BI-1
PROLIFERATIVE AND INFECTIVE FORMS OF *TRYPANOSOMA CRUZI* HAVE A DIFFERENTIAL ORGANIZATION

Porto RM, Elias MCQB, Schenkman S
Departamento de Microbiologia, Parasitologia e Imunologia, Unifesp, R. Botucatu 862, 8º andar, 04023-062 São Paulo, SP, Brasil

Trypanosomes undergo deep morphological changes when they transform from proliferative forms into infective forms. These changes are especially noticeable in the nucleus and correlate with decrease in the transcriptional rate. The nucleus of proliferative forms is round, contains a large nucleolus and small amounts of peripheral heterochromatin. The nucleus of trypomastigotes forms is elongated, the nucleolus disappears, and the heterochromatin occupies most of the nuclear space. As chromatin packing is related to transcription, we compared the chromatin structure in epimastigote and trypomastigote forms. Nuclei obtained from parasites disrupted by nitrogen cavitation remains intact and resemble the structure found in intact parasites. Treatment of the isolated nuclei with *Micrococcal* nuclease reveals a typical nucleosome structure as previously described. However, nuclei obtained from trypomastigotes were more susceptible to digestion with *Micrococcal* nuclease than epimastigote nuclei, although a larger part of trypomastigote DNA is totally resistant. This finding suggest that in trypomastigotes, most of the genome is arranged in a freely and accessible nucleosome structure, and part of it is complexed in a highly resistant domain, probably related to large amounts of heterochromatic material. In epimastigotes, the nucleosomal structure is probably protected from cleavage by the presence of the transcription and RNA processing machinery. In addition, we found that the total histones isolated from the purified nuclei differ in these life stages, which might be due to differential histone modifications or expression. Northern blots show that at least histone H1 is underexpressed in epimastigotes. Therefore, the chromatin may be differentially organized in proliferative and infective stages of *Trypanosoma cruzi*.

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BI-2
PROSPECTIVE EVALUATION OF POLYMERASE CHAIN REACTION OF *TRYPANOSOMA CRUZI* KINETOPLAST MINICIRCLE DNA AND NUCLEAR SATELITE DNA FOR DIAGNOSIS OF CHAGAS DISEASE IN PEDIATRIC POPULATION FROM ARGENTINA

Brandariz S, Altcheh J, Quintana F, Burgos J, Freilij H, Levin MJ, Schijman AG
INGEBI-CONICET, Servicio de Chagas del Hospital de Niños “Ricardo Gutierrez”, Buenos Aires, Argentina

Two hot start PCR procedures for amplification of *Trypanosoma cruzi* kinetoplastid 330 bp DNA and nuclear satelite 188 bp DNA were optimized for detection of *T. cruzi* in blood samples. Both PCR procedures were prospectively evaluated in a pediatric population followed by the Chagas service of the Ricardo Gutierrez Children’s Hospital in Buenos Aires. Hereby we analize the first 32 evaluated clinical cases, including 25 children from chagasic mothers, 3 children with toxoplasmosis, 4 normal children as controls. Clinical diagnosis of Chagas disease was assessed by microhematocrite in children younger than six months old (Group A, n = 10) and by conventional serology in older children and mothers (Groups B and C respectively). 2 ml of peripheral blood were withdrawn from group A; and 10 ml of blood from the other clinical groups. Blood was diluted in one volume of Guanidine - chloride-EDTA solution and stored at 4°C. Sera from all patients were also taken for conventional diagnosis and ELISA with recombinant antigens. PCR and recombinant ELISA were done blindly, results from the first 38 cases were finally compared with clinical diagnosis. Recombinant ELISA and conventional serology had 100% of correlation . Out of the 25 tested children born of chagasic mothers, one from group A and 13 from group B resulted positive by conventional methods. *T.cruzi* K-DNA as well as satellite DNA were amplified in the group A infected child, and furthermore in another 2 months old patient with negative microhematocrite born of a PCR positive chagasic mother; showing that PCR was more sensitive than hematocrite to detect parasitemia. 10 out of the 13 seropositive children from group B were PCR positive by both assays. showing that 77% of chronic infected children presented parasitemia. No false positive PCR results were obtained in the non chagasic patients. K-DNA and satelite DNA based PCR data were 100% concordant, analysis by both assays is important for confirmation of PCR results and exclusion of amplicon carry over sporadic contamination . As pediatric patients are being treated by parasiticidal drugs, basal PCR data on pretreated patients together with PCR to be performed during and after treatment will serve to monitor drug efficacy and clinical outcome in this population.

BI-3
POLYMERASE CHAIN REACTION BASED DETECTION OF *TRYPANOSOMA CRUZI* IN ARCHIVAL HEART NECROPSIES WITH MYOCARDITIS FROM ARGENTINIAN CHRONIC CHAGAS HEART DISEASE PATIENTS AT DIFFERENT STAGES OF DISEASE PROGRESSION

Schijman A, Brandariz S, Vigliano C, Viotti R, Lococo B, Leze M, Armenti R, Levin MJ
INGEBI-CONICET, FCEyN,UBA, Hospital Eva Perón, Servicio de Cardiología, Buenos Aires, Argentina

Trypanosoma cruzi nests and/or DNA were searched in archival heart necropsies with or without myocarditis from chronic chagas disease patients (ChD) who died at different stages of disease progression. Chagasic patients were classified in four groups: group 0, seropositive asymptomatic patients, group I, heart disease patients with abnormal electrocardiographic signs (ECG); group II, heart disease patients (ChHD) with abnormal ECG and cardiomegaly; and III, the same as group II but with complex ventricular arrythmias and heart failure as cause of death. Representative regions with and without inflammatory foci and myocarditis from fixed hearts of 15 seropositive patients and 7 seronegative patients (control group with non chagasic cardiomyopathies) were analyzed by histopathology and kinetoplastid DNA based PCR. Only one specimen of the 15 fixed chagasic hearts presented parasite nests at the site of myocarditis; in the other 14 seropositive cases, a 330 bp *T. cruzi* K-DNA fragment was detected by PCR in all tissue sections with focal or diffuse myocarditis but not in normal areas from the same hearts or in other organ necropsies from the same patients, neither in non chagasic hearts with myocarditis from the control group. In most heart sections from group III patients, a nuclear middle repetitive sequence, SIRE was also amplified. In summary, the finding of *T. cruzi* cells and/or kinetoplastid as well as genomic DNA found in heart tissue sections with inflammation and myocarditis from patients cursing different stages of ChD, strongly suggests that persistence of the parasite in the heart is involved in the mechanisms leading to heart injury typical of ChHD.

BI-4
PERFORMANCE OF XENOCULTURE FOR *TRYPANOSOMA CRUZI* ISOLATION FROM WILD MAMMALIANS CAUGHT IN THE SÃO PAULO STATE, BRAZIL

Nunes EV, Bisugo MC, Araújo MFL, Pinto PLS, Cunha EA, Taniguchi HH, Westphalen SR, Larosa L, Elias CR, Oliveira Jr. OC, Guilherme CS, Barata JMS, Tolezano JE
Seção de Parasitoses Sistêmicas, Instituto Adolfo Lutz, SP, Brasil

The trypanosomatides that infect vertebrates, primarily, are recognized as wild mammals parasites.Thus, the investigation related to the identification of these protozoan wild reservoirs deserve importance when the knowledge generation about the evolution and genetic diversity and the Public Health point of view are considered because a lot of species are responsable for human and domestic animals infections and patologies. These agents isolation and preservation are basic conditions for the development of studies relationed to the transmission standards, perpetuation and interrelation between enzootic and/or zoonotic cicles; new drugs resistence and susceptibility; new diagnostic metodologies, and others. In a previous study we had evaluated the viability of xenoculture utilization as laboratorial thecnique for *Trypanosoma cruzi* isolation from cronic Chagas' disease patients (24^{th} Annual Meeting on Basic Research in Chagas' Disease, 1997). In this paper we're presenting the evaluations related to the isolation of *Trypanosoma* from xenoculture utilization. In differents regions of São Paulo State (Eldorado City - Vale do Ribeira, Ilhabela City - North Shore and, Araraquara and Itupeva Cities - Southeast Region) were caught 124 wild mammals (marsupials, rodents and carnivorous). All of the animals were submetted to xenodiagnostic and 11 (8.9%) were positives for *Trypanosoma*. The xenoculture technique was done from triatomines intestinal trate cultivation. These triatomines were utilizated in xenodiagnostic accomplished on 124 animals. The xenoculture gave the *T. cruzi* like samples isolation possibility in 9 (81%) of the 11 xenodiagnostics recognized as positives. The xenoculture did not turn possible the isolation success of these protozoans from none animal recognized as negative for T*rypanosoma* in xenodiagnostic examination. The samples isolated in this study are preserved in liquid nitrogen for future studies.

BI-5
TRYPANOSOMA CRUZI INVASION INCREASES CALCIUM RESTING LEVEL IN CARDIOMYOCYTES

Garzoni L, Masuda MO*, Capela MAN**, Lopes AG*, Meirelles MNL
Laboratório de Ultra-estrutura Celular, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900, Rio de Janeiro, RJ, Brasil *Laboratório de Eletrofisiologia Cardíaca, IBCCF, CCS, UFRJ **Laboratório de Fisiologia Renal, IBCCF, CCS, UFRJ, RJ, Brasil

Cardiomyocytes (CM) are the main targets for *Trypanosoma cruzi* in the Chagas´ disease. It is generally accepted that calcium ions (Ca^{2+}) play an important role throughout the contraction-relaxation cycle of heart muscle

cells. Furthemore, existing literature shows the importance of calcium ions during the interaction of *T. cruzi* with different cell types. The aim of the present study was to analyze aspects related to the homeostase of the Ca^{2+} in cardiomyocytes and during its interaction with *T. cruzi*. The studied cells were obtained from the enzymatic dissociation of heart mice embryos. The detection of Ca^{2+} at the cell"surface was performed using Lanthanum Nitrate. To understand the role of the calcium ions in the *T. cruzi* infection, CM were treated with A 23187 calcium ionophore and Trifluoperazine, a calmodulin antagonist, with 5mM (5min) and 30 mM (2 h) and then they were infected with treated and untreated tripomastigotes and followed for 6 hours of interaction. Intracellular calcium activity was evaluated by fluorescence ratio microscopy in ATTO-FLUOR (Zeiss) System. CM were treated with Fura-2 AM and following they were incubated with the parasites.

Transmission electron microscope revealed lanthanum as an eletrondense precipitate which dislocates Ca^{2+} attached superficially at the cell membrane. Normal cells presented an electron dense deposit on their surface, including invaginations of the sarcolemma and T-tubules. *T. cruzi* infected cells displayed alterations in the sarcolemma pattern. The A 23187 treatment showed inhibition of 19,52%, 82.53% and 68,66% and the trifluoperazine showed 13.85%, 5.70% and 36.55% when CM, parasites and both were treated, respectively. The effect of the ionophore treatment was more pronounced when the parasite was treated. Calmodulin antagonist affected the invasion process in the three tested systems reducing the parasite infection. Exposition of cardiomyocites cultures that have been incubated with Fura-2 AM to *T. cruzi* induced increase in the resting calcium level.

Our data showed the participation of Ca^{2+} in the invasion process of cardiomyocytes. In addition, these results open new possibilities in the study of alterations caused by the *T. cruzi* in homeostase of Ca^{2+} during the cycle of contraction-relaxation of the muscle.

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BI-6
TRYPANOSOMA CRUZI INFECTION AFFECTS MRNA REGULATION IN HEART MUSCLE CELLS *IN VITRO*

Pereira MCS, Singer RH*, Meirelles MNL
Departamento de Ultra-estrutura e Biologia Celular, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil *Department of Anatomy and Structural Biology and cell Biology, Albert Einstein College of Medicine, Bronx, NY, USA

One of the most striking events in *Trypanosoma cruzi* – heart muscle cell (HMC) interaction is the disruption of the actin cytoskeleton, even in well formed sarcomeres (Pereira et al., 1993 J. Submicrosc. Cytol. Pathol. 25:559-569). Because of this cytoskeleton disruption, we hypothesized that the regulation of actin mRNA would likewise be affected. In this study, we investigated the regulation of actin mRNAs during the cytopathology induced in myocardial cells by *T. cruzi*. Oligonucleotides probes were labeled with CY3 or 5´end labeled with gP32 ATP for in situ hybridization and Northern Blotting analyses, respectively. Agarose gel electrophoresis and Northern Blotting was carried out using standards procedures. To detected endogenous actin mRNA, fixed cells were hybridized with 20ng of the probe in hybridization buffer.

The analyses of isoactin actin mRNAs expression during cardiomyogenesis *in vitro* revealed an decrease in both cytoplasmic isoforms (b and g) mRNAs concomitant with an increase in a-cardiac actin mRNA level. *T. cruzi* infection caused a reduction of 50% in the a-cardiac actin mRNA after 72h of interaction. In contrast, b-actin mRNA levels increased approximately 47% after 48h of infection. These effects were independent of *T. cruzi* RNA and did not result from changes in GAPDH mRNA, which was used as internal standard. Furthermore, in situ hybridization demonstrated that a-cardiac, g and b-cytoplasmic actin are located in different compartment within the cytoplasm during cell myogenesis, which corroborate with the concept that most mRNA are addressed to the functional protein compartment. Ribosome RNA 18S also co-localize with b-actin mRNA in motile cells, which support the concept of mRNA localization as a mechanism for targeting protein synthesis at their sites of action. After *T. cruzi* infection, b-actin mRNA is translocated from the periphery to the perinuclear region in highly infected cells.

Our data provide evidences that *T. cruzi* affects actin mRNA regulation. Further studies will be carried out to understand which mechanisms are involved in such changes in actin mRNAs levels. However, this is the first case of a pathological mechanism which disrupts the normal cytoplasmic localization of mRNA.

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BI-7
THE CIRCADIAN SYSTEM OF *TRYPANOSOMA CRUZI*- INFECTED MICE

Fernández Alfonso T, Celentano AM*, González Cappa SM*, Golombek DA
Departments of Physiology and *Microbiology, Faculty of Medicine, University of Buenos Aires, Argentina

Although Chagas disease is characterized mainly by its effects on the cardiovascular system, many reports have stressed effects on the nervous system. In particular, this disease affects the autonomic nervous system, but anoma-

lies in peripheral and central nervous system (including hypothalamic morphology) have also been reported. As this disease has severe effects on the life quality of patients, we decided to study changes in the circadian system of an animal model of Chagas disease; i.e., *Trypanosoma cruzi*-infected mice.

Male C57-BL6 mice (45 days old) were inoculated with CAI (50000 parasites, i.p) and RA (50 parasites, i.p) strains of *T. cruzi* or placebo, and parasitism was confirmed by blood specimen visualization 30 days after the injections. Mice were individually placed in cages equipped with a running-wheel (17 cm. diameter) whose revolutions were counted continuously and analyzed by a computer-driven system (Dataquest, Minimitter Co.). Data were further analyzed for circadian parameters with Circadia software.

RA strain *T. cruzi*-infected animals exhibited a significantly ($p<0.05$) decreased amplitude of circadian rhythms, both under light-dark (LD) and constant dark (DD) conditions, probably due to motor deficiencies. On the contrary, CAI-treated mice showed normal locomotor activity circadian rhythms with regards to period, amplitude and phase. In CAI treated mice, reentrainment to a 6-hr. phase shift of the LD cycle produced a readjustment of circadian phase that took from 5 to 12 days on average, with *T. cruzi*-infected animals taking a longer time to achieve a stable phase relationship with the zeitgeber. Under DD, 15-minute light pulses delivered at circadian time 15 (CT 15, with CT 12 defined as the time of activity onset) produced significant ($p<0.05$) phase delays of the locomotor activity rhythm, of a smaller amplitude in the infected animals.

Light pulses at this circadian time also induced the expression of the immediate-early gene c-fos in the hypothalamic suprachiasmatic nuclei (SCN), the site of a biological clock in mammals, as revealed by immunocytochemistry using an anti-Fos antibody (Cambridge Inc.). All groups exhibited light-induced Fos expression in the SCN.

The foregoing results suggest that the main effects of *T. cruzi* infection on the circadian system is an impairment of the motor output from the clock towards controlled rhythms. However, an effect on the visual sensitivity in general, and in the circadian range in particular, can not be discarded at the present time.

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BI-8
T-DAF: A TAXONOMIC MARKER FOR *TRYPANOSOMA CRUZI*

Cortez AP, Teixeira MMG, Mayer MG*, Kipnis TL**
Departamento de Parasitologia, ICB-USP *Laboratório de Genética, Instituto Butantan **Laboratório de Biologia do Reconhecer CBB, UENF, Campos, RJ, Brasil

Resistance to complement-mediated lysis of *Trypanosoma cruzi* trypomastigotes is due to the expression of complement-regulatory factors. One of this factors is a 87-93kDa molecule named Trypomastigote Decay Accelerating Factor (T-DAF) present only on the surface of trypomastigotes and capable of inhibiting complement activation in a manner functionally similar to the mammalian complement regulatory component DAF (Kipnis at al. 1986 *Braz J Med Biol Res 19*: 271, Joiner et al. 1988 *J Biol Chem 263*: 11327). A 285pb fragment previously isolated from a cDNA library showing genetic and functional similarities with the human C3 inhibitor DAF (Tambourgi et al. 1993 *Inf Immunity 61*: 3656) was used as a probe to screen a partial genomic library of *T. cruzi*. This screening resulted in a fragment of 3.0Kb (T-DAF 3.0Kb) that probably has the entire sequence of T-DAF gene (Mayer et al. 1997 *Mem Inst Oswaldo Cruz 92*: Suppl. I). We have tested 25 species of 8 trypanosomatids genera by slot blot hybridization of genomic DNA with 285pb fragment (T-DAF 285) and T-DAF 3.0Kb probes. Our results showed that under high stringent conditions T-DAF 285 hybridizes only with *T. cruzi* genomic DNA, and T-DAF 3.0Kb probe were *T. cruzi* specific under small stringent conditions. Both probes did not hybridize with any other species or genus of Trypanosomatidae family. Southern blot analysis of *T. cruzi* genomic DNA hybridized with T-DAF 285 revealed structural polymorphism of T-DAF gene in 15 strains analyzed. The hybridization with T-DAF 3.0Kb probe showed complex patterns. The amplification of T-DAF 285 fragment, by PCR, resulted in a 262pb product in all strains of *T. cruzi* analyzed. None of the strains of *T. rangeli*, species of *Leishmania* and *Crithidia* tested showed amplification. Taken together our results indicate that both fragments T-DAF 285 and T-DAF 3.0Kb are species-specific apparently with a high degree of preservation indicating that both sequences can be used as taxonomic markers of *T. cruzi*.

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BI-9
SYSTEMATIZED STUDY OF THE BRAIN AND SPINAL CORD IN ACUTE PHASE OF EXPERIMENTAL TRYPANOSOMIASIS CRUZI IN DOGS

Carneiro CM, Bahia MT*, Mendes DVM*, Silva EL*, Chaves M*, Veloso VM*, Lana M, Pittella JEH**, Tafuri WL*
Escola de Farmácia, Departamento de Análises Clínicas, UFOP *Instituto de Ciências Exatas e Biológicas, Departamento de Ciências Biológicas, UFOP **Faculdade de Medicina, Departamento de Anatomia Patológica e Medicina Legal, UFMG, Belo Horizonte, MG, Brasil

Because of its association with the serious forms of infection, central nervous system (CNS) involvement in the acute phase of Chagas disease has been investigated using several experimental models. The objective of the present work was to study the involvement of the brain and spinal cord of dogs during the acute phase of infection with Be-78 strain of *Trypanosoma cruzi*. Twenty young (2-month-old) mongrel dogs were used. Sixteen were inoculated through the conjuntival route with 2,000 metacyclic trypomastigotes per kilogram of body weight and the four that were not infected formed the control group. Parasitemia was determined daily starting on the fifteenth day post-infection using BRENER's method (1962). When they presented patent parasitemia, the animals were sacrificed in the acute phase of infection. The brain and spinal cord were collected *in totum*, fixed in 10% buffered formalin pH 7.2, sectioned in 0.5 cm thicknesses and embedded in paraffin. Five serial sections were obtained, stained with Hematoxylin-Eosin, Violet Cresyl, Glees-Marsland, Weil-Weigert and submitted to the peroxidase-antiperoxidase immunohistochemical technique to identify *T. cruzi* amastigotes. On average 75 sections of the brain and 350 of the spinal cord of each dog were analyzed. Histophatologic examination of the gray and white matter revealed glial nodules containing glial and mononuclear cells which were mainly lymphocytes. Parasites were observed in the gray and white matter; inside the glial nodules, in their proximity or without any relationship to them; free in the interstice; or inside glial cells. The Glees-Marsland and Weil-Weigert stains revealed uniform and normal patterns in all analyzed sections. Regressive alterations were verified only in the glial nodules. Analysis of variance was used to compare parasite frequency, glial nodules and perivascular mononuclear infiltrates in the gray and white matter of the brain and spinal cord. In the spinal cord significant differences were observed in: 1) tissue parasitism in the cervical and thoracic areas; 2) glial nodules in the cervical, thoracic, lumbar and sacral areas; and 3) perivascular mononuclear infiltrates in the thoracic area. No significant differences were detected in the brain. The acute nervous form of experimental *T. cruzi* infection with the Be-78 strain showed discrete multifocal encephalomyelitis with nodular arrangement in the inflammatory process. To better characterize CNS involvement in the acute phase of Chagas disease, other parasite strains should be studied.

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BI-10
ROLE OF PARASITE AND HOST GENETIC BACKGROUNDS IN THE DIFFERENTIAL TISSUE TROPISM OF *TRYPANOSOMA CRUZI* STRAINS

Andrade LO, Machado CMS*, Chiari E**, Pena SDJ, Macedo AM
Departamento de Bioquímica e Imunologia *Departamento de Morfologia **Departamento de Parasitologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil

Chagas disease, caused by the protozoan *Trypanosoma cruzi*, is characterized by a variable clinical course. The factors influencing the clinical manifestations of the disease are not completely understood, but most likely involve genetic variability of both hosts and parasites. In the past two years we tested the hypothesis that the genetic variability of *T. cruzi* might influence the pathogenesis of the experimental Chagas disease through differential tissue tropism of two clonal lineages (JG and Col1.7G2). LSSP-PCR analysis of parasite DNA extracted directly from different tissues of BALB/c chronically (3 or 6 months) infected with JG and/or Col1.7G2 showed clear differential tissue distribution of the two *T. cruzi* populations. After three or six months of inoculation we observed a clear predominance of JG strain in the heart, contrasting with the predominance of Col1.7G2 in the brain, rectum, diaphragm, esophagus and blood. Most important, there was strict correlation between the partitioning of the two populations and tissue injuries, supporting the idea of a major role of the *T. cruzi* genetic variability in determining the pathogenesis of Chagas disease. We next attempted to investigate the influence of host genetic background in the differential tissue distribution. For that, two other lineages of mice, the DBA/2 (inbred) and Swiss (outbred), were submitted to the described protocol. Data obtained from singly or doubly infected DBA/2 mice revealed basically the same results observed for BALB/c, with the difference that the differential tissue partitioning could only be detected after six months of inoculation. The observations on Swiss mice were different. After three months of inoculation, some JG and doubly infected animals did not present any longer parasite DNA in the analyzed tissues, suggesting that cure had spontaneously occurred. The number of possibly cured animals was higher after six months. In contrast, mice infected only with Col1.7G2 presented parasite DNA even after six months of infection. Thus, our data demonstrate once more the well known influence of the host genetic background in determining the susceptibility to infection. The demonstration of possible host effects on parasite tropism will depend on further studies.

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BI-11
POWER SPECTRAL ANALYSIS OF HEART RATE VARIABILITY IN CHAGAS' DISEASE IN HAMSTERS

Dias da Silva VJ, Guimarães AM, Della Libera G, Lages-Silva E, Chapadeiro E, Ramirez LE
Departamento de Ciências Biológicas, FMTM, Uberaba, MG, Brasil

The power spectral analysis (PSA) of heart rate variability (HRV) is an important tool used to evaluate the sympathetic and parasympathetic autonomic modulation on the heart. Recently, parasitological and morphological studies have characterized the hamster as a representative model of Chagas' disease. The aim of the present study was to investigate the cardiac autonomic modulation by means of PSA in Hamster with acute Chagas' disease.

Fifteen male golden hamster (100-150 g) were divided in two groups: control group (CON, n=7) and chagasic group (CHG, inoculated with 2 x 10^3 blood forms of the Vicentina strain of *Trypanosoma cruzi*, n=8). The infection was confirmed by positive parasitemia, using micro-hematocrite method. After 20 days, under anesthesia (sodium pentobarbital, 40mg/Kg, i.p.), cannulas and ECG electrodes were implanted in the animals to record arterial pressure (AP) and ECG, respectively. After 24 hours, direct AP and ECG were recorded in conscious animals during 30 minutes, using a acquisition data system (Aqdados, Lynx T.E., São Paulo) on a personal computer. Time series of heart rate (HR) were derived on systolic or R wave peaks. After a signal pre-processing, the power spectra were obtained using the fast Fourier transformer. Three spectral frequency bands were defined: low (LF, 0,01-0,19Hz), mid (MF, 0,20-0,60Hz) and high frequency (HF, 0,21-2,50Hz). In the end, the animals were sacrified and histo pathological studies were performed.

Mean values (±SE) of HR (bpm) and standard deviation of HR (bpm) were 358±19, 14±3*, 353±19 and 22±2 in CHG and CON groups, respectively. The power spectral densities (bpm.$Hz^{1/2}$) were, respectively: LF: 30,2±1,3, 29,6±1,4; MF:.1,3±1,6*, 3,5±2,5 and HF: 10,4±2,3* and 18,0±2,5 (* $p < 0,05$ versus CON group).

These data indicate that 1) acute Chagas' disease reduces the total variability of HR in hamsters 2) the spectral bands which contribute for this reduction are MF and HF bands. This bands were associated with sympathetic and parasympathetic modulation of the heart in others animal species. Then, these results suggest, in the first time, a cardiac autonomic dysfunction in acute Chagas' disease in hamsters.

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BI-12
PECULIAR BIOLOGIC, BIOCHEMICAL AND MOLECULAR BEHAVIOR OF A *TRYPANOSOMA CRUZI* STRAIN ISOLATED FROM A CHRONIC CHAGASIC PATIENT

Gomes ML, Toledo MJO, Nakamura CV, Bittencourt NLR, Chiari E*, Marques-Araújo S
Departamento de Análises Clínicas, Universidade Estadual de Maringá, Maringá, PR *Departamento de Parasitologia, ICB/UFMG, Belo Horizonte, MG, Brasil

The course of *Trypanosoma cruzi* infection varies widely. The reasons for this variability are unknown but there is evidence that parasite and host factors and their interaction influence the evolution of Chagas disease. Many studies have been conducted to identify correlations between the disease's clinical variations and intrinsic *T. cruzi* characteristics. Here, *T. cruzi* strains isolated from chronic chagasic patients were analyzed using biologic (infectivity, parasitaemia patterns, mortality, *in vivo* and *in vitro* susceptibility to benznidazole, metacyclogenesis capacity and growth in culture medium), biochemical (surface glycoproteins) and molecular (RAPD and Simple Sequence Repeat PCR - SSR-PCR) parameters. Strains had biologic characteristics that were homogeneous except for the PR-150 strain which presented peculiar behavior. Its infectivity was detected by haemoculture that turned positive late and using optical microscopy, we observed round agglutinated culture forms with short, not very evident flagellum. With maintenance, epimastigote forms that were more wide than long with short flagellum prevailed. The PR-150 strain was 100% resistant to benznidazole *in vivo* and *in vitro*. Surface glycoprotein constitution was also different between the PR-150 strain and the others analyzed. Genotypic analysis using RAPD and SSR-PCR profiles showed a large, genetically well-correlated group which contained a majority of the strains and a divergent group which included the PR-150 strain. It is worth noting that this strain was isolated from an asymptomatic subject born in Januária, MG, whose serum displayed 0% lithic activity (LMCo). The strain was infective to "vero" cells. In comparative molecular analyses using samples of *T. rangeli*, *Leishmania* and other *T. cruzi* isolates, the PR-150 strain grouped with the *T. cruzi* species. Our results suggest a possible correlation between biologic, biochemical and molecular characteristics in *T. cruzi* populations. One of our groups contained only a single strain. Could this be the result of the quality of the tools used or an extension of the parasite's variability? Additional studies with more strains and new approaches could clarify this situation and possibly infer if it has some significance in the clinical evolution of Chagas disease.

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BI-13
NUTRITIONAL STRESS REGULATES ADHESION TO SUBSTRATE AND METACYCLOGENESIS IN *TRYPANOSOMA CRUZI*

Figueiredo RCQ, Rosa DS, Soares MJ*
Departamento de Patologia e Biologia Celular, Centro de Pesquisas Aggeu Magalhães, Fiocruz, Recife, PE
*Departamento de Ultraestrutura e Biologia Celular, Instituto Oswaldo Cruz , Rio de Janeiro, RJ

Metacyclogenesis in *Trypanosoma cruzi* occurs naturally in the insect vector, where epimastigotes adhere to the intestinal surface before differentiating into the trypomastigotes. Adhesion and metacyclogenesis can be induced *in vitro* by incubating epimastigotes in TAU3AAG medium, where the cells adhere to the culture flask walls. Reservosomes are endosomal compartments in epimastigotes where proteins and lipids are accumulated, being absent in cell culture derived amastigotes and trypomastigotes. It has been suggested that nutrients accumulated in reservosomes are used as energy source during cell differentiation in *T. cruzi*.

We have investigate metacyclogenesis *in vitro*, by using TAU3AAG medium supplemented with different nutrients (10% fetal bovine serum or gold-labelled transferrin), in order to analyse the relationship between nutritional stress, adhesion to substrate, endocytosis and cell differentiation. For transferrin experiments, the cells were cultivated for 96 hours in TAU3AAG and then the supernatant was removed and incubated with transferrin-gold complex solution. Remaining adhered epimastigotes were cultivated for 48 hours with the tracer. As a control, the supernatant was removed and transferrin-free TAU3AAG medium was added to the adhered cells. After each experimental schedule, the parasites were fixed and processed for transmission electron microscopy.

Addition of serum to TAU3AAG medium inhibits the metacyclogenesis and promoted growth of epimastigotes. Ultrastructural analysis of the cells showed the presence of reservosomes at the posterior end. Few cells could be observed attached to substrate. The same result was observed when serum was added after 96 hr of cultivation. After this time approximately 50% of supernatant cells were under the epimastigote form. Gold-labelled transferrin was found in both adhered and free-swimming epimastigote forms, but not in the trypomastigote forms at any time of cultivation. Addition of transferrin-gold to TAU3AAG after 48 hr of cultivation decreased the metacyclogenesis rate and the adhesion.

Our results suggest that in *T. cruzi*, adhesion to substrate is triggered by nutritional stress. Supplementation of TAU3AAG with nutrients inhibited adhesion , reverted the metacyclogenesis and supported the epimastigotes growth. Our data suggest a close relationship between uptake of nutrients, adhesion and cell differentiation in *T. cruzi*.

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BI-14
NUMBER AND SIZE OF TRACHEAL GANGLION NEURONS IN ACUTE CHAGASIC WISTAR RATS

Furlani VCG, De Souza RR, Maifrino LBM, Liberti EA
Instituto de Ciências Biomédicas da Universidade de São Paulo, Instituto Dante Pazzanese de Cardiologia, Caixa Postal 66.208, 05388-970 São Paulo, SP, Brasil

Several works have shown that the infection with *Trypanosoma cruzi* had a marked influence in the number of neurons in Auerbach's plexus. However, little information exists about the effects of chagasic infection on tracheal ganglion neurons. In this work, the traqueal ganglion neurons of Wistar rats were stained by a histochemical technique in non infected and acute chagasic animals (Y strain of *T. cruzi*). The neurons were counted on whole mount preparations of the dorsal muscle layer of the trachea. The number of neurons in infected and non infected rats was not significantly different. In non infected rats the profile area of the ganglion neurons ranged between 130 μm^2 to 867 μm^2 (mean ± sd : 405 ± 42 μm^2). In acute chagasic rats nerve cells were markedly increase from 124 μm^2 to 1204 μm^2 (487 ± 49 μm^2). These results suggest a marked enlargement of neuronal cells bodies during the infection. We also have found degenerating neurons among others apparently non affected by the infecction. It seems that, contrarily to the neurons of the myenteric plexus, the tracheal ganglion neurons of the rat are not destroyed by the *T. cruzi*.

BI-15
NEUROLOGICAL MANIFESTATIONS IN PATIENTS WITH CHAGAS´ DISEASE AND ADQUIRED IMMUNODEFICIENCY SYNDROME

Velásquez J, Corti M, Perez Bianco R, Cermelj M, Candela M, De Tezanos Pinto M, Bellegarde E, Carnevale S, Vigna L, Levi Hara G, Kuo L
Academia Nacional de Medicina; Hospital "Francisco J. Muñiz", Hospital de Agudos "Carlos Durán", Departamento de Microbiología, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina

The purpose of this study is the description of neurological findings in the association Chagas´ disease-AIDS.

A retrospective study was carried out in eight HIV-infected patients with AIDS and Chagas´ disease. The parasitological diagnosis was achieved by observation of *Trypanosoma cruzi* in fluids and histological brain samples (direct method) and employing indirect hemoagglutination and indirect immunofluorescence in blood. The following neurological criteria were considered: focal or non-focal manifestations, computed axial tomography and nuclear magnetic resonance: normal, unique or multiple lesions with or without contrast enhancement.

The mean for age in the studied group was 26 years old (19 to 36) and all of them were males. Five patients were intravenous drug abusers with residence in endemic areas. The other three cases were hemophilic patients that had received multiple blood transfusions. Focal signs were present in four cases and non-focal signs in the other four patients. The image methods revealed unique or multiple lesions in seven individuals. The CD_4 count was less than $200/mm^3$ for all patients. Tripomastigotes were detected in cerebral spinal fluid of three patients and amastigotes were identified in histological samples of the other cases. Serological diagnosis was carried out only in three patients and thy resulted positive. Chagas´ disease is an important cause of neurological manifestation in those patients from endemic areas with HIV infection. The CD_4 count resulting less than $200/mm^3$ could be a parameter for beginning of prophylactic treatment and direct methods would be the choice for Chagas´ disease diagnosis in these patients.

BI-16
MOUSE PERITONEAL MACROPHAGES EXPRESSING A SIALOADHESIN PRESENTS A HIGHER ASSOCIATION INDEX AFTER INTERACTION WITH *TRYPANOSOMA CRUZI*

DaMatta RA, Santos CL, Seabra SH, De Souza W/*
Laboratório de Biologia Celular e Tecidual, Centro de Biociências e Biotecnologia, UENF Laboratório de Ultraestrutura Celular Hertha Meyer, Instituto de Biofísica Carlos Chagas Filho, UFRJ, Rio de Janeiro, Brasil

Mouse peritoneal macrophages cultured with homologous serum (HS) express, on its surface, a sialoadhesin that can aggregate erythrocytes (Crocker & Gordon 1988 *Immunology 65*: 515-522). To determine if the expressed sialoadhesin could influence the interaction of *Trypanosoma cruzi* and *Toxoplasma gondii* with peritoneal macrophages, these cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 2% HS (MøHS) or 2% fetal bovine serum (MøBS) for 48 hr, and interactions with the parasites performed. Interactions were performed for 2 hr with trypomastigotes and epimastigotes of *T. cruzi* and tachyzoites of *T. gondii*. Bloodstream trypomastigotes of the Y strain of *T. cruzi* were purified from blood harvested from mice on the seventh day post infection. Epimastigotes were obtained axenicaly after the fourth day in culture. Tachyzoites of the RH strain, were obtained by peritoneal washes of infected mice. After the interaction, cells were washed (to remove loose parasites), fixed with Bouin and stained with Giemsa. Association index (AI) was determined by multiplying the percentage of infected macrophages by the mean number of parasites per cell. MøBS exhibited an expected AI for trypomastigotes (6.9) and epimastigotes (17.3) and a high index (361) for tachyzoites. MøHS exhibited a higher AI for trypomastigotes (15.5) and epimastigotes (28.5) and also a similar index (324) for tachyzoites. These results indicate the possibility that macrophages cultured with HS express a sialoadhesin which could influence the interaction of both forms of *T. cruzi*. The fact that no difference could be detected in the AI for *T. gondii* between MøBS and MøHS, and that this parasite does not have neuraminic acid on its surface, suggests that the sialoadhesin expressed by MøHS could recognize the sialyl residues on *T. cruzi* surface resulting in a higher AI. Further studies have to be done to determine the survival of these parasites within the macrophages.

