important for it's physiopathology which is yet poorly understood. For that we think that the β adrenergic signal transduction system must be altered in some place of its pathways in *Trypanosoma cruzi* infected mice hearts, and these alterations should be different according to the infection phase or the infecting parasite strain. Therefore, we studied *T. cruzi* Tulahuen strain (Tul) (n=40) and SGO Z12 isolate (n=40) infected mice hearts in the acute phase of the experimental infection, determining: epinephrine (Epi) and norepinephrine (NE) plasma levels by HPLC-DE; cardiac β receptor's density and affinity through binding with ^{3}H/dihydroalprenolol, and their function using a no radioactive tracer; cAMP levels by RIE and the contractility of the heart as the physiologic response to the initial stimulus. Both infected groups' plasma catecholamines levels (pg/ml) diminished when compared with the uninfected (NI) group (NI Epi: 3999,75±579,73, NE: 5241,95±712,89; Tul Epi: 757±211, NE: 868±183; SGOZ12 Epi: 1356,58±512,02, NE: 651,92±179,48; $p<0,01$). The receptors' affinity (Kd in nM) (NI: 3,61±0,05) diminished in both infected groups (Tul: 5,63±0,26, SGO Z12: 5,72±0,57)($p<0,05$) while the receptors' density was augmented only in the SGO Z12 infected ones. The cAMP levels were higher in either infected group (Tul: 0,88±0,17 nM; SGO Z12: 2,63±0,40 nM) when compared with the uninfected ones (NI: 0,12±0,01 nM)($p<0,05$). The basal contractility however increased only in the Tul infected group ($p<0,05$) while the response to catecholamines remained unchanged. The SGO Z12 infected group presented a lower response to epinephrine ($p<0,05$) than the Tulahuen infected one. These results demonstrate that the β adrenergic signal transduction system is altered from the acute phase of Chagas disease and these alterations would be the early biochemical expression of the beginning of a sympathetic dysautonomy.

P25.
pH-SENSITIVE LIPOSOMES AS ANTI-AMASTIGOTE AGENTS

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The main obstacle for eliminating intracellular parasites is that hydrophilic or high molecular weigh drugs can not reach the cytoplasm where they have to exert their action, unless a specific way of transport exist. In this context, we designed and prepared pH-sensitive liposomes loaded with the hydrosoluble, low molecular weigh trypanocidal drug etanidazole (ETZ). pH-sensitive delivery strategy is based on the change of the liposomal membrane phase induced by the low pH in the endosomes/lisosomes, responsible of the concomitant delivery of the ETZ to the cytoplasm. ETZ was loaded in pH-sensitive large unilamellar vesicles (LUV) (dioleoyl-phosphatidylethanolamine: cholesteryl hemisuccinate, 6:4, mol:mol) prepared by extrusion through 200 nm policarbonate membranes followed by 5 freeze-thaw cycles. The non-encapsulated ETZ was removed by gel permeation chromatography. The resulting LUV-ETZ had a mean diameter of 380 ± 60 nm. The concentration of the resulting liposomal suspensions determined by HLPC was 0.51 mg ETZ /ml, at a 14% w/w drug/total lipid ratio. The endocytosis and intracellular fate of pH-sensitive liposomes loaded with the fluorophore/quencher pair HPTS/DPX was studied by fluorescence microscopy. We also determined the anti-amastigote activity (aa) in J774 murine macrophages infected with *Trypanosoma cruzi* amastigotes. Upon treatment with LUV-ETZ, no change in viability of healthy or infected cells was registered. The results showed that upon capture, a fast delivery of the liposomal aqueous content into the cytoplasm of non-infected macrophages as well as on infected macrophages occurred. On the other hand, we founded 77% aa after 2 h treatment with LUV-ETZ whereas the treatments with empty liposomes rendered nearly 0% aa. Due to the pharmacokinetic characteristics of ETZ, such high ETZ concentration along two hours used *in vitro* could never be sustained *in vivo.* On the contrary, the aa resulting from LUV-ETZ *in vitro* could *easily* be reproduced *in vivo*. Hence, our results point to LUV-ETZ as potential vehicles capable of delivering high amounts of ETZ to infected cells.

P27.
"VIVIR SIN CHAGAS" PROJECT: FOLLOW-UP OF INFECTED PATIENTS TREATED WITH BENZNIDAZOL AT THE CITY OF AÑATUYA, SANTIAGO DEL ESTERO, ARGENTINA

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Introduction: We started a prospective study in Añatuya, an endemic region under surveillance in Argentina. We followed 197 seropositive patients (15-45 years old) with no visceral symptoms, who received benznidazol treatment up to 60 days. **Methods:** Patients were analyzed at the begining (t0), at the end of treatment (t1) and at 3, 6, 12, 24 and 36 months post-treatment (t2, t3, t4 t5 and t6, respectively). The serology was followed-up by HAI and ELISA. The parasitemia was measured by PCR, based on *T.cruzi*- kDNA variable region. We report the evolution of 54 patients who were monitored until t6. **Results:** The PCR was positive in 72% of blood samples at t0. The cohort (54 patients) was classified ein three groups: G1 (PCR negative in all samples until t6; n=15), G2 (PCR positive only at t0; n=8) and G3 (patients with at least one PCR positive result during the post-treatment follow-up); n=31), we observed a seroconversion of 46.7% and 33.3% in G1 and G2 respectively but no seronegativation was achieved in G3 patients (by HAI test). Fourty eight of the 54 patients (88.9%) showed a decrease of ELISA titers, but no seroconversion was found. **Discussion:** All HAI seronegative cases were PCR negative during the 3 years of follow-up, suggesting that a low parasitic load may lead to a better post-therapy outcome. The post-treatment PCR positive cases are likely due to a capacity of the drug to reach amastigote nests in hidden reservoires. These results point to the neccesity to improve the available therapeutic regimens and to develop new drugs for etiological treatment of undeterminate or chronic Chagas disease patients.

P26.
TROPHOBLAST-*Trypanosoma cruzi* INTERACTION: ROLE OF THE PLACENTAL ALKALINE PHOSPHATASE IN HUMAN PLACENTA INFECTION

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Chagas' disease, endemic in 21 countries of Latin America, affects around 10 and 20 millions people. One of the infection mechanisms of this disease is the transplacental one. The congenital infection incidence vary between 1 to 9% of the chagasic pregnant women, and the same woman, in different pregnancies, can have child with our without the disease. As being the *Trypanosoma cruzi* an obligated intracellular parasite, must complete its life cycle in a host cell, but still now, that process is not well understood. The parasite adheres to specific receptors on the outer membrane of host cells before invasion, inducing changes on the protein pattern of the syncitiotrophoblast. Placental alkaline phosphatase (PLAP) (EC 3.1.3.1.), a glycoenzyme anchored to the membrane by a glycosyl-phosphatidylinositol (GPI) molecule, could be solubilized by Phospholipase C (PL-C) and acts as IgG receptor. In this work we observed that PLAP activity and its presence were altered by the parasite in cocultures of human placental villi and HEp2 cells with *T.cruzi,* whenever the enzyme was anchored to the membrane by its GPI molecule. The cells treated before the cultures with agents which affect PLAP or GPI (antibodies, PL-C, genistein, lithium) presented less parasitic invasion than the control ones. It was also observed a modification in the pattern of actine filaments of the host cells infected with the protozoo. Thus, it is conclude that PLAP participates in the process of *T. cruzi* invasion into placental syncitiotrophoblast cells, by a mechanism that involves hydrolysis of the GPI molecule, the activation of tyrosine kinase proteins, the increase of cytosolic calcium and finally the rearrangement of actine filaments of the host cells.

P28.
***IN VITRO* ABILITY OF *Trypanosoma cruzi* TO PRODUCE INFECTION OF CHORIONIC VILLI EXPLANTS FROM HUMAN NORMAL PLACENTAS**

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Transplacental infection by Trypanosome cruzi produce the congenital Chagas´disease. **Hypothesis:** As congenital cases incidence are low (0.5% to 4%) and placental subfractions alter the parasite cell, so the rate of penetration or sustained infection of *T. cruzi* into placental tissue might be diminished as it is affected by placental agents. **Materials and Methods:** chorionic villi explants from human normal placentas and VERO cells as controls were co-cultured with 10^6 trypomastigotes Tulahuen strain of *T. cruzi*, in 24 multiwells plaque containing 1.2ml MEM with inactivated FCS and antibiotics for 3h (n=6), 24h (n=7) and 72h (n=6). *T. cruzi* was detected by PCR, PAS/H and immuno-histochemistry and stereological analysis of tissues and cells was done. Also, endothelial Nitric Oxide Synthase (eNOS) was detected by immunohistochemistry. Alive parasites were counted in the culture media at the end of co-cultures. **Results:** *T. cruzi* area increased 2 to 3 times at 72h respect to 24h and 3h in placental tissues. Those areas were diminished ($p<0.01$) in comparison with those of VERO cells. There were 4.5 ± 2 amastigotes per nest in placentas and 54.79 ± 22.19 in VERO cells at 72h. 10% of placental explants were not infected according to PCR. There was no alive parasite in the culture media at 72h of placenta-*T. cruzi* co-cultures ($p<0.01$ compared to controls). Presence of *T. cruzi* increased the protein expression of eNOS in placental explants. **Conclusions:** *T. cruzi* is not able to produce a sustainable infection in chorionic villi explants from human normal placentas *in vitro* probably by eNOS increment. These results may explain at least some of the cases from most of the chagasic women that not transmit the *T. cruzi* to their offspring.

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P29.
EFFECT OF GENOTYPE ON THE NATURAL ENTEROPARASITOSIS IN MICE

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The host genotype constitutes an important factor to establish a parasitic infection. Differences observed in the quality and quantity of the natural enteroparasitosis in adult mice of lines CBi/C and CBi- from the CBi stock (IGE), moved us to study that trait in the (Cx-) F_1 hybrids and compare them with the respective parental lines. Nine male and 13 female F1, and 15 female and 15 male CBi/C and CBi- mice were studied. The F_1 parents were contemporaries to the animals from the parental lines. Faeces were examined microscopically to assess the quality and quantity of the parasitic infection. Differences among genotypes were analyzed statistically and were considered significant if $p>0,05$. The parasites found, the frequency of protozoa in both sexes (%) and the absolute (N) and relative to large intestine length (Nr) worm burden ($\bar{x}\pm ES$) are shown in the table:

Mice	CBi/C		F1 (C x -)		CBi-	
Parasites	F	M	F	M	F	M
T. muris (%)	100	100	92,3	100	0	0
S. muris (%)	40	53,3	0	0	100	100
N	56±10	80±11	33±6	122±34	44±7	426±66
Nr	5±0,9	7±1,1	3±0,7	10±2,3	6±0,9	47±7,5

The genotype influenced the host-parasite interaction, F_1 showed a different susceptibility from that of the parental lines, it was similar to CBi/C for *T. muris* and resulted resistant to *S. muris*. The worm burden showed sex effect in all genotypes; the females did not differ among them while CBi- males were different from CBi/C and F_1 males.

P30.
ACTIVATION OF *NF-kB* TRANSCRIPTION FACTOR IS INVOLVED IN THE INHIBITION OF CARDIOMYOCYTES APOPTOSIS FOLLOWING *Trypanosoma cruzi* INFECTION OR CRUZIPAIN TREATMENT

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Recently, we demonstrated that neonatal cardiomyocyte cultures infected with *T. cruzi* and subjected to serum starvation displayed a parasite dose-dependent increase in cell viability. Cruzipain (Cz) treatment also reproduces the antiapoptotic effect of the parasites through activation of ERK 1,2-MAPK and PI-3K/AkT but not p38-MAPK signaling pathways. The aim of the present work was to study the involvement of *NF-kB* in the cardioprotective effect of Cz. Cardiomyocytes from neonatal BALB/c mice were cultured in DMEM-0,1% FBS and then stimulated with 10 ug/ml of Cz or with Cz + 2,5 mM sulfosalazine (*NF-kB* inhibitor) for 24h or maintained in medium alone as control. The cell viability percentages determined by Trypan Blue exclusion staining were: 56±2% (control), 85±2% (Cz), 44±9% (Cz+inh). Apoptotic cell death rates assessed by flow cytometry were: 24±4%; 13±4% and 28±4% respectively. The activation of *NF-kB* was confirmed by immunofluorescence using an anti-p65 polyclonal antibody: Nuclear translocation was examined at 40 min after stimulation: 5±2% (control), 36±5% (Cz), 6±2% (Cz+inh) and 14±5% (infected with *T. cruzi*). We conclude that activation of *NF-KB* transcription factor is required for the antiapoptotic effect of Cz on cardiomyocytes.

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P31.
PHOSPHOLIPASE A_1 DISTRIBUTION CHANGES DRAMATICALLY THROUGHOUT T*rypanosoma cruzi* LIFE CYCLE

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We have previously examined the phospholipase acting on phosphatidylcholine (PC) in *Trypanosoma cruzi*. A soluble phospholipase A_1 (Plase A_1) isoform is the predominant form of the enzyme in epimastigotes and is located to the lysosoms (Wainszelbaum, 2001). Consisderably higher specific activity of Plase A_1 was also observed in homogenates of the infective amastigotes and trypomastigotes. We here report that Phospholipase A_1 specific activity in amastigotes and trypomastigotes is 10 to 15-fold higher than in epimastigotes and resides mainly on its membrane fraction. Our findings may have important biological consecuences. When [^{14}C-1]-oleic acid labeled Vero cells are incubated for 30 min in the presence of amastigotes, their lipids changes dramatically, with the appearance of free fatty acids, diacylglycerol and lysophosphatidylcholine. This indicates strong effects on membrane lipid composition in target host cells. Since diacylglycerol is increased, we investigated the possible activation of PKC in the host cells. We found that, indeed, this enzyme is clearly stimulated under the experimental conditions employed. In conclusion, the results presented here, support the view that Phospholipase A_1 from the infective stages, play a key role in the interaction with the host cells, possibly implicating PKC activation, which may mediate cell invasion.

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P32.
INVITRO* ANTIPARASITIC ACTIVITY OF CYCLOSPORIN A ANALOGS ON *Trypanosoma cruzi

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Cyclosporin A (CsA) and nonimmunosuppressive CsA analogs were evaluated against *Trypanosoma cruzi* and on *Tc*CyP19, a cyclophilin of 19 kDa. Two out of eight CsA analogs, H-7-94 and F-7-62 showed the best anti-parasitic effects on all *in vitro* assays. Their IC_{50} values were 0.82 and 3.41 micromolar respectively compared to CsA IC_{50} value 5.39 micromolar on epimastigote proliferation; and on trypomastigote lysis their IC_{50} values were 0.97 and 2.66 micromolar compared to CsA IC_{50} value 7.19 micromolar. CsA, H-7-94 and F-7-62 had a marked inhibitory effect on amastigote development on Vero cell and the inhibition of trypomastigote penetration was 56, 75.8 and 80.4 percent respectivaly at 25uM drug concentration. These two CsA analogs did not show toxic effects on mammalian cells when tested up to 50 uM concentration. The enzymatic activity of *Tc*CyP19 was inhibited by all CsA derivatives, suggesting this target is involved in the trypanocidal effects observed.

P33.

CONGENITAL CHAGAS' DISEASE: DETECTION AND MOLECULAR TYPING OF NATURAL POPULATIONS OF *T.cruzi* INVOLVED IN VERTICAL TRANSMISSION

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We report a prospective study of 60 chagasic pregnant women and a retrospective study of 6 mothers of newborns with congenital Chagas disease. **Methods:** We studied the evolution of the parasitemia in peripheral blood samples, collected in a trimestral basis from 17 women, by means of kDNA-targeted PCR. Seven of them have completed the follow-up (5 samples: 3 maternal blood specimens, placenta and newborn´s blood); other eight are currently under follow-up, while less than five specimens could be collected from the other patients. We characterized the parasite lineage directly from blood samples using miniexon-PCR and 24S rDNA-PCR. The parasite populations found in mothers and their newborns were also profiled by kDNA-PCR followed by double digestion with HinfI-AfaI and by LSSP-PCR from the same amplicons. **Results:** During pregnancy, the parasitemia was positive in 17.6%, 23.5% and 47% of the 17 women studied at the 1st, 2nd and 3rd trimestral periods, respectively. Five out of 32 (15.6%) collected placentas were PCR positive, only one of them belonged to a PCR positive blood sampled woman. Regarding transmission, 5 of 23 (21.7%) newborns were PCR positive; three of them born from PCR positive blood sampled mothers. These results show a higher parasitemia at the 3rd trimester of pregnancy but no relation in parasite detection between blood, placenta and newborns. Furthermore, we determined the lineage of parasite populations directly from 3 infected newborns and one mother. All of them were *T.cruzi* II. Moreover, in 6 retrospective cases (mother and newborn PCR positives) the kDNA profiles within each mother-newborn pair were almost identical, but different among the tested pairs.
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P34.

TRYPANOTHIONE SYNTHETASE: A KEY TARGET FOR DRUG DEVELOPMENT AGAINST AFRICAN TRYPANOSOMIASIS

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Protozoan parasites of the genus *Trypanosoma* and *Leishmania* are pathogenic for mammals and cause widespread diseases of man and his lifestock in developing countries. There is an urgent need for new chemotherapeutic against trypanosomiasis because the drugs currently available suffer from poor efficacy, development of resistance and substantial toxicity. In addition, vaccination seems rather a dream for African trypanosomiasis, since the parasites escape the immune response due to frequent antigenic variation on their surface. The trypanothione metabolism deserves particular interest in the search for novel trypanocidal drugs, since it is a trypanosomatid-specific and a vital redox compound. The biosynthetic steps and components involving synthesis of trypanothione in African trypanosomes have so far been unknown. Therefore, we used *T. brucei brucei*, the causative agent of cattle Nagana disease, as model to investigate how biosynthesis of trypanothione does occur and to proof its pivotal role to sustain essential cell functions. The results demonstrate that: i) a novel gene of *T. brucei brucei* encodes an enzyme, trypanothione synthetase (*Tb*-TryS), that catalyses both steps of trypanothione biosynthesis, ii) *Tb*-TryS does not have any significant sequence similarity to any known mammalian protein, iii) *Tb*-TryS is a protein of low abundance (0.005% total protein content), iv) *Tb*-TryS *in vivo* appears to be the only enzyme catalysing the entire synthesis of trypanothione, v) *Tb*-TryS, and hence trypanothione, are essential to support normal cell growth, viability and antioxidant defence in unstressed parasites. Thus, trypanothione synthetase qualifies as a most attractive drug target against African trypanosomes and offers an appealing therapeutic potential toward other trypanosomiasis.

P35.

TRYPANOTHIONE SYNTHETASE FROM *Crithidia fasciculata*: A NEW PARADIGM FOR THE BIOSYNTHESIS OF TRYPANOTHIONE AND A SUITABLE TARGET FOR TRYPANOCIDAL DRUG DEVELOPMENT

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Parasites belonging to the order Kinetoplastida, i.e. *Trypanosoma* and *Leishmania* species, comprise the causative agents of widespread and difficult to treat tropical diseases that affect human and domestic animals. Trypanothione is the major redox mediator unique to, and essential for members of this order. For this reason, the enzyme that effects its biosynthesis represents an important and potentially specific target for development of new chemotherapy. In this respect, the insect trypanosomatid *Crithidia fasciculata* has been chosen as a model of the mammal pathogens. However, due to conflictive data reported on trypanothione biosynthesis in this insect-pathogen and the novel biosynthetic pathway proposed for *T. cruzi* and *T. brucei*, the debate whether *C. fasciculata* were an adequate model for the mammal pathogens was raised. Therefore, we considered mandatory to elucidate the discrepancies set about trypanothione biosynthesis in *C. fasciculata* in order to answer this question. Our work unambiguously demonstrates that: i) trypanothione synthetase from *C. fasciculata* (*Cf*-TryS) can be heterologously over-expressed and obtained in an soluble/active form from *Escherichia coli*, ii) *Cf*-TryS synthesizes and degrades glutathionylspermidine and trypanothione, from and to glutathione and spermidine, like its counterparts in *T. cruzi* and *T. brucei*, and iii) *Cf*-TryS *in vivo* appears to catalyse more efficiently the glutathionylation of glutathionylspermidine rather than of spermidine. Thus, apart from solving the long-lasting controversy about trypanothione biosynthesis in *C. fasciculata*, and according to the catalytic features displayed by *Cf*-TryS, we finally conclude that this enzyme qualifies as a suitable model for the design of trypanocidal drugs.

P36.

INSIGHT TO THE CATALYTIC MECHANISM AND IDENTIFICATION OF STRUCTURAL/FUNCTIONAL ELEMENTS IN HIGHLY CONSERVED REGIONS OF TRYPANOTHIONE SYNTHETASES

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Parasites of the family *Trypanosomatidae* are responsible for some of the most devastating and prevalent diseases of humans and domestic animals. There is an urgent need for novel trypanocidal drugs considering that there is no prospect for a vaccine in the short-term, and the current chemotherapy is inefficient, toxic and costly. In this regard, metabolic pathways that are of vital importance for pathogens but absent in their hosts, like the biosynthesis and use of trypanothione deserve particular interest. Trypanothione synthetase (TryS) is a multifunctional enzyme that has been reported to catalyse the entire synthesis of trypanothione in *Crithidia fasciculata*, *Trypanosoma cruzi* and *T. brucei*, and has also been suggested in *Leishmania* spp. Moreover, TryS has recently been validated as a most attractive drug target for African trypanosomes. In order to progress further in the characterization of such relevant trypanocidal candidates, we conducted steady-state kinetic studies, limited proteolysis, and site-directed mutagenesis analysis on the available TrySs. Our studies suggest that: i) TrySs operate *via* a concerted-substitution mechanism for the ligation of glutathione to glutathionylspermidine, ii) TrySs posses in the C-terminal domain a module essential for synthetase activity, iii) TrySs contain non-regular secondary structures (resembling of Ω- and P-loop motifs) that interact with the substrates, iv) two arginine residues located in these motifs are essential in binding substrates and, hence, in enzyme function. Thus, these results constitute the first insight into the mechanism and amino acidic residues involve in catalysis in this family of enzymes.

P37.

NEW INTERPRETATIONS OF *Trypanosoma cruzi* PLA2 ACTIVITY

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From the begining our group has been focousing on molecular aspects of the interaction *T.cruzi*-host cells and the way they are involved in the pathogeny of Chagas´disease. A system that makes easy the analysis of surface contact is the interaction between live parasite and erythrocytes *in vitro*. We proved that *T.cruzi* induces red cell fusion, with changes in lipidic profile and phospholipid turnover that led us to propose the involvement of PLA2 activity. The hypothesis was reinforced after inhibition of fusion (50%) and hydrolisis of fluorescent and radioactive substrates (80%) when parasites were preincubated with gangliosides. The enzyme might have structural and functional homology with mammal PLA2 since the same inhibition was obtained when anti sPLA2 or anti cPLA2 antibodies or GM1 monosialoganglioside were present in the fusion medium. This also points to an extracellular location for the enzyme. Recently we have documented *in vivo* fusion of red blood cells in fresh mice blood at the moment of extraction with excess heparine, at 21ºC. Simultaneously thrombi with adhered movile parasites were observed. Mice plasma PLA2 activity (over fluorescent substrate) increased according to the size of the inoculum. Taken together, positive correlation between plasma PLA activity and parasitemia, and PLA2 participation in platelet activation, both favor the proposal that a parasite enzyme is involved.

P38.

SEQUENCE HETEROGENEITY IN THE SPLICED LEADER RNA GENE PROMOTER IN *Trypanosoma cruzi* CL-BRENER. A MOLECULAR TRACE OF THE HYBRID ORIGIN OF THIS STRAIN?

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Spliced Leader (SL) RNAs from trypanosomatids are encoded in highly repetitive genes, independently transcribed from their own promoters. Both gene and promoter sequences vary between species. *Trypanosoma cruzi* is divided into two phylogenetic lineages, *T. cruzi* I and *T. cruzi* II, which contain different SL RNA gene promoter sequences. Class I SL gene promoter sequences, found in *T. cruzi* II are highly conserved in the –80/+1 region, whereas Class II promoters from *T. cruzi* I are more variable. We cloned and sequenced different SL RNA promoter sequences from CL-Brener reference strain, belonging to *T. cruzi* II lineage. Surprisingly, we detected a sequence that differed within the –80/+1 region with the sequence previously reported for this strain. In order to further analyze the heterogeneity in the promoter region of CL-Brener strain, 1700 SL promoter sequences were obtained from 973418 *T. cruzi* genome project single pass sequences. A detailed analysis of these promoters discovered new divergent CL-Brener sequences with typical *T. cruzi* I features, showing *T. cruzi* I/*T. cruzi* II hybrid characteristics. Ten of these sequences were selected to be analyzed with Mr. Bayes program together with promoter sequences from both lineages. The program classified the data in two clades, grouping all *T. cruzi* II together with 8 of the new sequences in one, and *T.cruzi* I and 2 new ones in the other. The results presented herein show that sequence heterogeneity in SL RNA gene promoter not only exists between *T. cruzi* strains but also within CL-Brener strain. These CL-Brener "*T. cruzi* I-like" sequences could be considered a molecular trace of a hybrid origin of the SL RNA gene and a new evidence for the presence of sequences of *T. cruzi* I origin into a *T. cruzi* II strain.

P39.

HSP90 EXPRESSION AND INTRACELLULAR LOCALIZATION IS DEVELOPMENTALLY REGULATED IN *Toxoplasma gondii*

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Toxoplasma gondii is an ubiquitous obligate intracellular protozoan parasite that produces opportunistic infections in immunocompromised hosts. The *in vivo* bradyzoite differentiation is a stress-related response of *T. gondii* to environmental conditions such as the inflammatory response of the host to the tachyzoite stage. The induction of bradyzoite development *in vitro* has been associated to temperature, pH, and other kind of stress inductors, all known to stimulate the expression of heat shock proteins (hsps). The hsp90 expression profile of RH UPRT- *T. gondii* mutant was analyzed under stress (incubation 1 h at 44°C) and bradyzoite formation conditions (tissue culture under reduced CO2 atmosphere). RT-PCR assays using TgHsp90 and Tg-α-tubulin set of primers and Western blot analysis showed an increase in hsp90 mRNA and protein under both, stress conditions and bradyzoite induction. In addition, a differential intracellular localization depending on the parasite stage was shown by immunofluorescence microscopy imaging. In the tachyzoite stage most of the hsp90 protein was detected only in the cytoplasm. In the bradyzoite stage, although cytoplasmic localization still occurred, an abundant nuclear localization of hsp90 was evident. These results were corroborated using *T. gondii* PK cells, a cloned derivative of ME49 strain, and induction under alkaline stress. In addition, PK bradyzoites obtained from the brain of experimentally infected mice showed the presence of hsp90 in nuclei and cytoplasm, and lost the nuclear staining during tachyzoite conversion. Our results are consistent with hsp90 expression and subcellular localization being developmentally regulated in *T. gondii*.

P40.