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BI-17
MORPHOQUANTITATIVE AND ULTRASTRUCTURAL STUDIES OF THE MYENTERIC PLEXUS IN CHRONIC *TRYPANOSOMA CRUZI* INFECTED MICE

Moraes SRA, De Souza RR*
Departamento de Anatomia, CCB, UFPE *Departamento de Anatomia, ICB III, USP, São Paulo, SP, Brasil

The present study was undertaken to establish quantitative, morphological and ultrastructural features of the myenteric plexus of the cranial, middle and caudal parts of the Swiss mice esophagus chronically infected with the Y strain of *Trypanosoma cruzi*. The enteric nervous system was studied in whole-mount stained for NADH- diaphorase. Counts of neurons demonstrated in the *T. cruzi* infected animals a significant reduction in ganglion, neurons and neurons/ganglion number when compared with the controls ($p < 0.05$). In the cranial and middle parts of the esophagus the neuronal destruction by *T. cruzi* affected the three types (small, medium size and large neurons) of nervous cells but in the caudal part the neuronal destruction affected mainly medium size and large neurons. At the ultrastructural level the *T. cruzi* infected ganglia presented lesions in the ganglia and ganglion neurons. There were a increase of bundles of collagen fibrils in the sheath enveloping the ganglia of the myenteric plexus and alterations in neurons included vacuolization of mitochondria and hypertrophy of the RE. The study suggest that a denervation of the myenteric plexus in the striated muscle of the esophagus, similarly to that observed in the plexus of other parts of the digestive tract.

Supported by Capes.

BI-18
MORPHOLOGIC AND MORPHOMETRIC ANALYSES OF ESOPHAGUS OF DOGS EXPERIMENTALLY INFECTED WITH *TRYPANOSOMA CRUZI*

Santos CAB, Carneiro CM*, Bahia MT, Veloso VM, Lana M*, Tafuri WL
Instituto de Ciências Exatas e Biológicas, Departamento de Ciências Biológicas, UFOP *Escola de Farmácia, Departamento de Análises Clínicas, UFOP, Ouro Preto, MG, Brasil

Dogs have proven to be a good experimental model for the study of Chagas' disease. Three forms of the disease have been reproduced in dogs: acute symptomatic, indeterminate and chronic cardiopathy. However, the digestive form of the disease has not yet been detected. The objective of this study was to systematically analyze the esophagus of uninfected dogs and dogs infected with the Be 78 strain of *Trypanosoma cruzi* with emphasis on the morphometry of the Auerbach's plexus since intramural autonomic nervous system (ANS) lesions are an important factor in the development of mega syndromes. Fourteen young (60-day-old) mongrel dogs were used. Ten were inoculated via the conjunctival route with 2,000 metacyclic trypomastigotes per kg of body weight. Four uninfected dogs formed the control group. The animals were sacrificed during the acute phase of infection to study the intramural ANS. The esophagus was removed *in totum*, placed in saline solution, cut longitudinally and fixed in 10% buffered formalin, pH 7.2. Strips of 0.5 cm were taken from the upper, middle and lower regions of the esophagus and the esophageal-cardic junction. They were then routinely processed and semi-serially cut in 5 mm thicknesses with a 1:10 ratio for the esophageal-cardic transition region and 1:50 in the other regions. The fragments were stained with Hematoxylin-Eosin for histopathologic analysis. A KS300 (Zeiss) image analyzer was used for morphometric analysis. The esophagus had 6 layers: epithelium, lamina propria, submucosa, muscularis mucosae, muscle and adventitia. The Auerbach's plexus was distributed between the muscles themselves along the entire length of the organ becoming denser as it neared the esophageal-cardic junction. A significant difference was observed between the area and the diameter of the neurons present in the upper and middle thirds of the esophagus in both infected and control animals. Microscopic analysis showed the presence of amastigote nests in smooth and striated muscle cells and infrequently in Schwann cells. Whether related or not to the presence of parasites, myositis at times presented intense and extensive muscular cell destruction and when related to periganglionitis and ganglionitis, sometimes resulted in neuron destruction. No significant alterations in neurons were observed which is in line with the accumulated experience reported on the dog model which until now has not demonstrated clinical or morphologic development of the digestive form of the Chagas'disease.

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BI-19
^{60}CO IONIZING RADIATION EFFECTS IN THE PHYSIOLOGY AND CELLULAR INVASION OF *TOXOPLASMA GONDII* TACHYZOITES

Hiramoto RM, Galisteo Jr. AL*, Cardoso RPA*, Andrade Jr. HF*
Serviço de Radiobiologia IPEN/CNEN *Laboratório de Protozoologia, IMTSP, Av. Dr.E.C.Aguiar 470, 05403-000 São Paulo, SP, Brasil (E-mail: hfandrad@usp.br)

Toxoplasma gondii is an intracellular obligatory protozoon, with a complex life cycle, with felids as definitive host, and warm-blooded mammalian and birds as intermediate host. The infection is transmitted through the consumption of cysts on infected meat by or oocysts of cat's feces in contaminated food or water. Widespread among humans and generally asymptomatic, this agent could induce devastating disease in fetus, AIDS patients and recipients of organ transplants. The ionizing radiation was used to sterilized meat and immunized animals against *T. gondii*, with encouraging results, and here we study the physiological alterations and cellular invasiveness of 200 Gy irradiated tachyzoites. After the irradiation, we study the physiology of the agent by precursor incorporation in short term cultures, aside to metabolic assays using MTT(3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; Tyazolyl blue) as revealing agent of oxidative metabolism, and morphological study of its invasiveness on LLC-MK2 and human fibroblasts. The irradiated parasites presented the same oxidative response, protein synthesis(^{3}H-prolina) and nucleic acid(^{3}H-hypoxantina) incorporation as their non-irradiated counterparts. These irradiated parasites had the same capability of cell invasion and parasitic vacuole formation on both cells tested, as compared to non-irradiated agents. No further growth of the parasite was observed in those cultures, with some clearly degeneration of the irradiated agent after invasion, suggesting that the irradiation induced a mitotic death by double strand breaks in DNA. Those data reinforces the fact that, at those level of radiation, the only effect was the reproductive blockade of the parasite, with preservation of most of their metabolic and physiologic activity, an desirable effect in vaccine development.

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BI-20
A NEW MODEL FOR THE STUDY OF THE DAMAGE AND RECOVERY OF SYMPATHETIC NERVE TERMINALS INDUCED BY *TRYPANOSOMA CRUZI* AND OTHER PRO-INFLAMMATORY AGENTS USING THE RAT DUCTUS DEFERENS

Arantes RME, Galvão LMC, Santos MMF, Machado CRS
Departamentos de Morfologia e de Parasitologia, Instituto de Ciências Biológicas, UFMG, Belo Horizonte, MG, Brasil

We have developed and standardized an experimental model for studying inflammatory processes induced directly in the smooth muscle layer of the rat ductus deferens aiming at assessing their effects on the sympathetic innervation. Besides an easy surgical accessibility, the rat vas deferens has a rich post-ganglionic sympathetic innervation that remains unaffected by *Trypanosoma cruzi* infection (Y strain) via the peritoneal cavity. Adult Holtzman rats (180 animals) comprised the following groups according to the material injected directly in the ductal wall: alive trypomastigotes (Y strain), dead trypomastigotes, supernatant of *T. cruzi* culture, carrageenan and methylated albumin. In this later case sensitized and non-sensitized animals were used. Controls were provided by sham-operation, inoculation of sterile saline or culture medium. Ductus deferens fragments were withdrawn at 48 hours, 4, 8,10, 12, 14, 20 days and processed for histological study and for the histochemical demonstration of catecholamines by a glyoxylic acid technique. In groups inoculated with *T. cruzi* forms or culture supernatant the hearts were also histologically studied. Regarding the experiments with alive trypomastigotes, the kinetic of the inflammation was faster in the ductal smooth muscle than in heart. In the ductus, the most intense mononuclear exudate and parasitism occurred at days 8-12. Afterwards there was a fast recovery, the normal histological pattern being reached at day 20. By this time the heart still exhibited intense inflammatory infiltrate. The glyoxylic-acid-induced fluorescence showed intense and diffuse reduction of nerve terminals in the ductus at days 8 to 14. At day 20, the recovery of the normal pattern of innervation was still incomplete, in spite of the normal histology. All other procedures induced focal rarefaction of nerve terminals restricted to tissue damage, with faster recovery. We conclude that the nerve lesions occurred in parallel with the inflammatory process and that the regeneration of the smooth muscle was faster than that of the nerve terminals. Our model is suitable for *in vivo* interventions aiming at inhibiting hypothetical mechanisms supposed to be implicated in the pathology of Chagas' disease. This is our next objective.

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BI-21
A NEW TYPANOSOMATID BELONGING TO THE GENUS *CRITHIDIA* ISOLATED FROM *ZELUS LEUCOGRAMMUS* (HEMIPTERA: REDUVIIDAE)

Sá-Xavier C, Santos SM, Sousa MA
Coleção de Tripanosomatídeos, Laboratório de Transmissores de Hematozoários, Departamento de. Entomologia, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil

In this work we studied a trypanosomatid isolated by MP Deane and N Thomaz in 1991 from a predacious hemipteran (*Zelus leucogrammus*) captured in Belo Horizonte (MG, Brasil). After cloning in a 96-microwell plate (as described by AC Camargo et al. in 1996), a randomly chosen clone (code CT-IOC 193 in the Trypanosomatid Collection of the Oswaldo Cruz Institute) was characterized with regard to the growth and differentiation pattern in liver infusion-tryptose (LIT) medium at 27.3^{o}C. The cultures were studied from 48 to 144 hr, samples being collected at 24 hr intervals, either to determine the number of cells/ml and to prepare Giemsa-stained smears for morphological and morphometric studies. We evidenced that this trypanosomatid grew very easily, reaching ~0.8x10^{8}cells/ml within 72-96 hr. It was typically choanomastigote in shape, presenting the majority of the cells (60.3%, average from 48-144 hr data) with paranuclear kinetoplast; no cells with postnuclear kinetoplasts were found (metachoanomastigotes). In 1.6% of the population (mean percent from 48-144 hr), the flagellum was partially inside the cell body, resembling a question mark. In the majority of the dividing cells (54%) both kinetoplasts were placed at the side of the nuclei and, in 36%, at least one of the dividing kinetoplasts was paranuclear. Only 10% of cells in division had the kinetoplasts before the nuclei. The main evolutive stage of this organism (cells with paranuclear kinetoplast) presented 7.4 ±0.9 mm in length, 2.7 ±0.4 mm in width, flagellum of 6.9 ±1.7 mm, and nuclear index of 1.1±0.2 (mean and standard deviation). Several data from the morphobiological characterization of this clone were numerically coded for computational processing and compared with those from other choanomastigote-shaped species (10 *Crithidia* spp., *Proteomonas inconstans*, as well as the so-called *"Crithidia" deanei* which belongs to a new genus named *Angomonas* by Sousa & Côrte-Real, 1991). The phenetic analysis using the Jaccard or Simple Matching coefficients and the UPGMA clustering algorithm confirmed the affinities of this organism with the *Crithidia* species, mainly with *Crithidia hutneri*. The molecular analysis using contour-clamped homogeneous electric field (CHEF) electrophoresis showed that this organism has a unique DNA chromosomal banding pattern, distinguishing it from all *Crithidia* species analyzed herein, including *C. hutneri*. All these findings led us to consider that this trypanosomatid can be a new *Crithidia* species.

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BI-22
A ROLE FOR PHOSPHOINOSITIDE 3-KINASE IN THE INTERACTION OF *TRYPANOSOMA CRUZI* WITH MACROPHAGES

Todorov AG, Einicker-Lamas M, Silva LCF Guilherme AL*, De Castro SL**, Oliveira, MM
Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, 21949-900 Rio de Janeiro, RJ, Brasil *Departamento de Bioquímica Médica, ICB, Universidade Federal do Rio de Janeiro, Brasil **Departamento de Ultraestrutura e Biologia Celular, Instituto Oswaldo Cruz, Rio de Janeiro, Brasil

It is already well established that the *Trypanosoma cruzi* invasion in non-phagocytic cells occurs activating calcium and TGF-(signaling pathways in the host cells. As for the parasite interaction with phagocytic cells the above mentioned pathways were not observed, and its mechanism is not completely understood. We now propose that that one possible modulation for the interaction *T. cruzi* - macrophage implicates in the activation of the phosphoinositide-3 kinase (PI 3-K), recently recognized as an important metabolic pathway modulating many cellular processes. Pre-treatment of macrophages with the PI 3-K inhibitor, Wortmannin (1-20nM), blocked *T. cruzi* infection in a dose-dependent manner, reaching the maximum of 96% inhibition. To confirm the PI 3-K role during the invasion process, we determined the products of this enzime by TLC and HPLC techniques. Our results showed activation of the enzime in the infected macrophages as the amount of phosphatidylinositol-3,4,5-trisphosphate increased 16 fold, compared with the control value, followed by phosphatidylinositol 3,4-bisphosphate (9 fold) and phosphatidylinositol 3-phosphate (7Ýfold). These data suggest that PI 3-K, probably types I and II, are activated in the host cell during the *T. cruzi* invasion process.

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BI-23
ANALYSIS OF VAR SEQUENCES FROM NATURAL PARASITE POPULATIONS OF *PLASMODIUM FALCIPARUM* IN THE BRAZILIAN AMAZON

Kirchgatter K/*, Del Portillo HA
Departamento de Parasitologia, Instituto de Ciências Biomédicas II, Universidade de São Paulo *Laboratório de Malária, Divisão de Programas Especiais, Superintendência de Controle de Endemias, São Paulo, SP, Brasil

The *Plasmodium falciparum* var gene family encodes a predicted 200-350 kDa protein with two different exons: a highly polymorphic N-terminus displaying five Duffy binding like (DBL) domains followed by a transmembrane region and a conserved C-terminus. Expression of var genes on the surface of infected erythrocytes is directly associated with the phenomena of antigenic variation and cytadherence to postcapillary venules. Since both phenomena are responsible for most of the patophysiology of *falciparum* malaria, analysis of the var gene repertoire in natural parasite populations and how their expresion is controlled, are presently two of the most active research areas in malaria. PCR-sequence analisis of the N-terminal most DBL domain in the laboratory isolate 3D7 and field isolates from Kenya, Vietnam and Vanuatu, revealed that highly similar or divergent sequences can be detected in single isolates as well as in isolates from different regions. In the present study, the same set of primers and PCR amplification conditions were used to analyse the same N-terminal most DBL domain in 44 field isolates of *P. falciparum* from two different geographical regions of the Brazilian Amazon, Mato Grosso (MT) and Rondônia (RO). Genomic DNA pertaining to 20 patients from RO and 24 from MT, was extracted, PCR amplified, cloned and sequences from two individual clones from each patient, were generated. Clustal comparison of the nucleotide and deduced amino acid sequences demonstrated that 25% and 45.8% of the sequences generated from two independent clones of any one particular patient from RO and MT, respectively, were identical. Moreover, 22 different type sequences were found among the isolates from MT as opossed to only 16 detected in RO. This is likely due to the fact that the samples collected in RO were obtained in a shorter period of time (one month) as those of MT (over a year). Interestingly, identical sequences were observed between isolates from MT and RO but not between the Brazilian isolates and those from Asia and Africa. Similar analysis is now being performed with isolates from other regions to generate a DBL-1 sequence database from the Brazilian Amazon. This database will in turn form the basis of future studies on var gene expression.

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BI-24
ANIMAL MODEL OF CHAGAS' DISEASE: NATURAL *TRYPANOSOMA CRUZI* INFECTION OF BABOONS REARED IN CAPTIVITY

Ramos L, Hubbard GB, Argañaraz ER, Ford AL, VandeBerg JL, Teixeira ARL
Laboratory for Multidisciplinary Research in Chagas' Disease, University of Brasília, and Southwest Foundation for Biomedical Research, San Antonio, TX, USA

Trypanosoma cruzi infection causes Chagas' disease, a chronic ailment with protean manifestations, in humans and suitable hosts. A major limitation in unraveling its pathogenesis and thus, allowing development of methods for treatment and immunoprophylaxis, has been lack of an animal host presenting most, if not all, of these clinical features of Chagas' disease in humans.

We have preliminary results showing that *T. cruzi* infection is endemic in the baboon colony at the Southwest Foundation for Biomedical Research. Based on the Abbott diagnostic kit, the percent of seropositive baboons ranged from 9.4 to 22.5%, depending on age. Seroconversion generally occurred in juveniles, and seroconverted animals have remained positive throughout life. The serologic data have been confirmed by PCR from DNA samples obtained at necropsy or from buffy coat cells, with primers of *T. cruzi* kDNA or nuclear DNA. The amplification products obtained were shown to be specific by Southern hybridizations with the parasite DNA probes, and these results correlate with the serologic and pathologic data.

The histopathologic study of two juvenile baboons that died of acute Chagas' disease showed striking myocarditis with parasitic nests in the myofibers, thus showing tropism towards the heart cells. Mononuclear cell infiltrates, which were observed in several tissues and organs, were more conspicuous in striated and smooth muscles. The inflammatory cells invaded the parasympathetic and sympathetic ganglia and nerve fibers. In one acute case there were granulomatous lesions in the white and gray matter of the brain. Diffuse lymphoid tissue hyperplasia were also present.

Nine baboons were observed at necropsy with lesions consistent with chronic Chagas' disease as compared to lesions in humans; infection with *T. cruzi* was established by serologic, PCR and histopathologic study. In most of these cases, the heart appeared dilated and flacid. The microscopic study revealed heart cell lysis associated with mononuclear cell infiltrates, in the absence of the parasite. The immunohistochemistry study that revealed *T. cruzi* amastigote forms encysted in heart fibers did not show the parasite in sections of the hearts. Of interest, megaesophagus and megacolon were seen in two baboons with the chronic form of the disease. The microscopic study showed lymphocytic infiltrates of parasympathetic ganglia and neuronolysis. All of these findings have been described in human Chagas' disease.

BI-25
ASSESSMENT OF THE INFECTION RATE BY *LEISHMANIA CHAGASI* AMONG DOGS IN BARRA DE GUARATIBA, RIO DE JANEIRO, RJ, BRAZIL

Cabrera MA, Aguiar GM, Marzochi MCA*, Camacho LAB*, Xavier SCC, Paula AA, Queiroz JL, Gambardella S, Medeiros W, Jansen AM
Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro *Escola Nacional de Saúde Pública, Fiocruz, Rua Leopoldo Bulhões 1480, 21041-210 Rio de Janeiro, RJ, Brasil

Barra de Guaratiba is a coastal area of Rio de Janeiro city where visceral leishmaniasis is endemic. Despite the traditional control measures adopted by Ministry of Health active transmission among dogs can still be observed in this region. During 1996/98, 365 dogs were tested by an immunofluorescence assay and results showed a high serum prevalence (30.5%). Ten human cases were diagnosed in the area during this time. Infected opossums have been found in the proximity of households. Sand flies have been capturate in peridomiciliary and inside the forest with CDC light trap (April 1997 to March 1998). Among the sand flies capturated *Lutzomyia intermedia* (39.7%) and *Lu .migonei* (30.2%) were the most representative species. This fact confirm the high adaptability of those species to the environment changed by man. So, we decided to evaluate the infection rate by *Leishmania chagasi* according to area height, distance of houses from the forest, and restraining of the dog to the house and yard. The role of those covariates was also assessed in a multivariate model. The transmission probability near the forest (less than 50 m) was 12 fold higher (95% C.I. 4.6-13.8) than far from it (more than 100 m). Height showed a poor and nonsignificant association with rate of infection. To evaluate the transmission risk of distance controlled by the height, we utilized logistic regression, where the dependent variable was the serologic data and the independent variables was distance and height. In the subset of restrained dogs, the transmission risk in the area near the forest (<100 m) was 6.4 (95% C.I.2.37 -17.49) fold higher than far from it, and the probability of infection in high areas (> 100 m) is 8.4 (95% C.I. 3.03-23.50) fold higher than at the sea level. Our data suggest that wild animals could be a source of *L. chagasi* infection among dogs in this area and that *Lu. longipalpis* showed a certain degree of eclecticism as well to host as local sylvatic or household.

BI-26
AUTOCHTHONOUS HUMAN CUTANEOUS LEISHMANIASIS IN THE STATE OF SANTA CATARINA ARE CAUSED BY TWO DIFFERENT *LEISHMANIA* SPECIES

Grisard EC/*, Steindel M/**, Campbell DA*
Departamento de Microbiologia e Parasitologia, Universidade Federal de Santa Catarina, Florianópolis, SC, Brasil (E-mail: grisard@ccb.ufsc.br) *Department of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, CA, USA **Núcleo de Pesquisas em Dermatologia, Serviço de Dermatologia, HU, UFSC

Four *Leishmania* sp. strains (MHOM/BR/89/JSC89-H1, MHOM/BR/89/JSC89-H2, MHOM/BR/LSC/96-H3 and MHOM/BR/LSC/97-H4) were isolated from authocthonous human cases from non-endemic areas of the State of Santa Catarina, southern Brazil. Strains H-1, H-2 and H-3 were isolated from patients from the municipalities of Coronel Freitas, Quilombo and Chapecó in the western region of the state. Strain H-4 was isolated from a single case in the Piçarras municipality in the northeastern region of the state. All patients had a single lesion and revealed positive in skin biopsies and Montenegro test, being sucessfuly treated. In this work we have characterized these *Leishmania* sp. strains using the nuclear, multicopy mini-exon gene as a genetic marker. Parasites were harvested in the exponencial growth phase in LIT medium, washed twice in cold PBS pH 7.2 then mixed with 500ml of lysis buffer (10mM Tris-HCl pH 7.4, 10mM NaCl, 25mM EDTA, 1% SDS). DNA was obtained by phenol/chloroform extraction following ethanol precipitation. PCR amplification of the mini-exon gene was performed as previously decribed (Murthy et al. 1992 *Mol Cell Probes 6*: 237-243). Amplification products of strains H-1 and H-2 showed the same migration pattern of *L. amazonensis* control DNA, meanwhile samples H-3 and H-4 revealed amplification products that co-migrated with *L. braziliensis* control DNA. In order to confirm these results, hybridization assays were performed with group-specific probes described by Ramos et al. (1996 *Exp Parasitol 82*: 242-250). Strains H-1 and H-2 only hybridized with probe S-1595 specific to the New World dermotropic group (*L. amazonensis*) and strains H-3 and H-4 hybridized only with probe S-1593 specific to the *Viannia* Subgenus group (*L. braziliensis*). None of the strains were recognized by S-1698 probe specific to the Viscerotropic group. These results are in accordance with previous immunological characterization by monoclonal antibodies.

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BI-27
BINDING SITES DURING INTERACTION OF *TOXOPLASMA GONDII* WITH HUMAN ENDOTHELIAL CELLS

Stumbo AC, Barbosa HS*, Carvalho L
Laboratório de Cultura de Células, Depto de Histologia e Embriologia, IB, Universidade do Estado do Rio de Janeiro (E-mail: laiscar@uerj.br) *Laboratório de Ultra-estrutura Celular, Instituto Oswaldo Cruz, Rio de Janeiro, RJ, Brasil

Toxoplasma gondii, an obligate intracellular parasite, is able to infect a wide variety of host cells where it resides in a parasitophorous vacuole. Previous studies have suggested that events occurring during the initial formation of the parasitophorous vacuole (PV) are vital to the successful intracellular survival of *T. gondii*. We have shown that anionic sites located on the plasma membrane of the macrophage are interiorized together with the portion of the plasma membrane involved in the formation of the vacuole (Carvalho & De Souza 1990 *Eurp J Cell Biol 51*: 211-219).

In order to analyze which components of the human endothelial cell plasma membrane are internalized and become part of the membrane of the PV, we used cationized ferritin (CF) as a marker of cell surface anionic sites and ulex europeaus I lectin-complexed with colloidal gold (UEA I -Au) as a marker for human umbilical vein endothelial cells (HUVEC). Endothelial cells obtained from human umbilical cords were treated with FC or UEA-Au during 30 minutes at 4ºC and then incubated for 10 to 60 min at 37ºC with tachyzoites of *T. gondii*, fixed and routinely processed for transmission electron microscopy .After endothelial cell-parasite interaction, neither the anionic sites nor the UEA I lectin binding sites were seen on the parasitophorous vacuole membrane .However,both cationized ferritin and UEA-colloidal gold particles were observed in small cytoplasmic vesicles.

Supported by Uerj and Fiocruz.

BI-28
BIOCHEMICAL CHARACTERIZATION OF A *HERPETOMONAS* ISOLATED FROM THE FLOWER OF THE SQUASH *CUCURBITA MOSCHATA*

Fiorini JE, Teofilo VM, Nascimento LC, Faria-e-Silva PM*/**, Soares MJ***, Takata CSA****, Teixeira MMG****, Campaner M****, De Souza W**/*****
Departamento de Ciências Biológicas, Unifenas, Alfenas, MG *Departamento de Ciências Biológicas, EFOA, Alfenas, MG **Laboratório de Ultraestrutura Celular Hertha Meyer, IBCCF, UFRJ, Rio de Janeiro, RJ ***Departamento de Ultraestrutura e Biologia Celular, Instituto Oswaldo Cruz, Rio de Janeiro, RJ ****Departamento. de Parasitologia, USP, São Paulo, SP *****Laboratório de Biologia Celular e Tecidual, CBB, UENF, Campos dos Goytacazes, RJ, Brasil

Here we report on some morphological and molecular characteristics of a trypanosomatid previously isolated from the flower (petals and petioles) of *Cucurbita moschata* (Fiorini et al. 1995 *Mem Inst Oswaldo Cruz 90* Suppl. I: 258). Since the flagellate was isolated from plant parts it could have been placed in the genus *Phytomonas* according to the host origin criterion of Donovan (Donovan 1909 *Lancet 177*: 1195-6119). However, our findings argue against this placement. Firstly, the flagellate's DNA failed to hybridize with the SL3´oligonucleotide, which is complementary to mini-exon sequences that are considered diagnostic of the genus *Phytomonas* (Teixeira et al. 1996 *Exp Parasitol 84*: 311-319). Also, it was not recognized by specific anti-*Phytomonas* monoclonal antibodies (Teixeira & Camargo 1989 *J Protozool 36*: 262-264). Moreover, upon restriction enzyme analysis of the SSU of the ribosomal gene, a *Pvu* II site could be located at 360 bp downstream of the 5´end. This site is present in *Crithidia*, *Leptomonas* and *Herpetomonas* but never in *Phytomonas* (Camargo et al. 1992 *J Parasitol 78*: 40-48). For these reasons the squash flagellate can not be considered a *Phytomonas* sp.and since there are not choanomastigotes in their cultures it is neither a *Crithidia*. Thus it could either be a *Leptomonas* or a *Herpetomonas*. The flower flagellate was found to lack arginase but this does not permit to separate these 2 genera. However, the squash flagellate displays a few opisthomastigotes in culture, which favors its placement in *Herpetomonas*. The flagellate has a morphological peculiarity, which is unique among the known species of *Herpetomonas*. This is the presence, as detected by light and scanning electron microscopy, of a thin, rod-shaped, lateral expansion of the plasma membrane and subpellicular microtubules, which originates near the opening of the flagellar pocket at the anterior end of some flagellates.

Supported by CNPq, Pronex, Finep, Fapemig, Capes and Unifenas.

BI-29
BLASTOCRITHIDIA AND *CRITHIDIA* INTERACTION WITH *LUTZOMYIA LONGIPALPIS* AND *AEDES ALBOPICTUS* MIDGUTS

Lima PFN, Motta MCM*, Oliveira SMP**, Brazil RP***, Lima CD**** , Saraiva EMB
Laboratório Imun. das Leishmanioses, Departamento de Imununologia, Instituto de Microbiologia, UFRJ *Laboratório de Ultra-estrutura Celular Hertha Meyer, Instituto de Biofísica Carlos Chagas Filho,UFRJ **Departamento de Biologia, Instituto Oswaldo Cruz ***Laboratório de Leishmanioses, Centro de Pesquisa René Rachou, Fiocruz, MG ****Laboratório de Transmissores de Hematozoários, Departamento de Entomologia, Instituto Oswaldo Cruz, Rio de Janeiro, RJ, Brasil

Some monogenetic trypanosomatids harbor an endosymbiotic bacteria in their citoplasm. Its presence has been associated to nutritional advantages and morfological alterations in the host cell, as well as modification in the carbohydrate expression and surface charge at their hosts surface. Interaction studies between monogenetic trypanosomatids and hematophous insect guts are becoming important due to reported cases of AIDS patients, who contracted a disease caused by these protozoa, that is similar to diffuse leishmaniasis cutaneous infection. Recently it was demonstrated that endosymbiont harboring species interact more with insects cell lines when compared to aposymbiotic strains (Lima et al. 1997 *Mem Inst Oswaldo Cruz 92*: 71). In this work, we investigated the influence of symbionts in the interaction of the host protozoa to hematophagous Diptera guts. Groups of 7 to 10 isolated *Lutzomyia longipalpis* and *Aedes albopictus* adult females guts were opened longitudinally and allowed to interact with 10^6 endosymbiont bearing species of *Crithidia deanei* and *Blastocrithidia culicis* or *C. deanei* aposymbiotic strain. After 1 h at room temperature, guts were washed, homogenized and free pritozoa counted in a hemocytometer. The binding assays with *L. longipalpis* midguts showed an avarage number of 1,5 x 10^4 *B. culicis* harboring endosymbiot/gut and 4,4 x 10^3 *C. deanei*/gut, while *C. deanei* aposymbiotic strain did not interact. In the binding with *Aedes albopictus* midguts, it was found a mean number of 4,7 x 10^3 *B. culicis*/gut, 3,9 x 10^3 *C. deanei*/gut for, and only 3,2 x 10^2 *C. deanei* aposymbiotic strain /gut. Electron microscopy analysis of the interaction process revealed that the protozoa binds to midgut epithellium microvilli, mainly by insertion of its flagellum. Sometimes, at the point of adhesion, the flagellum is enlarged, and may also involves areas of protozoa cell body. Flagellum was also seen inserted between epithelial cells tight juctions, but no juctions between protozoa and gut membranes were observed. Despite not presenting.paraxial rod, these endosymbiont harboring trypanosomatids interacted with insect midgut epithelium as well as other trypanosomatids that have this structure.

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BI-30
CARDIOVASCULAR AUTONOMIC MODULATION IN THE EXPERIMENTAL CHAGAS' DISEASE IN SPONTANEOUSLY HYPERTENSIVE RATS

Machado CRA , Dias da Silva VJ, Chapadeiro E, Ramirez LE, Salgado HC*
Departamento de Ciências Biológicas, FMTM, Uberaba, MG *Departamento de Fisiologia, FMRP/USP, Ribeirão Preto, SP, Brasil

Some papers of the literature have demonstrated a low incidence of systemic arterial hypertension (SAH) in patients with Chagas' disease. In present study, we evaluated the arterial pressure (AP) and heart rate (HR) in young spontaneously hypertensive rats (SHR), which were inoculated with *Trypanosoma cruzi*. Eight-old weeks male SHRs (135-145 g) were submitted to AP and HR measurements, once a week, by means of the tail cuff method (Digital pressure meter, model LE-5000, Letica Scientific Instruments). After 4 weeks, the animals were inoculated with 2 x 10^6 blood forms of the Y strain of *T. cruzi* (CHG-SHR, n=6) or vehicle without parasites (CON-SHR, n=5). Following, AP and HR were measured twice a week during 5 months after inoculation (acute and chronic phases of the Chagas' disease). At the end of this period, the animals were canulated and direct AP was recorded. Cardiovascular autonomic modulation was investigated by means of the spectral analysis of AP and HR variabilities. At the end of the period, the animals present similar body weights. The systolic arterial pressure (SAP) decreases after inoculation in CHG-SHRs (147±3* versus 252±4mmHg in CON-SHR group in 3rd e 4th weeks after inoculation, *p<0,001). These values were mantained until the 9th week. Then, the SAP slowly rises until 12th week (178±3 versus 235±4mmHg , p<0,01). In the 5th month of inoculation the CHG-SHRs have their SAP lower than CON-SHRs. The HR was the same in two groups until 2nd week post-inoculation. Then, the HR increases in CHG-SHR group, reaching the higher values in the 3rd and 4th weeks (454±6 versus 393±4bpm in CON-SHR, p<0,01). Following, HR decreases until the 12th week (393±3 versus 379±8 in CON-SHR). The mid (MF) and high frequency (HF) bands of the spectrum of the SAP variability are reduced in the chronic CHG-SHR, which can indicate an impaired vasomotor sympathetic modulation. The MF and HF bands of spectrum of HR variability were normal. Young CHG-SHRs present a reduction of the systemic AP and tachycardic response during acute (4 weeks) and sub-acute (12 weeks) phases of the chagasic infection. The lower AP mantained in chronic phasis. A possible vascular sympathetic dysfunction evaluated by means the spectral analysis of SAP could be involved in this phenomenon.

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BI-31
CARDIOVASCULAR AUTONOMIC REFLEX CONTROL IN HAMSTERS INFECTED WITH *TRYPANOSOMA CRUZI*

Guimarães AM, Della Libera G, Chapadeiro E, Lages-Silva E, Ramirez LE, Dias da Silva VJ
Departamento de Ciências Biológicas, FMTM, Uberaba, MG, Brasil

The autonomic dysfunction is an important clinical manisfetation of the Chagas' disease. It can be associated with the pathogenesis of cardiac arrithmias and sudden death. In the present study, the autonomic control of the heart rate (HR) mediated by baroreceptor and Bezold-Jarisch reflexes was investigated. Fifteen male golden hamster (100-150 g) were divided in two groups: control group (CON, n=8) and chagasic group (CHG, inoculated with 2 x 10^3 blood forms of the Vicentina strain of *Trypanosoma cruzi*, n=7). The infection was confirmed by positive parasitemia, using micro-hematocrite method. After 20 days, under anesthesia (sodium pentobarbital, 40mg/Kg, i.p.), cannulas and ECG electrodes were implanted in the animals to record arterial pressure (AP) and ECG, respectively. After 24 hours, direct mean AP (MAP) and ECG were recorded in conscious animals, using a acquisition data system (Aqdados, Lynx T.E., São Paulo) on a personal computer. The baroreceptor reflex was tested by the injection of sodiun nitroprusside (16-32mg/Kg, i.v.) and phenylephrine (8-16mg/Kg, i.v.). Tachycardic (TRS) and bradycardic (BRS) response sensitivities were calculated using the index DHR/DMAP. The Bezold-Jarisch reflex were tested by the injection of serotonin (2-32mg/Kg, i.v.). Changes in HR and MAP were plotted in dose-response curves. In the end, the animals were sacrified and histo pathological studies were performed. Mean values (±SE) of HR (bpm) and MAP (mmHg) in CHG and CON groups were, respectively: HR: .520±32, 483±49 and 107±9 and 121±6. The BRS (bpm/mmHg) was significantly reduced in CHG group (- 5,43±1,3* versus -11,28±2,6 in CON group, * $p<0,05$). The TRS was reduced, but not significantly ($p=0,071$). The Bezold-Jarisch reflex did not differ between groups. These data indicate that the parasympathetic component of baroreceptor reflex is impaired, which suggest a lesion in the CNS, vagus nerve or intra-cardiac ganglionic plexus, during acute phasis of Chagas'disease.

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BI-32
CELLULAR DIFFERENTIATION AND GROWTH IN AXENIC CULTURE OF TRYPANOSOMATIDS ALLOCATED IN THE GENUS *LEPTOMONAS* (PROTOZOA: KINETOPLASTIDA)

Sousa MA, Santos SM, Sá-Xavier C, Branco DCB
Coleção de Tripanosomatídeos, Departamento de Entomologia, Instituto Oswaldo Cruz, Rio de Janeiro, RJ, Brasil

The genus *Leptomonas* includes monoxeneous parasites, mainly from insects, typically presenting the promastigote stage and, sometimes, flagellar "cysts". This genus has been considered a heterogeneous group by biochemical and molecular markers, possibly encompassing organisms from other taxa. In the present work we followed the cellular differentiation and growth in the Yaeger's LIT medium (27.3ºC) of several isolates allocated in this genus: *L. collosoma*, *L. seymouri*, *L. pyrrhocoris*, *L. costoris*, *L. lactosovorans*, *L. pulexsimulantis*, *L. mirabilis*, *Leptomonas* sp. isolated from *Oncopeltus fasciatus* (Romeiro *et al.* 1998), as well as the so-called *"Leptomonas" samueli*, considered a *Herpetomonas* by Camargo et al. in 1992. The cultures were started with $5x10^5$ cells/ml seeded in 4 ml-volumes of medium distributed in 16x150mm screwcap tubes and studied at 24 hr intervals, from 48-144 hr. The percentage of each evolutive stage was determined by examining at least 500 randomly chosen cells in Giemsa-stained smears from each time, excepting for *L. mirabilis* (100-200 cells/day). All species presented, besides the typical and predominant promastigotes, the paramastigote stage, this occurring at rates ranging from 0.6 (*"L." samueli*) to 38.2% (*L. mirabilis*). Cells with postnuclear kinetoplasts (opisthomastigotes) were also found in some species (mean): *L. lactosovorans* (0.2%), *L. pulexsimulantis* (0.2%) and *L. mirabilis* (1.2%), as well as in *"Leptomonas" samueli* (0.1%). Aflagellated cells were either absent or found in some species at rates £ 0.1% (*L. seymouri*, *L pyrrhocoris*, *L. collosoma*, *"Leptomonas" samueli*). In the other species, aflagellated cells having the kinetoplast either before or beside the nucleus were found at rates averaging from 0.3-2.8%. *"Leptomonas" samueli* and *L. lactosovorans* also presented aflagellated cells with postnuclear kinetoplast. Some species grew very easily (*L. seymouri*, *L. pyrrhocoris*, *L. collosoma*), others grew well, but at a lower extent (*"Leptomonas" samueli*, *L. lactosovorans*, *Leptomonas* sp. from *Oncopeltus fasciatus)*, while *L. costoris*, *L. pulexsimulantis* and *L. mirabilis* displayed very poor growth. Our results also suggest that *"L". samueli* can be a *Herpetomona*s, and that the so-called *L. lactosovorans* can belong to this genus as well, although this needs confirmation. Otherwise, despite the presence of opisthomastigotes in cultures of *L. pulexsimulantis* and *L. mirabili*s, their poor growth in LIT medium distinguishes them from the typical *Herpetomona*; this led us to consider them rather *incertae sedis* species, and arises the question that the genera presently accepted are insufficient to encompass the actual diversity of the trypanosomatids. Our results also evidence that the species here examined morphologically differ from well-characterized *Phytomonas* spp., although two of them present high rates of twisted cells (*L. pulexsimulantis* and *L. mirabilis*).

BI-33
CHARACTERIZATION OF A *HERPETOMONAS* SP. (PROTOZOA: KINETOPLASTIDA) ISOLATED FROM *NECTOMYS SQUAMIPES* (MAMMALIA: RODENTIA)

Sousa MA, Madeira MF*, Cupolillo E**, Santos SM, Sá-Xavier C, Soares MJ***, Brazil RP****
Coleção de Tripanosomatídeos, Departamento de Entomologia **Departamento de Imunologia ***Departamento de Ultraestrutura e Biologia Celular, Instituto Oswaldo Cruz, Rio de Janeiro, RJ *Departamento de Ciências Biológicas, Escola Nacional de Saúde Pública; Rio de Janeiro, RJ ****Centro de Pesquisas René Rachou, Fiocruz, Belo Horizonte, MG, Brasil

A trypanosomatid isolated from a hind-foot lesion of a rodent captured in the locality of Catimbau Grande, Rio Bonito (RJ, Brazil) was deposited in the Trypanosomatid Collection of the Oswaldo Cruz Institute, receiving the code number CT-IOC 219. It presented promastigotes and had been identified as *Leishmania* sp.; however, it was unable to infect hamster, otherwise easily growing in axenic cultures. In the present work, the original strain and a clone obtained from it (CT-IOC 220) were characterized by different approaches. Then, their growth at 27.3ºC in the Yaeger's LIT medium was followed from 48 to 144 hr, at 24 hr intervals, samples of each culture being collected for morphological studies. We evidenced that both isolates grew easily, reaching > $5x10^7$ cells/ml within 48-72 hr, and presented pro-, para- and opisthomastigotes in Giemsa-stained smears, as a typical *Herpetomonas* species. Promastigotes from the original isolate and its clone presented similar sizes, having respectively 10.5 ±1.8 and 11.1 ±2.0 mm in length, 2.4 ±0.4 and 2.3 ±0.4 mm in width, free flagellum of 8.8 ±2.5 and 9.0 ±2.5 mm, and 1.1±0.3 and 1.0 ±0.4 of nuclear index. Peculiar pairs of cells suggesting a sexual process and similar to those reported in other *Herpetomonas* spp. were also found. Murine macrophages cultured *in vitro* at 37ºC quickly internalized these parasites, destroying the majority of them within 24 hr; at that time no dividing amastigotes were found and several parasites were alive in the supernatant medium. The ultrastructural analysis of the promastigotes displayed the typical organization of the trypanosomatids. The original strain and its clone presented identical chromosomal DNA banding pattern when analyzed by contour-clamped homogeneous electric field electrophoresis, this pattern being greatly similar to that presented by a group of well-characterized *Herpetomonas* species (*H. muscarum muscarum*, *H. megaseliae*, three *Herpetomonas* spp. from plants and one from a phytophagous hemipteran), a noticiable feature shared by them being the lack of a chromosomal band around 2,200 kb. The similarity of these isolates with the above-mentioned *Herpetomonas* species was also confirmed by isozyme analysis. A possible infection of a mammal by a *Herpetomonas* was first noticed by McGhee and Cosgrove in 1980, the parasite having been isolated from the liver of a woman. More recently, other putative monoxeneous trypanosomatids were isolated from HIV-infected patients. All these findings reinforce the discussion whether some *Herpetomonas* species can include hosts other than insects in their life-cycle or whether, under special conditions, they can determine accidental and/or opportunistic infections.

BI-34
CHARACTERIZATION OF A REPETITIVE DNA SEQUENCE OF *TRYPANOSOMA RANGELI* AND APPLICATION IN PARASITOLOGICAL DIAGNOSIS

Vargas N, Souto RP, Zingales B
Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, Caixa Postal 26.077, 05599-970 São Paulo, SP, Brasil

Total DNA from *Trypanosoma rangeli* (San Agustin strain) was digested with *Taq*I. After electrophoresis in agarose gels, besides kinetoplast DNA, three bands (0.8Kb, 1Kb and 1.2Kb) showed intense ethidium bromide staining which suggested a high representation in the parasite genome. The 1.2Kb band was extracted from the gel and ligated to pBluescript previously linearized with *Acc*I. DNA from several recombinants was applied to Nylon membranes and hybridized to total labelled *T. rangeli* DNA (3xSSC, 2h at 65ºC). A positive clone named II-14 (1.230 Kb) was sequenced. It contained a region of 370 bp with 50% identity to *T. cruzi* trans-sialidase (gene TCTS154) and a 542 bp (P542) region that showed no homology with reported nucleotide or amino acid sequences. The P542 element is *T. rangeli*-specific, as assessed in dot blot assays with DNA from 15 trypanosomatid species (including *T. cruzi*). The P542 sequence is represented in 2,000 copies dispersed in *T. rangeli* genome and located in eight chromosomal bands (from 2.5 to 0.3 Mb). A PCR assay was standardized which showed the presence of the repetitive sequence in eight *T. rangeli* strains originary from different hosts of four countries of Latin America. The PCR assays allows the detection of one tenth of the nucleic acid content of one parasite cell by ethidium staining. The potential use of the P542 element for epidemiological studies was evaluated in samples containing DNA from the digestive tract and feces of *Rhodnius prolixus* and DNA from *T. rangeli* and/or *T. cruzi*. The PCR confirmed the presence of *T. rangeli* in all samples containing the parasite.

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BI-35
CHARACTERIZATION OF CELL SURFACE CARBOHYDRATES OF CULTURED *LEPTOMONAS WALLACEI* N. SP. ISOLATED FROM THE PHYTOPHAGOUS BUG *ONCOPELTUS FASCIATUS* (HEMIPTERA: LYGAEIDAE)

Romeiro A, Saraiva BEM*, Previato JO*, Mendonça-Previato L*, De Souza W, Attias M
Instituto de Biofísica Carlos Chagas Filho, CCS, UFRJ, 21949-900, RJ *Instituto de Microbiologia Prof. Paulo de Goes, CCS, UFRJ, 21949-900, RJ, Brasil

Leptomonas is a monoxenic genus of the family Trypanosomatidae which infects insects. *Leptomonas wallacei* n. sp. is a natural parasite of the intestinal tract of the hemipteran *Oncopeltus fasciatus*. The adhesion of the parasites to the gut epithelium of the bug is probably made by recognition of cell surface carbohydrates. For this reason, we have investigated the putative surface parasite sugar containing molecules by means of a set of 11 fluorescein labelled lectins specific for different sugars residues. The table below summarizes the results obtained.

Lectin	Specificity	Label
Bandeiraea simplicifolia-I (**BS-I**)	a-Gal, a-GalNAc	Positive
Maclura pomifera (**MPA**)	a-Gal, a-GalNAc	Positive
Concanavalia eosiformis (**Con A**)	a-Man, a-Glc	Positive
Helix pomatia (**HPA**)	GalNAc	Positive
Limulus poliphenus (**LPA**)	NeuNAc, GlucA	Positive
Triticum vulgaris (**WGA**)	$GlcNAc_2$	Positive
Arachis hypogea (**PNA**)	b-Gal(1-3)GalNAc	Negative
Bauhinia purpurea (**BPA**)	b-Gal(1-3)GalNAc	Negative
Bandeiraea simplicifolia-II (**BS-II**)	GlcNAc	Negative
Dolichos biflorus (**DBA**)	a-GalNAc	Negative
Glicine max (**SBA**)	GalNAc	Negative

Pure Con A and HPA agglutinated *L. wallacei* at minimum concentration of 0.24 and 4 mg/ml respectively. Pure PNA and WGA did not agglutinate the parasites. The a-L-fucose binding lectins, *Tetragonolobus purpureas* (Lotus) and *Ulex europaeus*-I (UEA-I) were also unable to agglutinate the cells. We also isolated phenol-aqueous extraction a carbohydrate-rich fraction from this parasite. After purification steps, the sugar composition was analysed by gas-liquid chromatography. Galactose (Gal), glucose (Glc) and mannose (Man), in a molar ratio of 6:6:1 were the major sugar components of this fraction. Inhibition studies of lectin agglutination, using the *L. wallacei* glycoconjugate are presently under investigation.

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BI-36
CHARACTERIZATION OF COLOMBIAN SYLVATIC *TRYPANOSOMA CRUZI* BASED ON RAPD, AND DIMORPHISMS OF BOTH RRNA AND MINI-EXON GENE SEQUENCES

Builes JJ, Mejía E, Moreno J, Jaramillo N
Departamento de Biología, Universidad de Antioquia, A.A. 1226, Medellín, Colombia

Trypanosoma cruzi exhibits a broad genetic diversity when studied by isoenzyme, schizodeme or RAPD analysis, but when analyzed with PCR amplification of sequences from the 24Sμ RNA and from the non-transcribed spacer of the mini-exon gene, dimorphism among all the *T. cruzi* isolates was encountered. That dimorphism allowed the identification of two major parasite lineages (Souto et al. 1996 *Mol Biochem Parasitol 83*: 141-152) which are strongly associated with domestic (lineage 1) and sylvatic (lineage 2) cycles.

Among the two known parasite lineages, only lineage 2 was detected in 36 sylvatic *T. cruzi* stocks obtained from a wide geographic area of Colombia, from 1 human individual, from 3 specimens of *Didelphis marsupialis*, from 1 sylvatic mouse host, and from 5 vectors (7 *Rhodnius prolixus*, 12 *R. pallescens*, 7 *Panstrongylus geniculatus*, 3 *Eratyrus cuspidatus*, and 1 *Triatoma dispar*). RAPD analysis showed high variability between all the Colombian isolates. However, when positioned in a tree built by the UPGMA method, they clearly grouped apart from the reference Brazilian clone Y, which is belong to lineage 1.

In conclusion, Colombian *T. cruzi* isolates were classified into lineage 2, and grouped with the predominantly sylvatic cycle of the parasite. It seems also that domestic genotypes are uncommon or absent in Colombia.

BI-37
CHARACTERIZATION OF PARASITOPHOROUS VACUOLE OF SKELETAL MUSCLE CELLS CONTAINING *TOXOPLASMA GONDII*

Andrade EF, Stumbo AC*, Marques AC , Carvalho L*, Barbosa HS
Laboratório de Ultra-estrutura Celular, Instituto Oswaldo Cruz, RJ *Laboratório de Cultura de Células, Departamento de. Histologia e Embriologia, IB, UERJ, Rio de Janeiro, RJ, Brasil

Toxoplasma gondii is an intracellular protozoan parasite that infects a wide variety of cell types in a broad range of host organisms (Joiner & Dubremetz 1993 *Infect Immunol 61*: 1169). This ubiquitous pathogen is a major source of congenital neurological abnormalities and recently there is an increasing interest in this pathogens because it is the most common opportunistic protozoan infection of the central nervous system and the skeletal fibers of individuals contaminated with HIV (Luft & Remington 1992 *Clin Infect Dis 15*: 211, Biggs et al. 1995 *J Immunol 154*: 6132). Our proposal is to analyze the participation of membranar components of skeletal muscle cells (SMC) in the formation of the parasitophorous vacuole containing *T. gondii.*

SMC were obtained from thigh muscles of 18-days-old mouse embryos. The tissue was dissociated in 0.05% trypsin and in 0.01% versene in PBS. The cells were resuspended in DME medium supplemented with horse serum, fetal calf serum, chick embryo extract, and then incubated at 37°C at 5% CO_2 atmosphere. For this study, the cultures were incubated with cationized ferritin (100mg/ml) as marker of anionic sites, for 30 min at 4°C and then infected with *T. gondii* (tachyzoites, RH strain) for different periods of time (15 min to 24h). The cells were fixed for 1h at 4°C in 2% glutaraldehyde + 4% paraformaldehyde in 0.1M cacodylate buffer containing 3.5% sucrose and 5mM $CaCl_2$, (pH 7.2), post-fixed in 1% O_sO_4 in the same buffer and processed as routine for transmission electron microscopy.

Our evidences showed that anionic sites localized on the membrane of SMC are not interiorized together with the parasite. The specificity of this finding is corroborated by the detection of ferritin cationized-containing cytoplasmatic vesicles that are not related to parasitophorous vacuole. These results demonstrate that the parasite utilize different mechanisms to invade different types of host cells: in SMC - not professional phagocytes - anionic sites are not present on the vacuolar membrane differently from the results described using macrophages (Carvalho & De Souza 1990 *Eur J Cell Biol 51*: 211).

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BI-38
CHARACTERIZATION OF STRAINS OF *ENTAMOEBA DISPAR* ISOLATED FROM AN ASYMPTOMATIC INDIVIDUAL IN DIFFERENT TIMES

Furst C, Gomes MA, Viana JC, Silva EF
Departamento de Parasitologia, ICB-UFMG, Caixa Postal 486, Belo Horizonte, MG, Brasil

Little is known about *Entamoeba dispar*, which is an amoebae commonly present in human intestinal tract. It is morphologically similar to *E. histolytica* but thought to be not able to invade tissues and to cause diseases even being able to produce erosion in the intestinal mucosa leading the individuals to feel symptoms at little like non dysenteric colitis formerly thought to be due *E. histolytica.* In this study we tried to clarify some aspects of pathogenicity of *E. dispar*. The stability of some factors related to pathogenicity as capability to infect and to produce lesions in experimental animals, capability to phagocyte erythrocytes, isoenzymes profile (zymodeme) and restriction fragment length polymorphism (RFLP) was investigated. It were isolated, at different times, three different samples from one asymptomatic individual. These strains, designated WIL1, WIL2 and WIL3, were cloned and monoaxenized with *Crithidia fasciculata*. The strains and clones were identified as *E. dispar* by RFLP of a 1970 bp genomic fragment and by electrophoretic mobility of a 482 bp genomic fragment. All the samples showed same results regarding the restriction pattern and electrophoretic mobility. By isoenzyme profile the strains were grouped in an *E. dispar* typical zymodeme. However, this zymodeme altered when the medium was changed for that usually utilized for axenic growth of amoebas. This new zymodeme do no belong to those previously known, revealing the unstable nature of zymodeme already demonstrated for *E. histolytica*. The characterization of strains regarding virulence was performed by inoculation into hamster liver and erythrophagocytosis. Although WIL1 has been able to infect the hamsters, only WIL2 produced lesion. WIL3 was not able to infect hamsters. All the strains showed little capacity to phagocyte erythrocytes, suggesting the disability of E. dispar to invade tissues.

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BI-39
CLINICAL AND PARASITOLOGICAL ASPECTS OF CANINE VISCERAL LEISHMANIASIS

Genaro O, Reis AB, Costa RT, Vieira EP*, Rocha MF**, França-Silva JC, Silva JC* da Costa CA*** Mayrink W, Arias JR****
Departamento de Parasitologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Caixa Postal 486, 31270-910 Belo Horizonte, MG *Fundação Nacional da Saúde, Minas Gerais **Universidade Estadual de Montes Claros, MG ***BIOBRAS S/A ****Pan-American Health Organization

The work described here was done in the urban area of Montes Claros, State of Minas Gerais, during the trial of a new diagnostic test for Visceral Canine Leishmaniasis. In the urban area, 1,798 dogs were studied. With the formal agreement, in writing, of their owners the animals were individually transported to the Zoonosis Control Center of Montes Claros. The dogs were examined clinically for signs of the disease and classified as asymptomatic, oligosymptomatic or symptomatic. Data were collected referring to gender, age and breed. They were then weighted and anesthetized with thionembutal. Skin biopsies from the tip of the ear were done and blood and bone marrow samples were drawn for serological (Indirect Immunofluorescence Assay, IFA) and parasitological surveys after Giemsa staining. Prevalence of the infection evaluated by IFA (titers ³1:40) was 18.5% (332/1798). Asymptomatic dogs were 57.2% (190/332), oligosymptomatic 25% (83/332) and symptomatic 17.8% (59/332). Among seropositive dogs, cutaneous parasitism was detectable in 70.8% of the animals (235/332), while 59.6% (198/332) of them were parasitologically positive at the bone marrow examination. Cutaneous and bone marrow parasitism coincided in 77.5% (183/236) of the IFA-positive cases. One dog was skin-positive and two dogs were bone-marrow positive but IFA-negative (repeated three-times). We noticed that, as the antibody titers increase, so do bone-marrow and skin parasitism, correlating clinically, as well. Among seropositive dogs presenting cutaneous parasitism, 62.1% (118/190) were asymptomatic, 71.1% (56/83) oligosymptomatic and 81.3% (48/59) symptomatic. Besides the mixed-bred animals, 17 breeds were recognized. Mongrel dogs contributed with 82.9% (1489/1798) of the animals studied, presenting a rate of infection of 17.3%. The infection rate among breeds having 10 or more individuals were: 42.9% among Dobermans, 30% among Brazilian Fila, 21.4% among Pinchers, 21.1% among Poodles, 20% among German Shepherds, 13.3% among Pekingese and 6.3% among Dachshunds. The age window in which the disease was most prevalent was that between 2 and 4 years of age (26.6%); the least affected ones were those under 6 months of age (7.3%) and over 10 years of age (7.4%).

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BI-40
CLINICAL SYMPTOMS AND HEMATOLOGICAL ALTERATIONS IN CATTLE NATURALLY INFECTED WITH *TRYPANOSOMA VIVAX* IN THE BRAZILIAN PANTANAL AND BOLIVIAN WETLANDS

Dávila AMR/****, Ramirez L*, Ortiz AG**, Pereira SR**, Souza SS*, Silva RAMS***
Laboratório de Sanidade Animal, EMBRAPA/Pantanal, Corumba, MS *Universidade Federal de Mato Grosso do Sul, CEUC/DAM, Av. Rio Branco 1270, Corumbá, MS **Universidade Estadual do Mato Grosso do Sul, Unidade de Aquidauana, Departamento de Zootecnia, Rodovia Aquidauana, CERA, km 12, Aquidauana, MS ***Laboratório de Sanidade Animal, EMBRAPA/Suinos & Aves, Br 153, km 110, 89700-000 Concordia, SC, Brasil
Present address: Lab. de Biologia Molecular de Tripanosomatídeos, DBBM, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil (E-mail: amrdavila@hotmail.com)

Non-tsetse transmitted trypanosomosis, caused by *Trypanosoma evansi* and *T. vivax*, occurs in various forms in South America, Africa and Asia (including China), and is a potential risk over 500 million cattle, 100 million buffalo and 12 million camels. Here, we report the clinical symptoms and the hematological changes observed in natural cases of bovine trypanosomosis due to *Trypanosoma vivax* in beef cattle from Bolivian wetlands and Pantanal, Brazil. The clinical symptoms were lachrymation, progressive weakness, marked weight loss, inappetence, diarrhea and abortions during the last third of pregnancy. The main hematological changes produced by *T. vivax* infections were anemia and severe leukopenia. The haematology examination showed the following parameters: erythrocytes (x 106/ml): 2.35 ± 0.55; haemoglobin (g/dl): 6.30 ± 2.61; PCV (%): 20.93 ± 7.16; MCV (fl): 109.04 ± 50.38; MCH (pg): 30.93 ± 18.50; MCHC (g/dl): 30.96 ± 15.98. Total leukocytes (x 103/ml): 1.26 ± 0.61; Neutrophils(%): 33.43 ± 9.55; Lymphocytes(%): 50.06 ± 9.02; Monocytes(%): 9.43 ± 2.49; Eosinophils(%): 6.91 ± 2.45; Basophils(%): 0.56 ± 1.50. The cattle presented macrocytic hypochromic anemia. The leukocyte changes were characterized decrease in the leukocyte count and relative monocytosis. We conclude that anemia and leukopenia are the principal signs of bovine trypanosomosis in the Brazilian Pantanal and Bolivian wetlands and could be the major causes of mortality of trypanosome-infected animals. The importance of trypanosomosis control in ensuring success of vaccination campaigns against foot and mouth disease in Brazil and Bolivia should be considered.

PCV: packed cell volume; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration.

BI-41
CLONAL POPULATION STRUCTURE OF COLOMBIAN SYLVATIC *TRYPANOSOMA CRUZI*

Márquez FE, Arcos-Burgos M, Triana CO, Moreno MJ, Mejía E, Jaramillo ON
Department of Biology, University of Antioquia. AA 1226, Medellín, Colombia (E-mail: njaram@matematicas.udea.edu.co)

Isoenzyme variability and evidence of genetic exchange were evaluated in 75 wild stocks of *Trypanosoma cruzi* obtained from different hosts from 5 geographical regions within the endemic area in Colombia. Cluster analysis of genetic variability was attempted. Thirty-three multilocus enzyme genotypes (clonets) were identified from 75 stocks; 27 of which clustered with zymodeme Z1, and 6 with zymodeme Z3. Two stocks isolated from human infections showed the potential risk to rural communities in Colombia. The stocks exhibited departures from Hardy-Weinberg expectations, including both fixed heterozygote and fixed homozygote demes, where both segregation and recombination were absent. To inspect for population subdivision which might falsely imply clonality in these stocks, Wright's *F* statistics were calculated. Theta values (F_{st}) were significantly different from 0 when 33 clonets, 27 Z1-like clonets, and 5 geographical sub-populations were compared; thus, a significant amount of divergence has occurred between and within them. In addition, linkage disequilibrium was detected for most possible pairwise comparisons of loci. In conclusion, the above results all support a scenario of long-term clonal evolution in Colombian sylvatic *T. cruzi* populations.

BI-42
COINFECTION OF CELLS WITH *TRYPANOSOMA CRUZI* AND *COXIELLA BURNETII*: DEVELOPMENT OF CL STRAIN METACYCLIC TRYPOMASTIGOTES INSIDE VERO CELLS LINES PERSISTENTLY INFECTED WITH *C. BURNETII*

Andreoli WK, Rabinovitch M, Mortara RA
Disciplina de Parasitologia, Escola Paulista de Medicina, UNIFESP, Rua Botucatu 862, 6º andar, 04023-062 São Paulo, SP, Brasil

The rickettsia *Coxiella burnetii*, the agent of Q fever, is an obligate intracellular *bacterium* that multiplies within vacuoles of phagolysosomal origin. Vero cells persistently infected with *C. burnetii* forms large acidified vacuoles that fuses with protozoan vacuoles (i.e, *Leishmania* species). Cell line infections of *Trypanosoma cruzi* metacyclic forms is characterized by formation of an acidified vacuole with recruitment of lysosomes. After about 2 hr the parasites leave the vacuoles to the cytosol and transforms into amastigotes and begin to multiply. Previous studies show that *T. cruzi* trypomastigotes fuses with *C.burnetii* vacuoles with no evidence if the parasites leave the vacuoles to the cytosol. In the present study, we show that vacuoles of metacyclic parasites from CL strain fuse with *C. burnetii* large vacuoles in about 3 hours of infection (@10^7 parasites/10^6 cells). Confocal Immunofluorescence with monoclonal antibodies (Mab 3F5) against metacyclic trypomastigotes shows that parasites that invade Vero cell persistently infected with *C. burnetii* colocalize only inside vacuoles. Monoclonal antibodies against parasite major surface glycoprotein Ssp-4 (Mab 2C2) indicate transfortmation of metacyclics to amastigotes in about 48 hr. Amastigotes multiplies inside *C. burnetii* vacuoles and about 144 hr after infection matures to trypomastigotes showing positive reaction to monoclonal antibodies against non-carbohydrate epitopes to *T. cruzi* intracellular flagellated forms (Mab 3B2). We concluded that *T. cruzi* matures inside large vacuoles and probably leaves to cytosol of persistently infected *C. burnetii* Vero cells after 160 hr of infection.

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BI-43
COMPARISON BETWEEN THE CONVENTIONAL TECHNIQUES IN THE DIAGNOSIS OF CUTANEOUS LEISHMANIASIS

Paiva XV, Rabello ALT*, Figueiredo EM, Silva ES, Brazil RP, Gontijo CMF
Laboratório de Leishmanioses *Laboratório de Pesquisas Clínicas René Rachou, Fiocruz, Belo Horizonte, MG, Brasil

The presumptive clinical diagnosis of leishmaniasis needs to be supported by laboratory results before the diagnosis can be confirmed. Usually cutaneous leishmaniasis (CL) diagnosis is performed by parasitological and immunological methods. Definitive diagnosis is realized when two methods are positives, including the clinical exam. In this study, we have compared the conventional techniques in CL diagnosis and evaluated which methods, alone or in combination can be useful in the diagnosis of CL. Patients exams from an outpatient clinic at Centro de Pesquisas René Rachou were analyzed. Between June/97 and July/98, 54 patients with confirmed CL realized 4 types of tests: imprint, culture, Montenegro skin test (ST) and immunofluorescence (IFI). The results showed that

ST was the most sensitive test (70.4%) followed by imprint (55.5%) and IFI (46.3%). Culture showed the lowest sensitivity (13%) and contamination was frequent. As expected, the combination of two methods (considering at least one positive) increased the positivity. When comparing the association of two methods (considering both positives), imprint + ST (35.2%) and IFI + ST (38.9%) showed the highest sensitivity and the difference was not statistically significant. In this study, Montenegro skin test has shown an efficient diagnostic method due to high positive index isolated or in combination with other methods. This is an important confirmation as Montenegro Skin Test is a very simple test, with rapid results, cheap and specialized personal is not necessary.

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BI-44
CONTROL OF THE CANINE VISCERAL LEISHMANIASIS IN MONTES CLAROS, MINAS GERAIS, BRAZIL

Genaro O, Costa RT, Vieira EP*, Rabelo LSS*, França-Silva JC, Silva JC*, da Costa CA, Giunchetti RC, Mayrink W
Departamento de Parasitologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Caixa Postal 486, 31270-901 Belo Horizonte, MG *Fundação Nacional de Saúde, Minas Gerais, MG, Brasil

Control of the canine visceral leishmaniasis in Montes Claros, MG, has been a permanent activity since 1994, as a joint initiative of the Fundação Nacional de Saúde (FNS) and Leishmaniasis Laboratory in the Department of Parasitology, UFMG. Montes Claros has a human population around 270,000 and a canine population of about one-seventh of that. Canine blood samples are routinely collected by the FNS personnel and sent weekly to Leishmaniasis Laboratory, where they are assayed for specific antibodies against *Leishmania chagasi* by the Indirect Immunofluorescence Assay (IFA). Discriminating titers in our conditions are ³1:40. Positive samples are re-assayed for confirmation. Weekly results are forwarded to FNS who apprehends seropositive animals for sacrifice. Control measures also include spraying of the neighborhoods epidemic for the canine infection or where human cases have been reported, with residual insecticides (Domitrine and K-Othrine). Data on the prevalence of the canine visceral leishmaniasis in Montes Claros are displayed in the following Table.

Years	Urban Areas				Rural Areas				Urban and Rural Areas			
	Dogs tested	Dogs positive	Prevalence (%)	Reduction in prevalence	Dogs tested	Dogs positive	Prevalence (%)	Reduction in prevalence	Dogs tested	Dogs positive	Prevalence (%)	Reduction in prevalence
1994	28,294	2,819	9.96	-	6,624	668	9,54	-	35,293	3,487	9.88	-
1995	27,471	1,635	5.95	41%	7,170	317	4,42	54%	34,641	1,952	5.63	43%
1996	29,912	1,521	5.08	50%	7,641	311	4,07	58%	37,730	1,832	4.90	50%
1997	33,759	791	2.34	76.5%	8,133	227	2.79	70.7%	40.874	1,314	3.21	67.5%

Measures implemented in Montes Claros reduced the prevalence of the canine visceral leishmaniasis in 43% after the first year of the program, 50% after the second year and 67.5% after the third year. The data here obtained demonstrate that it is possible to control the canine visceral leishmaniasis with the rapid diagnosis, the appropriate sacrifice of infected animals and the use of insecticides. Moreover, there is clear evidence that the control measures implemented in Montes Claros should be continued for several years to come in order for the effective control of the endemy to be eventually obtained.

Supported by Fundação Nacional de Saúde, Municipality of Montes Claros and UFMG.

BI-45
CRITHIDIA DEANEI ALFENASI ISOLATED FROM MULBERRY: SIMILARITIES AND DIFFERENCES WITH *CRITHIDIA DEANEI*

Fiorini JE, Nascimento LC, Faria-e-Silva PM*/**, Takata CSA***, Campaner M***, Teixeira MMG***, De Souza W**/****, Camargo EP***
Departamento de Biologia Celular, Unifenas, Alfenas, MG *Departamento de Biologia Celular, EFOA, Alfenas, MG **Laboratório de Ultraestrutura Hertha Meyer, IBCCF/UFRJ, RJ ***Departamento de Parasitologia, USP, SP ****Laboratório de Biologia Celular e Tecidual, CBB/UENF, Campos dos Goytacazes, RJ, Brasil

We have previously reported on the isolation and cloning from mulberry fruits of choanomastigotes containing a bacterium-like endosymbiont (Fiorini et al. 1995 *Mem Inst Oswaldo Cruz 90* Suppl. I: 247, Fiorini et al. 1996 *Mem Inst Oswaldo Cruz 91* Suppl. I: 99). Here we report on a comparative study of this flagellate and the original symbiont-bearing *Crithidia deanei* (Carvalho 1973 *Rev Patol Trop 22*:223-274). Our results indicate that the mulberry flagellate and *C. deanei* were very similar and indistinguishable in many aspects: a) they displayed similar nutritional characteristics when grown in a defined medium, requiring only nicotinamide and pantotenate as vitamins and methionine and tyrosin as aminoacids; b) their growth rates in different conditions

of pH, temperature and osmolarity, also were similar; c) both flagellates presented arginase and failed to hybridize with SL3, a synthetic oligonucleotide complementary to sequences of the mini-exon gene of the genus *Phytomonas*; d) by restriction analysis with different enzymes of the SSU and ITS of the ribosomal gene they displayed identical patterns; e) the mulberry flagellate lacked a *Pvu*II site at the SSU which also is characteristically absent in *C. deanei*, although occurring in *Crithidia* spp. (Camargo et al. 1992 *J Parasitol 78*: 40-48); f) they are morphologically identical. Nevertheless, there are also some differences between the 2 flagellates. After 48 hr of growth at 28°C, the presence of opisthomorphs in *C. deanei* cultures was never above 70% whereas, in cultures of the isolate from mulberry, opisthomorphs reached 100%. RAPDs analysis revealed that the 2 flagellates, although yielding identical patterns with 1 primer, yielded clearly distinguishable patterns with 3 other primers. RFLPs and RAPDs patterns of C.deanei and the mulberry flagellate were, in every case, quite distinct from those of *C. desouzai* and *C. oncopelti.* Mixing of cultures of the mulberry flagellates with cultures of *C. deanei* can be ruled out because at the time of isolation we did not keep cultures of C. *deanei* at our laboratory (Alfenas). Therefore, due to the similarities between the 2 flagellates, we conclude that the flagellate from mulberry fruits correspond to a re-isolation of *C. deanei.* But, since there are minor differences between the 2 flagellates, it seems more appropriate to consider the mulberry flagellate as a subspecies of *C. deanei*, to which we will refer to as *Crithidia deanei alfenasi.*

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BI-46
CYTOCHEMICAL LOCALIZATION OF CHITIN IN *TRITRICHOMONAS FOETUS*

Ferreira LN, Soares RMA, Santos ALS, De Souza W*, Alviano CS
Instituto de Microbiologia Prof. Paulo de Góes, CCS, UFRJ, Cidade Universitária, 21941-590 Rio de Janeiro, RJ, Brasil (E-mail: immgceu@microbio.ufrj.br) *Instituto de Biofísica Carlos Chagas Filho, CCS, UFRJ

Trichomoniasis is an infection of the genitourinary tract caused by the flagellated protozoan *Trichomonas vaginalis* in humans and *Tritrichomonas foetus* in cattle. In our previous studies, we report for the first time the occurrence of chitin in trichomonads. In the present work we investigated the localization of polysaccharide chitin of the surface structures of trichomonads, using a gold-labeled chitinase. *T. foetus* were grown in Diamond´s TYM medium and fixed for 60 min at 4ºC in 0.5% (v/v) glutaraldehyde, 4% paraformaldehyde in 0.1M cacodylate buffer, pH 7.2, supplemented with 5mM of $CaCl_2$. After fixation, the cells were incubated with recombinant chitinase-gold complex (1:20 dilution) in PBS, pH 6.0, overnight, at 4ºC. This was followed by post-fixation in OsO_4 1% with 0.8% (w/v) potassium ferricyanate. Thereafter they were dehydrated in acetone and embedded in Epon. This sections were stained with uranyl acetate and lead citrate and observed in Zeiss-900 electron microscope. As a result the transmission electron microscopy showed that the chitinase binding sites were detected at the parasite membrane since it have been observed labeling of the cell surface with enzyme-gold complex. The presence of chitinous structural components in *T. foetus* raise the possibility that inhibitors of chitin synthesis may have a role in prevention and control of this parasitic infection.