PROTEINS SIMILAR TO CYCLINS IN *Tripanosoma cruzi*

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In Trypanosomatids the cell cycle is under study and new results are actually presented. In *T. cruzi* we characterized two protein kinases related to cdc2 (CDK1) named TzCRK1 and TzCRK3 (with 33 and 35 kDa respectively). TzCRK3 control the stage G2 to M in synchronized epimastigote forms (Santori *et al*., 2002). Using the yeast two-hybrid system we have identified three new proteins TzCyc2, 4 and 5, able to associate to TzCRK1. These proteins present high homology to cyclins belonging to proteins of the PHO family that regulates phosphate metabolism in yeast. The deduced aminoacid sequence of TzCYC2 (230 aa) showed that the identity is limited to the cyclin-box domain (Gómez *et al*., 2001).
TzCyc2 was cloned in a bacterial vector pET22(b)+, with an Histidine tag. The recombinant protein was inoculated in rabbits and a specific serum was obtained. In a functional assay, *T. cruzi* cyclin 2 is able to rescue the growth of *Saccharomyces cerevisiae* mutants of G1/S cyclins. This result indicates that TzCyc2 could be involved in the cell progression cycle in the parasite. The availability of the complete genome database of *T. cruzi* (TIGR) allowed us to identified new putative cyclins genes using *T. brucei* sequences. The results indicate that *T.cruzi* has three proteins homologues to TbCyc 6, 8 and 9. T. brucei Cyc6 controls the transition G2/M, while TbCyc2 regulates the G1/S stage.
TzCyc 6 has been cloned and it is actually under study which is the better expression conditions. The main objective is to understand the function of these proteins in the cell cycle and in the different forms of the parasite.
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P41.

CHARACTERIZATION OF THE LONG-CHAIN *E*-ISOPRENYL DIPHOSPHATE SYNTHASE FROM *Trypanosoma cruzi*

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We report the cloning and sequencing of the gene encoding the *Trypanosoma cruzi* long-chain *E*-Isoprenyl Diphosphate Synthase. Multiple, almost identical, copies of the gene are found in two chromosomes of 1.1 and 0.8 Mb in CL Brener strain. The approximately 39 kDa protein is homologous to Isoprenyl Diphosphate Synthases from different organisms, showing the 5 conserved domains and the typical hydrophobic profile. The recombinant protein, expressed in soluble form in pET28, displayed enzymatic acitivity using several precursors. The length of the final product, as determined by Thin Layer Chromatography, is 9 isoprene units, suggesting that *T. cruzi* possess Ubiquinone 9, as described in *T. brucei*. By immunofluorescence, specific polyclonal antibodies generated a punctated pattern in the cytoplasm of the parasite, similar to the one produced with anti-Farnesyl Diphosphate Synthase Abs, opening the possibility that the enzymes of this biosynthetic pathway share the same intracellular compartment. Experiments are underway to determine the kinetic constants of the enzyme, confirm its location and measure its sensitivity to inhibitors. Ubiquinone or CoenzymeQ has a central role in energy production as well as in reoxidation of reduction equivalents. Since the parasite is affected by respiratory chain inhibitors, and considering the poor similarity to the mammalian enzyme, the *T. cruzi* enzyme could be proposed as a new chemotherapeutic target.

P42.

THE EVOLUTION OF PHOSPHATIDYLCHOLINE-PHOSPHOLIPASE SYSTEMS IN UNICELLULAR EUKARYOTES

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We have examinated the phospholidylcholine (PC) degrading enzimes in a number of unicellular protists cells obtained from axenic cultures. The organisms studied are thought to be closely related to the most primitive forms among eukaryotes. Data from this organisms, can thus offer clues on how enzymatic systems changed throughout evolution. In particular, we investigated the American parasitic flagellate *Trypanosoma cruzi*, the free living *Euglena gracilis*, and the ciliate *Tetrahymena termophila*.
Our studies, demosntraed that phospholipase A1 is ubiquotously present in all species studied, and is the only PC degrating phospholipase found in *T. cruzi* and *E. gracilis*. This agrees well with observations from other authors wich found a similar pattern among several African trypanosomes, some of which also displayed phospholipase A2. By contrast, *T. Termophila*, clearly shows the presence of acidic phospholipase C and neutral phospholipase D. We also observed for the first time, neutral phosphatidic acid phosphatase, which in conjunction with phospholipase D can generate diacylglycerol with a possible signaling role stimulating protein kinase C, as in higher eukaryotes. In conclution, our resarch supports the notion that phospholipase A1, which is generally also endowed of lysophospholipase activity is the primeval PC phospholipase among eukarytic cells and that phosphodiesteratic (phospholipases C and D) apperared at late stages in the evolution of this group.

P43.

CONSERVATION OF THE CONSTITUTIVE ACTIVITY MECHANISM OF SPECIFIC SR PROTEINS KINASES

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The regulation of the genetic expression in trypanosomatids is exerted mainly on the post-transcriptional level, including maturation, stability and translation of the mRNAs. Trypanosomatids are organisms that not only perform *trans*- but also *cis*-splicing (PolyA Polymerase gene). The *trans*- and *cis*-spliceosome contain conserved elements, that includes snRNPs (small nuclear RiboNucleoProteins) and non-snRNPs. From these last, SR proteins are the main components of this structure and together with their specific kinases constitute the "SR Network". Our group has identified and characterized for the first time components of this network in *Trypanosoma cruzi* and *Trypanosoma brucei*. The serine/arginine-rich protein (TcSR) and the specific SR kinases, TcSRPK and TbSRPK. These kinases belong to one (SRPK) of the four families chracterized in diferent organisms, extending them to all the eukariotic linage. Recently the structural mechanism of the constitutive activity of Sky1p, homolog protein of *S. cerevisiae*, has been characterized. In this work we show the modeling of the 3D-structure of the trypanosomatids kinases together with the characterization of the generated mutant proteins, demonstrating the conservation of such mechanism at this evolutive level.

P44.

EVALUATION OF TRYPANOSOMATID GROWTH IN DIFFERENT SYSTEMS AND CULTURE MEDIA

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In vitro cultivation of parasitic protozoas is valuable because it provides not only information about features of its development, but also permits to get new approaches in the study of biology, pathology, epidemiology and treatment of diseases caused by this organisms. Also is important, from the biotecnology point of view, for its use in the preparation of inmunologic tests. Exist different formulations of culture media for *in vitro* growth of this protozoas and all formulations use fetal bovine serum (FBS) as suplement. FBS is expensive, its quality is variable between different portions and further is a contamination sourse. It has been described the axenic culture of trypanosomatids in TC100 medium, generally employed for insect cell culture, with the addition of FBS. In our laboratory, we had developed a new serum-free medium for growing insect cells (UNL 8). In this work we analyse the use of this medium for culture of *Trypanosoma cruzi* (strain Tulahuen 0) in comparision with the TC 100 + 10% FBS and CIEN + 5% FBS media. The parasitic has been adapted fast in this serum – free medium. Maximum cell density reached in 100 mL shaker flasks with 10 mL of UNL 8 medium was 3,4 10^7 cell/ml and the specific growth rate was 0,124 $(days)^{-1}$. The kinetic parameters in TC100 +10% FBS and CIEN + 5% FBS were 2,85 10^7 cell/ mL, 0,47 $(days)^{-1}$ and 4,33 10^7 cell/ mL, 0,25 $(days)^{-1}$ respectly. Parasite culture in UNL 8 medium was scaled-up until 1L in a stirred tank bioreactor, improving significantly the kinetic parameters respect to low scale culture. UNL 8 is an optimum medium for culture of *T. cruzi*, it can be used in large scale and its cost is low respect to other culture medias. Also it permits growth of other trypanosomatids like the amphibian *Trypanosoma* mega.

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P45.
CLONING, EXPRESSION AND PURIFICATION OF *Trypanosoma cruzi* THIOREDOXIN

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All forms of life have developed enzymatic systems to detoxify reactive oxigen species (ROS). The thioredoxin (TRX)- thioredoxin reductase (TRXR) system plays an important rol in oxidative stress response in Archea, Eubacteria and Eukarya. In addition, TRX-TRXR system is envolved in a variety of cellular redox reactions, DNA synthesis and transcription regulation, cell growth and apoptosis. Thioredoxins are small proteins with a molecular mass of about 12 kDa. A redox active disulfide WCGPC motif is higly conserved amongst TRX's family. In this work we report the cloning of a gene encoding TRX in *Trypanosoma cruzi* (TcTRX), and the heterologous expression followed by purification of the recombinant protein. The results obtained by RT-PCR shows the presence of the mRNA of this gene. By Southern blot analysis we demostrated that the gene encoding TRX in *T.cruzi* is single copy. We used the pRSET A expression vector for *trx* sub-cloning and *Escherichia coli* BL21 (DE3) cells to produce the fusion protein. The recombinant six-histidine tagged TRX was purified by affinity metal chromatography. The deduced amino acid sequence of this protein has similarity to previously characterized *Trypanosoma brucei brucei* and *Leishmania* major TRXs. The purified TcTRX proved to be biologically active using an insulin reduction assay. By Western blotting TcTRX was recognized by antibodies raised against recombinant MBP-*T. brucei brucei* TRX. Epimastigotes of *T. cruzi* display strong immunoreactivity to the antibodies raised against MBP-TbbTRX, thus suggesting the occurence of TRX in this organism.

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P46.
ERITHROCYTE CARBOHYDRATE ANTIGENS: MOLECULAR MIMICRY OF *Ascaris lumbricoides*

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The last 20 years there has been publications about the fact that many parasites can carry blood group antigens,nevertheless the clinical significance is still unknown. The concept of mimicry were performed by Clegg *et al* on Schistosoma mansoni . The objective was to detect Erithocyte carbohydrate antigens (ABO and P System) in Ascaris lumbricoides extracts (AE).We worked with 50 AE The extracts were prepared by surgical remotion of the cuticle of adult specimens and refrigerated mechanical rupture for 5 days AE were centrifugated at 3000 rpm and the supernatns were kept at –20°C with a final concentration of timerozal 1:1000. Agglutination Inhibition (AI) and Haemoagglutination Kinetics (HK) tests were made with all the AE by using monoclonal antibodies in optimal concentrations (anti A, anti B, anti P y anti P_1). Suspensions of fresh red cells were used as a revealing system. In the serum of the children who sent the parasites the fenotype ABO was determined. P and P_1 antigens were determined in their erythrocytes. AI and HK tests showed: B epithopes: in all AE from B group patients (4) and AB group patients (3) A epithopes: in 1 of the 3 AE of AB group patients and in 3 of the 19 extracts from A group patients. No AE of 21 O group patients presented ABO epithopes. P and P_1 System epithopes: 18 AE presented P and P_1 epithopes and 3 extracts only P_1. These 21 patients had both epitophes in their erythrocytes. The presence of the same Erythrocyte carbohydrate antigens in A. lumbricoides and in its hosts suggests that the parasite might absorb them on its life cycle.Owing to the similarity between ABO and P System, we conclude that membrane glycolipids would be involved in the escape mechanism of the immune response.

P47.
INHIBITORY CAPACITY OF ASCARIS LUMBRICOIDES IN RELATION TO ABO SYSTEM EPITHOPES BY USING A LIGHT DIFFRACTION BY SUSPENDED PARTICLES METHOD (LIGTH SCATTERING)

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Innovative experiments have demonstrated that the parasites can acquire blood group antigens on their surfaces as a way of molecular mimicry. The aim was to determine the Inhibitory Capacity (IC) of Ascaris lumbricoides extracts in relation to ABO System epithopes.The extracts with A or B antigenic determiners were selected by Agglutination Inhibition and Haemagglutination Kinectic Test.The extracts were faced against anti A, anti B and anti AB antiserum by using red cell suspensions as a revealing system. A Manual Method of Quantification of Haemagglutination was made using Ligth Diffraction by suspended particles (light scattering) Inhibitory Power(IP) is defined as the relation between antiserum's titre against a control extract without ABO epithopes, and the antiserum´s titre against the analysed extract. All the extracts presented inhibitory capacity of agglutination reaction (values of IP exceeding 1 and values of IP/protein gram between $0{,}99.10^3$ y $10{,}35.10^3$) The affinity of the agglutination reactions with the monoclonal antiserum was greater than anti AB antiserum. The method used is simple, economic and easy to handle. It can be applied as a reference method because it has a great sensitivity and precision. It´s useful to analyze the erythrocyte antigenic reactivity, to control inmunohematologic reagents and to measure anticorps in immunized patients.In this experience the method permited to verify the IC of the extracts and to compare them(the same IP values corresponded to very different IC when they were related to the protein concentration of the extracts). The greater monoclonales antisera affinity of the agglutination reactions were verified. Another investigators have used this method to determine the bacterium haemagglutination capacity and this technique has an application in microbiological field.

P48.
MOLECULAR CHARACTERIZATION OF TWO NOVEL PHOSPHODIESTERASES FROM *Trypanosoma cruzi*

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In tripanosomatids, it has been reported that cAMP is involved in the differentiation pathway regulation between non-infective and infective forms of the parasite. Besides, previous reports have shown that cAMP downregulates cell proliferation. Phosphodiesterases (PDEs) are a key component in the regulation of intracellular levels of cAMP catalyzing its hydrolysis, and together with adenylyl cyclases, modulates biological responses mediated by this second messenger. Until now, there are just a few enzymes characterized that participate in the regulation of intracellular levels of cAMP in Tripanosomatids. Recently, four phosphodiesterases have been identified in *T. brucei,* and in our laboratory the first phosphodiesterase from *T. cruzi* has been described. All these phosphodiesterases belong to the class I of PDEs. In the present work we report the partial characterization of two novel phosphodiesterases found in the database of the genome project of *T. cruzi* named PDE-567 and PDE-703. The amino acid sequence of PDE-567 shows high identity value with TbPDE1 of *T. brucei* and PDEA of *Leishmania major.* PDE-703 it's also found in class I, but it's grouped together with type four of PDEs. Besides, this last one, has characteristic domains of the phosphodiesterases (FIVE and PDEaseI). These sequences were used to identify open reading frames, which were then amplified by PCR and subcloned into expression vectors. The expression of the recombinants proteins were confirmed by Western bolts assays. In phosphodiesterase activity studies using recombinant proteins it was determinated that PDE-703 is expressed in *E. coli* whit its catalytic activity.