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BI-47
CYTOSKELETAL CONJUNCTION DURING MORPHOGENESIS OF *TRITRICHOMONAS FOETUS*

Ribeiro KC, Benchimol M*
Laboratório de Microscopia Eletrônica Hertha Meyer, IBCCF, CCS, UFRJ, 21949-900 Rio de Janeiro, RJ, Brasil
*Universidade Santa Úrsula, Rua Jornalista Orlando Dantas 59, 222-31-010 Rio de Janeiro, RJ Brasil

This primitive anaerobic protozoa displays an unorthodox mitotic device which includes several cytoskeletal systems instead of an unique participation of a spindle mechanism. Its primitive character is reflected in its cell division process which is in part as prokaryotes and in part as eukaryotes. The closed mitosis with an extranuclear spindle presents a microtubular apparatus as eukaryotes and a membrane-dependent mode of genome segregation as prokaryotes. In this peculiar mitosis, the joint action of the microtubular spindle, the duplicated pelta\axostyle microtubular complex, and the costa together with the mastigont system interacts in the dividing cell to promote karyokinesis and citokinesis.

In order to approach this dynamic event “in vivo” videomicroscopy and computer animation were employed. Scanning electron microscopy and bright field optical microscopy were used to show cell morphology transition from interfase thru pre-mitosis and also along each of the four mitotic phases described. In addition, immunofluorescence microscopy was applied to demonstrate the axostyle and MTOC behavior during morphogenesis. The information obtained from the combined action of the spindle, mastigont, and skeletal systems could improve our understanding of this unorthodox type of mitosis where nuclear envelope breakdown does not occur and the spindle is totally extranuclear.

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BI-48
DEMONSTRATION OF ACTIVE PARASITEMIA IN PERIPHERAL BLOOD OF U.S. BLOOD DONORS SEROPOSITIVE FOR *TRYPANOSOMA CRUZI*

Leiby DA, Tibbals MA, Herwaldt BL*, Herron RM**
Transmissible Diseases Department, American Red Cross, 15601 Crabbs Branch Way, Rockville, MD 20855, USA *Centers for Disease Control and Prevention, Division of Parasitic Diseases, 4770 Buford Highway NE, Mailstop F-22, Atlanta, GA 30341, USA **Southern California Region, American Red Cross, 1130 So. Vermont Avenue, Los Angeles, CA 90006, USA

A large influx of immigrants to the United States from *Trypanosoma cruzi* endemic countries has raised concerns that transmission of the parasite may be occurring by blood transfusion. However, to date there have been only 4 reported cases of transfusion transmitted *T. cruzi* in the U.S. Several recent studies have identified seropositive donors in various locations across the U.S., suggesting a reservoir population for transmission of *T. cruzi*. Attempts to identify transmission through testing the recipients of blood from seropositive donors have proven unsuccessful. Thus, to clarify whether seropositive donors are parasitemic, we tested their peripheral blood for the presence of circulating parasites. *T. cruzi* seropositive blood donors, identified as part of a related study in Los Angeles, were asked to complete a questionnaire regarding potential exposure to *T. cruzi* and to provide a sample of blood for further testing. Blood samples were tested by polymerase chain reaction (PCR) for a 330 bp kinetoplast DNA product. Additionally, hemoculture assays were established to test for parasitemia. Thus far, 25 seropositive donors have been entered in the study and 16 (64%) have been found to be positive by PCR. Hemoculture assays have only been completed for 14 donors since these assays may take up to 16 weeks to complete. Of the 14 donors tested by hemoculture, 2 (17%) demonstrated the presence of active parasites. Both hemoculture positive assays were also PCR positive. When PCR negative and PCR positive donors were compared for various risk factors for infection, no differences regarding exposure to the vector, living in substandard housing, or time since immigration to the U.S. were apparent. These results demonstrate that a majority of *T. cruzi* seropositive blood donors have evidence of circulating parasites in their peripheral blood despite immigrating to the U.S. on average more than 20 years ago. This suggests that recipients of blood transfusions may be receiving parasites that do not produce active infections, or perhaps, they induce infections that have gone undetected.

BI-49
DETECTION OF *LEISHMANIA* IN BLOOD SAMPLES FROM DOGS USING POLYMERASE CHAIN REACTION

Silva ES, Gontijo CMF, Pirmez C*, Fernandes O**, Pacheco RS***, Brazil RP
Laboratório de Leishmanioses, Centro de Pesquisas René Rachou, Fiocruz (E-mail: silvarii@netra.cpqrr.fiocruz.br) *Laboratório de Imunopatologia ***Laboratório de Sistemática Bioquímica, Departamento de Bioquímica e Biologia Molecular **Departamento de Medicina Tropical, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil

Visceral leishmaniasis (VL), or Kala-azar, is a zoonosis in most regions in which it occurs. Dogs are the most important vertebrate reservoir, especially in areas were *Leishmania (L.) chagasi* and *Leishmania infantum* are the causative agents of the disease. In Brazil, foci of visceral leishmaniasis are found in rural and suburban areas although it has been reported in urban areas of Brazil in the past few years, including the State of Minas Gerais. In the present study, the authors describe the immunological and parasitological diagnosis from dogs, and molecular techniques such a specific DNA probes or the PCR of blood from dogs of Sociedade Mineira Protetora dos Animais in the Metropolitan Region of Belo Horizonte (MRBH). In addition, molecular characterization based on restriction fingerprints of kDNA, hybridization analysis and enzyme electrophoresis of *Leishmania* isolates from dogs was carried out. During the years of 1997, 663 domestic dogs, were studied. Peripheral blood samples with anticoagulated (with EDTA) were collected for serological diagnosis and detection of the parasites by PCR. Aspiration of tibia bone marrow were performed and material obtained for culture and parasitological survey. The indirect immunofluorescence test was positive in 139 (20.96%) of the 663 dogs examined. Anti *Leishmania* positive titers higher than 1:2560 were observed. The PCR was realized in 59 blood samples. In 31 (52.5%) the technique detected the presence of fragments with 120 bp, specific for *Leishmania*. Bone marrow aspirate were positive from 11 (91.6%) out of 12 dogs suspicious of visceral leishmaniosis. Two isolates were characterized by isoenzime analysis, kDNA restriction profile and molecular hybridization. The biochemical characterization using four different molecular techniques confirmed the identification of the isolates as *Leishmania (L.) chagasi*.

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BI-50
DIFFERENT SIGNAL TRANSDUCTION PATHWAYS ARE ACTIVATED IN *TRYPANOSOMA CRUZI* STRAINS WITH DIFFERENTIAL INFECTIVITY DURING HOST CELL INVASION

Cabrera A, Favoreto Jr. S, Dorta ML, Manque PM, Yoshida N
Departamento de Microbiologia, Imunologia e Parasitologia, Escola Paulista de Medicina, Universidade Federal de São Paulo, R. Botucatu 862, São Paulo, SP, Brasil

We have previously shown that treatment of metacyclic or tissue culture trypomastigotes of *Trypanosoma cruzi* (CL strain) with genistein, an inhibitor of protein tyrosine kinase, blocked the phosphorylation of p175, a 175 kDa protein undetectable in non invasive epimastigotes, and markedly decreased invasion of HeLa cells. Here we show that genistein does not affect the ability of metacyclic forms of the poorly invasive G strain to enter HeLa cells. Even without any activation, the levels of p175 phosphorylation in G strain metacyclic trypomastigotes were comparable to those observed in the CL strain upon interaction with HeLa cell extract. In contrast to what occurs in the CL strain, the p175 phosphorylation in G strain metacyclic forms was not significantly augmented above basal levels neither by HeLa cell extract nor by monoclonal antibody 3F6, an antibody directed to the metacyclic stage surface glycoprotein gp82 implicated in target cell invasion. Treatment of CL strain metacyclic forms with genistein also inhibited Ca^{2+} mobilization, indicating that tyrosine phosphorylation and Ca^{2+} signaling are associated events. On the other hand, we observed that treatment of G strain metacyclic trypomastigotes with 10 mM forskolin, an activator of adenylyl cyclase, resulted in about 2-fold increase in the rate of HeLa cell invasion. Such an effect was not detectable in the CL strain metacyclic forms. These data, taken together, indicate that *T. cruzi* strains with differential infectivity use different signal transduction mechanisms to enter host cells.

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BI-51
EFFECT OF BREFELDIN A ON INTRACELLULAR DEVELOPMENT AND SURVIVAL OF *TOXOPLASMA GONDII*

Oliveira AS, Melo EJT, De Souza W
Laboratório de Biologia Celular e Tecidual, LBCT, CBB/UENF, Campos, RJ, Brasil

Toxoplasma gondii, an obligate intracellular parasite of vertebrate animals, is able to infect cells from the vertebrate hosts surviving and multiplying within a parasitophorous vacuole until the lysis of the host cell. In the present study we decided to use brefeldin A, a drug which interrupts the traffic of vesicles between the endoplasmic reticulum and the Golgi complex inducing disorganization of the Golgi complex, to analyze the involvement of these components in the intracellular development of *T. gondii* .

Vero cells were cultivated in Linbro tissue plates that contained a sterile coverslip and 199 medium, and maintained at 37°C overnight. The cultures were then infected with tachyzoites of *T. gondii* and incubated for periods varying from 1 to 24 hr. Brefeldin A, at a concentration of 1mg/ml was added to some cultures. In order experiments control and infected cells were incubated for 1-5 min in the presence of TRITC-labeled brefeldin A and then observed in a Confocal Laser Scanning Microscope.

In control cells incubated for 10 min in the presence of brefeldin A staining of the perinuclear region was observed. In infected cells the intravacuolar parasites was stained, indicating that the drug reached the intravacuolar parasites. However, labeling of cells previously treated for 30 min with brefeldin A showed a diffuse labeling of the cytoplasm of the host cell and of the parasites, indicating that the drug induced the disruption of the Golgi complex. Incubation of infected cells in the presence of brefeldin A for 7 hr blocked the multiplication of tachyzoites within the parasitophorous vacuole. This effected could be reversed by removal of the drug following washing of the cultures. These observations suggest that intracellular development of tachyzoites of *T. gondii* depend of the integrity of the endoplasmic reticulum-Golgi complex system of the host cell.

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BI-52
EFFECTS OF DIFFERENTS FORMS OF THE CL STRAIN OF *TRYPANOSOMA CRUZI* IN THE MYENTERIC PLEXUS OF THE ESOPHAGUS IN CHRONIC CHAGASIC CH3 MICE

De Souza RR, Miyashiro PLS, Kitamura RJ, Maifrino LBM, Liberti EA
Instituto de Ciências Biomédicas, Universidade de São Paulo, Instituto Dante Pazzanese de Cardiologia, Caixa Postal 66.208, 05388-970 São Paulo, SP, Brasil

It is well known that different strains of *Trypanossoma cruzi* cause distinctive pathological pictures. The objective of the present paper was to observe the effects of different forms of the same strain of *T. cruzi* on the myenteric plexus of the mouse esophagus. The neurons of the myenteric plexus of the esophagus were stained by a histochemical technique in non infected and chronic chagasic CH3 mice with three differents forms of the CL strain of *T. cruzi* (blood stream, culture and culture + urine). The total number of myenteric neurons were counted on whole mount preparations of the esophagus muscularis externa. The density of the myenteric neurons fell in chronic chagasic animals inoculated with all kinds of *T. cruzi* forms. The decrease in the number of neurons with the culture + urine form of *T. cruzi* was significant (Table).

	Number of neurons (mean ± SD)	
Blood stream	195 ± 11	(p> 0.05)
Culture	134 ± 10	(p> 0.05)
Culture + urine	104 ± 11	(p< 0.05)
Control	259 ± 30	

The present findings suggest that different forms of the same strain of *T. cruzi* may have different effects on the neurons of the autonomic nervous system.

BI-53
ELECTROCARDIOGRAPHIC CHANGES IN HAMSTERS INFECTED WITH *TRYPANOSOMA CRUZI*

Ramirez LE, Silva Jr. EL Silva Jr. JR, Dias da Silva VJ, Lages-Silva E, Chapadeiro E
Departamento de Ciências Biológicas, FMTM, Uberaba, MG, Brasil

The experimental model of Chagas' disease in golden hamsters (*Mesocricetus auratus*) has been characterized. The aim of the present study was to evaluate the possible electrocardiographic (ECG) changes during acute phase of the experimental Chagas' disease in hamsters.

Twenty eight male golden hamster (100-150 g) were divided in two groups: control group (CON, n=13) and chagasic group (CHG, inoculated with 2 x 10^3 blood forms of the Vicentina strain of *Trypanosoma cruzi*, n=15). The infection was confirmed by positive parasitemia, using micro-hematocrite method. Under anesthesia (sodium pentobarbital, 40mg/Kg, i.p.), the animals had their ECGs recorded one day before (T0) and 20 days (T20) after inoculation, during acute phase of the disease. The ECG recordings were performed using nine leads (six classical frontal and three precordial leads) and an acquisition data system (Aqdados, Lynx T.E., São Paulo) on a personal computer. The following parameters were analysed: heart rate (HR), RR interval (RRi), amplitude (aP) and duration (dP) of P wave, PR interval (PRi), QRS duration (QRS), QaT interval (QaTi), P and QRS axis, J point changes, blocks and arrithmias. In the end, the animals were sacrified and histo pathological studies were performed.

Mean values of HR, RRi, aP, QaTi, P and QRS axis were not different among groups. Mean values (±SE) of dP (ms), PRi (ms) and QRS (ms) in CON-T0, CON-T20, CHG-T0 and CHG-T20 groups were, respectively: dP: 13,5±0,9, 12,9±1,0, 13,9±0,8 and 16,7±0,9*; PRi: 54,3±1,2, 54,5±1,1, 51,7±1,0 and 56,0±1,1*; and QRS: 18,6±0,9, 16,7±1,0, 16,6±0,9 and 22,1±0,8* (* $p < 0,05$ versus the others). Arrithmias, bundle branch blocks, pathological Q waves and important repolarization changes were not found.

In conclusion, acute Chagas' disease in hamsters was characterized by the following ECG changes: dP, PRi and QRS enlargements, probably associated with atria enlargement, atrio-ventricular and intraventricular conduction defects caused by an acute myocarditis. Similar ECG changes are observed in acute myocarditis of the human Chagas' disease.

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BI-54
ELECTRON MICROSCOPIC STUDY OF THE BLOODSTREAM TRYPOMASTIGOTE FORMS OF *TRYPANOSOMA CRUZI*

Corrêa AFS, Meuser M*, Soares MJ
Laboratório de Biologia Celular de Microrganismos *Laboratório de Ultra-estrutura Celular, Departamento de Ultra-estrutura e Biologia Celular, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil

Trypanosoma cruzi presents during its life cycle distinct evolutive stages (amastigotes, epimastigotes and trypomastigotes), adapted for living in different hosts and environments. Studies on the fine structure and cell biology of *T. cruzi* are focused mainly on the epimastigotes, since they can be easily grown *in vitro*. Studies on the trypomastigote forms are mostly restricted to parasites obtained *in vitro*, and little is known on the cell biology of the bloodstream trypomastigotes, due to difficulties in obtaining such evolutive stages. Ultrastructural data have shown

that *T. cruzi* presents cytoplasmic organelles common to other eukaryotic cells, besides some specific structures, such as the glycosomes and the reservosomes of epimastigotes.

Since few data is available on the bloodstream trypomastigotes morphology, in this study we have analyzed the cell biology of these forms. Parasites (*T. cruzi*, strain Y) were isolated from albino Swiss mice, at the peak of parasitemia (7th day post-inoculum), and processed for routine scanning and transmission electron microscopy.

Preliminary results demonstrate the presence of high number of lipid droplets, glycosomes and endoplasmic reticule profiles. Differently from what has been observed with the epimastigote forms, the Golgi complex is not located at the flagellar pocket opening, but usually close to the nucleus, suggesting that both structures are not tightly bound and/or present different migration rates. The delocalization of the Golgi complex from the flagellar pocket further suggests that epimastigotes and trypomastigotes present diverse exocytic activities. Two distinct organelles are also found in great numbers: electrondense granules, probably a reserve of iron or phosphate, and round electronluscent vacuoles, which may correspond to precursors of reservosomes of the epimastigote forms. Further studies are being carried out in order to characterize the nature of these cytoplasmic inclusions and the functions of the Golgi complex.

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BI-55
ENCEPHALIC PARASITISM IN RATS EXPERIMENTALLY INFECTED WITH Y STRAIN OF *TRYPANOSOMA CRUZI*: IMPORTANCE OF AGE AND INOCULUM SIZE

Da Mata JR, Chiari E, Machado CRS
Departamentos de Morfologia e Parasitologia, Instituto de Ciências Biológicas, UFMG, Belo Horizonte, MG, Brasil

Chagas' disease is more severe in less than two-year-old children and young experimental animals. The present work aims at assessing the brain parasitism in 10-day-old and 30-day-old Holtzman rats inoculated with two inocula (5,000, 250,000) trypomastigotes per 50 g of body weight, ip) of *T. cruzi*. The animals were killed 13 days after inoculation under anesthesia by intracardiac perfusion of saline followed by fixative (4% phosphate-buffered paraformaldehyde). Slices through the different regions of the brain were processed for paraplast or glycolmethacrylate embedding. Four-µm-thick sections were stained with routine histological techniques or immunohistochemically for detection of *T. cruzi* antigens. In animals inoculated with 5,000 trypomastigotes (both ages) few amastigotes were detected only by the immunohistochemical technique. The inoculation of 250,000 clearly showed more intense parasitism in 10-day-old animals in comparison with the older ones. At both ages, the amastigote nests were particularly numerous in the molecular layer and around the Purkinje cells of the cerebellar cortex, being easily found in other cerebellar regions as well as in both white and gray matter of the cerebral hemispheres. The amastigote nests seems to be restricted to glial cells. Cerebral areas devoid of blood brain barrier (area postrema, pineal, median eminence, choroid plexus) showed low, if any, parasitism. Preliminary data obtained in studies of the brain permeability to Evans blue showed defective or absence of blood brain barrier in the 10-day-old animals. In conclusion, our findings support the importance of age and inoculum size for the central nervous system parasitism. No preference for the areas devoid of blood brain barrier was found. However the higher parasitism in 10-day-old animals could involve the incomplete development of such barrier.

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BI-56
ENTAMOEBA DISPAR: GENETIC POLYMORPHISM AND VIRULENCE

Gomes MA, Furst C, Valle PR, Ludgero ML, Silva EF
Departamento de Parasitologia, ICB, UFMG, Belo Horizonte, MG, Brasil

Recently one amoebae commonly found in human intestinal tract was admitted as a new specie, the *Entamoeba dispar* described by Brumpt in 1925. This amoebae was considered to be responsible by the infection of most of the asymptomatic individuals that were previously assigned to *E. histolytica*. However, non-dysenteric colitis, the main clinical manifestation of symptomatic amoebiasis in Brazil, has been attributed to *E. dispar*. Thus, not only distribution but the pathogenicity of this amoebae should be more investigated. In order to contribute in this aspect we studied the genetic polymorphism of 3 isolates of *E. dispar* took from one individual at different times, WIL 1, WIL 2 and WIL 3. These isolates were cloned and reisolated from hamster liver trying to increase it virulence *in vivo*. To identify the isolates, clones and reisolates, the 1970 bp DNA fragment derived of the small-subunit rRNA genes (SSU-rDNA) was submitted to digestion with *Taq* I restriction enzyme. All of the isolates showed the same pattern of restriction, being classified into cluster of *E. dispar*. The capability of these isolates to produce tissue lesions was accounted by inoculation into hamster liver. Only WIL2 produced lesions into inoculated animals. Genetic polymorphism among isolates, clones and reisolates was showed by low stringency PCR using a single primer (LSSP-PCR). The DNA fragment analyzed was 1970 bp, the same used for *E. dispar* identification. A distinct fingerprint

was obtained for each isolate. Clones and reisolates from WIL1, WIL2 and WIL3 showed similar fingerprint for each ones, suggesting that these isolates were originate from an unique infection and that they were formed by homogeneous population of amoebae. These results intensify the commensal behavior attributed to *E. dispar*. The 1970 bp DNA fragment can be used as target to study the inter-specific polymorphism in *Entamoeba*.

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BI-57
ENTEROCYTOZOON BIENEUSI AND COINFECTIONS IN PATIENTS WITH AIDS AND CHRONIC DIARRHEA

Velásquez J, Mariano M, Peralta M, Carnevale S, Oelemann W, Ibañez C, Chertcoff A, Bozzini J
Servicio de Microscopía Electrónica, Instituto Nacional de Enfermedades Infecciosas, ANLIS. Buenos Aires, Argentina *Instituto de Microbiologia, CCS, Universidade Federal de Rio de Janeiro. Rio de Janeiro, Brasil

Our report concerns patients with AIDS who had chronic diarrhea with *Enterocytozoon bieneusi* and co-infections. The present study included 118 adult patients with AIDS and chronic diarrhea. Among them we describe 8 patients with *E. bieneusi* and co-infections. The examination of stool specimens included trichrome blue and acid-fast preparations of direct and concentrated samples. We carried out videoesophagogastroduodenoscopy (VEDA) to visually inspect the mucosa and to obtain biopsy specimens and luminal fluid. Duodenal biopsy sections were stained with hematoxylin-eosin, Giemsa and Azur II for light microscopy. Transmission electron microscopy of duodenal biopsy specimens were used for identification of microsporidia. A PCR assay with *Trypanosoma cruzi*-specific primers was employed in order to confirm the species of amastigote-like forms observed by light microscopy. This method was carried out in paraffin-embedded formalin-fixed biopsy specimens.The average age of the subjects was 30 years (range: 24-37). Among of the 8 described patients, 7 presented infections caused by *E. bieneusi* and one associated pathogen: *T. cruzi* (3/8), *Histoplasma capsulatum* (2/8), *Cryptosporidium* sp. (1/8) and Uncinarias (1/8). In one patient, three associated protozoa were identified as the infection cause: *Isospora belli*, *T. cruzi* and *E. bieneusi*. VEDA revealed mucosa changes in the 8 cases: granular duodenum (6/8), jasper duodenum (1/8) and erosion (1/8). Pathogens observed in histological sections included: *Enterocytozoon bieneusi* (8/8), *T. cruzi* (4/8), *Histoplasma capsulatum* (2/8) and *Isospora belli* (1/8). *T. cruzi* infections were positive by PCR in the four cases that revealed amastigotes by histology. In feces, spores were identified in 2 patients, *Cryptosporidium* sp. in 1 patient and Uncinarias in another one.VEDA and biopsy specimens worked together might be important tools to document infections due to microsporidia, Coccidia, disseminated micosis and Chagas´ disease. The identification of one etiological agent does not rule out the presence of associated pathogens.

BI-58
EPIDEMIOLOGICAL ASPECTS OF TOXOPLASMOSIS ON PREGNANT WOMEN IN THE REGION OF ALTO URUGUAY RIVER IN THE STATE OF RIO GRANDE DO SUL, BRAZIL

Spalding SM, Alves AS*, Vicente RT*, Costa TODA*, São José ASDE*, Velloso CFP, Ribeiro LC**, Munaro N***, Miranda S, Amendoeira MRR*
Lacen, Porto Alegre, RS *ENSP, Fiocruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil **Hospital da Criança Santo Antônio, Porto Alegre, RS ***Del. Reg. de Saúde de Erechim, RS, Brasil

Toxoplasmosis, a worldwide distributed parasitic disease causing subclinic or inapparent infection in immunocompetent individuals is known to cause severe damage in fetuses or newborn from mothers acutely infected during pregnancy. One can acquire the *Toxoplasma gondii* through diverse transmission mechanisms, such as, ingestion of cysts from undercooked or raw meats, oocysts from cats feces contaminating raw vegetables, earth manipulation, among others. The pathogenicity of congenital infection is related with pregnancy stage in which the women had contact with the parasite. This study is based on this facts and in the high toxoplasmosis prevalence in Alto Uruguay river (northeast of the state) area, along with the greatest ocular acquired toxoplasmosis incidence of the world. Samples of pregnant women from sanitary unites of the Eleventh Regional Heath Department were analyzed by IFI and ELISA for IgG and IgM immunoglobulins dosage. Epidemiological data studies of the parasites were correlated with serological results from the pregnant women group. Out of 620 pregnant women 63.76% live in urban area and 36.24% in rural area; 453 (73.06%) were sera positive. In urban area the soropositivity was 57.46% while in rural area was 77.71%. When the risks of parasite transmission were considered, we registered the relative risk factors of the infection, as following: 2.58 of pregnant women that lives in rural area; 1.73 patients who had contact with others animals except cat; 1.38 among those who had contact with cats; 1.34 for those who handled earth; 0.98 for eating raw undercooked meat; 0.98 for those who drink raw milk and 0.92 for those who eat raw inlaid. Only 3 (0.48%) related raw vegetables and sera positive. The results suggest that is the most important transmission mechanism on region studied is the ingestion of the ooscysts that have been excreted from infected cats feces. The educational preventive measures has been recommended to pregnant women to prevent toxoplasmosis infection.

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BI-59
EPIDEMIOLOGICAL STUDIES OF PHYTOPHAGOUS HEMIPTERA IN THE STATES OF PARANÁ, SOUTH OF SÃO PAULO AND MATO GROSSO DO SUL

Cavazzana Jr. M, Santos MA, Baccan GC, Ogatta SFY, Romão C, Takimoto CA, Ono CJ, Almeida ML, Batistoti M, Kaneshima EN, Costa C, Jankevicius JV, Itow Jankevicius S
Universidade Estadual de Londrina, CCB, Dep. de Microbiologia, 86051-900 Londrina, PR, Brasil

In this study, 408 phytophagous hemiptera of ten different genera were examinated and 226 (55.4%) were harbouring trypanosomatids in salivar gland or digestive tract or both, and 15 (6.6%) insects were infected with choanomastigote forms and 211 (93.4%) were infected with promastigote forms. The insects were collected in Parana, south of São Paulo and Mato Grosso do Sul states from 1983 to 1996 and among them 28 (12.4%), 96 (42.59%), 66 (29.2%) and 36 (15.9%) belonging to the family Lygaeidae, Coreidae, Pentatomidae, Pyrrhocoridae family respectively. The genera *Oncopeltus* and *Pachibrachius* spp both Lygaeidae accounted for 11.9% (27) of the infection with trypanosomatids. The genera *Leptoglossus zonatus*, *Phthia picta*, *Teognis* sp, *Megalotomus* sp and Coreidae (genus without identification) all Coreidae accounted for 27.9% (63), 6.3% (12), 0.88% (2), 0.88% (2) and 7.5%, of the infection with trypanosomatids, respectively. The genera *Nezara viridula*, *Euchistus herus* and *Piezodorus guildini*, all Pentatomidae accounted for 18,6% (42), 5,3% (12), 5,3% (12) of the infection with trypanosomatids, respectively. The genus *Dysdercus* (Pyrrhocoridae) accounted for 15.9% (36) of the infection with trypanosomatids. Sbravate et al (1989) demonstrated the prevalence rates of flagellates in bugs as being Pyrrhocoridae 44%, Coreidae 40%, Lygaeidae 19% and Pentatomidae 18%. Our results showed the prevalence rates of Coreidae 42.5% followed by Pentatomidae 29%, Pyrrhocoridae 15.9% and Lygaeidae 12.4%. Our results also indicated that *Leptoglossus zonatus* (Coreidae) has had a progressively population increase and today it constitutes the dominant insect in cultures such as soybean, bean, maize, fruits, etc., showing a great capacity of adaptation and locomotion. The control of these insects in the agriculture is important because of grains and fruits damage as well as the transmission of microorganisms such as fungi bacteria and virus to plants.

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BI-60
EPIDEMIOLOGICAL STUDY OF CANINE TEGUMENTARY LEISHMANIASIS IN INOÃ DISTRICT (MARICÁ MUNICIPALITY, RIO DE JANEIRO, BRAZI)

Horta FT, Serra CMB*, Madeira MF**, Macedo RMS**, Duarte R***, Pacheco RS****
Departamento de Patologia Clínica, UFF *Laboratório de Protozoologia **Laboratório de Imunodiagnóstico, ENSP, Fiocruz ***Laboratório de Sistemática Bioquímica, DBBM, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil

Occurrence of American Tegumentary Leishmaniasis (ATL) has been observed in many regions of the State of Rio de Janeiro. Changes introduced by humans in natural environments favor the installation of non-sylvan foci, thereby leading to the involvement of various species of animals domestics, mainly canids and equids.

The frequent presence of household dogs with infection rates associated with the human disease in endemic areas for ATL, as well as the presence of the same genotypic pattern of the parasite circulating in human and canine populations prove the dog's involvement in the peri and intradomiciliar transmission cycle of disease.

In 1997, three cases of human have been noted, being (Antonio Pedro Hospital, Niterói, RJ) from Inoã District. That place is located near by regions natural complex of mountains. The increase of local population and disordered occupations has been observed and resulted in a progressive degradation of environment. In attention of the human cases, veterinarians have observed frequently dogs with suggestive lesions and/or positive serology.

In order to establish the situation of leishmaniasis in this region, a clinical, serological and parasitological study was initiated with 55 dogs. The serological survey was done by collecting blood samples of the dogs in the femur vein of each animal. Individual data about race, age, sex, residence and owner's name of the dogs were obtained during the survey.

The results showed humoral immune response in 5 animals (9%); 1 positive by indirect immunofluorescence reation (IFAT) and 4 positive by enzyme-linked immunosorbent assay (ELISA). Suggestive cutaneous leishmaniasis lesions have been observed in 7 animals (12%). In one seronegative dog by two methods was possible to isolate the parasite from ulcerated scrotum lesion by biopsy. Phenotypic characterization from isolated was compatible with pattern strain of *Leishmania (Viannia) braziliensis*.

Further studies will be carry out to evaluate of phlebotomine fauna as well its association of dogs in order to studying the dynamics of leishmaniasis transmission in this area.

BI-61
EVALUATION OF FLOW CYTOMETRY FOR *LEISHMANIA* SPP. MORPHOMETRY

Batista LN, Bernardes FC, Ferreira SAC de P**, Steindel M/*, Lima HC/*
Departamento de Microbiologia e Parasitologia,UFSC, Caixa Postal 476, 88040-900 Florianópolis, SC, Brasil *Serviço de Dermatologia, Núcleo de Pesquisas em Dermatologia, HU, UFSC **HEMOSC, Florianópolis, SC, Brasil

The leishmaniases refers to various clinical syndromes that are caused by *Leishmania* spp. parasites. These diseases are transmitted by the bite of female sandflies, whose habitat are tropical and subtropical areas. The protozoa installs in the mononuclear-phagocyte system of some mammals, including human being. After the infection, different clinical forms of disease are established by the host immune response and the parasite interaction. Direct demonstration of parasite in biopsies specimens is the most method used for parasite diagnosis. However, isolation of the microorganism in axenic culture media is of utmost importance to allow species identification and characterization using different approaches. In the past, biological behaviour and morphometry of cultured parasites where the most common methods used for *Leishmania* identification. However, traditional morphometry is tiredness and has been replaced by more sofisticated and precise methods. In this study, we evaluated dimensions of culture promastigotes forms using the traditional methodology and flow cytometry technique. Three standard *Leishmania* strains (LV-39 *L. major,* PH8 *L. amazonensis* and L 2904 *L. braziliensis*) and four isolates from patients (two *L. braziliensis* and two *L. amazonensis*) were cultivated at 26ºC in Schneider's insect medium supplemented with 5% of heath inactivated calf serum. After 48 and 96 hr, 50㎖ of each culture was collected, fixed in 50㎖ of paraformaldehyde 2%, ressuspended in 500㎖ of PBS and read in a Flow Cytometer Becton & Dickinson. Additionally, culture promastigotes were fixed with methanol onto glass slides and stained with Giemsa. Standard measurements were done in 100 parasites for each strain in an optical microscope using a micrometric ocular and a 100X objective. Data analysis was done using ANOVA for comparison of the traditional and flow cytometry techniques. Data analysis shows that both methods were able to distinguished the different *Leishmania* species. The correlation between these two methods were satisfactory when the diameter of the parasite was compared. Therefore, the flow cytometry represents a good method for evaluation of the diameter. Moreover, it may be applied as an easier and faster method to *Leishmania* differentiation. However, we recognize the limitation of this method for only experimental conditions nowadays.

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BI-62
EVIDENCES OF THE PROKARYOTIC NATURE OF THE OUTER MEMBRANE OF ENDOSYMBIONTS FROM TRYPANOSOMATIDS

Motta MCM, Morgado-Díaz JA*, Cavalcanti D, De-Simoni GS*, De Souza W
Laboratório de Ultraestrutura Celular Hertha Meyer, Instituto de Boofísica Carlos Chagas Filho, UFRJ, Rio de Janeiro, RJ *Departamento de Bioquímica e Biologia Molecular, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil

The nature of the outer membrane of endosymbionts from trypanosomatids is controversial. Some authors consider that this membrane was acquired from the host in an early stage of cell entry while others propose that it derived from the prokaryote itself. We used two approaches to analyse this question: the analysis of the effect of Polymixin B, an antibiotic which exert lethal action only in Gram-negative bacteria by acting in the outer membrane surface, thus altering the osmotic properties and morphology of the prokaryote, and biochemical analysis of isolated endosymbionts. Electron microscopic analysis of *Blastocrithida culicis* incubated in the presence of 100 ㎎/ml of polymixin B showed that the endosymbiont undergoes morphological changes. This process initiated with an increase in the space between the two membranes which outline the symbiont, forming a "bubble-like" structure. Sucessive passages in culture medium containing the same drug concentration untied the external membrane and induced the loss of all the prokaryote envelope. This process culminated in a complete degradation of the symbiotic bacteria in the host cytoplasm.In order to investigate the nature of the endosymbiont outer membrane proteins, we carried out TX-114 extraction of a symbiont fraction. Upon electrophoresis, 10% SDS-PAGE, the hydrophobic phase yielded five bands with molecular weight of 60, 45, 35, 20 and 18 kDA by silver staining. The 35 kDA membrane protein may likely correspond to one of the well-characterizad porines displayed in the outer membrane of Gram-negative bacteria.

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BI-63
EXOERYTHROCYTIC DEVELOPMENT OF *PLASMODIUM GALLINCEUM* SPOROZOITES IN A PRIMARY CULTURE OF CHICKEN EMBRYO FIBROBLAST: IMMUNOFLUORESCENCE AND ELECTRON MICROSCOPY STUDY

Couto-Lima D, Lourenço-de-Oliveira R*, Meirelles MN, Porrozzi R
Laboratório de Ultra-estrutura Celular, Departamento de Ultra-estrutura e Biologia Celular *Laboratório de Transmissores de Hematozoários, Departamento de Entomologia, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil

Studies on exoerythrocitic (EE) development of *Plasmodium* sp. have demonstrated the crucial role of this stage to control malaria infection. Antibodies and T cells against EE forms can fully protect animals and humans by inhibiting/killing EE development. However, a safety and efficient sub-unit, recombinant or DNA vaccine have not been achieved. One of the reasons might be the poor knowledge regarding the biology of this no symptom "silent" part of the complex life cycle of *Plasmodium*. *P. gallinaceum* is a chicken parasite and has been pointed as phylogenetic closely related to *P. falciparum* (McCutchan et al. 1996 *Proc Natl Acad Sci USA 93*: 11889-11894). In the case of mammalian malaria, sporozoites target cells are hepatocytes while in birds they invade and develop in a variety of cell types, including macrophages, endothelial cell and fibroblast. Only in avian malaria a secondary EE cycle can be originated by merozoites from both, exoerythrocytic and erythrocytic cycles suggesting that the cells types and parasite form involved share receptor and ligands. In the past years attempts of cultivating EE forms from *Plasmodium gallinaceum* sporozoítes have been achieved and the potential of antibodies tested (Rocha et al. 1993 *J Euk Microbiol 40*: 64-66, Ramirez et al. 1995 *J Euk Microbiol 42*: 705-708).

We obtained a primary culture of embryo chicken fibroblast from enzymatic dissociation of skeletal muscle and sub-cultivation was performed in order to purify the culture from muscle cell contamination. Sporozoites obtained from infected *Aedes fluviatilis* were added to the cultures and assayed for immunofluorescence to circumsporozoite protein and electron microscopy.

Three hours after sporozoite inoculation to the cultures, they were seen attached strongly to the cell surface. After 24 hr sporozoites appeared transformed into trophozoite and the fluorescence was preferable in the periphery. By 48 hr schizonts of different sizes were observed. The transmission electron microscopy revealed that prior the penetration sporozoites attach also to the cell surface with their body instead by the apical portion. The subsequent steps are under investigation and it may contribute for the understanding of the malaria parasites invasion mechanism and molecules involved during their interation with the host cell.

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BI-64
EXPERIMENTAL INFECTION OF TOMATOES (*LYCOPERSICON ESCULENTUM*) AND MAIZE (*ZEA MAYS*) WITH TRYPANOSOMATIDS ISOLATED FROM INSECTS

Cavazzana Jr. M, Batistoti M, Romão C, Takimoto CA, Baccan GC, Jankevicius JV, Ogatta SFY, Yang AV, Itow Jankevicius S
Departamento de Microbiologia, Universidade Estadual de Londrina, Centro de Ciências Biológicas, 86051-900 Londrina, PR, Brasil

In the nature several insects of Hemiptera order are able to harbor and to transmit trypanosomatids to plants and other insects living on those plants. Cultures of economic interest such as soybean, bean, maize, tomatoes, grape, orange, apple, mulberry, Surinan-cherry, etc. were shown to be infected and the role of phytophagous hemiptera Coreidae (*Phthia picta*) and Pentatomidae (*Nezara viridula*,) as vectors was also demonstrated. In this study we evaluated the possibility of monoxenous trypanosomatids to survive and to multiply in vegetable hosts by experimental infection and their capacity to develop heteroxenous cycle in the nature. Maize grains and tomatoes fruits cultivated in greenhouse and protected against insects naturally infected with trypanosomatids, were experimentally infected with strains of trypanosomatids with promastigotes and choanomastigotes forms, by using *Leptoglossus zonatus* as the vector insect. The trypanosomatids were isolated from phytophagous hemiptera of several genera collected in regions of São Paulo and Paraná. All the strains of trypanosomatids used in this study were previously characterized by enzymatic profile of urea cycle and showed a profile similar to that of genera *Phytomonas*, *Leptomonas*, *Crithidia* and *Herpetomonas*. The insect *L. zonatus* was experimentally infected by feeding on mature tomatoes infected with each strains. The experimental infection showed that all strains were able to develop in the insect *L. zonatus* and to migrate for salivary gland, including the trypanosomatids with choanomastigote forms. All the insects tested were infected. It was demonstrated that 100% of the strains of trypanosomatids were transmitted to tomatoes and 85% to maize. These results suggested that trypanosomatids of *Leptomonas*, *Herpetomonas* and *Crithidia* genera as well *Phytomonas* genus can grow in plant hosts and our results also showed that these genera can also develop a heteroxenous and monoxenous cycles in the nature. Moreover it is suggested that tomato and maize can be reservoir of pro and choanomastigote trypanosomatids and with aid of hemiptera phytophagous can be dispersed to other hosts and reservoirs.

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BI-65
EXPRESSION OF GALACTOSYL RESIDUES DURING THE *IN VITRO* INFECTION OF *TRYPANOSOMA CRUZI* IN HEART MUSCLE CELLS

Barbosa HS, Soeiro MN, Guimarães EV, Rodrigues RM, Meirelles MNL
Laboratório de Ultra-estrutura Celular, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil

We have accumulated data in the last years, which have demonstrated the participation of galactosyl residues during the invasion of *Trypanosoma cruzi* in heart muscle cells [HMC] (Barbosa & Meirelles 1992 *Parasitol Res 8*: 404, Barbosa & Meirelles 1993 *J Submicrosc Cytol Pathol 25*: 47). To study the expression of the galactosyl residues during the intracellular development of the parasite in HMC, we employed cultures infected with blood-stream forms, Y strain, for periods up 24 to 96 hours. The infected cells were fixed in 4% PFA + 0.1% glutaraldehyde in PBS, embedded in Lowicryl resin and processed for transmission electron microscope. The ultra-thin section were incubated with TBS + Tween + BSA for 30 min, labeled with RCA-lectin gold particles complex diluted in the same buffer for 1 hour at room temperature and analyzed under Zeiss EM10C TEM.

The present results showed that non-infected cultures displayed few RCA-Au particles over the sarcolemma of HMC suggesting a weak expression of galactosyl residues at the cardiomyocytes surface. The marker was also found in cytoplasmatic vesicles and was enriched in cell-cell adhesion regions. After *T. cruzi* infection, no considerable alteration of RCA binding on the HMC surface was detected, however a higher positive intracellular labeling usually close to the parasites was observed in the cytoplasm of 72-96 hr infected cultures. Likewise, intracellular parasites also displayed gold particles, but there was a difference to the RCA labeling related to the parasite evolutive stage. Cultures infected for 48 hr showed intracellular amastigotes were little reaction with RCA lectin. The expression of galactosyl residues was more intensive after transformation of the amastigotes into trypomastigotes 72-96 hr after host cell invasion. The labelling was observed on the surface of parasite, more expressive in the flagellar pocket and also on the flagellar membrane. Our results demonstrate for the first time the expression of galactosyl residues in *T. cruzi* during the infection of heart muscle cells, which has been described to have an important role in the process of parasite-host cell interaction.

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BI-66
EXPRESSION OF THE ENDOPLASMIC RETICULUM ENZYMES IN THE *TRYPANOSOMA CRUZI* INFECTED AND NON INFECTED CARDIOMYOCYTES

Silva DT, Meirelles MNL
Laboratório de Ultra-estrutura Celular, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil

We have previously shown that Ca^{++} ATPase of the sarcoplasmic reticulum (SERCA) is involved in the process of invasion of cardiomyocytes by *Trypanosoma cruzi*. Treating cardiomyocytes (CM) with 1, 2 and 4mM of Thapsigargin, a tumor-promoting sesquiterpene lactone that binds to all SERCA ATPases and causes an irreversible inhibition of their activity, we found an inhibition of 64 to 68% in the *Trypanosoma cruzi* CM invasion. Also, this drug affected the beginning of the proliferative stage of the intracellular forms of the parasite. Here we investigated whether the Glucose-6- Phosphatase (G6Pase), an enzyme that control the rate of breakdowm of carbohydrates via glycolysis, is affected in *T. cruzi* infected CM.

To investigate the distribution of glucose-6-phosphatase, the cells were fixed with 1% GA and 1% PFA for 20' at 4°C and incubated in a modified cytochemical medium containing 3 mM glucose-6-phosphate and 2mM $CeCl_3$.The control was performed in absence of the substrate. G6Pase activity was localized in SR cistern of the CM and within the nuclear envelope. Profiles of the SR were seen encircling the mitochondria and myofibrils. Infected CM did not display G6Pase in the SR profiles. In the intracellular parasites the activity was localized in the Golgi complex, in the flagellar pocket and in the plasma membrane, but no reaction was observed in the reticular structure. Heavily infected CM showed G6Pase distribution on the sarcolemma. Similar results were previously observed when we treated heavily infected CM with potassium iodide-osmium tetroxide solution.

Glucose-6-phosphatase is a multifunctional enzyme distributed in a variety of cell types of various organs, but in muscle cells it seems acts specifically at glucose-6-phosphate hydrolysis. This enzyme is tightly bound to the endoplasmic reticulum membrane. We observed that non infected cells from a infected culture expressed a normal G6Pase activity, but in infected cells this activity was not detected. This observations suggest a possible effect of *T. cruzi* infection in the host cell glucose metabolism.

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BI-67
FIBRONECTIN AND LAMININ EXPRESSION DURING *TRYPANOSOMA CRUZI*-HEART MUSCLE CELLS INTERACTION *IN VITRO*

Calvet CM, Pereira MCS, Nogueira AR, Meirelles MNL
Laboratório de Ultra-estrutura Celular, Departamento de Ultra-estrutura e Biologia Celular, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil

Extracellular matrix (ECM) is involved in many cellular processes including adhesion, differentiation and cell recognition. It has been demonstrated that fibronectin (FN) mediates the adhesion of pathogens to the host cell with an subsequent increase of infection. Chronic Chagas's diseases promotes an enhancement of extracellular matrix accompanied by tissue fibrosis as consequence of chronic inflammation (Andrade et al. 1989 *Am J Trop Med Hyg 40*: 252, Sanchez et al. 1993 *Am Heart J 126*: 1392). Our interest is to analyze the expression of fibronectin (FN) and laminin (LM) during acute phase of experimental Chagas's diseases *in vitro* and its specific interaction via integrin receptors with the cytoarchiteture organization.

To investigate the expression of ECM during *Trypanosoma cruzi*-heart muscle cells interaction, uninfected and trypomastigotes-infected cells grown on coverslips were fixed with 4% PFA in PBS and incubated with anti-fibronectin or anti-laminin antibodies in a 1/500 dilution. After rinsing, the cell were incubated in a 1/200 dilution of TRITC or FITC-coupled anti-rabbit IgG. Indirect immunofluorescence was performed after 24, 48, 72 and 96 hr of *T. cruzi* – heart muscle cells interaction. We also analyzed cryosections of normal and infected murine heart tissue at the peak of parasitemia.

An enhancement of FN expression was observed during heart muscle cells myogenesis, showing an intrinsic network. After 24 and 48 hr of *T. cruzi* interaction no substantial change in FN expression was detected. However, at later time of infection (72 and 96 hr) its expression decreased, showing a weak or no fluorescence signal in highly infected cells. In contrast, no significant change in LM expression was observed even in highly infected cells. Normal heart cryosections displayed an intense FN and LM labeling. In infected tissue, FN expression was decreased while LM expression was significantly enhanced. Our data demonstrates a distinct pattern of ECM expression during cardiac cell myogenesis and suggests changes in the FN and LM expression which was induced by *T. cruzi* infection.

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BI-68
GALACTOSE BINDING PATTERN ON *TRYPANOSOMA CRUZI* INFECTED AND NON INFECTED MOUSE HEPATOCYTE PLASMA MEMBRANE

Soares R, Santos CL, Cruz WB, Meuser M, Porrozzi R, Meirelles MNL
Laboratório de Ultra-estrutura Celular, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil

We have previously described that *Trypanosoma cruzi* infection in mouse hepatocyte cultures allow not only the invasion but also the development of the complete cycle of the parasite (Porrozzi et al. 1997 *Mem Inst Oswaldo Cruz 92*: 117-120). Here we investigate whether macromolecules on the cell membrane , especially carbohydrate-containing compounds, that are responsible for a number of specific functions ocurring at cell surface, could be involved in recognition events of the *T. cruzi*-hepatocyte interaction. The presence of the galactose specific receptor has already been described in rat liver cells (Kempka & Kolb-Bachofen 1985 *Biochem et Biophy Acta 1008*: 114).

Hepatocytes obtained by collagenase digestion of mouse embryos livers were partially purified by centrifugation and cultivated as long term cultures in a defined medium. To investigate the surface distribution of galactose residues we incubated normal hepatocyte cells for fluorescence, and scanning electron microscopy (SEM) with RCA 120 - FITC or coupled to 15nm colloidal gold particles, respectively. *T. cruzi* infected and non infected cells were studied by transmission electron microscopy (TEM) with two treatments: fixing the cells with glutaraldehyde (GA) for one hour, after exposing to the galactose- colloidal gold marker; incubating the cells *in vivo* with RCA 120 colloidal gold for one hour, at 4°C, following fixation with GA.

Hepatocytes cultures preserved their original poligonal shape and bile canaliculi as characteristic of well differentiated liver parenchymal cells. Fluorescence partterns showed uniform distribution in the whole surface of cells. SEM microscopy displayed colloidal gold particles almost uniformly over the entire hepatocyte membrane. The TEM on normal and fixed *T. cruzi* infected cells shawed a regular distribution of galactose residues in its surface; *in vivo* cells colloidal gold particles were seen in pits and invaginations of its surface but with a less expression. When we observed *T. cruzi* infected cells under the two treatments, rare gold particles were observed over the hepatocyte plasma membrane. These differences in the expression of galactose residues between normal and infected cells seems to be directely related to the presence of the parasite. New protocols are being done in order to understand the decrease in the galactose binding sites in *T cruzi* infected hepatocyte cells.

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BI-69
GENETIC DIVERSITY OF *PLASMODIUM VIVAX* MSP-1 GENE FROM THE BRAZILIAN AMAZON FIELD ISOLATES BY PCR-RFLP: ANALYSIS OF CLINICAL RECRUDESCENT VIVAX SAMPLES

Jeovanio-Silva AL, Laserson K*, Fontes CJ**, Duarte EC***, Kain CK****, Barnwell J*****, Wirth FD*, Zalis MG
Laboratório de Biologia da Malária, Instituto de Biofísica Carlos Chagas Filho, UFRJ, RJ, Brasil *Department of Tropical Public Health, Harvard School of Public Health, Boston, MA, USA ** Faculdade de Ciências Médicas, Universidade Federal de Mato Grosso, Cuiabá, MT ***Fundação Nacional de Saúde, Coordenadoria Regional de Mato Grosso, MT, Brasil ****Tropical Disease Unit, Toronto Hospital, Toronto, Canada *****Departament of Medical and Molecular Parasitology, NYU Medical Center, New York, USA

In order to extend epidemiological and molecular data on the biology of the *Plasmodium vivax* parasite, we analyzed the genetic polymorphism of Brazilian field isolates from the State of Mato Grosso (Brazilian Amazon region). Variable regions of the PvMSP-1 gene of 50 samples were amplified by PCR, showing heterogeneity of fragments which were distributed in four allelic groups which showed diversity of prevalence and a low frequency of mixed infections (16%). The digestion of 46 samples by the endonuclease RsaI, visualized by ethidium bromide stained 3% low melting agarose electrophoresis gel, allowed the observation of two stronger bands that enabled us the identification of three restriction patterns (Restriction Fragment Length Polymorphism). In addition, three recrudescence samples of *P. vivax* were also analyzed by the same methodology. The majority of the PCR fragments wich the same size showed the same RFLP pattern, but our results suggests that different RFLP patterns could also be presented .The PvMSP-1 PCR study in recrudescent samples showed both the same and different RFLP patterns from the pre-recrudescent sample. This data shows the low polymorphism of South American vivax strains and the preliminary results do not correlate MSP-1 with clonal selection in chloroquine or primaquine resistance.

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BI-70
GLYCOCONJUGATES OF *TRYPANOSOMA CRUZI* STRAINS ISOLATED IN THE NORTHWEST OF THE STATE OF PARANÁ

Nakamura CV, Abreu Filho BA, Bittencourt NLR, Araujo SM, Gomes ML, Toledo MJO, Nakamura TU, Dias Filho BP
Departamento de Análises Clínicas, Centro de Ciências da Saúde, Universidade Estadual de Maringá, 87020-900 Maringá, PR, Brasil

The purpose of the present study was to compare the glycoconjugates composition of nine *Trypanosoma cruzi* strains isolated from chagasic chronic patients, opossum or reduviid insect, in northwest of the Paraná state, Brazil. For the experiments, the flagellates epimastigotes forms (036, 150, 168, 1256, G1, G3, F3, F47, N914A and Y strains) were grown in LIT medium. Cells were solubilized overnight at 0ºC in 10 mM Tris saline buffer, pH 7.4, containing 2% Triton X-114. Insoluble material was removed from the lysate by centrifugation at 20,000 g for 20 min. The hydrophobic and hydrophilic phases were separated with a 6% sucrose cushion (1:1.5, vol/vol), and analysed in 15% SDS-PAGE gels. Gels were stained by soaking in 0.25% Coomassie blue R-250 or in periodic acid-Schiff reagent. The glycoconjugates of all *Trypanosoma* were preferentialy found in the hydrophobic phase rather than in hydrophilic phase. Relatively simple glycoconjugates profiles with only 3 to 6 major bands were observed in all sample. Only minor quantitative differences in the glycoproteins profiles (43-82-kDa) of the *T. cruzi* 150, 168, 1256, and Y strains isolated from human, G1 and G3 strains isolated from opossum, and F3 and N914A strains isolated from insect were detected. The 036 and F47 strains isolated from human and insect, respectively, produced at least one additional major band (32- kDa). Glycoprotein band with 82-kDa was observed in *T. cruzi* 150 and G1 strains. One broadly stained glycoconjugate band with apparent molecular weight of 14-kDa was present in all ten *T. cruzi* preparations. These results are very similar to that described by Branquinha et al. (1995 *Current Microbiol 30*: 77-82) who suggest that Triton X-114-extracted glycoconjugates could be useful markers for trypanosomatid taxonomy. Morever, for the first time, Parana state authoctonous strains (reservoir and insect) were analysed by this technique. Studies are in progress aiming to determine the release of this glycoconjugates by exogenous specific phospholipase and the occurrence of phospholipase-resistant forms.

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BI-71
HARVESTING OF *ISOSPORA BELLI* OOCYSTS FROM FAECAL SAMPLES USING A GRAVITY FLOATATION RECOVERY TECHNIQUE

Leite SM, Fonseca RA,Torno CO, Silva E,Garcia ZM, Cuba Cuba CA
Departamento de Patologia e Parasitologia Médica, Universidade de Brasília, 70910-900 Brasília, DF, Brasil

A supply of human coccidian sporulated oocysts is an initial requirement in investigations aiming at the isolation of viable coccidial sporozoites and further experimental studies. *Isospora belli*, a recognized opportunistic pathogen protozoa in HIV+/AIDS immunocompromised patients, is prevalent in Brazil and other tropical and subtropical countries. In Brasilia, DF we found in 5.3% of our documented series of these patients. To recover significant number of oocysts: sporoblasts and sporocysts of *I. belli*, relatively clean from faecal human detritus, applying a gravitiy floatation technique in stools of AIDS patients. Taking advantage of the technique described by AR Jackson (1964 *Parasitology 54*: 87-93) for studies on *Eimeria* (Sporozoa, Coccidia) we adapted the gravitiy floatation Perspex sheet recovery technique, with minor modifications, to isosporiasis faecal samples.Briefly, we pour out into stainless circular plates (11 cm diameter, 2 cm depth) a mixed suspension of a positive *I. belli* faecal sample with equal amount of a concentrated sucrose solution (150 g of sucrose in 100 ml of water/phenol). A sheet of OFREX (AV write –on film projection) cut to fit exactly the surface area of the plate is floated on the mixture during 2 hours. The oocysts accumulate in the layer of solution in contact with the sheet. As the sheet is carefully lifted-off, the oocysts are retained in the film of fluid held to the OFREX *by surface tension.* Then the oocysts are washed off with a known amount of saline and counted in (Neubauer chamber or under 22 x 22 coverslip, 5 ul of concentrated sediment) and adjusted to number/ml. A total 114,900 clean oocysts (80% sporocysts and 16% sporoblasts) were recovered from 5.5 ml of the same faecal preserved sample after three consecutive harvesting assays. Some variation of the amount of oocysts recovered were observed among the consecutive sampling (X=38,300 ± 18,663) probably related to sampling procedures and the process counting. The method described allows to obtain reproducible high yield of ooocyst of *I. belli* reasonably free of faecal debris. If carried out on preserved samples ($K_2Cr_20_7$-2.5%, suspension), sporulated forms (sporocysts) are easily collected. Restirred, again the same sample provides further harvest.

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BI-72
HEART DENERVATION IN ACUTE EXPERIMENTAL CHAGAS' DISEASE IN RATS AND RESPONSE TO NEUROTOXINS

Teixeira Jr. AL, Fontoura BF, Freire-Maia L, Camargos ERS*, Machado CRS*, Teixeira MM
Laboratório de Imunofarmacologia e Farmacologia Cardiovascular, Departamento de Farmacologia, ICB-UFMG
*Laboratório de Neurobiologia, Departamento de Morfologia, ICB-UFMG, Avenida Antônio Carlos 6627, 31270-901 Belo Horizonte, MG, Brasil

In the acute phase of experimental Chagas' disease in rats, there is a severe noradrenergic and cholinergic denervation of the heart demonstrated by morphological and biochemical methods. The aim of the present study was to determine whether autonomic denervation, as demonstrated morphologically, was accompanied by impairment of heart function. In order to stimulate the release of cathecolamines and acetylcoline from nerve endings and hence activate the heart, we employed the crude venom from *Tityus serrulatus* scorpion. Holtzmann rats aged 27-30 days were inoculated intraperitoneally with 300,000 tripomastigotes of the Y strain of *Trypanosoma cruzi*. Control and infected animals were killed 20 days after inoculation. We have previously shown this to be the time of maximal denervation of the heart. The hearts were isolated and perfused with Locke solution using Langendorff's method. Cardiac contraction and eletrocardiogram were recorded simultaneously. Before the perfusion, auricular appendages were removed for histochemical visualization of noradrenergic (glyoxylic acid-induced fluorescence) and cholinergic (acetylcolinesterase activity) innervation. In control animals, the administration of venom induced initially a significant bradycardia followed by oscillation of heart rate. The minimum (62 ± 4 % fall) and maximal (36 ± 11% increase) heart rates after the injection of the venom were used as an index of cholinergic and adrenergic stimulation, respectively. In infected animals, noradrenergic and cholinergic denervation was almost complete, as assessed morphologically. Nevertheless, in these animals we observed a similar initial decrease of heart rate (61 ± 12%) and a similar late increase of heart rate (41 ± 14%). Despite the noradrenergic and cholinergic heart denervation in acutely infected rats, the effects evoked by the crude venom were not statistically different from the control group. The results suggest that the morphological alterations in the acute phase of experimental Chagas' disease in rats are not accompanied by a significant change in heart rate following the activation of adrenergic and cholinergic nerve endings with scorpion venom.

BI-73
HEMATOLOGICAL AND BLOOD CHEMISTRY CHANGES DUE TO TRYPANOSOMOSIS IN DAIRY CATTLE FROM THE PANTANAL OF NABILEQUE, BRAZIL

Dávila AMR/+, Ramirez L*, Ortiz AG**, Pereira SR**, Souza SS*, Silva RAMS***
Embrapa/Pantanal, Corumba, MS *UFMS/CEUC/DAM, Av. Rio Branco 1270, Corumbá, MS **UEMS, Unidade de Aquidauana, Aquidauana, MS ***Laboratório de Sanidade Animal, EMBRAPA/Suinos & Aves, Br 153, km 110, 89700-000 Concordia, SC, Brasil
+Present address: Lab. de Biologia Molecular de Tripanosomatídeos, DBBM, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil (E-mail: amrdavila@hotmail.com)

Bovine trypanosomosis due to *Trypanosoma vivax* has been reported as a wasting disease in the Pantanal, Brazil. We evaluated some pathophysiological changes in bovines from the Nabileque sub-region naturally infected by *T. vivax*. In this study the prevalence observed for *T. vivax* was 40.50% (17/40). The principal haematological changes produced by *T. vivax* infections were anaemia and leukopenia. The cattle presented macrocytic hipochromic anaemia. The haematological examination showed the following parameters: erythrocytes ($x10^6$/ml): 3.11 ± 1.12; haemoglobin (g/dl): 7.74 ± 1.41; PCV (%): 29.60 ± 4.43; MCV (fl): 98.07 ± 26.60; MCH (pg): 27.04 ± 7.23; MCHC (g/dl): 27.36 ± 3.22. Total leukocytes ($x10^3$/ml): 1.54 ± 0.81; Neutrophils (%): 35.62 ± 10.44; Lymphocytes (%): 44.43 ± 10.85; Monocytes (%): 11.87 ± 4.61; Eosinophils (%): 7.5 ± 4.58; Basophils (%): 0.56 ± 1.50. Blood chemistry parameters (mean ± SD) were: total proteins (g/dl): 7.84 ± 1.24; total lipids (mg/dl): 136.81 ± 86.12; blood urea nitrogen (mg/dl): 23.40 ± 8.73. Normal values*: total proteins: 7.56 ± 0.5*; total lipids: 34.8**; blood urea nitrogen: 6.0-27*. Increase in serum values of total lipids was observed. No changes in serum total protein values and blood urea nitrogen were observed. According to some authors, in addition to the changes observed in glycogen, lipid, and protein metabolism in an African trypanosome infection, there are strong indications of endocrine dysfunction. Although we are showing evidences of haematological and pathophysiological changes in the lipids metabolism of infected bovines from the Pantanal, further studies should be done to know more about the disease in South America.

PCV: packed cell volume; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration. *Coles (1984), **Kolb (1974)

BI-74
HEMATOLOGICAL, CELULAR AND HUMORAL ALTERATIONS IN *CALOMYS CALLOSUS* INFECTED WITH CG STRAIN OF *TRYPANOSOMA CRUZI*

Santello FH, Dost CK, Toldo MPA, Levy AMA, Santos DR, Souza AM, Garia TAR, Prado Jr. JC
Faculdade de Ciências Farmacêuticas, USP, Av. do Café s/n, 14049-903 Ribeirão Preto, SP, Brasil

Unicellular organisms morfologically similar to *Trypanosoma cruzi* circulate in nature from vertebrate to invertebrate hosts. The importance to identify these flagellates from triatomines and reservoirs implies the utilization of different criteria. In this work it was studied some biological features of a silvatic *T. cruzi* strain (CG) isolated from *Panstrongylus megistus* were studied. Hemogram, myelogram, peritoneal macrophage profile and complement mediated lysis were done. *Mus musculus* infected with the CG strain showed absence of parasitemia. For this reason, an alternative model was used. One month old male *C. callosus* weighing 20-25gr. were inoculated i.p. with 4,000 blood trypomastigotes of CG strain. The samples were collected on days 11^{th}, 15^{th}, 19^{th} (peak of parasitemia) and 32^{nd} days after infection. Celular response: At the peak of parasitemia, peritoneal macrophages reached the highest values and showed a more intense citoplasmatic basophilia denoting a high state of activation. Hematological alterations: This strain seems to cause slight hematological alterations. Hematocrit and erythrocyte values were unchanged throughout the observed period. On 19^{th} day, a non-significant drop on the levels of hemoglobin levels was noted, when compared to control groups. Unexpectedly lower platelet counts were observed during the ascendent phase of the infection (15^{o}day). Myelogram: On 19^{th} day, the infected group showed an increase of the mononuclear cells (lynphoblast and pro-lymphocite) and some cells with basophilic cytoplasm, probably plasmocytes. Humoral response: On 19th day p.i., lytic antibody levels reached the highest values (67% of lysis) decreasing to lower levels on 32th day (46%). At this period scarce number of parasite could be seen in circulation, but sufficient to maintain the production of lytic antibodies in the late phase of the disease (32^{nd} day). Analyzing these data, we conclude that CG strain has a similar behavior to that of several strains like Colombiana, F, Costalimai among others which express the ability to elicit the immune response here represented by a higher activation of macrophages, presence of significant lytic antibody titers, discrete hematological changes, negative mortality rate and a certain tissue damage. In previous experiments, despite the fact that *M. musculus* showed negative parasitemia, liver, heart and spleen imprints displayed some intracellular and free amastigotes an d rare loose intermediate forms, confirming the behaviour of a typical *T. cruzi* strain.

BI-75
HISTOPATHOLOGIC ANALYSIS OF *TRYPANOSOMA CRUZI* POPULATIONS ISOLATED FROM VERTEBRATE HOSTS AFTER 8 AND 17 YEARS OF INFECTION

Veloso VM, Carneiro CM*, Andrade EM, Soares KA, Lana M*, Tafuri,WL, Chiari E**, Bahia MT
Instituto de Ciências Exatas e Biológicas, Departamento de Ciências Biológicas *Escola de Farmácia, Departamento de Análises Clínicas, UFOP **Instituto de Ciências Biológicas, Departamento de Parasitologia, UFMG, Belo Horizonte, MG, Brasil

Biologic and molecular studies of different *Trypanosoma cruzi* isolates have reported strong polymorphism. In 1990 Carneiro et al. verified that successive passages of *T. cruzi* strains in mice induced selection or changes in the population. One explanation for genetic variations could be that natural selection occurs during *T. cruzi* infection in vertebrate hosts because of immune response. To verify this phenomenon, comparative studies were performed using Colombiana strain and 2 isolates from Pinscher dogs infected with this strain 8 (Co-A) and 17 (Co-B) years ago. Biologic parameters (infectivity, parasitemia, mortality and histopathology) were comparatively evaluated in Swiss mice inoculated through the intraperitoneal route with 5,000 trypomastigotes of the 10th, 15th and 20th passages in mice of the Co-A and Co-B isolates as well as the original Colombiana strain. Parasitemia was evaluated daily according to the method described by Brener in 1962. Histopathologic studies were carried out during peak parasitemia (acute phase) and on the 100th day of infection (chronic phase). Necropsy was complete with systematic collection of brain, heart, endocrine and exocrine glands and digestive and genito-urinary tracts. During the acute phase, inflammatory infiltrates composed predominantly of mononuclear cells associated or not with intact or disrupted amastigote nests (pseudocysts) were observed. These findings were most striking in the heart, followed by smooth and skeletal muscles, digestive and genito-urinary tracts and glands. Parasitism and inflammation were not observed in the brain. There were no differences in tissue tropism between Colombiana strain and the two isolates (Co-A and Co-B). All lesions were more intense in animals infected with Colombiana strain compared to Co-B and Co-A caused lesions with intermediate intensity. During the chronic phase animals infected with Co-B had more moderate lesions than animals infected with Co-A. These data agree with parasitologic results that revealed important differences related to peak parasitemia and mortality rates which were, respectively: $1.2x10^6$ and 100% for Colombiana strain; $9.6x10^5$ and 35% for Co-A; and $4.0x10^5$ and 5% for Co-B. These results suggest that *T. cruzi* undergoes changes and/or selection throughout the infection in vertebrate hosts. This selection could depend on the parasite-host relationship. Further biochemical and genetic characterization of the three populations will be done.

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BI-76
HUMAN CUTANEOUS LEISHMANIASIS IN THE NORTHEASTERN REGION OF SANTA CATARINA, BRAZIL

Steindel M/***, Lima JH/***, Marcondes CB, Cório da Luz A*, Eger-Mangrich I, Grisard EC/**
Departamento de Microbiologia e Parasitologia, Universidade Federal de Santa Catarina, Florianópolis, SC, Brasil (E-mail: ccb1mst@ccb.ufsc.br) *Fundação Nacional de Saúde, SC **Department of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, CA, USA ***Núcleo de Pesquisas em Dermatologia, Serviço de Dermatologia, HU, UFSC, Florianópolis, SC, Brasil

An autochthonous case of american cutaneous leishmaniasis (ACL) was detected in a 45 year-old woman from Piçarras municipality, northeastern region of the State of Santa Catarina. The patient presented a single ulcer in the distal part of the right leg, which yielded a positive biopsy with the presence of amastigote forms, as well as a positive Montenegro skin test. After a 30-days treatment with Glucantimeâ and local heat, the lesion healed. Biopsy material was inoculated in hamster footpads, and culture of the lesion in Schneider's Insect Medium at 26ºC was positive. Promastigote forms were harvested in the exponential growth phase, washed in PBS pH 7.4 and submitted to indirect immunofluorescence assays (IFA) against a panel of monoclonal antibodies (Mab). In addition, parasite DNA was extracted and PCR amplification of the mini-exon gene was performed as described previously (Murthy et al. 1992 *Mol Cell Probes 6*: 237-243). Amplification products were submitted to hybridization with probes specific to the New World dermotropic group (*Leishmania amazonensis*), to the *Viannia* Subgenus group (*L. braziliensis*) and to the Viscerotropic group. The strain was named MHOM/BR/LSC/97-H4 and only reacted in IFA with the anti-*L. braziliensis* B-12 Mab. The mini-exon gene amplification product showed the same migration pattern of the *L. braziliensis* control strain, and hybridized only with the S-1593 probe that is specific for the *Viannia* group. A survey was undertaken in two localities of the Piçarras municipality (Morretes and Medeirinhos) using the Montenegro skin test. Two men out of 72 inhabitants were revealed positive, one from each locality. Both individuals had always lived in this region and denied having signals compatible with ACL. A preliminary study on the Phlebotomine sandfly fauna was performed in these areas using CDC-like traps for three nights. To date, among 20 insects collected, 19 were identified as *Lutzomyia neivai* and one as *L. lanei*. Our results confirms a new case of ACL due to *L. braziliensis* at the northeastern region of the State. These data together with the reported outbreak of ACL in the western region of the Santa Catarina State (São Thiago & Guida 1990 *Rev Soc Bras Med Trop 23*: 201-203), suggest that new ACL cases might be expected in other regions of this state.

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BI-77
HUMAN CYCLOSPORIASIS DIAGNOSIS - REPORT OF A CASE IN SÃO PAULO, SP, BRAZIL

Fernandes AOP, Carollo MCC, Braz LMA, Amato Neto V, Villela MSH, Pinto THL
Laboratório de Investigação Médica, Parasitologia, Instituto de Medicina Tropical, FMUSP, Av. Dr. Enéas Carvalho de Aguiar 500, 1º andar, sala 9, 05403-000 São Paulo, SP, Brasil

For several years, *Cyclospora cayetanensis* was taken as a "cyanobacterium-like body" or a large *Cryptosporidium*. Only as recently as 1993, Ortega et al., succeeded at inducing the sporulation of the *Cyclospora* oocysts and noticed that they presented, when mature, two sporocysts, each one containing two sporozoites inside. The oocysts are 8 to 10 mm in diameter. Clinical manifestations most commonly observed during the cyclosporiasis are: fatigue, nausea, abdominal cramps, anorexia, weight loss of 5% to 10%, vomiting and diarrhea. By means of this communication we have reported the finding of *C. cayetanensis* in a feces examination at the request of an individual in São Paulo, Brazil. In April, 1998, the Parasitology Laboratory of the " Instituto de Medicina Tropical de São Paulo", received a sample of feces from a 30 years old man. This happened during the comparative study of applied techniques to diagnosis the criptosporidiasis. The analysis of the pasty sample was done, by the modified Kinyoun staining method, and acid-fast organisms stained deep pink, of 8 to 10 mm in diameter, were observed. To verify sporulation of the possible *C. cayetanensis* oocysts, a solution of 2.5% potassium dichromate was added and maintained at 28°C for seven days. After this, spherical bodies, containing two sporocysts; which were not seen before in the fresh sample, were seen in the light microscope. Thus, because of this feature, it was possible to confirm that it was *C. cayetanensis* oocysts. In Brazil, in 1995, Araújo et al. identified *Cyclospora* in patient feces with the human immunodeficiency virus (HIV). In 1997, Yal et al. identified the protozoan, for the first time, in dog feces. Through this report, we wish to call attention to this protozoan, which must be infecting more people than is realized, and to point out that it is becoming imperative to bring this out by means of techniques which already exist and which are not necessarily complex. Besides this, we wish to note that it is important not to forget, that now with the constant rise of immunocompromised patients, and the increased influence of HIV and subsequent AIDS, that cyclosporiasis should not be overlooked as an opportunistic infection.