P49.
CHARACTERIZATION OF TBP AND BRF LIKE FACTORS FROM GIARDIA LAMBLIA

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The TATA Binding protein (TBP) is an initiation factor that participates in the transcription together with the three eucaryotic RNA polymerases. Other initiation factors are also necessary for the polymerases to bind promoter sequences, among them is TFIIB, a RNA pol II initiation factor, and its homologue factor BRF which acts in the ARN pol III dependent transcription. The regulation of transcription initiation is not well understood in Giardia lamblia. Neither the composition of the initiation complex nor its mechanism of action are known, mostly because the characterized promoter sequences have poorly defined consensus regions.
Exhaustive searches in the available genomic sequences of G. lamblia allowed us the identification of genes corresponding to homologues of the eucaryotic factors TBP and BRF (glTBP and glBRF). Northern blot experiments showed that both transcripts are present in the parasites, with a higher expression on trofozoites than during the induction of encystment. Electroforetic mobility shift assays performed with the recombinant proteins and different probes showed that both factors can bind double stranded DNA without an apparent sequence specificity. Two hybrid experiments demonstrated that these factors interact with each other. With these two hybrid experiments we intend to define which domains are involved in the binding and run a comparative analysis with the interactions characterized for the eucaryotic homologues. DNA-protein and protein-protein interactions involving glTBP and glBRF represent the first experimental data that contributes to the understanding of the divergent mechanisms of transcription initiation regulation in Giardia lamblia.

P50.
IDENTIFICATION, CHARACTERIZATION AND PURIFICATION OF A PUTATIVE THIOL CONTAINING NADPH DEPENDENT REDUCTASE FROM *Trypanosoma cruzi*

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The protozoon *Trypanosoma cruzi*, ethiological agent of Chagas' disease, has a particular glutathione (GSH) cycle, since it synthesizes a GSH analog called trypanothione. With the aim to characterize new enzymes of this metabolism, a gene codifying for a novel *T. cruzi* protein containing the glutaredoxin (Grx) pattern CXXC was cloned. This protein, denominated *Tc*Grx, shows homology to prostaglandin (PG) E synthases-2 and glutathione-S-transferases (GSTs). A serum anti-recombinant *Tc*Grx recognized by western blot two proteins in epimastigote lysates, one of them with the expected molecular weight of 35 kDa and the other of 32 kDa, which were denominated $TcGrx_{35}$ and $TcGrx_{32}$, respectively. $TcGrx_{32}$ was present in epimastigote cytosol, while $TcGrx_{35}$ was in the non-soluble fraction. In *T. cruzi* mammalian stages, tripomastigote and amastigote, only $TcGrx_{35}$ was found. In order to select putative $TcGrx_{32}$ substrates, its capability to bind certain compounds was tested by affinity chromatography. $TcGrx_{32}$ bound to thiopropyl-agarose, confirming its thiol containing protein nature. It did not bind to neither GSH nor S-hexyl GSH-agarose, which does not rule out it uses GSH, but suggests it may use another thiol as substrate. Finally, $TcGrx_{32}$ bound to cibacron blue-agarose and eluted specifically with NADPH co-purifying with four proteins. This suggests $TcGrx_{32}$ is a NADPH dependent reductase. $TcGrx_{32}$ biochemical characterization will determine its involvment in GSH and / or PG metabolisms. $TcGrx_{32}$ sequencing will allow to define if both proteins are *Tc*Grx isoforms or they are different proteins which cross-react with the anti-*Tc*Grx serum.

P51.
BONE MARROW MYELOID CELLS FROM *Trypanosoma cruzi* INFECTED MICE KILL IMMATURE B CELLS THROUGH A MECHANISM INVOLVING PROSTAGLANDIN-E2

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A deficient humoral immune response may be originated at different levels of B cell development and, in case of infections, could have strong consequences in the control of microorganism replication and pathology development. We investigate the influence of *T cruzi* infection on the central B cell compartment. Mice ongoing the acute phase of the infection present a marked cellular hypoplasia in bone marrow (BM), which mainly affects immature $B220^{low}HSA^{high}$ B (imB) cells. This BM hypoplasia is not due to an enhanced migration to periphery since splenic imB cells are also reduced, but it could be a consequence of the increased apoptosis observed in the imB cell population. Even tough BM hypoplasia is transient and coincident with the presence of parasite in blood; apoptosis is not a direct effect of parasite-cell interaction, since *T. cruzi* trypomastigotes incubated with normal BM cells do not induce imm B cell elimination. By co-cultures, we demonstrated that infected mice BM cells secrete a soluble factor that kills imB cells. This factor is not IFN-γ, TNF-α, TGF-β nor H_2O_2 but it is a product of the ciclooxigenase (COX) pathway since indomethacin treatment restrains the apoptosis observed. Furthermore, infected mice BM lose their killing activity when depleted from Mac+, but not Thy1.2+, cells. This effect is directly related to PGE2 level that is high in undepleted or Thy^+-depleted infected mice BM but reduced after Mac^+ cells depletion. Our findings indicate that *T. cruzi* infection induces myeloid cells to secrete PGE2 that eliminates imB cells. This event would affect the progression of the humoral by reducing the development of Ab-secreting cells and allowing an uncontrolled parasite replication

P52.
PHENOTYPIC CHARACTERIZATION OF MEMORY CD8+ T LYMPHOCYTES IN THE PERIPHERAL BLOOD OF CHRONIC CHAGASIC PATIENTS

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We have demonstrated previously that the phenotype of peripheral blood *T. cruzi*-specific CD8+ T cells from chronic chagasic patients is enriched in early differentiated (CD27+CD28+) memory T. cells (Albareda *et al*, Medicina 63:515, 2003). In the present work, we determined by flow cytometry the impact of *T. cruzi*-specific memory (CD8+CD45RA-) T lymphocytes on the total CD8+ T cell population in chronic chagasic patients with different cardiac dysfunction. The results of this analysis showed that the percentage of memory CD8+ T cells in chagasic patients was similar to that of non-infected controls. The total memory CD8+ T cell population in the patients was enriched in fully and early differentiated subsets (CD27-CD28- y CD27+CD28+ respectively). In contrast, the memory CD8+ T cell population in the peripheral blood of non-infected individuals was enriched in early differentiated memory T cells (CD27+CD28+), with decreasing numbers of the other memory subsets. The analysis of CCR7 expression in the total CD8+ memory T cell population showed that the percentage of CD8+ memory T cells with CCR7- phenotype (homing to peripheral tissues) in chronic chagasic patients was significantly higher than in the controls ($p<0.05$). Altogether these results demonstrate that the *T. cruzi*-specific memory CD8+ T cell profile in chronic chagasic patients is not reflected in the total CD8+ memory T cell compartment. However, the more "effector" profile of the CD8+ T cell population in chronic chagasic patients respect to non-infected controls demonstrates the impact of *T. cruzi* infection on the immune system of the host.

P53.
DIFFERENT HUMORAL RESPONSES IN BOVINES INFECTED WITH A PATHOGENIC OR AN ATTENUATED *Babesia bovis* STRAIN

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We have investigated the early humoral response developed in bovines infected with two different *Babesia bovis* strains (R1A and S2P) towards two antigens that have been postulated as vaccine candidates for bovine babesiosis, due to their high degree of conservation among strains and their possible participation in erythrocyte recognition and invasion: Merozoite Surface Antigen-2c (MSA-2c) and Rhoptry-Associated Protein-1 (RAP-1). R1A is an attenuated strain and is used in live vaccines against B. bovis in Argentina, while S2P is a pathogenic isolate from Salta. Two bovines were inoculated with R1A and two with S2P merozoites, and serum samples were obtained at different time points between days 0 and 66 post-infection. Recombinant forms of MSA-2c and RAP-1 were obtained in a prokaryotic system, purified by affinity chromatography; and used to develop indirect ELISAs. Using these tests, it was observed that anti-MSA-2c IgG antibodies could be detected earlier (10 dpi) in animals infected with S2P than in those infected with R1A (28 dpi). After that, titers remained high until the last observation day (66 dpi) in both cases. On the other hand, while detectable anti-RAP-1 antibody titers appeared at about the same time in both cases, striking differences were found at later stages: in R1A infections, anti-RAP-1 titers descended to undetectable levels around day 55 or 60 dpi, and remained low until the end of the experiment, but no titer decreases were observed in S2P infections. These results indicate important differences in the early stages of infection and/or antigen expression by attenuated and pathogenic strains of *B. bovis*.
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P54.
TNFα AND IL-1β EFFECTS ON THYMUS APOPTOSIS DURING *Trypanosoma cruzi* ACUTE INFECTION IN C57BL/6 MICE

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Severity of experimental *Trypanosoma cruzi* (Tc) acute infection in C57BL/6 (B6) mice is associated with increased serum levels of TNFα, IL-1β and thymic atrophy. Though both cytokines are related with apoptosis induction in thymocytes, we proposed to study whether in deficient receptor mice for both pro-inflammatory cytokines (TNF-$R_{1+2}^{(-/-)}$ and IL-1R $^{(-/-)}$) could have some modification in the thymic atrophy accompanying such an infection. In view that both cytokines plus IL-6, can increase plasma corticosterone levels (CT) which is able by itself to induce thymocyte apoptosis, we analysed circulating levels of the four mediators in Tc-infected B6 and KO mice. Results (mean ± sem, n=4-5) at day 15 pi: Parasitemia (parasites/50 fields) B6 195±10, TNF-$R_{1+2}^{(-/-)}$ 951±129 ($p<0.014$ vs B6), IL-1R $^{(-/-)}$ 212±9; TNFα(pg/ml) B6 588±98, TNF-$R_{1+2}^{(-/-)}$ 2990±328 ($p<0.014$ vs B6), IL-1R$^{(-/-)}$ 579±96; IL-1β(pg/ml) B6 82±13, TNF-$R_{1+2}^{(-/-)}$ 724±320 (vs B6 $p<0,014$), IL-1R$^{(-/-)}$ 709±75 (vs B6 $p<0,014$); IL-6 (pg/ml) B6 527±74, TNF-$R_{1+2}^{(-/-)}$ 1666±588 (vs B6 $p<0,014$) IL-1R$^{(-/-)}$ 899±172 (vs B6 $p<0,029$); CT (ug/dl, relative increase day15/day0 pi) B6 3,06±0.2, TNF-$R_{1+2}^{(-/-)}$ 18.5±6.6 (vs B6 $p<0,01$), IL-1R$^{(-/-)}$ 5.8±3.8; Apoptosis (relative increase day15/day0 pi) B6 2.8±0.7, TNF-$R_{1+2}^{(-/-)}$ 5.3.0±1.0 ($p<0.05$ vs B6), IL-1R$^{(-/-)}$ 3.45±0.2. Acute infection in TNF-$R_{(1+2)}^{(-/-)}$ mice was associated with a significant increase in CT plasma levels and thymus apoptosis, whereas infection parameters in IL-1R$^{(-/-)}$ mice were similar to those observed in B6 mice. The remarkable increase of thymocyte apoptosis in absence of TNFα signal, could be due to an excessive HPA activation mediated by IL-1β and IL-6. These results extend the redundant effects of these pro-inflammatory cytokines in activating HPA axis within the setting of acute Tc infection.

P55.
GALACTOSIL RESIDUES IN *Trypanosoma cruzi* AND ANTIGALACTOSE ANTIBODIES IN INFECTED PATIENTS

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Antigalactose antibodies are natural antibodies whose level in serum is increased in patients with *T. cruzi*. Galactosil residues of membrane glycoconjugates are significantly antigenic determinants of *T. cruzi*. The red cell B receptor has the same antigenic determinant. There exists a basal level in title 1/16, which corresponds to natural immunity. The aim of our work was: a) to show the presence of galactosil residues in a *T. cruzi* aqueous extract by inhibitions of hemaglutination and b) to evaluate antigalactose antibody levels in infected patients, by agglutination in an enzymatic environment.
Methods: a) monoclonal antibodies (anti A and anti B), a *T. cruzi* aqueous extract and a red cell suspension (groups A and B) 5% in saline solutions were used. Antigen dilutions were confronted with constant quantities of antibodies at their best dilution. Then, a red cell suspension was added and incubated. Finally, it was centrifuged. B) 103 positive chagas sera were used (HAI and ELISA) and 50 non – reactive sera were analysed to confirm the cutting title.
The samples were obtained from Centenario Hospital patients. Then, serum dilutions were confronted with a B group red cell suspension, previously treated with papain (enzymatic medium). The last dilution which presented macroscopic agglutination was considered to be the title. Results: a) when the antigens were confronted with antibody B and group B red cells, the agglutination was inhibited.b) a percentage of 91.26 +/- 5.45% of serum with title bigger than 1/16 was found.
T. cruzi galactosil residues were found both directly due to their presence in antigen and indirectly through antigalactose antibodies.

P56.
HISTOPATHOLOGICAL ANALYSIS ASSOCIATED TO THE INOCULUM IN THE ACUTE PHASE OF EXPERIMENTAL INFECTION WITH *Trypanosoma cruzi* IN RATS: AGE-ASSOCIATED ALTERATIONS

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Inoculation at weaning (W; aged 21-28 days; 1.10^6 trypomastigotes; Tulahuen strain; s.c. route) with Trypanosoma cruzi in inbred 'l' rats results in a self-resolving acute infection characterized by marked parasitaemias, whereas challenge to adult rats (A; aged 70-120 days; 7.10^6 trypomastigotes; Tulahuen strain; s.c. route) gives place to a mild disease with extremely low parasitaemias. Previous work has shown that the increased resistance in adult rats seems to be the result of an earlier production of specific antibodies. W and A rats (n=25) were infected on one flank (IF) with the parasites and on the other flank with saline (CF). Skin and regional lymph node samples, obtained at 5 min, 1, 4, 24 and 48 h post-infection, were fixed in formaline, embedded in paraffin and stained with Haematoxylin and Eosin or Giemsa. Uninfected controls were included for the study of lymph nodes and spleen. In the skin from A rats we found: vasodilation at 1 h p.i., an important acute inflammatory infiltrate (peak of acute inflammation) at 4 h p.i. and presence of lymphocytes at 24 h p.i. In the skin from W rats we observed: vasodilation of a similar intensity to that found in A rats at 1 h p.i.; acute inflammatory infiltrate (peak) at 24 h p.i. At 48 h p.i. both groups of rats presented a lymphocytic inflammatory infiltrate of similar intensity. Numerous mast cells were identified by Giemsa staining. At 48 h p.i. lymph nodes presented lymphoreticular hyperplasia and the spleen, white pulp hyperplasia. In addtion to the production of specific antibodies, the greater resistance of A rats to *T.cruzi* infection would also be related to a faster development of the peak inflammatory infiltrate, and this could potentially favour a faster resolution of the acute phase of the infection.