BI-78
IDENTIFICATION OF *LEISHMANIA* ANTIGEN IN THE KIDNEY OF NATURALLY INFECTED DOGS WITH VISCERAL LEISHMANIASIS

Costa FAL, Goto H*/**, Mathias R*, Silva SMMS, Klein RP, Sousa MCBB****, Guerra JL****
Departamento de Clínica e Cirurgia Veterinária, Centro de Ciências Agrárias, UFPI, 64049-550 Teresina, PI, Brasil *Instituto de Medicina Tropical de São Paulo (LIM-38/HC-FMUSP) **Departamento de Medicina Preventiva, FMUSP ***Departamento de Morfofisiologia Veterinária, Centro de Ciências Agrárias, UFPI ****Departamento de Patologia, FMVZ, USP, São Paulo, SP, Brasil

In the city of Terezina, State of Piauí, visceral leishmaniasis is endemic and the dog is the main domestic reservoir. We previously reported different renal alterations as severe as in humans in naturally infected dogs (Costa et al. 1997 *Mem Inst Oswaldo Cruz 92* (Suppl I): 117). However the detection of *Leishmania* in histological sections by traditional staining was negative. In the present study to ascertain that the renal changes in these dogs are actually related to visceral leishmaniasis *Leishmania* antigen was searched using specific immunohistochemistry.

Thirty naturally infected dogs with positive sorology for visceral leishmaniasis were studied. The diagnosis was certified by culture of *Leishmania* from the bone marrow, spleen and popliteal limph node samples. Kidney samples were embedded in parafin and the sections were processed by specific and sensitive streptavidin-peroxidase immunohistochemistry to detect *Leishmania* antigen using polyclonal mouse anti-*Leishmania* antibody.

The renal histopathology was characterized by different glomerular alterations: minimal changes, chronic, mesangioproliferative and membranoproliferative lesions. Interstitial mononuclear cell infiltrate with different intensity was also found. Under light microscopic examination characteristic dark brown peroxidase staining of antigenic material with granulous aspects was observed. The positive reaction was scarse and observed in the cytoplasm of phagocytic cells of glomeruli and in the interstitial localization and in the tubular walls. It was only detected as antigenic material and no whole amastigotes could be seen in any of the sections. No positive staining could be seen in control sections.

Dogs with visceral leishmaniasis naturally infected present renal changes clearly related to the presence of *Leishmania* antigen. However there is discrepancy between the scarcity of the antigenic material and the severity of the lesions. Therefore pathogenesis of these lesions has to be clarified in the future studies.

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BI-79
IDENTIFICATION OF TELOMERE-BINDING PROTEINS IN *TRYPANOSOMA CRUZI*

Melo-Godoy PD, Ejchel TF, Freitas-Junior LHG, Schenkman S
Departamento de Microbiologia, Imunologia e Parasitolgia, Universidade Federal de São Paulo, R. Botucatu 862, 8º andar, São Paulo, SP, Brasil

When *Trypanosoma cruzi* differentiate from proliferative (epimastigotes or amastigotes) forms into non-replicative and infective stages (trypomastigotes) it undergoes biochemical and morphologic changes, which are related to differential gene expression. While proliferative forms display a round nucleus with a large nucleolus and small amount of heterochromatic regions, infective forms display an elongated nucleus containing a large proportion of heterochromatic material and no evident nucleolus. As heterochromatin is related to telomeric regions in other eucariotes, here we investigated whether these changes may be related to differential expression of proteins with capacity to bind the telomere portion of chromosomes. A probe derived from *Trypanosoma brucei* telomere hybridized with *T. cruzi* telomeres indicating that it can be used to detect telomere-binding proteins in this parasite. An oligonucleotide containing *T. brucei* telomere sequences was then used in gel retardation analyses of DNA-protein interactions in the presence of nuclear extracts of *T. cruzi* epimastigotes and trypomastigote forms. Our results show the presence of proteins in both stages that bind specifically to single strand probes. Therefore, the nuclear modifications found during differentiation are not related to a differential binding to single strand telomere sequences.

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BI-80
IMMUNOCYTOCHEMICAL TECHNIQUES REVEAL NEW ASPECTS IN TRYPANOSOMATIDS WHICH HARBOUR ENDOSYMBIONTS

Motta MCM, De Souza W, Thiry M*
Laboratório de Ultraestrutura Celular Hertha Meyer, Instituto de Biofísica Carlos Chagas Filho, UFRJ, Rio de Janeiro, RJ, Brasil *Université de Liège, Belgique

Some protozoa of the Trypanosomatidae family harbour an endosymbiotic bacteria in the cytoplasm. The prokaryote supplies the protozoa with essential nutrients, while it obtains ATP from the host glycosomes. Furthermore, the presence of the symbiotic bacteria is related to morphological and physico-chemical changes in the host. The TDT (Terminal deoxynucleotidyl Transferase) is an immunolabeling technique which allows single and double DNA labeling at 3'ends. This method can be combined with acetylation allowing the identification of different nucleolar components, a clear visualization of ribosomes and the identification of cellular sites containing DNA. In this study the TdT was applied on ultrathin sections of two endosymbiont bearing species: *Blastocrithidia culicis* and *Crithidia deanei*. The results showed an intense labeling in the nucleus, mainly associated with the peripheral condensed chromatin, and also in the kinetoplast DNA fibers. With regard to the endosymbiont, the application of the TdT method after the acetylation technique, permitted a better distinction of the genetic material in the symbiotic bacteria. The DNA was seen disributed in an electron dense area, probably corresponding to the bacterial chromosome, which sometimes is seen attached to the symbiotic envelope. In a previous study, the genetic material was considered to be distributed through all the electron lucid area present in the protozoa matrix. *Crithidia desouzai* is a endosymbiont-bearing specie which also presents virus-like particles (VLPs) in the cytoplasm. Some years ago, it was proposed that these VLPs did not present deoxyribonucleoproteins, being probably constituted by RNA. Recently the TdT method and the use of anti-RNA antibodies confirmed this prediction. Using transmission electron microscopy analysis no labeling was detected in VLPs when the TdT technique was applied, however such particles were labeled by anti-RNA antibodies.

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BI-81
INTERACTION OF CHRONIC CHAGASIC PATIENTS' SERA WITH CARDIAC ADENOSINE RECEPTOR

Farias de Oliveira S, Almeida NA, dos Santos Costa PC, Pedrosa RC*, Campos de Carvalho AC*, Masuda MO
Instituto de Biofísica Carlos Chagas Filho *Hospital Universitário Clementino Fraga Filho, UFRJ, Rio de Janeiro, RJ, Brasil

It has been shown that sera from chronic chagasic patients (CrCh) induce alterations in cardiac electrogenesis by activation of muscarinic and b-adrenergic receptors. In a previous report we demonstrated that sera from 13 out of

25 CrCh patients were able to induce bradycardia and atrioventricular conduction block (AVB) in the presence of atropine, a muscarinc antagonist. In the present work we investigate the for interaction of these sera with the adenosine receptor in isolated rabbit heart since adenosine is known to depress AV conduction and pacemaker activity.

ECG was continuously monitored in isolated rabbit hearts during perfusion with Tyrode (Ty) solution (in mmol/L NaCl 2.7, glucose 9, $NaHCO_3$ 18, KCl 2.7, NaH_2PO_4 1.8, $MgCl_2$ 0.5, $CaCl_2$ 2.7) equilibrated with a mixture of 95% O_2 and 5% CO_2, at 35± 0.5ºC. The experimental protocol consisted of 30 min control perfusion with Ty containing DCPCX (10^{-6} M) , a antagonist of A1 receptors, followed by another 30 min perfusion with Ty containing DCPCX + serum (diluted 1:100 v:v) and a washout period of 30 min with Ty containing DCPCX. Nine out of 11 non-muscarinic sera tested had their ability to induce bradycardia blocked by DCPCX. In addition 2 out of 7 sera that induce AV block had the effect reversed by the A1 receptor blocker.

We conclude that sera from a fraction of CrCh patients are able to depress the electrogenesis in the isolated rabbit heart through activation of A1 receptors.

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BI-82
INTERACTION OF *PHYTOMONAS* SP. WITH *AEDES ALBOPICTUS* CELL CULTURE

Miguens FC, da Cunha M, Gomes RA, Keller DG, De Souza W*
Laboratório de Biologia Celular e Tecidual, CBB/UENF, Av. Alberto Lamego 2000, 28015-620 Campos dos Goytacazes, RJ *Laboratório Hertha Meyer, Instituto de Biofísica Carlos Chagas Filho, UFRJ, Rio de Janeiro, RJ, Brasil

Phytomonas can be defined as promastigote parasites that lack arginase, do not have opithomastigote stages, and infecte plants and insects. They have been known as parasites of laticiferous plants, except cassava, and pathogenic for several palm trees. Trypanosomatids of the genus *Phytomonas* have been reported in 158 species of 9 plant families. Several species of phytophagous insects, mainly Heteroptera, have been imputed as vectors of these trypanosomatids. *Phytomonas davidi*, cultured from laticiferous Euphorbiaceae, and *Phytomonas serpens*, isolated from Solanaceae, had been cultured in different culture medium. However, ATCC CCL 126 cells from *Aedes albopictus* in Mitsuhashi and Maramorosch insect medium supplemented with 10% fetal bovine serum has been used as an alternative approach to isolate *Phytomonas staheli* . In this study, we report our preliminary results of the interaction of *P. davidi* and *P. staheli* with *Ae. albopictus* cell line grown in MM medium supplemented with 10% fetal bovine serum. *P. davidi* were obtained from laticifers tubes of *Chamaesyce thymifolia* and inoculated in cell culture. After 30 min, 1, 2 and 6 hr samples were washed in PBS. Then, samples were fixed with 2.5% glutaraldehyde + 2% formaldehyde in 0.1M phosphate buffer and observed by light or electron microscopy. Similar experiments were carried out with coconut juice containing *P. staheli.* The washed material was also fixed and observed. During the whole period of interaction, *Phytomonas* were alive and presented high motility. A small number of protozoa was found adhered to *Ae. albopictus* cells. Most of them, were adhered by the flagellum. We never found flagellates in cell cytoplasm or in endocytic vacuoles. A large number of trypanosomatids could be found free in culture medium. None of them present alterations in their ultrastructure. In conclusion, we believe that a monolayer of *Ae. albopictus* grown in MM medium can be a good substrate to isolate *Phytomonas*.

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BI-83
JG STRAIN OF *TRYPANOSOMA CRUZI* FAIL TO INDUCE CARDIAC SYMPATHETIC DENERVATION IN YOUNG RATS

Silva GC, Camargos ERS, Chiari E, Machado CRS
Departamentos de Morfologia e Parasitologia, Instituto de Ciências Biológicas, UFMG, 31270-901 Belo Horizonte, MG, Brasil

Previous studies have demonstrated that several strains or clones of *Trypanosoma cruzi* are able to induce severe (Y, ABC, CL-Brener) or moderate (Col 1.7G2) acute myocarditis in rats. In all animals cardiac sympathetic denervation was also observed. Now we test the JG strain that was recently isolated from a patient with chagasic megaesophagus. Previous study had demonstrated that in BALB/c mice this strain showed tropism for the heart muscle, causing prominent myocarditis. Holtzman rats aged 27-29 days were inoculated with 1, 000 or 10,000. Trypomastigotes/50 g of body weight, ip) and sacrificed 21, 35 and 45 days after inoculation for histological (heart, esophagus and intestine) and histochemical (heart) studies. Parasitemic curves showed few circulating trypomastigotes (maximum of 14 or 80 parasites/5 µl, respectively), no parasites being found after day 29. Histological analysis showed absence of parasitism and inflammatory processes in the esophagus and rectum of all animals at different periods of the acute phase. In the heart only discrete and diffuse inflammation was observed in ventricles and right

auricular appendages (days 35 and 45 days) in accordance with a low myocardial parasitism. The histochemical method for visualizing the sympathetic nerves (glyoxylic acid-induced fluorescence) showed that the density of noradrenergic nerve terminals in *T. cruzi*-infected rats was similar to that of controls. From the five *T. cruzi* strains or clones already tested in rats, the JG strain was the first to fail in inducing moderate or severe myocarditis. The myocarditis induced by the other *T. cruzi* isolates (inoculum = 10,000) was always accompanied by sympathetic denervation. Because of these results, we studied the effect of JG strain in BALB/c mice (1,000 trypomastigotes/ 50 g of body weight). In this animal patent parasitemia was found till day 100 after inoculation, the highest values being found at 23 and 29 days (mean value of 1,200 and 1,300 trypomastigotes/5 µl, respectively). At day 36 of infection all mice presented moderate denervation in the auricular appendages coexistent with moderate to intense inflammatory process.

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BI-84
LECTIN-BINDING SITES IN FIBROBLASTS FROM PRIMARY CULTURES DURING ITS INTERACTION WITH *LEISHMANIA AMAZONENSIS* PROMASTIGOTE FORMS

Côrte-Real S, Soeiro MN, Moreno MLV, Airano RC, Almeida DS, Meirelles MNL
Laboratório de Ultra-estrutura Celular, Departamento de Ultra-estrutura e Biologia Celular, Instituto Oswaldo Cruz, Av. Brasil 4365, 21945-900 Rio de Janeiro, RJ, Brasil

Lectins are useful tools for the detection and localization of carbohydrate residues. The mechanisms that govern the invasion of parasites of the genus *Leishmania* in fibroblasts are yet poorly studied. This work analyses the presence of Con A and RCA binding sites at the fibroblasts surface during the early events of *Leishmania amazonensis* promastigotes invasion.

Primary cultures of fibroblast were obtained from skin (SF) and skeletal muscle (SMF) by enzymatic treatments and were infected for 2h with *L. amazonensis* promastigote forms. For the detection of mannosyl and galactosyl residues, infected and non-infected cultures were incubated with ConA-FITC or RCA 120- TRITC (50mg/ml) during 60 mim/4°C, respectively. Afterwards, the cultures were fixed with 2% paraformaldehyde for 5min/4°C, and further incubated with DAPI for DNA detection. As controls, competition assays were performed incubating the cells with specific sugars.

Mannosyl residues were seen over the surface of fibroblasts from both non-infected SF and SMF using both cultures, no considerable difference was noticed on mannosyl labeling due to promastigotes infection. On the other hand, attached parasites displayed a stronger signal than to both host cells. The parasite surface labeling displayed a discontinous labeling similar to patches. The flagellar pocket and also the posterior end of the parasite displayed a higher fluorescence intensity.

The expression of galactosyl residues seemed to be different between non-infected SF and SMF, being more intense in the latter cells. In infected SMF, RCA 120 binding sites seem to be decreased while no significant alteration was detected in infected SF cells. Extracellular parasites were devoid of RCA 120 labeling.

Our previous data showed that Con A binding sites participate in the attachment of promastigotes to fibroblasts. Here we demonstrate that after 2 hr of interaction, the mannose content of fibroblasts seems to be the same as in non-infected cells. Our preliminary studies suggest that the down-modulation of the galactosyl residues is directly related to the stage of parasite-SMF interaction. Other studies are underway to better clarify the events related with the whole process of promastigotes invasion into fibroblasts.

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BI-85
LEISHMANIA (*VIANNIA*) *BRAZILIENSIS* EXPERIMENTAL INFECTIONS IN THE ASIAN RHESUS MONKEYS (*MACACA MULATTA)*

Teva A, Oliveira-Neto MP, Amaral VF, Silva AJ, Pereira MS, Carvalho-Paes LE, Coutinho SG*, Pirmez C**, Ferreira V***, Grimaldi Jr G
Departamento de Imunologia, HEC *Departamento de Protozoologia **DBBM, Instituto Oswaldo Cruz ***Departamento de Primatologia, Fiocruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil

Nonhuman primates are emerging as invaluable tools employed in studying human diseases. As nonhuman primate host responses to *Leishmania* are very similar to those observed in humans, primate models could provide an indication of the potential success and/or limitations for human vaccine against leishmaniasis. This will be specially important in the case of *L.. braziliensis* complex parasites where the monkey models studied to date are susceptible to infection and the murine model is limited.

In this study, laboratory-bred and –reared adult rhesus macaques of both sexes were infected using *L* .(*V.*) *braziliensis* isolates originating either from localized cutaneous (strain MHOM/BR/97/SIS) [group A; N=5] or mucosal (strain MHOM/BR/95/OSC) [group B; N= 5) leishmaniasis patients from Rio de Janeiro. All the monkeys inoculated intradermally in the upper eyelid area with 10(7) virulent promastigotes of either parasite strain developed skin lesions at the site of inoculation. Variation in the level of susceptibility between individual monkeys was observed, but earlier infection onset and larger size of ulcerating nodules was found in group A. An erythematous-papular nature, were first visible at 1-2 wk p.i. Lesion development progressed rapidly and all lesions ulcerated (after 3 to 6 weeks p.i.) and was subsequently followed by regression and healing. Some monkeys developed satellite lesions peripheral to the resolving primary nodule (N=2) or metastases in the extremities (N=2). Most of the primary lesions had disappeared from 8 of the infected monkeys by 18 weeks, whereas satellite/metastatic lesions persisted at this time.

Level of IgG antibodies to promastigote antigens rose during active infection and then declined. Parasite-specific lymphoproliferative responses of PBL from monkeys were negative iat the initiation of infection, but positive reactions (SI = 16) developed as early as 6 weeks p.i. (mean SI values were not significantly different between groups) and subsequently continued to increase, peaking at 27 weeks p.i. (SI = 28) after which levels persisted (B) or declined (A) beyond selfe-cure. In addition, strong leishmanin skin test (LST reaction size = 10-30 mm) positivity to homologous antigen was detected in 7 (70%) animals, during active infection and following self-cure. There was no conspicous correlation between the LST positivity reaction sizes and the level of antigen-stimulated cell responses. Further studies are in progress to (a) determine whether the presence of proliferative (and/or IL-2, IFN-g responses) and a skin test response are sufficient indicators of self-healing and (b) for testing if these responses elicit substancial resistance to a subsequent homologous challenge with virulent parasites.

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BI-86
LEISHMANIA AMAZONENSIS AND *L. BRAZILIENSIS* PROMASTIGOTES CAN RESIDE INTRACELLULARLY IN HUMAN MACROPHAGES AND RESPOND TO IFN-b AND TGF-b TREATMENT

Silva MP, Barral A, Barral-Netto M, Van Weyenbergh J
LIMI, Centro de Pesquisas Gonçalo Moniz , Fiocruz, Salvador, BA, Brasil

Leishmania are protozoan parasites that are transmitted in the promastigote form by a phlebotomine vector to the human host, where they differentiate into the amastigote form, preferentially residing in cells from the macrophage lineage. *Leishmania* promastigotes are considered to exist exclusively extracellularly and amastigotes intracellularly, although both forms can be grown axenically. To our knowledge, intracellular promastigotes have never been documented for any of the *Leishmania* species. When infecting human macrophages (derived from peripheral blood of healthy donors) with stationary phase *L. amazonensis* promastigotes, and examining by ordinary light microscopy, we readily observed large phagosomes with vividly moving intact promastigotes in a small number of cells (<0.1 %), whereas with *L. braziliensis* fewer and smaller phagosomes containing promastigotes were observed. The number of cells displaying visible promastigotes increased with time and reached a plateau between 48 to 72 hr, although live intracellular promastigotes could be observed for up to five days following infection. The presence of intact intracellular promastigotes was confirmed and quantified on cytospin preparations stained with hematoxylin/eosin. Macrophages infected with *L. amazonensis* were found to contain 5 to 10 intact promastigotes per phagosome in 10-20% of the cells. Treatment with IFN-b or TGF-b increased the number of intracellular promastigotes with ± 50%, similar to what we previously demonstrated for normal amastigote parasite burden. Conversely, IFN-g treatment diminished the number of intracellular promastigotes, but increased the size of promastigote-containing phagosomes, rendering them more easily visible by light microscopy. However, IFN-b, IFN-g or TGF-b treatment did not alter the promastigote/amastigote ratios (1/20 approximately), indicating that intracellular promastigotes behave similarly to amastigotes in response to these cytokines. Interestingly, intracellular promastigotes were easily detected when macrophages were cultured in medium containing human serum and virtually undetectable in cultures supplemented with fetal bovine serum, suggesting a stimulatory or inhibitory factor in human or bovine serum, respectively, that seems to act specificically upon the promastigote form of *Leishmania*.

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BI-87
LEPTOMONAS SP. ISOLATED FROM THE SANDFLY *LUTZOMYIA AYROZAI* (DIPTERA: PSYCHODIDAE)

Sousa MA, Barrett TV***, Naiff RD***, Branco DCB, Sá-Xavier C, Santos SM, Cysne L*, Brandão A**
Coleção de Tripanosomatídeos, Laboratório de Transmissores de Hematozoários, Departamento de Entomologia *Departamento de Protozoologia **Departamento de Bioquímica e Biologia Molecular, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil ***I.N.P da Amazônia, Caixa Postal 478, 69011-970 Manaus, AM, Brasil

Trypanosomatids belonging to the genera *Leishmania*, *Endotrypanum* and *Trypanosoma* have been isolated on several occasions from phlebotomines, but few reports exist on presumed monoxenous parasites isolated from these hosts. In the present work, we characterized two isolates (IM 3943 e IM 3944) obtained from *Lutzomyia ayrozai* captured in August 1993 in Porto Urucu (State of Amazonas, Brazil) with a view to determine their generic identification. Both isolates presented promastigotes in axenic cultures and were unable to infect hamsters. Then, they were cloned in a flow cytometer and a clone from each one (IM 3943-c and IM 3944-c) was studied with regard to the capacity of multiplication within macrophages *in vitro*, growth, morphological differentiation and division process in the Yaeger's LIT medium at 27.3ºC (from 48-144 hr). A morphometric analysis of the predominant stage found in these cultures was carried out as well. The kDNA minicircle size of both isolates was estimated and compared with those from two reference strains of *Endotrypanum* and with data published on *Leishmania* species.

These clones were unable to multiply within macrophages and grew well in LIT medium, reaching the maximum growth ($3x10^7$ cells/ml) within 72-96 hr. As seen in Giemsa-stained smears, slim promastigotes greatly predominated and were very similar in both clones. Paramastigotes also could be found, although at low percent (averages: 0.1% in IM 3943-c and 1.1% in IM 3944-c). Aflagellated cells were absent. No opisthomastigote was seen, then discarding the possibility of these clones belonging to the *Herpetomonas* genus. During the division process, in both clones, the nuclei were predominantly placed at different planes. However, in IM 3944-c, the majority of dividing cells (93%) presented the two kinetoplasts before the nuclei, while in IM 3943-c the kinetoplasts presented two main positions: (1) both being placed before the dividing nuclei (45% of the dividing cells) and (2) one kinetoplast placed before the nuclei, while the other was paranuclear (49%). The promastigotes of IM 3943-c and IM 3944-c were similar in size, having respectively 13.0 ±3.2 and 14.3 ±3.5 mm in length, 1.7 ±0.2 and 1.6 ±0.2 mm in width, free flagellum of 12.9 ±4.3 and 13.3 ±4.3 mm, and 1.5 mean nuclear index. The kDNA minicircle size of these clones was estimated at 2.3 kb, then being markedly higher than those of *Endotrypanum* and *Leishmania* spp. (0.8-0.9 kb). All these findings led us to consider these isolates as varieties of a same species, which do not belong to the *Leishmania* or *Endotrypanum* genera, otherwise having morphological features of a *Leptomonas* sp. The IM3943-c and IM3944-c cultures are deposited in the Trypanosomatid Collection of the Oswaldo Cruz Institute under the codes CT-IOC 100 and CT-IOC 058, respectively.

BI-88
LIPID TRAFFIC IN VERO CELLS INFECTED WITH *TOXOPLASMA GONDII*

Melo EJT, Oliveira AS, De Souza W
Laboratório de Biologia Celular e Tecidual, LBCT/CBB/UENF, Campos, RJ, Brasil

Toxoplasma gondii is an intracellular parasite widely distributed among the vertebrate animals. Tachyzoites of *T. gondii* multiply within parasitophorous vacuole. During its development and multiplication host cells structures change their position. In a previous study we have shown that intravacuolar parasites are able to capture metabolic of C_6-NBD-ceramide. In the present study we explored this point further analyzing, using fluorescence microscopy, and the incorporation by the parasites of some labeled lipid analogues.

Vero Cells were cultured in Linbro tissue plates that contained a sterile coverslip (3-5 10^5/well) and maintained at 37°C overnight. The cultures were then infected with tachyzoites (RH strain). Cells were incubated in the presence of labeled lipids (C6-NBD-Ceramide; C5-sphingomieline, Br2-C5-ceramide, C6-NBD-sphingomieline) for 1-5 hr and then observed in a Zeiss Confocal Laser Scan Microscope. Fluorescence indicated of the presence of C_5-SM and C_6-NBD-SM was initially observed in the plasma membrane (1 hr incubation) and later in the cytoplasm of the host cell. No labeling of the parasites located within the parasitophorous vacuole was observed. However, extracellular parasites were labeled. Infected host cells incubated in the presence of Br_2-C_5-ceramide and C_6-NBD-ceramide initially the perinuclear region was stained (1 hr incubation). After 5 hr incubation a intense labeling was observed in cytoplasm of host cell and intravacuolar tachyzoites. However, only the plasma membrane of intravacuolar parasites was stained with Br_2-C_5-ceramide. These observations suggest that there is a selective incorporation of host cell lipids in *T. gondii* containing parasitophorous vacuoles.

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BI-89
MACROPHAGE *IN VITRO* INFECTION WITH *LEISHMANIA (L.) AMAZONENSIS* AND *LEISHMANIA (V.) PANAMENSIS*

Matta VLR, Gomes CMC*, Ura D.M, Laurenti MD, Corbett CEP
Laboratório de Patologia de Moléstias Infecciosas (LIM/50 HC-FMUSP), Departamento de Patologia, FMUSP, São Paulo, SP, Brasil *Universidade Federal do Maranhã, São Luís, MA, Brasil

We have been working with a strain of cutaneous leishmaniasis characterized by isoenzyme and hybridization as *Leishmania (Viannia) panamensis*, HSJD-1 strain. However this strain showed unexpected pattern of biological behaviour with ulceration and metastatic lesion in mice tail after six month of infection, in contrast with a local cutaneous lesion caused by panamensis species. A new characterization identified this strain as *Leishmania (Leishmania) amazonensis* by monoclonal antibodies (Dr JJ Shaw) and by hybridization (Dr S Uliana).

In order to study better the behaviour of this leishmania strain, we compared the BALB/c perioneal macrophages infection with *L. (L.) amazonensis* (HSJD-1) and with a WHO strain of *L. (V.) panamensis* (MHOM/PA/91/CIDEP002). The infection index were determined at 6 post infection.

The infection index post 6 hr with *L. (L.) amazonensis* were higher (69.12%) comparing to *L. (V.) panamensis* (32%).

The higher *in vitro* macrophage infection using HSJD-1 strain corroborates with the new characterization and the *in vivo* behaviour of the classical *L. (L.) amazonensis* infection in which we observed a higher hind footpad swelling than *L. (V.) panamensis* and characterized by intense vacuolated macrophage infiltrate with high amount of parasites.

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BI-90
MAPPING OF HOST CELL RECOGNITION SITE OF *TRYPANOSOMA CRUZI* SURFACE MOLECULE GP82

Manque PM, Eichinger D*, Ruiz R, Araya JE, Juliano MA*, Juliano L*, Silveira JF, Yoshida N
Departamento de Microbiologia, Imunologia e Parasitologia *Departamento de Biofísica, Escola Paulista de Medicina, Universidade Federal de São Paulo, R. Botucatu 862, São Paulo, SP, Brasil ** Department of Molecular and Medical Parasitology, New York University, New York, USA

Gp82, a surface glycoprotein expressed specifically in *Trypanosoma cruzi* metacyclic trypomastigotes, has been implicated in the process of mammalian cell invasion. The portion of gp82 that binds to target cells appears to be the central domain of the molecule, comprising aminoacids 224-357. In order to map within this central region the binding site for host cell receptor, we have: i) generated by site-directed mutagenesis a set of pGEX constructs containg the nucleotide sequence of gp82 with various deletions, ii) transformed bacteria with the various plasmid constructs to produce recombinant proteins iii) purified the mutated proteins and tested them for binding activity, capacity to inhibit host cell invasion and reactivity with monoclonal antibody 3F6, an antibody that inhibits parasite internalization. We observed that all mutated recombinant proteins, including the one containig the largest deletion (amino acid 257-321) bound to HeLa cells and was recognized by monoclonal antibody 3F6. Based on this result, we are producing additional truncated gp82 recombinant proteins with deletion spanning aminoacids 257-363 and 321-363. In parallel, we synthesized a set of 20-mer peptides with 10 overlapping amino acids corresponding to residues 314-363 (based on the G strain sequence). One peptide contained a stretch of 10 amino acids based on the sequence of the highly invasive CL strain and not shared by the poorly invasive G strain. These peptides were used for inhibition assay of HeLa cell invasion. The penetration of CL strain metacyclic forms were into HeLa cells was maximally inhibited (~80%) by the peptide containg the strain-specific sequence. This peptide failed to inhibit the internalization of G strain metacyclic trypomastigotes.

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BI-91
METACYCLOGENESIS IN *TRYPANOSOMA CRUZI* IS INFLUENCED BY ATTACHMENT TO THE SUBSTRATE

Martins SCF, Soares MJ
Laboratório de Biologia Celular de Microrganismos, Departamento de Ultra-estrutura e Biologia Celular, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil

The life cycle of *Trypanosoma cruzi* includes living in the digestive tract of an invertebrate host (triatomine bugs). In the rectum of these insects the parasites transform from epimastigotes to metacyclic (infective) trypomastigote forms. In this site the epimastigotes are found attached to the rectal wall by the flagellum. This attachment to the substrate seems to be essential to the multiplication and differentiation of parasites of the *Trypanosoma* genus.

In *T. cruzi*, attachment can be induced in vitro by incubating epimastigotes in TAU3AAG, a chemically defined liquid medium that can also promote their differentiation into trypomastigote forms. To test the role played by adhesion to a substrate on the cell differentiation process (metacyclogenesis), epimastigote forms of *T. cruzi*, strain Dm28c, were allowed to adhere to different inert substrates and then the differentiation rate was analyzed after incubation of the parasites for 96 hours in TAU3AAG medium. After this time the supernatant was collected and the percent of trypomastigote forms was estimated by cell counts in a Neubauer chamber. The following substrates were used: chitin, chitosan flakes and glass coverslips coated with glycol chitosan, poly-L-Lysine or bovine serum albumin (BSA). Uncoated coverslips were used as a control.

Our results demonstrate that higher differentiation rates are obtained in vitro when parasites are incubated with substrates that promote higher adhesion rates. An exception to this rule occurred with poly-L-lysine, a highly positively-charged substrate: although it has been the substrate with the highest adhesion rate, it presented the lower metacyclogenesis rate (similar to that obtained with negatively charged substrates), since the parasites were strongly bound to the substrate due to the surface charges. Higher differentiation rates occurred with chitosan, a positively charged chitin derivative. Our data support the hypothesis that in *T. cruzi* adhesion to a substrate is an important stimulus for the cell differentiation process.

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BI-92
MICROMORPHOLOGY OF THE PREDOMINANT CILIATOFAUNA OF RODRIGO DE FREITAS LAKE, RIO DE JANEIRO

Moreira MGG, Silva-Neto ID da
Laboratório de Protistologia, Departamento de Zoologia, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Ilha do Fundão, 21941-590 Rio de Janeiro, RJ, Brasil

The Rodrigo de Freitas Lake, coastal and tropical, placed in an urbanized area of Rio de Janeiro, has a diameter of 2 km in its largest width, it presents muddy fund with a 4 m maximum depth. The lake is marginated by swamp vegetation and it is affected by the entrance of salted and of fresh water, modifying a lot of its interior. For six months mensal collections of water and mud samples were made in different points of the lake, for exam of the fauna of predominant ciliates. The ciliates were cultivated in laboratory with grains of rice, wheat and diatoms in petri plates with sterilized water of the collected place.

The identification of the ciliate was made through silver impregnation techniques (Protargol) Silva-Neto (1996 *Mem Inst Oswaldo Cruz 91*: 64). Among the ciliates species belonging to the groups Karyorelictea, Hymenostomatida, Heterotrichida, Prostomatida, Gymnostomatida and Hipotrichida, found in the lake, highlighted the ciliate Hipotrichea, *Holosticha* sp. for a detailed study of its structures whose characteristics are different from the species of the genere. The ciliary structures will be shown through schematic drawings and complemented with observations on Scaning Electron Microscopy. The species of predominant ciliates in the collected samples of the lake, belong mainly to Heterotrichida, Hipotrichida and Hymenostomatida.

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BI-93
MICROMORPHOLOGY, MORFOGENESIS AND REGENERATIVE ASPECTS OF THE CILIATE *PSEUDOKERONOPSIS* SP. (HIPOTRICHEA, UROSTYLINA) ON THE OPTICAL MICROSCOPE AND SCANNING ELECTRON MICROSCOPY

Matos A BA, Silva-Neto ID da
Laboratório de Protistologia, Departamento de Zoologia, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Ilha do Fundão, 21941-590 Rio de Janeiro, RJ, Brasil

The ciliate *Pseudokeronopsis* sp.,collected from mangroves of Ilha do Fundão, Rio de Janeiro, was studied on the optical microscope, through the silver impregnation technique (Protargol) modified by Silva-Neto (1996 *Mem Inst Oswaldo Cruz 91*: *64*) and on Scanning Electron Microscopy for the study of its structures.

Pseudokeronopsis sp. it has a size, *in vivo*, of 160-250 mm length and of 28-58 mm width. The body of the ciliate is dorso-ventral flattened, its form is oval-prolonged, *in vivo*, and ellipsoid after the fixation. In the dorsal face, 4 kineties are disposed like a meridian. In the ventral face it has around 32-42 cirrus midventral and 3-4 transverse cirrus. Beside the external paraoral, which is shorter than the internal paroral, there is a buccal cirri. The front cirrus are arranged as a bicorona with 5-7 cirrus each. In the left margin are found about 46 cirrus aligned and in the right

margin about 51 cirrus. Therefore after these last ones are found slightly deslocated to the left, two frontal cirrus. The adoral zone membranelles is constituted of 37-63 membranelles. The nuclear apparatus is constituted of 5 micronuclei and of 72-136 segments macronucleus. The cytoplasm has a yellow-green coloration and it's very granulated. A contractile vacuole is usually found in the right and medium board of the cell.

We verified that the regenerative process seems to be an important form of preservation of this ciliate population. The ciliate presents a number variable of 3 or 4 transverse cirrus and an interruption among the 11^{th} and 12^{th} membranelles. These characteristics are different from the other species of the genera *Pseudokeronopsis.* The morphologic and morphogenetics features and aspects of the regenerative processe will be shown supported by of drawings and micrographics.

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BI-94
MICROSATELLITE ANALYSIS OF 54 STRAINS AND CLONES OF *TRYPANOSOMA CRUZI*

Oliveira RP, Melo AIR, Macedo AM, Pena SDJ
Departamento de Bioquímica e Imunologia, UFMG, Caixa Postal 486, 30161-320 Belo Horizonte, MG, Brasil

Recently we described the isolation and characterizations of the first polymorphic microsatellites in the genome of *Trypanosoma cruzi*. We now have used the PCR amplification of eight distinct (CA)n microsatellites to study 54 strains and clones of the parasite and obtained valuable new information about its population structure. Identically to our previous observation, all clones examined exhibited in some microsatellites the presence of two alleles after PCR amplification, demonstrating that *T. cruzi* is diploid. However fifteen strains showed more than two allele peaks in many loci, suggesting they are composed of more than one clonal sub-population. Interestingly, the multiple-allele pattern was preferentially observed among those isolated from non-human sources. No single multilocus genotype was observed more than once among the strains analyzed. The phylogenetic analysis of *T. cruzi* based on microsatellites using parsimony method revealed a great genetic distance among strains. Although the strain dispersion profile in the Wagner network was in general agreement with the species dimorphism found by PCR amplification of the divergent region of the rRNA 24Sa gene, as described previously, the results obtained with this larger set of strains strongly suggests that the so-called group 2 has two subdivisions.

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BI-95
MICROSCOPIC ASPECTS OF THE LIVER OF MICE INOCULATED WITH *TRYPANOSOMA CRUZI* AND SUBMITTED TO ALTERATIONS IN IRON LEVELS

Carneiro CM, Amaral JF*, Santos ACC**, Silva ME*, Bahia MT**, Afonso LCC**, Tafuri WL**, Pedrosa ML**
Escola de Farmácia, Departamento de Análises Clínicas, UFOP *Escola de Nutrição, UFOP **Instituto de Ciências Exatas e Biológicas, Departamento de Ciências Biológicas, UFOP, Ouro Pret, MG, Brasil

Iron plays an important role in the pathology of several diseases. In previous studies we showed that higher iron levels in hosts were related to higher parasitemia and mortality. In the present study we evaluated the effects of iron deprivation and supplementation in Swiss mice infected with the Y strain of *Trypanosoma cruzi*. Thirty-day-old male mice were kept in stainless steel cages and fed a semi-purified diet either with or without ferrous sulfate. Animals were divided into four groups according to the type of treatment they received: 1) control diet according to A. O. A. C. (C); 2) control diet plus intraperitoneal injections of iron-dextran (ID); 3) iron deficient diet (NI); and 4) iron deficient diet plus intraperitoneal injections of desferrioxamine (DF). The animals were sacrificed two weeks after being infected with *T. cruzi*. All animals were examined by complete necropsy including histopathologic studies of all organs. The liver was sectioned after being fixed "in totum" in 10% buffered formalin pH 7.2. The fragments were processed for paraffin embedding and 3-mm-thick sections were obtained and stained with Hematoxylin and Eosin. Histopathologic examination revealed the following: C-group had discreet Kupffer cell parasitism (one nest per 100 fields at 40X), discreet focal inflammatory reaction which was predominantly lymphocytic and discreet hydropic degeneration of the hepatocyte. ID- group had intense parasitism of Kupffer cells (more than 10 nests per 100 fields at 40X) and hepatocytes, intact or disrupted nests, intense focal lymphoplasmohistiocitary inflammatory reaction reminiscent of granuloma at times and moderate intra and extracellular accumulation of iron ions. NI- group had accentuated Kupffer cell parasitism (8 nests per 100 fields at 40X), intact or disrupted nests, with or without predominantly macrophage inflammatory reaction, eventual granuloma with no giant cells and binucleated, acidophile, hypertrophied Kupffer cells and hydropic degeneration of hepatocytes in systematized foci. DF- group had accentuated Kupffer cell parasitism (7 nests per 100 fields at 40X), intact and disrupted nests, with or without predominantly lymphocytic inflammatory reaction, intense diffuse hydropic degeneration of the hepatocyte and frequent giant cells with four or more nuclei. These results demonstrate that in tissue, an excess of iron promotes more intense parasite multiplication and more serious lesions. In the absence of iron the parasite is favored again.

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BI-96
MINI-EXON GENE SEQUENCE POLYMORPHISM AMONG *TRYPANOSOMA RANGELI* STRAINS

Grisard EC/**, Romanha AJ*, Campbell DA**
Departamento de Microbiologia e Parasitologia, Universidade Federal de Santa Catarina, Brasil (E-mail: grisard@ccb.ufsc.br) *Centro de Pesquisas René Rachou, Fiocruz, Belo Horizonte, MG, Brasil **Department of Microbiology and Immunology, UCLA School of Medicine, USA

Trypanosoma rangeli is one of the two parasites of this genus that can infect humans as well as domestic and sylvatic animals in Central and South America. *T. rangeli* shares several mammalian hosts and triatomine vectors with *T. cruzi*, the etiological agent of Chagas disease. It is very important in the epidemiology of South American trypanosomiasis because *T. rangeli* infection induces a humoral immune response with high antibody levels that cross react with *T. cruzi* in immunological assays. In this work, we have studied *T. rangeli* strains isolated from different geographical regions using the nuclear, multicopy mini-exon gene as a molecular marker. After amplification of the whole mini-exon gene repeat of *T. rangeli* SC-58 (Brazil) and H8GS (Honduras) strains using oligonucleotides ME-R/ME-L as previously described (Murthy *et al*., *Mol. Cell. Probes* 6, 237-243, 1992), products were cloned and sequenced. Described sequences from *T. rangeli* BG-60 from Costa Rica, San Agustin from Venezuela and *T. cruzi* CL, Y and Tulahuen strains were used for comparison assays. *T. rangeli* strains are highly conserved, however, the non-transcribed intergenic spacer contained variable microsatellite repeats. In accordance with previous results using isoenzyme, RAPD and kDNA analysis, the SC-58 strain proved to be genetically distinct from the others. When compared with *T. cruzi* strains at the mini-exon gene level, all *T. rangeli* strains formed a separate group.

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BI-97
MONOCLONAL AND MIXED EXPERIMENTAL INFECTIONS WITH DIFFERENT *TRYPANOSOMA CRUZI* GENOTYPES IN MICE: ACUTE AND CHRONIC PHASES

Lana M, Pinto AS*, Bastrenta B**, Barnabé C**, Noel S**, Tibayrenc M**
Departamento de Análises Clínicas, Escola de Farmácia, Universidade Federal de Ouro Preto, 35400-000 Ouro Preto, MG, Brasil *Departamento de Microbiologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, 31270-010 Belo Horizonte, MG, Brasil **Centre d Études sur le Polymorphism des Microorganisms (CEPM), Unité Mixte de Recherche, No. 9926, CNRS/ORSTOM, BP 5045, 34 032, Montpellier, Cedex 1, France

Eleven mixtures involving 8 different stocks attributed to 19/20 (3 stocks), 32 (3 stocks), 39 (2 stocks) major clonal genotypes of *Trypanosoma cruzi* were used to infect groups of 30 days old male Balb/C mice. Animals were inoculated by intraperitoneal route with ($5,0 \times 10^5$ - monoclonal infection and $1,0 \times 10^6$ - mixed infection, $5,0 \times 10^5$ of each clone) purified metacyclic trypomastigotes originated from culture in LIT medium. During the acute phase the mice were examined daily to determine the MP (maximum of parasitemia), %MPH (percentage of mice with positive hemoculture) and the %MPP (percentage of mice with patent parasitemia). Thirty days (acute phase) and 90 days (chronic phase) after inoculation mice blood was obtained to hemoculture in LIT medium. Parasites isolated were checked for the identification of the clones that were present by their isoenzymes and randonly amplified polymorphic DNA (RAPD) profiles. The use of both techniques together may it possible to detect 27.7% and 32% of mixed infection respectively during the acute and chronic phases. In the chronic phase, only the mixtures with 19/20 + 32 genotypes were still present.Three analysis were performed during the acute and chronic phases: (i) behavior of pure clones; (ii) behavior of mixtures by comparison with pure clones; (iii) behavior of actual mixtures by comparison with theoretical mixtures (arithmetic mean of the results observed for each clonal genotype of the mixture taken separately). The parameters cited above were compared either by standard deviation or by X^2 test or by Yates X^2 test.

Results showed that in the first comparison (i) , despite notable standard deviation, there was significative statistic differences between the 3 clonal genotypes. For MP and %MPH the decreasing value order was 19/20 > 32 > 39 and 32 > 19/20 > 39 for % MPP. In the second and third comparisons (ii) and (iii), the results confirm data obtained from similar experiments in *T. infestans*. In several cases the mixtures were statiscaly different from monoclonal infections and also from the theoretical mixtures. This shows that the behavior of the mixtures were not a simple justaposition of the behavior of each clone taken separately. We noted a paradoxal effect of possible interaction (potencialization) in the case of 32 + 39 mixtures (in which the %MPH was significantly higher that of the pure 32 clone, which is higher than that of the pure 39 clone. The role of possible interactions of different *Trypanosoma cruzi* genetic clones in the same patient on the pathogenesis of Chagas'disease still has to be evaluated.

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BI-98

OCCURENCE OF NATURAL *LEISHMANIA (LEISHMANIA)* INFECTION IN *PROECHIMYS IHERINGI* IN AN ENDEMIC AREA OF HUMAN AND CANINE AMERICAN CUTANEOUS LEISHMANIASIS DUE TO *LEISHMANIA (VIANNIA) BRAZILIENSIS* IN ILHABELA, SÃO PAULO, BRAZIL

Tolezano JE, Taniguchi, Bisugo MC, Araújo MFL, Cunha EA, Elias CR, Larosa R
Seção de Parasitoses Sistêmicas, Instituto Adolfo Lutz, Av. Dr. Arnaldo 355, 01246-902 São Paulo, SP, Brasil

This communication has the aim to report the concomitant occurrence and circulation of two *Leishmania* species in an endemic area of human and canine american cutaneous leishmaniasis (ACL) in the State of São Paulo. Since the beginning of ninenties, several human and canine cases of ACL has been notified in the north shore of São Paulo State region, all of them has been associated with *L. (V.) braziliensis*. In Ilhabela, a city of this region, investigating silvatic reservoir of *Leishmania*, we caught a *Proechimys iheringi* naturally infected by *L. (Leishmania)* in a farm where at least two human and two canine cases of ACL due *L.(V.) braziliensis* were diagnosticated. The parasite shows biological behavior in hamsters and in acellular media as *a* subgenus *Leishmania* (*Mexicana* complex). The biochemical and molecular chacterization can confirme the similarity to *L.(L.) amazonensis* or *L.(L.) forattinii.* Entomological investigations in this locality showed an apparent absolute predominance of *Lutzomyia intermedia*, a know anthropophilic sandfly, in the domiciliary and peridomiciliary levels, where probably the transmission of *L.(V.)braziliensis* to man and dogs take place. In the forest, near than 10 or 15 meters from the houses, *Lu. intermedia*, *Lu. shannoni* and *Lu. monticola* were the more frequent sandflies. The concomitant existence of this silvatic cicle of *L. Leishmania* near to human presence pointed to the risk of transmission to man of two different *Leishmania* species.

BI-99

ON THE DIFFERENTIATION BETWEEN *TRYPANOSOMA RANGELI* AND *TRYPANOSOMA CRUZI* BY COMPLEMENT SENSITIVITY: VARIABLE BEHAVIOR OF *T. RANGELI* CULTURE STAGES IN THIS TEST

Sousa MA, Almeida RF, Santos SM, Sá-Xavier C
Coleção de Tripanosomatídeos, Departamento de Entomologia, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil

Trypanosoma rangeli and *T. cruzi* are human parasites which can share triatomine bugs as vectors and mammalian hosts. Then, it is desirable to know markers which make possible to distinguish these species in mixed infections, since *T. cruzi* can be a human pathogen. These species differ from each other with regard to the majority of their evolutive stages, the development pattern in the insect, the transmission mode to vertebrates, as well as by some biochemical and molecular markers. Their different behavior in the complement lysis test was first reported by Schottelius (1982 *Tropenmed Parasit 33*: 147-150), evidencing that *T. rangeli* was not lysed by several fresh mammalian serum (guinea-pig, rat, rabbit and human). In these experiments, 50 ml of a heavily concentrated parasite suspension (10^8cells/ml) from a 4-day old culture was incubated with 100 ml of fresh serum (1 hr, room temperature). In another study, Carreira (1987 Thesis, Univ. Federal de Minas Gerais, MG, Brazil) reported that: (1) *T. rangeli* could be lysed by guinea pig complement and that the lysis resistance was gradually acquired in culture, (2) two parasite strains analyzed showed different lysis %. In the present work, the complement sensitivity of six well-characterized *T. rangeli* strains was reviewed using an assay based on Nogueira et al. (1975 *J Exp Med 142*: 224-229). Then, 150 ml of a parasite suspension having 1-2x10^6cells/ml was incubated with 50 ml of fresh undiluted guinea pig serum during 30 min at 37^{o}C. The experiments were carried out using parasites from 4- to 17-day-old cultures in NNN+LIT. Heat-inactivated guinea pig serum (68^{o}C, 1hr) was used as control. *T. cruzi* Y strain was included in all experiments to confirm the lytic activity of the fresh serum. The results from 10 experiments are presented in table, evidencing the variability of *T. rangeli* strains in the complement lysis test; however, no clear correlation was observed between the lysis % and the parasite growing phase in culture. Some experiments were performed using C2-depleted human serum evidencing that, at least partially, the alternative pathway of complement activation can be involved in *T. rangeli* lysis. A morphometric analysis of the H 14 strain epimastigotes which had been incubated with heat-inactivated serum (control tube) and of the survival ones after incubation with fresh serum (test tube), evidenced the disappearance in the test tube mainly of the greatest forms, which are typical of *T. rangeli* in axenic cultures.

Species	*T. cruzi*	*Trypanosoma rangeli*					
Strains	Y	San Agustín	Choachi	Macias	R 1625	H 14	SC 58
lysis %: mean, SD (range)	99.5 ± 0.7 (98.0-100)	74.0 ± 17.0 (50.1-99.8)	52.5 ± 21.6 (10.1-84.1)	34.9 ± 26.0 (0-69.4)	40.2 ± 24.2 (0-66.3)	83.3 ± 22.7 (23.4-99.5)	79.6 ± 27.8 (28.2-99.5)

BI-100
PARTIAL CHARACTERIZATION OF THE ENDOCYTIC SYSTEM IN *ENTAMOEBA HISTOLYTICA*

Batista E, De Menezes LF, De Souza W
Laboratório de Ultraestrutura Celular Herthe Meyer, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, RJ, Brasil

Entamoeba histolytica is an intestinal protozoan that parasites humans worldwide, causing a potentially fatal disease - amebiasis. It presents a monogenic evolutive cycle, starting by the ingestion of contaminated food and water with cystic forms, which upon reaching the digestive tract, liberate the motile forms, the trophozoites. These can live in the intestine as simple commensal or invade the mucosa, compromising internal organs, mainly the liver. This parasite is characterized by the absence of typical eucaryotic organelles such as mitochondria and peroxisomes and the presence of disperse elements of the Golgi complex and the endoplasmic reticulum. Little is known about the endocytic pathway in this organism despite the presence of a multitude of vesicles. Some of these vesicles have been considered phagocytic, pinocytic, residual bodies and autophagic vacuoles. The objetive of this work is to characterize the endocytic pathway of *E. histolytica* using a morphological approach integrating cytochemical detection of acid phosphatase, a lysosomal marker, and incorporation of labeled molecules which can be visualized by confocal laser scanning and transmission electron microscopy. The trophozoites were grown in TYI-S-33 medium supplemented with 10% bovine serum at 37ºC. For identification of acidic compartments, the cells were incubated with 20mg/ml acridine orange. Endocytic compartments were identified by incubation with 2mg/ml lucyfer yellow at 37ºC. For acid phosphatase cytochemistry, the cells were incubated in a medium containing 1,5 mM of $CeCl_3$, 3mM of Na-b-glicerophosphate plus 5% sucrose in 10mM sodium acetate buffer, pH-5,0. Gold-labeled proteins were also used to identify the components of the endocytic pathway. The cells were starved for 60 min at 37ºC in PBS and incubated with gold-labeled bovine albumin and human lactoferrin diluted in a proportion of 1:1 with PBS, for 5, 15 and 60 min. About 62% of the vacuoles were labeled with acridine orange and lucyfer yellow. Many, but not all, vacuoles presented reaction product indicative of acid phosphatase activity. The results obtained show that not all vacuoles found in trophozoites of *E. histolytica* are involved with endocytic processes.

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BI-101
PHYLOGENETICAL RELATIONSHIP AMONG *ENTAMOEBA HISTOLYTICA* STRAINS BASED ON RAPD AND RAP-PCR

Valle PR, Silva EF, Pires EM, Soares RPP, Pesquero JL, Gomes MA
Departamento de Parasitologia, ICB, UFMG, Belo Horizonte, MG, Brasil

The lack of a borderline to define differences between species in protozoan parasites makes this theme polemic. The morphological character usually used with this objective has been shown not efficient since it some times put together organisms a little different regarding biochemical parameters. More recently it has been utilized techniques that make possible classify the protozoan based on molecular structure like randomly amplified polymorphic DNA (RAPD) and the RNA arbitrarily primed (RAP-PCR). By these methods it is used primers randomly in a polymerase chain reaction (PCR) being the template cDNA in the case of RAP-PCR and genomic DNA in the case of RAPD. In this study we determined the fenotypical and genotypical variability of strains of *Entamoeba histolytica*. Further this variability was used for the stablishment of phylogenetical relationship between strains. In both methods we used 5 different primers which produced reproducible polymorphic pattern between strains. Analysis of RAP-PCR phenogram showed no relationhip between the estimated distance for each strain and the other characters like clinical findings, sorology, virulence and geographical origem. On the other hand, the trees obtained by RAPD are in agreement with these parameters. Thus, we may conclude from these results that the analysed phenotype – protein expression – is not a good criterion to set organisms belong the same specie differently for RAPD which seem to be a good parameter since it encompass a wide range of amoebae genome and therefore more appropriated to determine phylogenetical relationships.

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BI-102
PLASMODIUM VIVAX MSP1: EXPRESSION OF THE CARBOXYL-TERMINAL 19-KDA FRAGMENT IN *SACCHAROMYCES CEREVISAE*

Domingues FC, Hashizume AT, Laurino JP, Rodrigues MM, Castilho BA, Soares IS/*
Departamento de Microbiologia, Imunologia e Parasitologia, UNIFESP, Escola Paulista de Medicina, São Paulo *Universidade Federal do Pará, Belém, PA, Brasil

One of the most promising vaccine candidates against the erythrocytic stages of malaria is the merozoite surface protein 1 (MSP1). The C-terminal region of MSP1 is of special interest with respect to vaccine development. Immunization with recombinant proteins based on the sequence of $MSP1_{19}$ of human, non-human primates and rodents species of malaria can provide remarkable protection against experimental infection with blood stages of *Plasmodium* sp.

In earlier studies, using bacterial recombinant proteins based on the sequence of the N- and C-terminal regions of the MSP1 of *Plamodium vivax*, we have established that $PvMSP1_{19}$ is highly immunogenic for humans during natural vivax infection. To further explore the immunogenic properties of $PvMSP1_{19}$ as a vaccine candidate, we have produced $PvMSP1_{19}$ (residues Thr_{1615} to Ser_{1704}) in the yeast *Saccharomyces cerevisae* as glutathione S-transferase (GST) fusion proteins using a galactose-inducible promoter (pEG-KT). Two different plasmids were generated harboring the sequence corresponding to the Belém or Asian alleles of $PvMSP1_{19}$. After establishing the basic aspects for optimal recombinant fusion protein expression, the antigens were purified from cell extracts by affinity chromatography using glutathione agarose beads. By ELISA, we observed that both antigens were specifically recognized by serum from rabbits immunized with a baculovirus derived $PvMSP1_{19}$ or a mouse monoclonal antibody. Most relevant, the recombinant proteins were well recognized by human antibodies from *P. vivax*-infected individuals from the north of Brazil.

BI-103
PRELIMINARY RESULTS OF THE EFFECTS OF ANTI-HELMINTIC DRUGS AGAINST *GIARDIA LAMBLIA*

Campanati L, Green H*, Gadelha APR, Bernardo LC, Mattioli GM, Monteiro-Leal LH
Universidade do Estado do Rio de Janeiro, Av. Prof. Manoel de Abreu 48, 3º andar, 20550-170 Rio de Janeiro, RJ, Brasil *University of Maryland, Baltimore, USA

Giardia lamblia is a parasitic protozoa that causes giardiasis, a disease that affects thousands of people all around the world. In this study we used video-microscopy associated to image-processing systems (KS-300/400) and transmission electron microscopy to analyze the effects of many anti-helmintic drugs against this protozoa. Several drugs used in the treatment of helmintic diseases, such as the bezimidazoles, target the protein, tubulin. Because the *Giardia* cytoskeleton is composed mainly by microtubular structures, the effects of these drugs against it have been extensively studied. Among the effects are the disassembly of the adhesive disk, the organelle that the cell uses to attach itself and the lost of shape. One drug, used in the treatment of pinworm seems to affect the lateral flange, a membrane projection of *Giardia* and in certain concentrations, affects the adhesive disk and the number and size of vesicles, and changes the cell aspect. Another drug, the metronidazole, changes the cell shape and kills the cells in only a few hours. All these drugs have in common, the inhibition of cell growth *in vitro*. We are also testing these drugs during the interaction between *Giardia* and IEC-6, an intestinal epithelial cell line.

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BI-104
PREVALENCE STUDY OF CANINE LEISHMANIASIS IN GUARATIBA, RIO DE JANEIRO

Duarte R, Theophilo FAO, Mendes da Cruz DA, Madeira MF*, Ferreira FC**, Marzochi MCA
Laboratório de Imunodiagnóstico *Laboratório de Protozoologia, ENSP, Fiocruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil *Centro de Controle de Zoonoses, Secretaria Municipal de Saúde, Rio de Janeiro, RJ, Brasil

The leishmaniasis is an endemic disease in several areas of Brazil, several focuses of the disease has been found in the State of Rio de Janeiro. The discussion regarding the importance of the domestic dog as the main source of the disease maintenance in peridomestic surroundings, and following increases human cases, stays the same for many decades. Although several studies have already been made to define the importance of sick animals extermination attempting infection control. Serological tests were made for the evaluation of disease prevalence and the domestic dog participation in the cycle maintenance of leishmaniasis in Guaratiba. Samples of blood have been collected from domestic dogs of the area, through veined puncture. A total of 128 samples were picked and tested by indirect imunofluorescence reaction (RIFI) and ELISA. Domestic dogs that present positive reaction are examined, a liver

puncture is made, and a skin fragment is collected for culture. From the 128 collected samples, 8 (6.2%) were positive for RIFI and ELISA, 7 with high titles (1:640) and one with a low title (1:40). Six of these domestic dogs (4.7%) had parasites isolated in culture medium, but the parasites are still in characterization phase. Five of them had the isolated parasites of seemingly healthy skin. Clinical symptoms and serological titles point for the visceral leishmaniasis. These results suggest that the domestic dog is aiding in the maintenance of leishmaniasis peridomestic cycle at this specific area. The presence of animals with healthy appearance even with present parasites in healthy skin reinforces this hypothesis. New collections are being accomplished for the area evaluation. It will be fundamental the standardization of another more sensitive methods that give us the exact idea of the problem magnitude.

BI-105
ROLE OF POLYAMINE BIOSYNTHESIS ON THE INTERACTION BETWEEN *ENTAMOEBA HISTOLYTICA* AND *ENTAMOEBA MOSHKOVISKII* AND CULTURED EPITHELIAL CELLS

Linhares ABR, Yarlett N*, Silva-Filho FC
Laboratório Biologia da Superfície Celular, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brasil *Haskins Laboratories, Pace University, NY, USA

DL-a-difluormethylornithine (DFMO), a specific and irreversible inhibidor of ornithine descarboxylase (ODC) and 1-4-diamino-2-butanone (DAB), a putrescine analogue, were used to inhibit the amebae biosynthesis of this diamine. In amebae, as well as in many other cell types, polyamines play pivotal roles in cell growth and differentiation, regulating replication, transcription and protein synthesis. *Entamoeba moshkovskii* is a free-living protozoan used as an experimental model in parasitology research because of its similarity with the pathogenic *Entaboeba histolytica.* Adherence to the intestinal mucosa and subsequent disruptions of the intestinal mucosal barriers are important mechanisms by which *E. histolytica* causes invasive disease. *E. moshkovskii* (FIC) and *E. hitolytica* (HM-1:IMSS) were cultivated in the axenic TYI-S-33 medium suplemented or not with 20mM DAB and 30mM (DFMO). These protozoa were allowed to interact por 1h with MDCK cell monolayers. The results obtained in these studies revealed that both species attach to epithelial monolayers but only the amebae not treated with each one of DAB and DFMO damaged epithelial monolayers.

Surpported by Finep, CNPq-Pronex, Faperj, FUJB-UFRJ, and Capes.

BI-106
STRUCTURE-ACTIVITY RELATIONSHIP OF NEW GROWTH INHIBITORS OF *TRYPANOSOMA CRUZI*

Szajnman SH, Cinque GM, Zhong L*, Docampo R*, Rodriguez JB, Gros EG
Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón 2, Ciudad Universitaria, 1428 Buenos Aires, Argentina *Laboratory of Molecular Parasitology, Department of Pathobiology, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, 2001 South Lincoln Avenue, Urbana, IL, 61801, USA

As a part of our ongoing program aimed at the search for new chemotherapeutic agents to control *Trypanosoma cruzi* proliferation, we designed, synthesized and biologically evaluated a new series of compounds taken 4-phenoxyphenoxyethyl thiocyanate as lead drug. This compound had previously proved to be an extremely potent growth inhibitor of *T. cruzi* growth against epimastigotes and against the intracellular forms of the parasite. It is interesting to pointing out that this compound was much more active than two well known antiparasitic agents: nifurtimox and ketoconazole. In an attempt to improve the inhibitory action observed several structural modifications were made, mainly at the aromatic moiety, keeping the thiocyanate group at the polar extreme, which is responsible for the ultrapotent activity observed. There is tantalizing evidence to believe that these drugs block the ergosterol biosynthetic pathway. A structure-activity study is presented.

BI-107
THE GENETIC DIVERSITY OF *PLASMODIUM FALCIPARUM* FIELD ISOLATES FROM THE BRAZILIAN AMAZON

Sallenave-Sales S, Ferreira da Cruz MF, Bezerra da Rocha L**, Durlacher R*, Pang L*, Daniel-Ribeiro CT, Zalis MG**
Departamento de Imunologia, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil *USA Medical Research Unit **IBCCF, UFRJ, RJ, Brasil

Epidemiological studies carried out in malaria transmission areas has demonstrated a genetic diversity and complexity of *Plasmodium falciparum* populations in infected individuals. This parasite dynamics needs to be

understood in order to improve the establishment of efficient control measures. In this work, the degree of clonal diversity of 79 isolates from Peixoto de Azevedo (MT-Brazil), was analyzed by amplifying the central variable region of MSP-2 gene. The PCR fragments were first analyzed in a 2% agarose gel, and then by typing using specific probes corresponding the two main allelic families of MSP-2: FC27 and 3D7. Twenty seven isolates (34%) presented mixed infections, while fifty two (66%) presented only one fragment. Three different allelic variants were detected: the 520bp (51%) and 500bp (4%), which hybridized with FC27 probe and 620bp (45%) which hybridized with 3D7 probe. Twenty two fragments (21%) failed to hybridized with any probe. In order to verify microheterogeneities in the sequence of amplified products, we performed a SSCP technique (Single Strand Conformational Polymorphism). Each PCR product was digested with RsaI restriction enzyme, denatured and analyzed in a silver stained polyacrilamide gel. This approach confirmed the results observed in agarose gel and with the Southern Blot analysis using the families specific probes. The sequencing of each allelic type has been done.

Supported by Fiocruz /CNPq.

BI-108
THE ROLE OF PLATELLET ACTIVATING FACTOR (PAF) ON *LEISHMANIA (LEISHMANIA) AMAZONENSIS* INFECTION

Chebabo R, Lombardi RA, Lonardoni MVC*, Jancar S*, Corbett CEP
Laboratório Pat. Mol. Infecciosas (LIM-50) HC-FMUSP São Paulo, SP, Brasil (E-mail: ccorbett@usp.br) *Instituto de Ciências Biomédicas, USP, São Paulo, Brasil

The inoculation of promastigotes in the vertebrate host promotes acute inflammatory reaction in which humoral and cellular factors have possible role in the control or evolution of the disease. The PAF is a mediator of the acute inflammation, which can act in the *Leishmania* infection as chemiotatic for neutrophils and macrophage as well as increasing CR1 and CR3 expression in macrophages. It also stimulate the oxidative burst in neutrophils and macrophages. In adition, the PAF can induce production of citokines. In this work we studied the possible role of the PAF in the evolution of *L. (L) amazonensis* infection. Four mice, C57BL/6, treated with the PAF antagonist, BN52021, and non treated were infected with 10^6 promastigotes of *L. (L) amazonensis* in hind footpad and fragments from the subcutaneous inoculation site were collected after 5 weeks of the infection for histopathological analysis and the parasite burden, evaluated by limitant dilution. The hind footpad swelling was measured weekly. The treated group showed greater swellig than the control group up to 5 weeks. At light microscopy the treated group showed a more intense inflammatory infiltrate constituted mainly by macrophages with parasitophorous vacuoles filled with preserved parasite. Areas of necrosis were also found. The control group showed degenerative aspect of the parasites inside macrophages. In addition, the parasite burden was higher in the BN52021 treated group. The group with parasite inoculation only showed polymorphouss mononuclear infiltrare made up by macrophages, lymphocites and plasma cells. These preliminary data suggest that PAF could participate in the control of the infection caused by *L. (L.) amazonensis in vivo.*

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BI-109
THE ROLE OF SERUM ON THE MACROPHAGES INTERACTION WITH *LEISHMANIA IN VITRO*

Ura DM, Laurenti MD, Romeo S*, Goto H**, Corbett CEP
Laboratório de Patologia de Moléstias Infecciosas (LIM/50), Departamento de Patologia FMUSP, São Paulo, SP, Brasil *Universidade de Roma "La Sapienza" **Departamento de Medicina Preventiva FMUSP, São Paulo, SP, Brasil (LIM/38).

Formerly, we have studied the role of complement on the interaction between *Leishmania* parasites and inflammatory cells *in vivo.* Hamsters infected with *L. (L.) chagasi* showed that complement is important for parasite escape leading to visceral dissemination (1996 *Int J ExpPathol 77*:15-24). Opposite results have been observed in tegumentar experimental model, where mice depleted in complement by CVF and infected with *L. (V.) panamensis*, showed increased number of parasites in the lesion.

Now, in order to study the role of complement on macrophage/parasite interaction *in vitro*, we used cultures of mice peritoneal macrophages infected with *L. (V.) panamensis* promastigotes (MHOM/PA/91/CIDEP002). We analyzed the parasite adherence and uptake after 30 min of infection and the parasite survival after 48 hr of infection. The parasite uptake was high in the fresh serum presence, however it was even higher in the presence of heat inactiveted serum. The highest parasite adherence was observed using heat inactivated serum and the enhanced parasite survival was seen at the same condition too. These data were confirmed by the macrophage infection index after 30 min and after 48 hr which was always higher in the presence of heat inactiveted serum. Our preliminary data showed that the complement has an important role in the *L. (V.) panamensis* macrophage infection *in vitro*, decreasing the parasite adherence, uptake and survival.

Supported by Fapesp, Finep and LIM/50 HC-FMUSP.

BI-110
TOXOPLASMA GONDII AND MILK. SURVIVAL AND INFECTIVITY OF ME-49 STRAIN CYSTS IN ARTIFICIALLY INFECTED BOVINE MILK

Mayrbaurl-Borges M, Cardoso RPA, Araujo Fo. OF, Andrade Jr. HF
Laboratório de Protozoologia, Inst. Medicina Tropical de São Paulo, FMUSP, São Paulo, SP, Brasil (E-mail: – hfandrad@usp.br)

Toxoplasma gondii is an Apicomplexa protozoon with a complex life cycle, involving felines and warm blood animals. High prevalent, the infection is usually asymptomatic, except in the eye, or in intrauterine infections or in immunossupressed patients. This infection is transmitted mainly by ingestion of oocysts, in raw vegetables or water contaminated with cat feaces or, cysts, in undercooked products from animal origin, like meat and milk. We study the artificial infection of bovine milk with cysts of ME-49 strain of *T.gondii*, to evaluate the survival and infectivity of this infective form, as a possible contaminant of raw milk. Sterilized bovine whole milk was infected with cysts of ME-49 strain from brains of infected C57Bl/6j mice, and stored at 4ºC up to 20 days. This mixture was used to orally infect groups of C57Bl/6j mice by gavage (12 cysts/mouse). Cysts maintained and equally stored in sterile PBS were used as controls. The mortality, morbidity and serological evidence of infection were monitored by 40 days after the infection. Storage in milk induces a high mortality(100%/O days and 50%/5 and 10 days) of infected mice in short storage periods, declining thereafter, but without losing its infectivity, as detected by neurological symptoms and specific antibody production by ELISA assays. Storage in PBS resulted lower and erratic mortality in short periods, but this mortality was not found after 10 days of storage, with also a loss of infectivity(75%/10 days and 50%/20 days). Histopathological analysis of brains of milk stored cysts infected mice showed large areas of necrosis of brain tissue, with many and frequently seen cysts, as compared to a more restrict pattern in PBS-stored cysts infected mice. Anti-*T.gondii* IgG antibody titers of survivors, after 40 days of infection, were higher than in PBS infected animals, discarding those without signs of infection. All these data suggests that the survival of cysts of *T.gondii* are improved when stored in bovine milk, that could be ascribed to a less acidic environment, probably due a buffering effect of milk proteins, during cyst rupture in stomach after infection, with higher bradyzoite survival and more aggressive infections.

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BI-111
TRYPANOSOMA RANGELI: INTRA-SPECIFIC VARIABILITY AS ANALYZED BY PULSED-FIELD GEL ELECTROPHORESIS AND LOCALIZATION OF HOUSEKEEPING GENES

Toaldo CB, Sousa MA*, Steindel M**, Tavares CC
Departamento de Biologia Celular e Genética, IBRAG, UERJ, Rio de Janeiro, RJ, Brasil *Coleção de Tripanosomatídeos, Departamento de Entomologia, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro **Departamento de Microbiologia e Parasitologia, UFSC, Florianópolis, SC, Brasil

Trypanosoma rangeli is a non pathogenic parasite of humans and of a variety of domestic and wild mammals, being transmitted by triatomine bugs. It is found in Central and South Americas, sometimes in mixed infection with *T. cruzi*. This can be a problem to epidemiological studies, and then it is necessary to know as many specific markers as possible to help in the differential diagnosis of these species. In the present study we have analyzed the molecular karyotype of nine *T. rangeli* strains using contour-clamped homogeneous electric field electrophoresis (CHEF) followed by molecular hybridization using housekeeping genes as probes. These strains were isolated from different geographical regions of South and Central Americas. Two well-known *T. cruzi* isolates (CL Brener clone and Y strain) were included in this study as references. At least 20 chromosomal bands were observed in *T. rangeli* karyotype, these ranging from ~370 kb to 3,200 kb or more. In *T. cruzi* the lower band had ~550 kb. The majority of *T. rangeli* strains presented a distinct chromosomal banding pattern, excepting those from Brazil (SC58 and SC61), which were very similar to each other. The molecular hybridization using b-tubulin genes as probe displayed two groups of strains in *T. rangeli*. One including the SC58, SC61 and Pepita Gonzales strains, which presented these genes in chromosomal bands ranging from ~650 kb to ~750 kb. The other group, comprising the R1625, H9, H14, Macias, Choachi and San Agostin strains, showed that b-tubulin genes were located only in chromosomes arrested in the compression zone (above 1,000 kb), which was the same result observed for the two *T. cruzi* isolates. On the other hand, location of genes encoding heat shock protein of 70 kDa (HSP 70) and actin revealed great similarity among *T. rangeli* strains, discriminating them from *T. cruzi* isolates analyzed herein. In all *T. rangeli* strains, HSP 70 genes were detected in chromosomes of smaller size (~1,020 kb- ~1,300 kb) than in *T. cruzi* isolates (~1,500 kb). The actin genes were located in chromosomal bands ranging from ~2,350 kb to ~2,700 kb in all *T. rangeli* strains, while in *T. cruzi* they were found in smaller chromosomes (~1,500 kb and ~785 kb). Further studies will be undertaken to analyze the location of these two later genes in other *T. cruzi* isolates aiming to confirm whether they can be useful molecular markers to the differential diagnostic of *T. cruzi* and *T. rangeli*.