P57.
ANTIBODY INDEPENDENT PROTECTION INDUCED BY IMMUNIZATION WITH LIVE TRYPOMASTIGOTES OF AN ATTENATUED STRAIN OF *Trypanosoma cruzi*

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Axenic cultures of the attenuated TCC strain of *Trypanosoma cruzi* (Lineage *T. cruzi* I) have been widely used in our laboratory as live immunogens against a virulent challenge in animal models. In previous works we have demonstrated that this protective efect is principally due to trypomastigotes forms present in low number in the axenic cultures. The previous immunization with two low doses of TCC trypomastigotes (250 or 2500 parasites) induce a decrease in the parasitemia levels after a challenge with virulent parasites. In the present work we show the results obtained from the specific antibodies search by ELISA in trypomastigotes TCC immunized mice and from the serum passive transference to naïve mice. No differences were observed in antibodies levels between the control group and mice (Swiss and Balb/c) inoculated with two doses of 250 and 2500 trypomastigotes TCC at day 15 post immunization. We neither observed a significant specific antibody level at day 50 post inoculation using an only immunizing dose of 500 trypomastigotes TCC. However, mice inoculated with the same unique dose of cultured-derived trypomastigotes belonging to other *T. cruzi* I starins showed significantly higher antibody levels when compared with the non-immunized control group at day 50 post immunization. The serum transference of Balb/c mice immunized with 2500 trypomastigotes TCC to naïve mice did not protect them from a later subsequent virulent strain challenge. Mice immunized with TCC trypomastigotes forms developed a delay type hypersensibility (DTH) reaction suggesting the protection induced by TCC trypomastigotes could be mediated mainly by a cellular response.

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P58.
DIFFERENTIAL EXPRESSION OF B CELL ACTIVATION MARKERS IN MICE IMMUNIZED WITH CRUZIPAIN, A MAJOR *Trypanosoma cruzi* ANTIGEN

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Differences in the susceptibility degree to *Trypanosoma cruzi* infection have been reported. BALB/c mice are susceptible to infection and autoimmunity, whereas C57BL/6 mice are resistant. Previously we demonstrated that BALB/c mice immunized with cruzipain (Cz) showed an increase in spleen B lymphocytes (LB), it was related with the presence of autoantibodies to cardiac myosin. In C57BL/6 mice immunized with Cz these alterations were absent. The purpose of this work was to study B cell activation markers such as a) CD23 and b) MHC-II. Spleen cells (SC) were obtained at day 14 after the third immunization, from BALB/c and C57BL/6 mice immunized with: Cz + CFA (immune group, n=4) and OVA + CFA (control group, n=4). Each group received three *i.d.* injections containing 10 mg of Cz or OVA. a) CD23 expression was analyzed in SC obtained from both mouse strains and cultivated for 24 h. b) MHC-II expression was analyzed in purified LB, obtained from BALB/c mice and incubated with LPS or medium for 24 h. LB purification was done using Dynabeads mouse pan T (Thy1.2) and plastic adherence. Results: We demonstrate that in BALB/c mice, Cz immunization increased the number of SC with CD23+ marker in BALB/c mice compared with C57BL/6 SC (23 ± 4% vs 8 ± 4%). Purified LB from BALB/c immune mice when were stimulated with LPS or medium alone showed an increased expression of MHC-II. Mean values for fluorescence intensity ± SD were, immune vs control, 776 ± 70 vs 530 ± 6 (medium), 795 ± 50 vs 596 ± 30 (LPS).
Conclusion: BALB/c, a mouse strain susceptible to infection and autoimmunity when is immunized with Cz, shows a LB higher activation compared with control ones. In addition, these cells show a higher activation state compared with the ones derived from C57BL/6, a mouse strain resistant to infection and autoimmunity.

P59.
THE EFFECT OF TEMPERATURE ON SYNTHESIS OF PROTEINS IN *Toxocara canis*

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Introduction: The success of L2 of *T.canis* in survival and evading immunity within the mammalian host is due to their ability to Slough off surface antigen. This antigen is associate with the esophage glands and the cuticle. **Aim:** study the effect of temperature on synthesis of proteins in L_2 of *Toxocara canis.* **Materials and methods**: T.canis adult were recovered from naturally infected dog, The eggs were resuspended in a 1% formalin solution and pipetted into sterile flask at 25ºC until second stage (L_2) larvae developed. The L_2 were suspended in culture medium RPMI 1640, and incubate at 37ºC for 24 hours. Infective larvae were maintained at 37ºC, 38ºC y, 39ºC during 1,2,3 y 4 hours. Then they were centifugate at 3000 RPM for 10 minutes. TES products were collected and concentrate using PEG 8000and stored a –70ºC. Somatic extract was washing three time, then were homogenizated in Ommimizer. After centifugate at 3000 RPM, a supernatant was collected and concentrates with PEG 8000. The proteins concentration were determinates using the Bradford method. **Results**:

Proteins concentration in somatic extract of *T.canis*

Time / Temperature	1hour	2 hours	3hours	4hours
37ºC	0,81ug/ml	1,36ug/ml	1,22ug/ml	1,22ug/ml
38ºC	0,63ug/ml	1,41ug/ml	1,27ug/m	1,09ug/ml
39ºC	0,54ug/ml	0,4ug/ml	0,45ug/ml	0,49ug/ml

Protein concentration in TES of T.canis

Time / Temperature	1hour	2 hours	3 hours	4 hours
37ºC	0,5 ug/ml	0,63 ug/ml	0,58 ug/ml	0,4 ug/ml
38ºC	0,86 ug/ml	1,36 ug/ml	1,27 ug/ml	1,09 ug/ml
39ºC	0,25 ug/ml	0,31 ug/ml	0,77 ug/ml	0,77 ug/ml

Discussion: High level of proteins were found in TES and in somatic extract when these product were incubates 2 hours at 38ºC. Probably, the effect of temperature on level of protein could have a relation with the probability that, the parasite complete its life's cycle or remain as migrant larvae.

P60.
Trypanosoma cruzi INFECTION INDUCED DENERVATION AT SPLEEN LEVEL. EFFECTS ON CYTOKINES AND ANTIBODIES PRODUCTION

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Current evidence indicates that noradrenaline (NA) modulates antibody (Ab) synthesis in the spleen. The aim of this study was to evaluate NA spleen content and its possible relationship with Ab production during acute *T. cruzi* (Tc) infection in C57BL/6 mice. Since sympathetic denervation may also affect inmune system activation during this acute infection, we analyzed plasma levels of most relevant cytokines in this protozoan infection. Chemical denervation was carried out at birth with 6-OH-dopamine neurotoxin (Den) and 60 days later mice were infected with 100 trypomastigotes of Tulahuen strain (DenTc).
Spleen NA contents in the experimental groups (ng/g spleen, mean±sem; n=4-7) at day 17 post-infection were as follows: Control (Co): 267±42, Den: 15±2 (vs Co $p<0.028$), Tc: 20±2 (vs Co $p<0.006$), DenTc: 5±2 (vs Den $p<0.017$, vs Tc $p<0.001$). Plasma levels of IL-6, IL-10 and IFNγ were significantly increased in DenTc mice with respect to Tc. In contrast no changes were observed in plasma levels of TNFα and IL-1β in the same experimental groups. Analysis of specific Ab revealed the following results: IgG_{2a} (OD) Co: 0.16±0.06, Den: 0.18±0.01, Tc:0.64±0.1, Den Tc:0.61±0.07, IgM (OD) Co:0.08±0.02, Den: 0.09±0.04, Tc:0.85±0.1; DenTc: 0.65±0.07. Tc acute infection diminished spleen NA contents in infected mice as did chemical denervation. Furthermore, changes in cytokines levels involved in class switching were not accompanied by differences in specific IgG_{2a} and IgM levels between Tc and DenTc groups.

P61.

NORADRENERGIC NEUROTRANSMITION IN CENTRAL NERVIOUS SYSTEM IN C57BL/6 MICE INFECTED WITH *Trypanosoma cruzi*

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It is known that the immuno-endocrine and neural systems are profoundly interrelated. The aim of this study was to analyze the noradrenaline (NA) content in the central nervous system (CNS) tissues and their possible relationship with some immuno-endocrine parameters during acute *Trypanosoma cruzi* infection in C57BL/6 mice.
The analysis focused on plasma levels of pro-inflammatory cytokines (IL-1β, IL-6 and FNTα; ELISA, pg/ml); corticosterone (CT; RIA, ug/dl); as well as NA and their metabolite 3-metoxi,4-hidroxifeniletilenglicol (MHPG) (HPLC, ng/g tissue) in brain stem (BS), hypothalamous (Ht) e hippocampus (Hc). Results: (mean±sem, n=4-7; *=p<0,05) IL-1β Control (Co): nd, Tc: 89.5±6*; IL-6 Co: nd, Tc 163 ± 85*; FNTα Co: 13±1, Tc: 357 ± 42*; CT Co: 2.14±1.24; Tc: 9.3±1.77*; NA (BS) Co: 716±10, Tc 640±22*; NA (Ht) Co: 1500±118, Tc: 1370±58; NA (Hc) Co: 399±47, Tc: 295±26*. NA utilization was valorated as MHPG/NA ratio in BS Co: 0.076±0.009, Tc: 0.060±0.004*; Ht Co: 0.048±0.004, Tc: 0.058±0.006; and Hc Co: 0.054±0.008, Tc: 0.048±0.006. Conclusion: infected mice showed significantly increased levels of pro-inflammatory cytokines and CT, in presence of a reduced NA content and utilization in BS, with a diminished NA content being only found in Hc. There were no between group differences in NA content or utilization in Ht.

P62.

BCG AS HETEROLOGOUS EXPRESSION VECTOR FOR *Babesia bovis*

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The protozoo *Babesia bovis* is the causative agent of babesiosis a tick-borne diseases that is a major cause of loss to livestock production in Latin America. Vaccination against *Babesia* represents a major challenge against cattle morbidity and mortality in enzootic areas. The live vaccine being used contains attenuated strains of *Babesia bovis* and *B. bigemina*. The main disadvantages of these vaccines are cost, inadequate or variable efficacy, relative instability with the requirement of cold chain for distribution and risk of side reactions. The main goal of this work is to develop new vaccines against tick-born disease based on the BCG vaccine strain of the bovine tubercle bacillus - recombinant or rBCG -. The rhoptry-associated protein 1 (RAP-1) from *Babesia bovis* is the best characterized antigen to be investigated as a vaccine component. We have developed rBCG strains expresing Rap-1 using two different vectors. Stability of both strains was tested in bovine macorphages. Immunogenicity assays in mice are being performed in order to predict the usefullness of the system.

P63.

THE INHIBITION OF INTRACELLULAR SIGNALS THAT INDUCE ARGINASE FAVORS THE CONTROL OF *T. cruzi* GROWTH OF THE PARASITE IN *ex vivo* MACROPHAGES OF INFECTED MICE

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We demonstrate that cruzipaina (Cz), induce alternative activation of macrophages (Mϕ) through the induction of arginase (Arg), besides this profile of activation induced by Cz favors the intracellular growth of the parasite. This effect was reverted for NOHA, an Arg inhibitor. The induction of Arg mediated for Cz involves the activation of multiple intracellular signals like Tyrosin Kinases (TK), Protein Kinase A (PKA) and p38 MAPKinase, their inhibition, modifies the balance iNOS/Arg in favor of iNOS, therefore these signals are relevant in the control of the parasite replication in Mϕ activated with Cz and infected with *T. cruzi*. Due to that these studies were performed *in vitro* using principally a cellular line of Mϕ, the objective of this work was study the pathways of activation of Mϕ in a model of *T. cruzi* infection. For that, BALB/c mice were infected with 500 tripomastigotes and the activity and expression of Arg were studied in peritoneal Mϕ at 8, 15 and 19 days p.i. Also we evaluated the effect of the inhibition of intracellular signals on the parasite growth in spleen Mϕ. The activity and expression of Arg I were higher at day 19, where it was evaluated the effect of the treatment of the Mϕ *ex vivo* with the inhibitor of Arg, PKA and p38 MAPK. They were able to decrease the parasite growth in infected Mϕ, similar to *in vitro* assays. In contrast, the inhibitors of iNOS, PKC and p44/p42 MAPK could not control the parasite replication. The identification of antigens that induce Arg would help to find optimal targets to inhibit this pathway improving the control of the *T. cruzi* growth in Mϕ.

P64.