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BI-112
ULTRASTRUCTURAL STUDIES ON THE NUTRIENT UPTAKE IN LOWER TRYPANOSOMATIDS

Meirelles RMS, Soares MJ.
Laboratório de Biologia Celular de Microrganismos, Departamento de Ultra-estrutura e Biologia Celular, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil

Transport of proteins and nutrients into and outward eukaryotic cells is an essential step for several functional processes. Studies on *Trypanosoma brucei*, *Trypanosoma congolense*, *Trypanosoma cruzi* and *Leishmania* have demonstrated that these parasites are able to ingest proteins such as LDL, albumin and transferrin by receptor-mediated endocytosis and/or fluid-phase pinocytosis.

In the present work we have performed an ultrastructural and cytochemical study to analyze the ingestion of proteins in different species of monogenetic trypanosomatids, since little is known about endocytosis in these protozoa. *Crithidia deanei*, *Leptomonas colossoma* and *Herpetomonas muscarum muscarum* were maintained at 28º C in LIT medium for one day and then incubated for one hour with gold-labeled transferrin or albumin. Thereafter, the parasites were processed for routine transmission electron microscopy. Some parasites were incubated in a medium specific for the detection of acid phosphatase activity, containing sodium b-glycerophosphate, cerium chloride and 0.1M Tris-maleate buffer, pH 5.0.

Our results showed the absence of coloidal gold particles (8-10 nm in diameter) inside cytoplasmic vacuoles or specialized organelles in all tested parasites. However, the marker could be detected inside the flagellar pocket, but it was not bound to the pocket and flagellar membranes, suggesting absence of receptors for these proteins. Acid phosphatase activity (detected as an electrondense product) was detected inside the flagellar pocket, but not in the cytoplasm, indicating that this digestive enzyme is secreted to the extracellular medium. In addition, gold particles at the flagellar pocket were always colocalized with the acid phosphatase reaction, further suggesting that some kind of extracellular digestion of nutrients occurs in these protozoa.

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BI-113
VIDEO-MICROSCOPY ANALYSIS OF THE INTERACTION OF *TOXOPLASMA GONDII* WITH HOST CELLS

Monteiro-Leal LH, Stumbo AC, Campanati L, Barbosa HS*, Carvalho L
Departamento de Histologia e Embriologia, IB, UERJ, Av. Prof. Manoel de Abreu 48, 3o andar, 20550-170 Rio de Janeiro, RJ, Brasil (E-mail: laiscar@uerj.br) *Laboratório de Ultra-estrutura Celular, Instituto Oswaldo Cruz, Rio de Janeiro, RJ, Brasil

Toxoplasma gondii, agent of human and animal toxoplasmosis, infects a wide variety of eukariotic cells. Tachyzoites of *T.gondii* penetrate hosts cell through a process in which specialized organelles located in the anterior portion of the protozoa are involved. With the use of video-microscopy associated to analogue and digital image processing devices, we were able to follow the interaction process of *T.gondii* with different host cells, such as Human Umbilical Vein Endothelial Cells (HUVEC), Mouse Peritoneal Macrophages (MPM) and Skeletal Muscle Cells (SMC). Using this system, we observed that, sometimes, the *Toxoplasma* adheres to the host by its conoidal region and invades the cell, but occasionally, the parasite adheres and despite its movements, its does not penetrate the host cell. Besides, after several minutes it is common to see the parasite detaching from the target cell. Once in a while, the *Toxoplasma* keeps itself attached for several minutes (observed for more than 7 min)and then suddenly invades the cell (less than 30 sec). The process of invasion was followed using neutral red to analyze the behavior of the lysosomes from host cell.

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BI-114
VISCERAL LEISHMANAISIS IN RIBEIRÃO DAS NEVES, MUNICIPALITY OF METROPOLITAN REGION OF BELO HORIZONTE, MG, BRAZIL

Silva ES, Gontijo CMF, Santos SG*, Amorim VD*, Lemos FL*, Pirmez C**, Fernandes O***, Brazil RP
Laboratório de Leishmanioses, Centro de Pesquisas René Rachou, Fiocruz, Belo Horizonte, MG, Brasil (E-mail: silvarii@netra.cpqrr.fiocruz.br) *Secretária Municipal Saúde, Ribeirão das Neves **Laboratório de Imunopatologia, DBBM ***Departamento de Medicina Tropical, Instituto Oswaldo Cruz, Rio de Janeiro, RJ, Brasil

Visceral leishmaniasis in Brazil is a peri-urban and rural zooanthroponosis. Canines are considered a major reservoir of the infection. Blood samples in the filter paper were collected for serological diagnosis of dogs from different district in the municipality of Ribeirão das Neves, Minas Gerais, Brazil. Indirect immunofluorescent test (IFT) was used for, detection of antibodies of all studied animals with an IFT Kit for canine leishmaniasis (Fiocruz/ Bio-Manguinhos). In 7 dogs, peripheral blood samples with anticoagulated were collected for detection of the parasites by PCR. Aspiration of tibia bone marrow was obtained also for culture and parasitological survey. At the moment the indirect immunofluorescence test was positive in 280 (5.2%) out of 5266 dogs examined. The majority the positive cases come from district border with Belo Horizonte municipality. The IFT positive dogs were confirmed with detection of *Leishmania* parasites in 7 dogs examined. The PCR was realized in 02 blood samples and 2 bone marrow and we detected the presence of fragments with 120 bp, specific for *Leishmania*. The nature of the PCR product was identified by hibridization with specific *Leishmania* probes. The present study confirm the presence the *L. chagasi* as a responsable specie for canine visceral leishmaniasis in the RMBH.

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BI-115
EVALUATION OF PCR FOR DIAGNOSIS OF LEISHMANIASIS IN BOLIVIAN PATIENTS

Revollo S, Martinez E*, Bellido T, Sejas R, Gonzales G, Torrez M*, De la Riva J*, Harris E**, Belli A**
Instituto de Servicios de Laboratorio de Diagnóstico e Investigación en Salud (SELADIS), Facultad de Ciencias Farmacéuticas y Biquímicas *Instituto Boliviano de Biología de Altura, Facultad de Medicina, Universidad Mayor de San Andrés, Av. Saavedra Nº 2224, La Paz, Bolivia **Program in Molecular Parasitology, University of California at San Francisco, CA 94143-0422, Box 0422, USA

The leishmaniase consist of a group of disease with different clinical manifestations (Cutaneous, mucocutaneous and viceral), which represent a severe public health problem in endemic tropical countries world-wide. Leishmaniasis is a major cause of disease and morbidity in Bolivia, where there are an estimated 1800 new cases annually. Epidemiological studies have demostrated the presence of different species of *Leishmania* in the same geographical region in Bolivia. In recent years, DNA-based techniques have shown high potential for diagnosis and genetic characterization of various parasites. Sensitivity of molecular techniques has improved with the advent of the polymerase chain reaction (PCR). This method is capable of identifying the parasite with high sensitivity in samples such as dermal scrapings, biopsies, aspirates, and peripheral blood mononuclear cell (PBMCs) of the patients.

In this report, we have studied 30 patients with leishmaniasis from Inquisivi (La Paz), area where both *L. braziliensis* and *L. amazonensis* have been proven to circulate sympatrically. Two PCR protocols were used: 1) *L. braziliensis* complex PCR, described by Lopez et al. in 1993 that amplifie 70 bp fragment of the kinetoplast minicircle specific to members of the subgenus *Viannia*, which includes the *L. braziliensis* complex, and 2) multiplex PCR, developed by Harris et al. in 1998, which amplies fragments of different sizes from the variable region of the Spliced Leader RNA gene repeat . This assay differentiates among the three complexes of *Leishmania* found in the New World (*L. braziliensis, L. mexicana* and *L. donovani*) based on the size of the product and the sequence specificity of the 3' primer.

Dermal scraping were collected from the border of lesions, placed in 5% Chelex 100 [or Tris-EDTA (TE)], and boiled for 10 min. Our preliminary results show that 16/30 (53.3% were positive by the *L. braziliensis*-complex PCR, and 19/30 (63.3%) were positive for *L. braziliensis* and 2/30 (6.6%) were positive for *L. mexicana* using the multiplex PCR. Microscopical examination of stained scrapings yielded positive results for 6/30 (20%) of the patients.

These preliminary results demostrate that PCR method is more efficient that the conventionel method (microscopical examination). The multiplex PCR is more sensitive that *L. braziliensis* complex PCR, moreover, it can be used for identifying *Leishmania* complexes. We are currently using this method to conduct larger molecular epidemiological studies in Bolivia.

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BI-116
DETECTION OF *TRYPANOSOMA CRUZI* IN VECTOR AND HOSTS BY POLYMERASE CHAIN REACTION AMPLIFICATION OF NUCLEAR DNA

Revollo S, Riveiro E, Micayo J, Garcia G, Soto ML, Alcazar JL*, Flores M**, Illanes M, Collazos M, Harris E***
Instituto de Servicios de Laboratorio de Diagnóstico e Investigación en Salud (SELADIS), Facultad de Ciencias Farmacéuticas y Bioquímicas, Universidad Mayor de San Andrés, Av. Saavedra, Nº 2224, La Paz, Bolivia *Agroquímico, ORSTOM, Universidad Mayor de San Simón, Cochabamba, Bolivia **ORSTOM, Instituto Boliviano de Biología de Altura (IBBA), CP9214, La Paz, Bolivia ***Program in Molecular Parasitology, University of California at San Francisco, CA 94143-0422, Box 0422, USA

In the recent past, several groups developed molecular tests for the detection of *Trypanosoma cruzi*, including a variety of nuclear and kinetoplast DNA probes. PCR amplification of nuclear DNA sequences appears to be the most sensitive method for *T. cruzi* detection.

In this study we describe the detection of the 188 bp amplicon using a PCR assay developed by Moser et al. in 1989, in peripherical blood of chagasic patients and feces of triatomes collected in a endemic zone of Bolivia. On one hand, 30 children seropositive for *T. cruzi* [Test ELISA (+) e IFI (+)] were studied with parasitological methods, including: PCR, xenodiagnosis and Buffy coat. On the other hand, 50 triatomes isolated from houses of seropositive children were studied by the following methods: PCR, culture in Liver Infusion Midium suplemented with 10% Fetal Bovine Serum (LIT/10% FBS) and microscopic observation. The results show that 33.3% of peripheral blood samples were PCR positive, while conventional methods (xenodiagnosis and Buffy-coat) resulted in 13.3% and 10% positivity, respectively. The detection of *T. cruzi* in triatomes was positive in 56.9% by PCR, 51.7% by culture, and 41.4% by microscopic observation.

These results demostrate that PCR method is more efficient than conventionel methods for detecting *T. cruzi* in host as well as vectors samples. We are currently using this method for use in diagnosis of Chagas disease and molecular epidemiological studies in Bolivia.

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BI-117
METACYCLOGENSIS IN *LEISHMANIA CHAGASI*: ULTRASTRUCTURAL ANALYSIS AND BINDING CHARACTERIZATION TO THE INSECT-VECTOR MIDGUT

da Silva LHP, Oliveira SMP**, da Cunha-e-Silva NL*, Saraiva EMB
Laboratório de Imunobiologia das Leishmanioses, Departamento de Imunologia, Instituto de Microbiologia *Laboratório de Ultraestrutura Celular Hertha Meyer, Instituto de Biofísica Carlos Chagas Filho, UFRJ, Rio de Janeiro, RJ, BRasil **Departamento de Biologia, Instituto Oswaldo Cruz, RJ, Brasil

Promastigote forms of *Leishmania* are able of differentiating from a non-infective (procyclic) to an infective (metacyclic) form within the vector‘s alimentary tract and in axenic culture. Metacyclic promastigotes obtained from sandfly or from stationary growth phase are far more infective to vertebrate host than their procyclic counterparts obtained during logaritmic growth phase. The development of such infective promastigotes was first demonstrated for *L. major* and *L. donovani*, two Old World species.

We characterized the metacyclogenesis in axenic culture of *L. chagasi*, the causative agent of visceral leishmaniasis in the New World, by resistance to complement lysis, infectivity for macrophage *in vitro*, loss of PNA binding sites and a typical morphology by Giemsa staining (da Silva et al. 1997 *Mem Inst Oswaldo Cruz 92*: 83). In this work, we characterize the metacyclic forms of *L. chagasi* by ultrastructural analysis and binding to *Lutzomyia longipalpis* midgut, its insect-vector. *L. chagasi* was mantained at 26°C in Schneider´s medium supplemented with 10% fetal calf serum and 2% human urine. Promastigotes taken from logaritmic growth phase and stationary phase purified with PNA were used for binding assays to *L. longipalpis* midgut and for transmission electron microscopy. 3-5 days-old non blood feeded female sandflies were dissected in PBS. The isolated midguts (7-10/group) were opened along the length of the abdominal segment and incubated with promastigotes (106 cells) for 45 min at room temperature. The guts were repeatedly washed with PBS to remove free promastigotes and then each individual gut was homogenized and released promastigotes counted. An average of 8000 log phase promastigotes remained bound per midgut, whereas the binding of metacyclic forms was 1600 per midgut. The ultrastructural analysis by transmission electron microscopy showed that PNA (-) metacyclic promastigotes have a thick coat, long flagella and a slender cell body. Log phase promastigotes in contrast, present a large cell body and a thin cell coat.

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BI-118
TRYPANOSOMA CRUZI: IMPACT OF CLONAL EVOLUTION OF THE PARASITE ON ITS BIOLOGICAL AND MEDICAL PROPERTIES

Revollo S, Oury B*, Laurent JP*, Barnabe C*, Quesney V*, Carriere V**, Noel S*, Tibayrenc M*
Instituto de Servicios de Laboratorio de Diagnostico e Investigacion en Salud (SELADIS), Facultad de Ciencias Farmaceuticas y Biquimicas, Universidad Mayor de San Andrés, Av. Saavedra 2224, La Paz, Bolivia *Centre d’Etudes sur le Polymorphisme des Microorganismes (CEPM), Unité Mixte de Recherche Centre National de la Recherche Scientifique (CNRS)/Institut Français de Recherche Scientifique pour le Development en Cooperation (ORSTOM) 9926, BP 5045, 34032 Montpellier Cedex 01, France **Laboratoire d’Epidemiologie des Maladies a Vecteurs (LEMV), ORSTOM,BP 5045, 34032 Montpellier Cedex 01, France

Trypanosoma cruzi natural populations are subdivided into natural clones that can exhibit considerable genetic differences. It has been proposed that *T. cruzi* clonal structure has a major impact on this parasite's biological properties. The present work aims at testing this hypothesis. Twenty-one stocks which represent a broad sample of geographics, ecological cycles, and hosts, were selected among 4 major clones and characterized by Multilocus Enzyme Electrophoresis (MLEE) with 22 genetic loci and random amplification of polymorphic DNA (RAPD) with 10 primers on the one hand and by 14 different biological parameters on the other hand. These parameters were related to: (i) growth kinetics of epimastigotes and amastigotes; (ii) infection of culture cells by amastigotes; (iii) viability of extracellular trypomastigotes; or (iv) sensitivity of epimastigotes, trypomastigotes, and amastigotes to Benznidazole and Nifurtimox. MLEE and RAPD results exhibited parity to each other, as previously noted and showed that the 21 stocks were distributed into 3 main genetic groups, 19/20, 32, and 39, corresponding to the major clones 19, 20, 32, and 39 previously described on the basis of 15 isozyme loci. Most biological parameters showed a strong correlation to the genetic distances evaluated from either MLEE or RAPD, which favors the working hypothesis. The only exception came from drug sensitivity estimated on trypomastigote forms. The overall results made it possible to firmly reject the null hypothesis that there is no relationships between evolutionary distances and biological differences in *T. cruzi* natural clones.

Suported by UNDP/World Bank/WHO/TDR

BI-119
MORPHOMETRIC CHARACTERIZATION OF A STRAIN OF *TRYPANOSOMA CRUZI*

Santello FH, Dost CK, Albuquerque S, Carraro-Abrahão AA, Toldo MPA, Rissato e Garcia TA, Prado Jr. JC
Faculdade de Ciências Farmacêuticas, USP, Av. do Café s/n, Ribeirão Preto, 14049-903 São Paulo, SP, Brasil

Due to the morphological heterogeneity of the different strains of *Trypanosoma cruzi* they can be separated in groups based on distinct criteria such as morphology, curve of parasitemia, virulence, mortality and tissue tropism. It is well known that, this morphological intra-specific variation changes during the evolution of the experimental infection as well as its tissue distribution. In this experiment we did the morphometry of a strain of *T. cruzi* isolated from a *Panstrongylus megistus* captured in Ribeirão Preto, São Paulo, Brazil. *Mus musculus* and *Calomys callosus* weighing 25-29g were inoculated with 4000 metacyclic trypomastigotes and the parasitemia was evaluated. *M. musculus* showed negative results, but *C. callosus* displayed a patent parasitemia with peak on 15th day after inocule. The following table shows the morphometric values and the parasitemia curve respectively.

Parameters	8th day	10th day	14th day	19th day
Flagellum Length	10,53	10,71	9,62	9,43
Anterior End (NA)	9,48	9,16	9,22	6,33
Posterior End (PN)	12,99	11,44	10,34	10,83
Body Width	2,38	2,64	2,74	2,99
Body Length	21,63	20,65	19,58	17,2
Total Length	31,35	31,40	29,19	26,6
Nucleus Width	1,67	1,63	1,54	1,88
PN/NA	1,60	1,41	1,20	1,86
Kinetoplast (post.extr.)	2,89	1,64	1,76	1,31

According to the data, it seems that slender and longer forms are more frequent in the early phase of infection, while a slight tendency to find broad forms can be seen in the late phase. Imprints of heart, liver and spleen were analyzed and both *M. musculus* and *C. callosus* were positive for intracellular and free amastigotes, trypomastigotes and some intermediate forms. The presence of amastigotes in both experimental models and the absence of parasites in the blood circulation of *M. musculus* lead us to conclude that this strain shows a silvatic behaviour, by the fact that it developed a marked parasitemia in *C. callosus*, that it is known to be an excellent reservoir for Chagas' disease in South America as well as for other pathogens like Machupo virus, *Yersinia pestis* among others. Further investigations are being done by analyzing the histopathological smears of several organs including thymus.

BI-120
CHARACTERIZATION OF A MONOCLONAL ANTIBODY (LUCA-D5) AGAINST *LEISHMANIA AMAZONENSIS* LIPOPHOSPHOGLYCAN

Chaves CS, Sacks DL*, Saraiva EMB
Laboratório de Imunobiologia das Leishmanioses, Departamento de Imunologia, Instituto de Microbiologia, Universidade Federal do Rio de Janeiro, UFRJ, RJ, Brasil *NIAID-NIH, Bethesda, MD, USA

Promastigote forms of *Leishmania* are able to differenciate from a procyclic (non-infective) to a metacyclic (infective) stage within the alimentary tract of their sandfly vector or in axenic cultures, in a process named metacyclogenesis. Alterations on the lipophosphoglycan (LPG), the major glicoconjugate present in promastigote forms, is used to purify metacyclic forms from stationary growth phase cultures. Trying to isolate *L. amazonensis* infective forms, monoclonal antibodies (MoAb) against procyclic LPG were produced: hybridomas were prepared by fusion of SP2/0 myeloma cells with splenic lymphocytes from Balb/c mice intraperitoneally immunized with LPG isolated from *L. amazonensis* ("Josefa" strain) procyclic promastigotes in Ribi adjuvant. Anti-LPG producing hybridomas were selected by procyclic agglitination; one MoAb designated LuCa-D5 was recloned by limited dilution and expanded in pristane treated Balb/c mice. LuCa-D5 is a IgG3 (k), isotyped by ELISA technique. In this study, we characterize the species specificity of LuCa-D5 antibody by microagglutination assays with live promastigotes from 5 different *L. amazonensis* strains, 18 other *Leishmania* species and 6 non-*Leishmania* parasites from the Trypanosomatidae family. Positive agglutination was only observed with *L. amazonensis* "Josefa", PH8 and M2269 strains in titres from 400 to 1600. Indirect Immunofluorescence and ELISA assays are being performed to further characterize these reactions. In order to characterize the anti-carbohydrate specificity of this MoAb, we are using 39 different mono and oligosaccharides in ELISA inhibition assays. From these, D-manosamin (6mM) and fructose-6-phosphate (25mM) were the more potent inhibitors.

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BI-121
EXPRESSION OF GALACTOSYL RESIDUES DURING THE *IN VITRO* INFECTION OF *TRYPANOSOMA CRUZI* IN HEART MUSCLE CELLS

Barbosa HS, Soeiro MN, Guimarães EV, Mendonça RR, Meirelles MNL
Laboratório de Ultra-estrutura Celular, DUBC, Instituto Oswaldo Cruz, Av. Brasil 4365, 212045-900, Rio de Janeiro, RJ, Brasil

We have accumulated data in the last years, which have demonstrated the participation of galactosyl residues during the invasion of *Trypanosoma cruzi* in heart muscle cells [HMC] (Barbosa & Meirelles 1992 *Parasitol Res 8*: 404, 1993 *J Submicrosc Cytol Pathol 25*: 47). To study the expression of the galactosyl residues during the intracellular development of the parasite in HMC, we employed cultures infected with bloodstream forms, Y strain, for periods up 24 to 96 hr. The infected cells were fixed in 4% PFA + 0.1% glutaraldehyde in PBS, embedded in Lowicryl resin and processed for transmission electron microscope. The ultra-thin section were incubated with TBS + Tween + BSA for 30 min, labeled with RCA-lectin gold particles complex diluted in the same buffer for 1 hr at room temperature and analyzed under Zeiss EM10C TEM. The present results showed that non-infected cultures displayed few RCA-Au particles over the sarcolemma of HMC suggesting a weak expression of galactosyl residues at the cardiomyocytes surface. The marker was also found in cytoplasmatic vesicles and was enriched in cell-cell adhesion regions. After *T. cruzi* infection, no considerable alteration of RCA binding on the HMC surface was detected, however a higher positive intracellular labeling usually close to the parasites was observed in the cytoplasm of 72-96 hr infected cultures. Likewise, intracellular parasites also displayed gold particles, but there was a difference to the RCA labeling related to the parasite evolutive stage. Cultures infected for 48 hr showed intracellular amastigotes were little reaction with RCA lectin. The expression of galactosyl residues was more intensive after transformation of the amastigotes into trypomastigotes 72-96 hr after host cell invasion. The labelling was observed on the surface of parasite, more expressive in the flagellar pocket and also on the flagellar membrane. Our results demonstrate for the first time the expression of galactosyl residues in *T. cruzi* during the infection of heart muscle cells, which has been described to have an important role in the process of parasite-host cell interaction.

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BI-122
EXPRESSION OF THE ENDOPLASMIC RETICULUM ENZYMES IN THE *TRYPANOSOMA CRUZI* INFECTED AND NON INFECTED CARDIOMYOCYTES

Silva DT, Meirelles MNL
Laboratório de Ultra-estrutura Celular, Instituto Oswaldo Cruz, Av. Brasil 4365, 212045-900, Rio de Janeiro, RJ, Brasil

We have previously shown that Ca^{++} ATPase of the sarcoplasmic reticulum (SERCA) is involved in the process of invasion of cardiomyocytes by *Trypanosoma cruzi*. Treating cardiomyocytes (CM) with 1, 2 and 4mM of Thapsigargin, a tumor-promoting sesquiterpene lactone that binds to all SERCA ATPases and causes an irreversible inhibition of their activity, we found an inhibition of 64 to 68.% in the *T. cruzi* cardiomyocytes invasion. Also, this

drug affected the beginning of the proliferative stage of the intracellular forms of the parasite. Here we investigated whether the Glucose-6- Phosphatase (G6Pase), an enzyme that control the rate of breakdowm of carbohydrates via glycolysis, is affected in *T. cruzi* infected CM.

To investigate the distribution of glucose-6-phosphatase, the cells were fixed with 1% GA and 1% PFA for 20' at 4°C and incubated in a modified cytochemical medium containing 3 mM glucose-6-phosphate and 2mM $CeCl_3$.The control was performed in absence of the substrate. G6Pase activity was localized in SR cistern of the CM and within the nuclear envelope. Profiles of the SR were seen encircling the mitochondria and myofibrils. Infected CM did not display G6Pase in the SR profiles. In the intracellular parasites the activity was localized in the Golgi complex, in the flagellar pocket and in the plasma membrane, but no reaction was observed in the reticular structure. Heavily infected CM showed G6Pase distribution on the sarcolemma. Similar results were previously observed when we treated heavily infected CM with potassium iodide-osmium tetroxide solution.

Glucose-6-phosphatase is a multifunctional enzyme distributed in a variety of cell types of various organs, but in muscle cells it seems acts specifically at glucose-6-phosphate hydrolysis. This enzyme is tightly bound to the endoplasmic reticulum membrane. We observed that non infected cells from a infected culture expressed a normal G6Pase activity, but in infected cells this activity was not detected. This observations suggest a possible effect of *T. cruzi* infection in the host cell glucose metabolism.

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BI-123
TRYPANOSOMA CRUZI INFECTION AFFECTS MRNA REGULATION IN HEART MUSCLE CELLS *IN VITRO*

Pereira MCS, Singer RH*, Meirelles MNL
Departamento de Ultra-estrutura e Biologia Celular, Instituto Oswaldo Cruz, RJ, Brasil *Department of Anatomy and Structural Biology and cell Biology, Albert Einstein College of Medicine, Bronx, NY, USA

One of the most striking events in *Trypanosoma cruzi* – heart muscle cell (HMC) interaction is the disruption of the actin cytoskeleton, even in well formed sarcomeres (Pereira et al. 1993 *J Submicrosc Cytol Pathol 25*: 559-569). Because of this cytoskeleton disruption, we hypothesized that the regulation of actin mRNA would likewise be affected. In this study, we investigated the regulation of actin mRNAs during the cytopathology induced in myocardial cells by *T. cruzi*. Oligonucleotides probes were labeled with CY3 or 5´end labeled with gP32 ATP for in situ hybridization and Northern Blotting analyses, respectively. Agarose gel electrophoresis and Northern Blotting was carried out using standards procedures. To detected endogenous actin mRNA, fixed cells were hybridized with 20ng of the probe in hybridization buffer.

The analyses of isoactin actin mRNAs expression during cardiomyogenesis *in vitro* revealed an decrease in both cytoplasmic isoforms (b and g) mRNAs concomitant with an increase in a-cardiac actin mRNA level. *T. cruzi* infection caused a reduction of 50% in the a-cardiac actin mRNA after 72 hr of interaction. In contrast, b-actin mRNA levels increased approximately 47% after 48 hr of infection. These effects were independent of *T. cruzi* RNA and did not result from changes in GAPDH mRNA, which was used as internal standard. Furthermore, in situ hybridization demonstrated that a-cardiac, g and b-cytoplasmic actin are located in different compartment within the cytoplasm during cell myogenesis, which corroborate with the concept that most mRNA are addressed to the functional protein compartment. Ribosome RNA 18S also co-localize with g-actin mRNA in motile cells, which support the concept of mRNA localization as a mechanism for targeting protein synthesis at their sites of action. After *T. cruzi* infection, b-actin mRNA is translocated from the periphery to the perinuclear region in highly infected cells.

Our data provide evidences that *T. cruzi* affects actin mRNA regulation. Further studies will be carried out to understand which mechanisms are involved in such changes in actin mRNAs levels. However, this is the first case of a pathological mechanism which disrupts the normal cytoplasmic localization of mRNA.

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BI-124
REACTIVE AMYLOIDOSIS IN EXPERIMENTAL NEW WORLD CUTANEOUS LEISHMANIASIS

Cupolilo SMN, Gonçalves da Costa SC*
Departamento de Patologia, Universidade Federal de Juiz de Fora, MG, Brasil *Laboratório de Imunomodulação, Departamento de Protozoologia, Instituto Oswaldo Cruz, Av. Brasil 4365, 212045-900, Rio de Janeiro, RJ, Brasil

Systemic amyloidose has been described in man and classified as heredofamilial, immunoglobulin derived (or primary) and reactive (or secondary). reactive amyloidosis occurs in association with rheumatoid arthritis, neoplastic and chronic infections diseases such as tuberculosis, leprosy and schistosomiasis. Reactive amyloidosis has been described in various vertebrate species and it has been demonstrated among hamsters and mice chronically infected with *Leishmania*. It was previously described that Swiss outbred OF1 mice chronically infected with *L. amazonensis* MHoM/BR/76Ma-5, present renal and hepatosplenic amyloidosis. It has been demonstrated that different mice straises present consistent differences in serum level of acute phase proteins. The present investigation covered different inbred mice strains infected with *L. amazonensis*. Susceptible (BALB/c) and relatively resistant (C_{57}Bl/6) female mice were infected with 10^6 amastigotes into the left hind footpad and time course infection followed during 10 months. Because of the relation between amyloidosis and aging, control uninfected groups were investigated too. Clinical observations were performed by accompaniment for metastatic lesions formation and other aspects. Fragments of tissues were taken from the spleen, liver, kidney, lymph nodes and lesions and fixed in Milloning's buffer. The material was then routinely processed for paraffin embedding. Five mm thick sections were stained with hematoxylin-eosin and Gomori. Amyloid deposits were indentified by polarization microscopy of sections stained with Congo red. *L. amazonensis* infection in mice showed metastatic lesions and visceralization and was frequently associated with systemic amyloidosis. The animals developed the nephrotic syndrome with an increment of the mesangial matrix and proliferation of the kidney cells. Spleen and liver sections showed diffuse amyloid deposits. The results of the present study show that *L. amazonensis* infection in mice could reproduce a reactive amyloidosis that is similar to those observed in experimental Kala-azar in the golden hamsters or to those observed in man in association with several infections. Amyloidosis was observed in OF1 outbred mice as well as C_3H He/N and C57Bl/6 mice infected with *L. amazonensis*. Since TNF stimulated hepatocytes to produce acute phase proteins, its kinetics has been followed in different strains of mice infected with *L. amazonensis*.

BI-125
STUDY OF ACUTE CHAGASIC MICE UNDER CYCLOSPORIN A THERAPY: MODULATION AND CONFOCAL ANALYSIS OF THE INFLAMMATORY REACTION

Calabrese KS, Paradela ASRC, Zaverucha do Valle T, Tedesco RC*, Silva S*, Mortara RA*, Gonçalves da Costa SC Laboratório de Imunomodulação, Departamento de Protozoologia, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil *Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal de São Paulo, Escola Paulista de Medicina, Divisão de Parasitologia, São Paulo, SP, Brasil

Cyclosporin A (CsA) is an immunosuppressive drug that has its main action on inhibiting the transcription of some interleukines by T-$CD4^+$ lymphocytes. The *in vivo* effects of CsA on *Trypanosoma cruzi* infection were examined using different schedules of the drug in mice infected with the Y strain. Mice receiving 50 mg/kg every other day (eod) of CsA showed the most severe parasitemia and mortality rates. In contrast, mice receiving a single dose of CsA (200mg/kg) two days before or five days after infection had significantly lower parasitemia than control groups. CsA does not alter the splenomegaly induced by *T. cruzi* which reachs the peak on day 12 post-infection. In parallel to this severe splenomegaly the outbred OF1 mice infected with *T. cruzi* showed a progressive thymic atrophy detected by a drastic thymic weight loss. Low therapeutic CsA treatment (10mg/kg given eod) did not alter significantly the atrophy of thymus promoted by *T. cruzi* infection. In order to evaluate the effect of the drug on tissue, fragments of mice hearts were analyzed using specific monoclonal antibodies (Mabs) against CD4+ and CD8+ T-cells as well as macrophage receptors (Mac1). Cell nuclei were stained by 4,6-Diamino-2-phenylidole fluorescent binding probe for DNA (DAPI) and parasites were visualized after reaction with human chagasic sera and anti-human IgG coupled to Cy3. Immunohistochemical features were investigated with a confocal scanning light microscope. Not only inflammatory reactions but also amastigotes were found in the myocardium of mice that had been treated with a single dose of 200mg/kg of CsA before or after infection with *T. cruzi* Y strain. However, CsA given in therapeutic doses (10mg/kg given eod) prevented the colonization of the heart by parasites during the first twelve days of infection and only a myocarditis was observed. The inflammatory infiltrate was composed mostly by macrophages, some of them colonized by amastigotes. The results of the immunofluorescence analyzed by confocal microscope revealed that macrophages are a major component of the inflammatory infiltrate in all groups of CsA treated mice and also in the control group, while a small number of lymphocytes that are immunoreactive for CD4+-T cell antigens was observed.

BI-126

CLINICAL EPIDEMIOLOGICAL EVALUATION OF THE ASSOCIATION BETWEEN CUTANEOUS LEISHMANIASIS AND LEPROSY IN THE MUNICIPALITY OF BURITICUPU, MARANHÃO, BRAZIL

Costa JML, Melo LS, Silva AR, Ferreira LA, Rebêlo JMM,Gama MEA, Saldanha ACR, Barral A
Núcleo de Patologia Tropical e Medicina Social, Departamento de Patologia, Universidade Federal do Maranhão, São Luis, MA, Brasil

Cutaneous leishmaniasis (CL) and leprosy, are endemic diseases in the State of Maranhão and share many analogies for to be both granulomatous diseases, compromised cutaneous tissue and presented a great spectrum of clinical pictures, in addition occur mainly population with low social economic conditions. The region of Buriticupu (Amazonian of the State of Maranhão) constituted in the area of great importance for both diseases in the state. Recently observed patients that shown clinical and laboratorial picture alike with the association between these diseases. In spite of this constatation and unusual report in the literature about the association of CL and leprosy, intended relate the observations in seven patients come from this region that had this association. All patients had diagnosis of mucocutaneous leishmaniasis and leprosy in the health section of Federal University of Maranhão, localized in the Buriticupu municipality, in the period of January 1993 to September 1997, considered endemic area for both diseases. In the study existed predominance of male sex (71.3%), age between 9 to 64 years old being 57% landworkers, with poverty social economic. In relation to clinical of the patients noted the presence of cutaneous envolviment in all patients in respect of CL with 90% of ulcerated lesions, about leprosy 7 patients had dimorfic aspect and only one indeterminate clinical form. Used therapy with meglumine anthimoniate associated therapy for leprosy (dapsone and multidrugtherapy) with good results. Observed a new fact, the association of cutaneous leishmaniasis and leprosy, although exist reports in the literature of the association of granulomatous diseases caused for distinct parasites.