NEW LIPOSOME FORMULATION THAT ENHANCES MICE HUMORAL RESPONSE TOWARDS AN EXPERIMENTAL DNA VACCINE FOR BOVINE BABESIOSIS

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We have investigated the use of liposomes made of crude egg yolk lipids for the presentation of a DNA vaccine based on the gene that codifies for *Babesia bovis* Merozoite Surface Antigen 2-c (MSA-2c). Truncated msa-2c was cloned into pCI-neo vector. Total egg yolk lipids were extracted and liposomes containing control pCI-neo or msa-2c-pCI-neo were prepared using an established protocol that included: the preparation of a lipidic film, the formation of multilamellar vesicles by rehydration and vortexing, the formation of unilamellar vesicles by sonication, lyophylization in the presence of plasmid DNA, rehydration, and ultracentrifugation to get rid of non-encapsulated DNA. A ratio of 16 umoles of phosphatidylcholine (PC) per 125 ug plasmid was used, considering that egg yolk total lipids contain 20% PC. Groups of 4 mice were intradermally inoculated at days 0 and 15 with 25 ug of naked or liposome encapsulated control pCI-neo vector, or msa-2c-pCI-neo. Mice were bled at 15 and 30 days, and antibody titers were determined by ELISA, using purified recombinant MSA-2c, as antigen. Significant anti-MSA-2c antibody titers were already observed at 15 days in 75% of the mice inoculated with msa-2c-pCI.neo, while 100% had significant titers at 30 days. On the other hand, no animal inoculated with naked msa-2c-pCI-neo showed a significant response at 15 days, and only 50% did at 30 days. These results show the effectiveness of our liposome preparation to intensify the humoral response of a DNA vaccine for bovine babesiosis. Given the low cost of these liposomes, our work can also be of broader interest in the field of veterinary medicine.
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P65.
CYSTS OF *Physaloptera* sp. (NEMATODES) IN STOMACHS OF *Physalaemus biligonigerus* (ANURA: LEPTODACTYLIDAE) POPULATIONS THAT INHABIT TRANSGENIC SOYBEAN CULTIVATED AREAS OF CORDOBA PROVINCE (ARGENTINA)

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Beginning the years 90´, scientific and naturalists began to find frogs with body malformity members in the North American wetlands. Start then on, it has been trying to determine the reasons of the problem. These anuran malformations show certain similarities with those that the Thalidomide in human caused decades ago. These events, partly, were related with the infestation caused by trematode parasites. In the same sense, the list of the immunology suppression is also investigating to that can be subjected the amphibians for the continuous exposure to pesticides, and its relationship with the p arasitic disease. As part of a monitoring of wild populations of amphibians of the east-center of Argentina Provinces, the infestation by *Physaloptera* sp. (Nematode) larval cysts are described, in stomachs of adult specimens of *Physalaemus biligonigerus* (Anura: Leptodactylidae), coming from fields cultivated with transgenic soybean in Río Primero (31º 14´46´´S - 63º 33´8´´W, Córdoba). The specimens of *P. biligonigerus* (n = 19) were collected with wet pit fall traps inside cultivations of soybean in the 2002-2003 field survey, between December to March, respectively. The frogs presented an average weight of 9 g DS 1.79 g with 84.21% percentage of infestation. The cysts were registered on the outer section of the stomach wall, in an average number of 49 per stomach. This record is considered the first registration of the parasite for these vertebrates. In addition, we are continuing with studies on the taxonomic identity and with analyses of the inmunitary system. This investigation allows us to explain the important infestation of encountered populations and if this, has relationship with the plaguicides exposure that is subjected the wild populations that inhabit the extensive cultivations.

P66.
OUTBREAK OF TRIQUINOSIS IN THE SUBURBAN AREA OF ROSARIO

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Introduction: Parasitic zoonosis of worldwide distribution produced by Nematelminto (TrichinelIa spiralis) Transmitted to the man by the ingestion of contained larvate weave cysts in cooked crude meats or bad. In Argentina they take place frequently I appear epidemic sporadic, associated to practices of clandestine tasks of pigs without veterinary inspection. **Objective:** To analyze a bud of triquinosis in the conurbano of Rosary in 2002 being shown to the clinical aspects and the importance of the monitoring epidemiologist. **Material and Methods:** Were attended in the Service of infectology of the Provincial Hospital of Centenary 38 patients, 32 of which they presented/displayed compatible symptomatology with Triquinosis. 81.57% Myalgias; 47.3% Periorbital edema; 47.3% Fever; 44.7% Headache; 26% Vomiting; 26% Abdominal Pain; 10.5% Diarrhea. And 6 were asyntomatic. The period of incubation oscillated in the different cases between 7 and 32 days all had ingested products of pig of the same origin (clandestine tasks). To all was made serology to them (IFI) presenting/displaying the following results. 7,9 % positives (1º shows); 26,3% positives (2º shows); 65,8% positives (3º shows); 35 of the patients made treatment with Tiabendazol and in the 3 rest the treatment with imidazólicos was contraindicated, sending the symptoms in tot. **Conclusion:** To emphasize the importance of an opportune diagnosis of this parasitism before the existence of centers of clandestine task of pigs in peripheral zones of great large cities to execute the monitoring measures epidemiologist and to prevent this pathology.

P67.
AMERICAN TEGUMENTARY LEISHMANIASIS IN BELLA VISTA (CORRIENTES, ARGENTINA)

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An epidemiological survey about the tegumentary leishmaniasis was performed in Bella Vista municipality, distant 140km to the Southwest from Corrientes city (28 and 29° of south latitude and 58 and 59° of North longitude). This was a response at the request of the Honorable Deliberative Council of Bella Vista to the CENPETROP (Declaration N° 013/2003) for carry out a situation diagnosis of the second epidemic outbreak due to leishmaniasis, during August 2003. The diagnosis was performed in 23 patients that lived in the neighborhood "La Florida" in Bella Vista city by the Montenegro skin intradermic reaction, apposition smear and NNN cultivations. All the people suffered different varieties of the cutaneous form, (probably of the subgeneus *Viannia* of the *Leishmania braziliensis* complex) and the lesions evolution time was about 30 and 240 days. The disease affected both sexes and all the ages of family groups. Two sisters of eight months and two years old were affected, what suggests the transmission in their domicile. All responded satisfactorily to the specific treatment (Glucantime® 20mg/kg/día) with a previous evaluation of the heart, hepatic and renal systems. With the Rioux and Shannon traps, 15 *Lutzomyia (Nyssomia) nievai* of the *intermedia* complex were collected in the peridomicile of a sick person and it is probably that this specie would be one of the transmitters in that area.

P68.
INCIDENCE AND PREVALENCE OF *Trypanosoma cruzi* INFECTION IN DOGS FROM A RURAL AREA UNDER ENTOMOLOGICAL SURVEILLANCE

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Dogs are one of the main domestic *Trypanosoma cruzi* reservoirs in rural areas of the Argentinean Chaco and they represent a risk factor of infection to humans cohabiting with them. Besides, because of their high susceptibility to *T. cruzi* infection, high abundance and their close relation with their owners; they have been suggested as natural sentinels of the vectorial transmission of *T. cruzi* in rural areas under entomological surveillance. The aims of the present study were to 1) determine the incidence and prevalence rate of *T. cruzi* infection in dogs from a rural area under regular entomological surveillance activities since 1992; 2) detect new infected native-born dogs and explain the possible transmission routes involved. Overall prevalence of *T. cruzi* infection, diagnosed by ELISA, IFAT and IHA or xenodiagnosis was of 4,7% among 256 dogs examined in November 2002. In native dogs the age-prevalence rate was of 4,3% in dogs younger than 1 year, nil in dogs from 1 to 3 years-old and showed an increasing trend from 4,0% to 16,7% in dogs from 4-5 years old and older. Of 81 dogs surveyed in May 2000 and November 2002 no seroconversion was registered. This nil incidence is a consequence of diminishing the abundance of *T. infestans* caused by a massive spray done in 1992 combined with community-based regular surveillance and selective sprayings. We identified the external clinical aspect of the dog, cohabiting with one or more infected dogs and the *T. cruzi* infection status of the mother as significant risk factors associated with *T. cruzi* infection; determined by a bivariated analysis of the odds ratios. In the absence of seroconversions among dogs, we could expect the absence of seroconversion among humans.

P69.

SPATIO-TEMPORAL ANALYSIS OF REINFESTATION BY *Triatoma infestans* (HEMIPTERA: REDUVIIDAE) FOLLOWING INSECTICIDE SPRAYING IN A RURAL COMMUNITY IN NORTHWESTERN ARGENTINA

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The spatio-temporal reinfestation patterns by *Triatoma infestans* following a blanket insecticide spraying in the rural community of Amamá, northwestern Argentina, were analyzed using geographic information system (GIS), satellite imagery, and spatial statistics. Domestic and peridomestic reinfestation by triatomine bugs was monitored from 1993 to 1997. *T. infestans* was detected at least once in 75% of 2110 sites evaluated. The prevalence of sites positive at least once for *T. infestans* during the study period increased sharply from 1993-1995 (0.6-2.9%) to November 1997 (32%). The initial source of *T. infestans* was a pig corral in southern Amamá one year post-spraying. Subsequent infestations were clustered around this initial focus at a distance of ~400 m starting in 1995. In 1996, clustering was maximized in sites within the same or in neighboring compounds at distances of 25-175 m. The reinfestation process in the northern section apparently did not originate from this pig corral and was independent of the southern sources, as also supported by wing geometric morphometrics. Application of focal spatial statistics in this study allowed for the identification of *T. infestans* propagation epicenters. Targeted surveillance of key peridomestic sites, such as goat and pig corrals, using low-cost sensing devices and improved treatment regimes are recommended. An effective control program on the community level will be based on the spraying of actual epicenters and sites within a 450 m of these epicenters to prevent the propagation of *T. infestans*.

P70.

CASES OF TOXOPLASMOSIS ATTENDED IN THE SERVICE OF INFECTOLOGIA OF A GENERAL HOSPITAL IN PERIOD 2002 – 2003

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Introduction: The toxoplasmosis, it is a cosmopolitan zoonosis of frequent presentation that affects to the man and homothermous animals. The acute infection in inmunocompetentes patients generally is non-symptoms, only in some clinical manifestations appear. In pregnancy the acute infection, it produces fetopatía, pronouncing itself in its slighter forms, like coriorretinitis in 2º or 3º decade of the life. In inmunocomprometidos, it can affect SNC in its more frequent form. **Objectives:** To present/display the cases of toxoplasmosis attended in during Years 2002 - 2003 in the service of Infectología of a general hospital. **Clinical Cases:** *Coriorretinitis:* 13 patients with reactivation of ocular focus of toxoplasmosis. Rank of ages between 20 and 50 years. IgM (IFI) negative in the total, IgG (IFI) average 1/64. Bottom of eyes (90%) cicatricial injuries, vitreítis (50%), typical focus of toxoplásmica coriorretinitis (100%).Other causes discarded - election treatment was made. *Syndrome of mass occupant in brain in HIV/AIDS:* 5 patients with sign focal neurological, none HAART, non prophylaxis AIDS, smaller count of 200 CD4 of céls/mm≈. Serology negative for Chagas, IgG (IFI) toxoplasmosis positive (average 1/32) IgM (IFI) toxoplasmosis negative. TAC and RMN characteristic images. Treatment of election. TAC of control favourable answer. *Clinical features of acute toxoplasmosis aguda:* 4 inmunocompetent patients with generalized lymphadenopathy. IgM (IFI) positive, rest of negative serology. *Toxoplasmosis acute in the pregnant woman:* 34 patients with twin samples by HAI with values between 1/256 and1/1024 between 1º and 2º show. IgM was made (ISAGA) obtaining positive 29,4%, 27.2% made election treatment. RN serology negative IgM. **Conclusion:** To emphasize the importance of the diagnosis of toxoplasmosis in its different forms from presentation.

P71.

INTESTINAL PROTOZOOS IN A SANITARY REGION OF THE PROVINCE OF CORRIENTES

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The intestinal parasitisms, by its high prevalence, the diversity of its clinical manifestations, their effects on the nutricional and immune condition of the population and their cosmopolitan distribution, represent a true problem of public health. The province of Corrientes is organized in five sanitary Regions according to the level of complexity of the public health centers. Sanitary Region 1, located to the northwest of the province, includes localities like Empedrado, Ita Ibate, Itati, San Cosme and Riachuelo. With the objective to determine the prevalence of the enteroparasitos, its distribution by locality, to determine the most affected locality in the region and to propose prophylaxis measures, the study was made between March and December of 2003, in kids from 0 to 12 years who went in espontanea form to infantile dining rooms. Serial samples were taken from matter fecal, using SAF like conservant, and brushed perianal. The samples applied method of concentration by sedimentation of Richie. 353 cases were found of enteroparasitos on a total of 402 samples, that showed a general prevalence of 87.8%, Riachuelo was the highest prevalence locaty (92,7%). The most frequent protozoo was Blastocystis hominis (59,2%), follow that: Giardia lamblia (36,6%), Entamoeba coli(29,5%), Iodameba bütchlii (22,7%), Entamoeba histolytica/ dispar (7,7%).In the perianales brushes were found protozoos opportunists like Amoebas of free life (10,5%). Blastocystis hominis and Giardia lamblia were in highest prevalence in the localities of Empedrado and San Cosme. This sanitary region presents high indice of enteroparasitosis, that put the equipment of health in front of a real challenge for the control and prevention, as also it suggests the necessity to improve tie works and public services like elimination and depositon of excrete, sweepings, potable water and characteristic of the house.

P72.

TEGUMENTARY LEISHMANIASIS IN BELLA VISTA CITY, CORRIENTES. ARGENTINA

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Leishmaniasis, fundamentally a skin disease, is a clinical signs produced by different species of Leishmania. The three clinical varieties are cutaneous, mucocutaneous and visceral. The disease was recognized in 1985 as an edemoepidemic pathology in various regions of northern Argentina. The object of this study was to detect the cases of Leishmaniasis in a rural area from Corrientes, find the epidemiology factors and suggest preventative actions. Between the months of August and October of 2003, a clinical epidemological study was performed on suspect patients from the city of Bella Vista and the surrounding areas. Samples of the lesion were taken for the direct parasitology diagnosis, in addition to clinical exams and immunology tests. 40 patients participated in the study, 34 of which (85.0%) cases of Leishmaniasis tested positive in the diagnostic clinic-epidemiologic exam, Montenegro skin test and direct parasitologic diagnostic test. The sensitivity from the Direct Parasitologic Exam was 74.0 % (25/40). The clinical variation of the location of the lesion and theraputic diagnostic parameters are similar to those that were found in other regions of Argentina. The epidemic outbreak could be associated with environmental factors, and it is suggested that a sanitary education for primary care personal medical and the people, because the infection stars when the man irruption the vector habitat.

P73.

POLYMORPHIC MICROSATELLITE MARKERS IN THE CHAGAS' DISEASE VECTOR *Triatoma infestans* (HEMIPTERA: REDUVIIDAE)

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Triatoma infestans is the principal vector of Chagas' disease in the Southern Cone of South American countries. The long-term effectiveness of the control campaigns is greatly dependent upon the knowledge of the vector population structure. With the purpose to analyze natural populations of this vector using polymorphic molecular markers as microsatellites, we identified and characterized these genetic markers from *T. infestans*. Ninety-three microsatellite loci were isolated from partial genomic libraries of which thirty were amplified. Ten of the polymorphic microsatellite loci for which different allele types could be resolved clearly were selected for genotyping. The degree of intra-population variation in these loci was determined using 34 specimens of *T. infestans* collected from different houses or peridomiciliary sites of the locality of Chancaní (Pocho, Córdoba, Argentina). The number of alleles per locus ranged from 5 to 19, and the observed heterozygosity ranged from 0.242 to 0.938, suggesting a high degree of intra-population variation in isolated loci. Four loci showed significant heterozygosity deficits, which may reflect population subdivision or the presence of null alleles. The variability of these microsatellite markers provides a valuable molecular tool for population genetic studies in *T. infestans*.

P74.

COAGULATION PROCESS IN THE TREATMENT OF WATER AS A BARRIER TO AVOID WATERBORNE OUTBREAKS OF CRYPTOSPORIDIOSIS

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Cryptosporidium is one of the microorganisms of main concern from the point of view of Public Health, being a priority problem for water treatment plants and water regulatory institutions. Due to its small size and resistance to chlorination, *Cryptosporidium* removal during the drinking water treatment process is a hard task. The effectiveness of the different coagulants commonly used in such a process for the removal of oocysts was analyzed. The Jar test was used in the experience. It was found that: 1) coagulants with the addition of coadjuvant polymers cause an oocyst removal higher than 2 log; 2) a low value in turbidity does not necessarily mean an optimum parasite removal, and 3) the addition of polyelectrolite to ferric chloride diminishes the variability both in final turbidity and *Cryptosporidium* removal.

P75.

ATIPICAL LOCATIONS IN PRIMARY HIDATIDOSIS

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Introduction: The hidatidosis is a world-wide zoonosis that affects mainly, cattle agricultural regions. Being located (hidatídic cyst) frequently in liver or lung (primary filters), if such they are crossed can be located in any organ of the economy, acquiring characteristic different in bone and encephalic. **Clinical Cases:** -*Man of 47 years*, native of Santiago del Estero (Argentina), right coxalgia of 6 months of evolution. X-rayses, TAC and RMN of pelvis: Mass that jeopardizes ileón, pubis, coxofemoral and sacred joint. RMN of dorsal column osteolíticas alterations in D4, D5 and D6. Abdominal TAC: hepatic quística image. Bony biopsy: it confirms hidátide. Positive DD5. Treatment: Albendazol in cycles without surgical treatment. -*Woman of 28 years*, native of Cuzco (Perú), lumbociatalgia and hematúricos episodes of 3 years of evolution. Abdominopelviana Echografy and TAC: quísticas images in kidney and right ovary. Positive DD5. Albendazol treatment in cycles and right nephrectomy. Positive Pathological anatomy for hidátide. Evolution. Resolution of anexial hidatidosis. -*Woman of 45 years*, native of Valparaiso (Chile), periodic rash and taquiarrytmia of 10 years of evolution. ECG: negative waves T in anteroseptal face. Echocardiogram: quística image in the interventricular septum, type 3 of Gharbdi that was corroborated with RMN. Negative seriatim DD5. Albendazol treatment in cycles, waiting for surgical resolution. -*Woman of 50 years*, native of Between Rivers (Argentina), antecedent of puncture of hepatic hidatídico cyst for 10 years, multiorganic dissemination of hidatide is stated according to diagnosis by images (lung, spleen, ovary, kidney). Positive DD5. At the moment with Albendazol in cycles with bad evolution. **Conclusion:** Our province does not count on official data that they define the problem in his real magnitude; the prevalence of hidatidosis in bovines is of 31.4% (Data collected by seizer). Before the currents imigratorias from areas of high endemicidad, they cause that we must suspect this pathology in non-habitual locations.

P76.

FLIGHT INITIATION OF *Triatoma infestans* IN EXPERIMENTAL HUTS UNDER NATURAL CLIMATIC CONDITIONS

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Flight dispersal of *Triatoma infestans*, main vector of Chagas' disease, plays a central role in house reinfestation after spraying with pyrethroid insecticides. Simplified experimental disigns with laboratory individuals found that flight initiation decreases with nutritional status and increases with temperature and age. The aim of this work was to analize flight initiation in relation to sex, nutritional status (measured as the weight:length ratio), adult age and the presence or not of a host not accessible for feeding. The experiments were carried out in experimental huts under natural climatic conditions in Cordoba Province, Argentina, during the months of February and March with average temperatures during flying period of 26°C. Aproximately 35 adults (2:1 Ma:Fe) captured from chicken houses in Santiago del Estero, Argentina, and individually marked were released in each hut. The flying individuals were registered during three consecutive nights being returned to the hut every morning. This design was repeated with a group of known age (1 to 5 weeks). In average, 44% of males and 68% of females initiated flight each night. The probability of flying a given night increased if the individual had flown the previous night. Flight initiation was positively assocciated with age and tended to decrease with weight:length ratio. These results show that individuals from natural populations, under natural climatic conditions, reach higher values of flight initiation than those obtained previously under similar temperatures and more artificial conditions. In contrast to previous works, sex appeared as an important factor in the study of flight dispersal of *T. infestans*.

P77.
MORPHOLOGY, INFRACILIATURE, AND ECOLOGY OF PLANKTONIC CILIATES (PROTOZOA, CILIOPHORA) FROM A TEMPORARY POND IN BUENOS AIRES PROVINCE, ARGENTINA

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Ciliated protozoans are very well represented among the microfauna from freshwater biotops and despite of their great diversity and the roles they play in the communities they live, only a few Argentinian investigators have intended to study them. The scope of this work is to communicate morphological, infraciliature's, biometrical, and ecological data of some planktonic ciliates from a temporary freshwater pond located in Magdalena, Buenos Aires province, Argentina. Qualitative samplings were carried out from August to December 2003. Physico-chemical characteristics of the environment were also registered at the sampling site, by using a multiparameter sensor. Taxonomic identifications were made *in vivo* and after revealing argentophilic structures by the protargol technique according to Wilbert's modification. Stained specimens were meassured, illustrated, and photographed under the light microscope. The following species were recorded: *Didinium nasutum* (Müller, 1773) Stein, 1859; *Halteria grandinella* (Müller, 1773) Dujardin, 1841; *Limnostrombidium pelagicum* (Kahl, 1932) Krainer, 1995; *Rimostrombidium brachykinetum* Krainer, 1995; *Strobilidium caudatum* (Fromentel, 1876) Foissner, 1987; *Linostomella vorticella* (Ehrenberg, 1833), Aescht in Foissner, Berger & Schaumburg, 1999, and *Hypotrichidium conicum* Ilowaisky, 1921. With the exception of *D. nasutum*, *H. grandinella,* and *L. vorticella*, the remaining species are new records for the ciliated microfauna from Argentina.

P78.
ALARMING LEVELS OF INFECTION FOR ENTERAL PROTOZOA IN CHILDREN OF BURRUYACU, PROVINCE OF TUCUMÁN, ARGENTINA

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As Tucumán presents an important deficit in the potabilización of the water in the rural areas, we wanted to see the level of infections for enteroparásitos protozoa, which are transmitted mainly by water or contaminated food. The study was carried out in the School Nº 325 of the department Burruyacu. It was carried out serial coproparasitologic exams and anal swabs to 150 boy, those that had an understood age between 4 and 14 years. The samples of grounds were analyzed carrying out direct observations in fresh, with coloring (Lugol, Carbolficsina) and carrying out investigation of intestinal coccidios for permanent colorations (Ziehl Neelsen), later on the samples were concentrated by the Ritchie modified method. The anal swabs were centrifuged and observed to the microscope the silts. The level of observed parasitism was of 93,3%, superior to that of other studies carried out in the province (70 to 80%). The prevalencia of the enteral protozoa was the following one: *Blastocystis hominis* 76%, *Entamoeba coli* 54,6%, *Giardia intestinalis* 35,3%, *Endolimax nana* 33,3%, *Chilomastix mesnilli* 5,3% and *Iodamoeba bütschlii* 2%. The prevalencia of the helmintos was: *Hymenolepis nana* 8,7%, *Ascaris lumbricoides* 6% and *Trichuris trichiura* 5,3%. 76 children only gathered the anal swabs, being observed a prevalencia for *Enterobius vermicularis* of 61,8%. When analyzing these results it shows the importance of taking measures with regard to the appropriate potabilitation of the consumption water, it is known that the cloration like method of potabilitation of the water is not enough to eliminate the risk of transmission of the intestinal parasites, because the viability of the cysts, ooquistes and eggs of the same ones are not affected. It is necessary to carry out the complete potabilitation (flocculation, filtration and cloration).

P79.
LEVELS OF INFECTION BY ENTEROPARASITES IN CHILDREN OF YERBA BUENA, PROVINCE OF TUCUMÁN, ARGENTINA

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The Province of Tucumán has high indexes of poverty and infantile malnutrition, as the infection by intestinal parasites are closely related with these two parameters we decide to study the levels of infections for enteroparasites in the city of Yerba Buena. It was carried out serial coproparasitologics exams (three samples, using SAF like conservative liquid) and anal escobillado (technique of the gauzes, three days) to 70 children that converged to two dining rooms of the area, those that had understood ages between 1 and 12 years. The samples of fecal matter were processed being carried out a direct exam in fresh and with coloring (lugol and carbolfucsina), it was investigated by means of permanent colorations (Ziehl Neelsen) *Cryptosporidium spp* and, to all they were carried out the method of concentration of Ritchie. The samples of anal swabs were centrifuged and it was observed the silts. The 84% of the studied population was parasited. The prevalencia of the different enteroparasites protozoa was: *Blastocystis hominis* 44,3%, *Giardia intestinal*is 25,7%, *Endolimax nana* 25,7%, *Entamoeba coli* 14,3%, *Chilomastix mesnili* 2,9% and Iodamoeba bütschlii 1,4%. The prevalencia of the helmintos was: *Ascaris lumbricoides* 31,4%, *Trichuris trichiura* 18,6%, *Strongyloides stercoralis* 2,9% and *Hymenolepis nana* 1,4%. Of the total of 70 children only studied 34 (48,7%) they gathered the anal swabs, giving positive result for *Enterobius vermicularis* 11 samples, 32,3%. The level of children poliparasitados is of 44,3%. The level of parasitism is very high, but it coincides with other studies carried out in the province. What we observe when comparing with previous studies carried out for other investigatior, is the increase of the prevalencia of the protozoa, what is correlated with the big problems that there is in the province with regard to the potabilitation of the drink water.

P80.
POPULATION STATISTICS OF *Triatoma rubrovaria* BLANCHARD, 1843 (HETEROPTERA: REDUVIIDAE) UNDER LABORATORY CONDITIONS

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The aim of this work was to obtain *T. rubrovaria* population parameters in order to contribute to the knowledge of its demographic characteristics. The investigation was carried out from October 2000 through February 2003 in the Arthropod laboratory, Corrientes, Argentina. Eggs were grouped to form 5 cohorts of 100 eggs each. Insects were fed on chickens (*Gallus domesticus*). The bugs were checked weekly and were kept under controlled temperature (28 ± 3°C) and relative humidity (63 ± 10%). A life table was constructed and other vital statistics were calculated and recorded The higher mortality was registered in the first through the fourth nymphal stadium. From the fifth nymphal instar the number of individuals showed a constant decrease. Life expectancy dropped linearly after overcoming the critical stages with the largest mortality risks. Adults mean survival was 50.2 weeks. The first oviposition was 40.6 weeks. The fecundity was 859.6 eggs with a weekly average number of eggs per female of 22.8. The reproductive period was 37.7 weeks. The generation time was 55.3 weeks and the net reproduction rate was 133.7. The intrinsic rate of weekly increment was 0.088. The adults carried 70% out of the total reproductive value. *T. rubrovaria* had a long survivorship as imago, a late first reproduction and a low intrinsic rate of natural increase.

P81.
SEROLOGICAL SURVEY OF CANINE VISCERAL LEISHMANIOSIS AT THE ARGENTINE BORDER OF PARAGUAY RIVER EMPLOYING THE K39 ANTIGEN

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The spread area of Canine Visceral Leishmaniasis (CVL) has extented to the countries limiting Argentina in recent years. An important outbreak has been detected in Asunción city and surroundings with the possibility that reservoirs and vectors may reach some Argentinean localities. The K39 antigen has proved to be a useful tool for tracking L. donovani group dispersion (*L. chagasi* and *L. infantum*) in human and canine populations. We have used this antigen in ELISA and immunochromatography dip sticks (Kalazar Detect INBIOS, USA) in laboratory assays and field surveys. Reactivity rates were relatively low in apparently normal dogs from Salta city (1/13, 8%), or carrying Tegumentary Leishmaniosis (2/11, 18%), or naturaly infected by *Trypanosoma cruzi* in the Chaco province (2/12, 17%). Reactivity was comparatively higher in dogs experimentaly infected by *L. infantum* which had not been developed pathological symptoms (6/17, 26%). All CVL-carrying dogs from Asunción city displayed positive reaction. A serological, parasitological and clinical survey of 107 dogs was undertaken in argentine areas of potential risk for spread (Clorinda, Puerto Pilcomayo, city of Formosa and borders of the rivers Pilcomayo and Paraguay), detecting a global seropositivity of 12% (13/107), not associated to CVL symptoms or parasite isolation. This data suggest a possible occurence of CVL cases in this zone. However, parasitological or molecular techniques would be neccesary for validating these results, due mainly to the false seropositive percentage among healthy or *T. cruzi* infected dogs. Animals carried, or simply walking, from Asunción city to Argentina may be a potential disease spreading factor.
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P82.
STRONGYLOIDIASIS IN A RURAL AND URBAN AREA OF THE CORRIENTES PROVINCE, ARGENTINA

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Publications about *Strongyloides stercoralis* prevalence in the Argentina and specially in the northeast are quite scarce. We present the results of a survey in a rural area and the symptomatic cases from an urban area of Corrientes province. Stool samples were collected over a period of six consecutive days in a vial with 5% formol solution. Each sample was processed using the technique of Hoffmann Pons and Janer A fresh sample was taken in order to conduct the coproculture using the method of Harada and Mori. The anal mucus was collected according to Graham's technique. We carried out a survey in the rural places of Costa Grande, Km 89 and Laguna Negra (San Luis del Palmar, Corrientes) in the second semester of 2002 on household sanitary conditions and the prevalence of intestinal parasites. A total of 148 individuals of both sexes and all the ages were examined and in 86% (127) of the samples one or more parasite and commensal species were found. *S. stercoralis* was detected in 19% (28) of different age groups and both sexes. Between February 1989 and March 2004, 1.012 persons of both sexes and all the ages groups were attended in the CENPETROP. One or more parasites and commensal species were observed in 60% (611), *S. stercoralis* was found in 8% (48) and 19% (9) of the infected were children smaller than four years age and among them a girl of six months. Serious clinical manifestations were presented in two women with hyperinfection. In 43% (20) the eosinophilia was between 11 and 82%. The prevalence of Strongyloidiasis in the rural area (19%) and the frequency of symptomatic cases (8%) in the urban zone should be considered high.

P83.
PARASITOSES IN AN ABORIGINAL POPULATION OF THE ARGENTINE NORTH

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The aboriginal comunities of the north of our country constitute population groups relatively closed and faithful to their habits and customs. Their houses posses neither running wather nor sanitary facilities theirs floors are soil and they are completely opened on the outside. Since this habitat is propitious to parasitoses development and maintenance, the aim of this work is to detect chagasic, toxoplasmic, hidatidic infections and intestinal parasitoses in the population under study. We worked with an aboriginal group of the etnia coya of Los Naranjos (sub area Los Cerros, Salta, 1400 m. above see level). For the systemic infections 42 samples of a total of 308 inhabitants (13,6%) were studied by serology (with comercial kits). The intestinal parasitoses were investigated in 60 childrens under 15 years old of a total for the groups of 130 (46%). Parasitologic analisys were carried out of stool samples by conventional way (3 samples) and Kinyou stained. The finding of the serology showed 66,6% (28/42) of prevalence for *T. gondii* while there were negative for *T. cruzi* and *E. granulosus*. The parasitologic analisys of sool indicated that 68.3% 41/60) were parasitised which 39% (16/41) were presenting polyparasitism. The prevalent species were *Giardia lamblia, Strongyloides stercoralis* and *Ascaris lumbricoides*. With previous microscopy Kinyou stained 11,6% *Cryptosporidium sp* were detected and 3,3% of *Isospora belli*; in all the cases the presence of coccidios was accompanied by at least another pathogenic genre. These finding indicate that more studies must be carried aut in order to know the pollution grade of the soils in Los Naranjos.

P84.
INTESTINAL PASITOSES FRECUENCY IN A PAEDIATRICS POPULATION OF MENDOZA, ARGENTINA

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Intestinal parasitoses constitute a public health problem that mainly affects childrens compromising their normal growth and development. The aim of this work is to know the frecuency of IP, its distribution by sex and age, its relation with the cause for consultation, nutricional state and sociocultural factors in childrens aged 2 to 5. A total of 67 childrens of the population of the pediatric service of helth center nº 16 Guaymallen(Mza) were aleatority studied. The IP were investigated by direct methology (Telemann method and Graham test). For the investigation of the clinical, enviromental and sociocultural parameters, a clinical-epidemiological survey was elaborated and carried out. The results indicated that 36% of the childrens was presenting IP without age differences and being males the most affected (47%). The most frecuent agents were *O. vernicularis* (67%), *B. hominis* (25%) and *G. lamblia* (25%). The 81% went for a control of healthy child and 19% for other illnesses (angina, urinary infection, etc.) Symptoms related to IP as nasal and/or anal itching (57%), hiporexia (54%), abdominal pain (40%) and irritability (30%) were observed despite the fact that none of these were cause of consultation. Having analysed the childrens with IP it was observed that 71% were eutrophic, 21% presented undernoushment grade 1 and 8% overweigth. The sociocultural characterization showed that 68% of the homes had bathrooms, 79% running water inside the house and only 10% had soil floor. The 86.5% of the parents had finished the primary school, as mimimun. We believe that IP is not investigated properly despite their high frecuency. We want to point out the necessity that IP research should constitute a routine in the pediatrics consulting room.

P85.
CHAGAS' DISEASE: PRESENT SITUATION IN INDIGENOUS POPULATIONS OF CHACO AND FORMOSA, ARGENTINA

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Indigenous populations are frequently organized as sort reservations, somehow isolated from other communities and often in a rural location. The enviroment in wich these populations develop (precarious houses and almost null sanitary infractructure), favor hight risk conditions to acquire many infectious deseases, like Chagas. The aim of this study was to evaluate the epidemiological charasteristics of Chagas'desease in native groups from Chaco and Formosa. Between march and november of 2003, 369 serum samples of individuals belongins to Toba (112) and Wichi (120) ethnic groups from Chaco were studied, as well 58 serum samples from Pilagá natives from Formosa. All samples were tested by indirect hemagglutination (HAI – Wiener lab. Arg.) and indirect inmunofluorescence tests (IFI). All samples reactive to both methods with titles equal or greater to 1/32 were considered positives for Chagas'disease. Over all prevalenece rate was 55,83%, widely highter than the infection rate for general population of Argentina (< 8%). Prevalence for natives from Formosa was 48,28%, this value is lower than 61,00% obteined in a study of native communities from Formosa (Wichis and Pilagas) in previous report. In natives from Chaco the prevalence found 57,23% is similar to the value obtained in a study of rural communities (natives and creoles) in 1999-2000 (53,20%). The prevalence found ascended with the age, due to a longer contact time with the vector. In Misión Nueva Pompeya the value found 69,17% is significantly higher ($p<0,001$; OR=0,44) than the prevalence (49,40%) from theother Localities (El Sauzalito, Pampa del Indio, Estanislao del Campo).The prevalence rates found in this work show the need to introdute vectorial control works, taking in to consideration political and sanitary interventions, toward often forgotten communities.

P86.
CHAGAS' DISEASE IN A RURAL AREA IN PROVINCE OF CORRIENTES, ARGENTINA

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Chagas' disease continues being a public health problem in many countries of Latin America, 90 years ago of its description. The distribution of morbi-mortality is directly associated to poverty, whit an special impact in the rural population.
The aim of this study was to evaluate the epidemiological charasteristics and the prevalenece rate of Chagas' desease in a rural area of Corrientes, Argentina. Between June and December of 2003, were estudied 200 serum samples conserved in SEROKIT® and epidemiological data of Departments of San Miguel, General Paz and Empedrado from Corrientes. All samples were tested by indirect hemagglutination (HAI- Wiener lab. Arg.) and ELISA.
Over all prevalenece rate was 10,00%, widely higher than the prevalence rate for general population in Argentina (< 8%). The most affected group were the 16 – 30 age range (11,76%) and more than 60 age range (25,71%). The department with greater prevalence was General Paz (21,95%), in concordance with the house caracteristics (straw ceiling, walls of marinates and soil floor), distant less than 50 mts from the forest, with firewood deposits and hen houses in nearless, without fumigation antecedents in the last five years and to disown the characteristics of the vector. The enviroment in wich these populations live favor a hight risk conditions to acquire many infectious deseases, like Chagas; the precarious houses also favor the establishment of the vector, in addition with a poor access to primary medical attention services, reflecting a high prevalence of Chagas disease. The way to follow by the health care equipment must be the education as a main strong sanitary component.

P87.
SPATIAL PATTERNS OF COMMUNITY REINFESTATION BY *Triatoma guasayana* (HETEROPTERA: REDUVIIDAE) IN RURAL NORTHWESTERN ARGENTINA

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Triatoma guasayana is a potential substitute for *Triatoma infestans* as a vector of *Trypanosoma cruzi*, the causal agent of Chagas disease, in the Chaco region of Argentina and Bolivia. The spatial distribution of *T. guasayana* in the rural community of Amamá in northern Argentina over the 10 years that followed a blanket spraying with deltamethrin in October 1992 is described and analyzed using very high spatial resolution satellite imagery (1-4 m^2), GIS and spatial statistics. Site-specific domestic and peridomestic reinfestation by triatomine bugs were monitored by various methods semi annually from October 1993 to October 2002. The reinfestation by *T. guasayana* started with the finding of only adult bugs in a few domestic and peridomestic ecotopes. In both the southern and northern extremes of Amamá overall bug abundance was significantly clustered and predominantly peridomestic. The identified source of reinfestation in the northern cluster was a colony in a wood pile, whereas no potential peridomestic source was found for the southern cluster. Houses closer to the edges of the community were invaded significantly more by flight-dispersing *T. guasayana* bugs. Active dispersal from the hypothesized source and from the surrounding sylvatic environment, and passive transport of bugs into and from wood piles appear to be the most likely mechanisms underlying the observed spatial pattern of *T. guasayana*. The absence of persistent domestic colonizations indicates that to date there is no increasing trend toward domesticity of *T. guasayana* in the study area. The clustering zones can be considered high risk areas where *T. guasayana* invasion from the sylvatic environment is expected to be higher and in which the introduction of sylvatic *T. cruzi* is more likely to occur.

P88.
VECTOR URBAN TRANSMISSION OF CHAGAS DISEASE

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An entomological study was carried out in two peri-urban zones (Z1 and Z2) of San Juan and Mendoza Capital cities of Argentina. In both, during a survey dates were registered about inhabitants, domestics animals and house characteristics. A man/hour entomological evaluation was development in the houses and was carried out xenodiagnosis test in dogs. In laboratory, *Trypanosoma cruzi* infection and blood meal of collected bug, were determined. *Triatoma infestans* infestation and *T.cruzi* infection were 51.6% (16/31) and 6.4% (7/109) in Z1, and 7.4% (2/27) and 43.6% (24/55) in Z2. The results of xenodiagnosis were 8.6%(5/58) and 50%(6/12). It was detected that in Z1 the prevalent blood meal was double on dog/human (53%), while in Z2 the blood meals were majority simple on dog (79.6%). *T.cruzi* infection rate were similars to the average of parasited dogs like it was observed in other endemic rural zones of Argentina. Popular costumes in both zones, like disorder and domestic animals sleeping inside houses, make possible the colonization, vector development and potential vector transmission of Chagas disease. In the cities official control methods are rarely apply because of transfusions were considered the main mechanism of transmission. *T. Infestans* in the cities could been involved in an urban vectorial transmission of Chagas disease.

P89.

AMERICAN TEGUMENTARY LEISHMANIASIS IN THE PROVINCE OF CORRIENTES, ARGENTINA

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The leishmaniasis has increased in the last years in the Argentina northeast. From 1980 we began researches about leishmaniasis in this region of the country in collaboration with university institutions from France and Brazil and their objective was to demonstrate the existence of the disease in the patients, the probable transmitters and the environment where they lived. The following laboratory tests were used: a) Montenegro skin intradermic reaction; b) apposition smear; c) NNN cultivations; d) inoculation in *Mesocricetus auratus*. Sandfly captures were performed with the Rioux and Shannon traps. The cases of leishmaniasis reported were from 15 of the 25 departments from the Corrientes province irrigated by the Paraná river basin (Corrientes, Empedrado, San Luis del Palmar, Mburucuyá, Bella Vista, San Roque, Concepción, Caá-Catí, Lavalle, Ituzaingó, San Cosme, Mercedes and Goya) and as those from the Uruguay river (San Martin and Monte Caseros). Leishmaniosis was diagnosed in 85 individuals from eight months to 84 years old of both sexes. Only the cutaneous form was observed (ulcerous, ulcerousnodular, verruciform, vegetanting, disseminated, mucocutaneous and mucous). A total of 1 106 *Lutzomyia* were collected (937 *Lu.* (*Nyssomia) nievai* of the *intermedia* complex; 144 *Lu. migonei* (group *migonei*); 21 *Lu. cortelezzii* and 4 *Lu. (Pintomyia) shannoni).* None of the females had natural *Leishmania* infections, but *Lu. intermediate* and *Lu. migonei* were experimentally infected with *L. (Viannia) braziliensis*. We concluded that the american tegumentary leishmaniasis is endemic in the four cardinal points of the Corrientes province.

P90.

TOXOPLASMOSIS IN PREGNANT WOMEN FROM A HEALTH CARE CENTRE IN CORRIENTES CITY. ARGENTINA

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Toxoplasmosis is one of the best known of parasitical diseases; with oral, placental and organ transplant transmission. The Toxoplasma gondii infection during pregnancy can have a serious evolution to the unborn child, or in severe immunosuppression cases in which can develop a chronic Toxoplasmosis reactivation. Severity in neonates has an inverse proportion related with the time of infection during pregnancy. The aim of this study was to evaluate the prevalenece rate of Toxoplasma gondii infection in pregnant women from a health care centre and to relate that in acute cases and reactivations with women's age and gestation stage.
The population studied corresponds to a media-high social class; 535 serum samples, personal and epidemiological data were taken. All samples were tested by indirect hemagglutination (HAI Wienner Lab) and, in same cases, IFI to IgG and IgM. The general seroprevalence founded was of 60,2% (322/535). The most frequently founded titles were with HAI 1/64 (36,6%), 1/32 and 1/128 (20,2%). There were 139 cases with larger or equal titles to 1/128, just 87 (62,5%) IFI to IgG and IgM were made, 91,9% (80/87) were positives to IgM. The immunological scar clinical presentation were founded in 92,5% cases (298/322), 17 reactivations (5,3%), 13 (76,5%) in the first gestation trimester and 7 acute cases (2,3%), where the primary infection mainly occurred during the second gestation trimester (42,8%). The population with medical care was 93,5% (500/535), and the 100% live in adequate health-hygienic conditions, and don't have cultural predisposal habits. The largest ages rage was between 26-35 years and, consequently, it was the most affected (67%). The high prevalence in this population reflects a sanitary educational deficit; and the most frequent titles founded suggest a new concept of study and control in pregnant patients.