B-U-1 – *TRYPANOSOMA CRUZI* INCORPORATES PHOSPHOLIPIDS FROM *RHODNIUS PROLIXUS'* MIDGUT

[1]Folly E, [4]Dutra PML, [2]Lopes AHCS, [3]Oliveira MM and [1]Atella GC
[1]Deptº de Bioquímica Médica, ICB/CCS; [2]Deptº de Microbiologia Geral, IM/CCS; [3]Instituto de Biofísica Carlos Chagas Filho - UFRJ; [4]Deptº de Patologia e Laboratórios, FCM - UERJ, Rio de Janeiro,Brasil.

In insects the importance of lipid metabolism has been widely recognized. Neutral lipids and fatty acids constitute the major lipids of insect hemolymph whereas phospholipids are the main lipid class found in the midgut. In insect hemolymph lipophorins are the molecules responsible for lipid transfer between the tissues involved in lipid absorption, storage and utilization. We have previously demonstrated that *Rhodnius prolixus'* midgut are able to take up ^{14}C-oleic acid from lipophorin preferentially at 10th after a blood meal. The fatty acids are used to synthesizes neutral lipids (40%) and phospholipids (60%). The neutral lipids are released to hemolymph but phospholipids remained in the organ and are used to synthesizes midgut's membranes.

When *Rhodnius prolixus* are infected with *Trypanosoma cruzi* these parasites multiplies and develops in intestine of insect attached to the membranes. Now we are studying the capacity of *T. cruzi* to incorporate phospholipids from *R. prolixus'* midguts membranes. The infection of *Trypanosoma cruzi* increases 2-fold the ^{3}H-palmitic acid incorporation by midgut from hemolymphatic lipophorin. The time course was linear up to 30 minutes. The ^{3}H-palmitic acid was used to synthesizes mainly phospholipids membranes (70% from total lipids). In order to separate the parasites from membranes after ^{3}H-palmitic acid incorporation the luminal content was subject to a Percoll gradient. In the infected midguts the ^{3}H-phospholipids were found associated with *T. cruzi*. These data indicate that *R. prolixus'* midgut can provide essential lipids which will perhaps constitute parasite membranes.

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B-U-2 – SPECIFIC INHIBITORS OF THE *TRYPANOSOMA CRUZI* PROLYL-ENDOPEPTIDASE (TC 80) ARREST TRYPOMASTIGOTES ENTRY INTO HOST CELLS

Grellier, P.[1], Vandeville, S.[2], Joyeau R.[3], Frapier, F.[3], Teixeira, A.R.L.[4], Schrével, J.[1], Sergheraert, C.[2], and Santana, J.M.[4]
[1, 2]Laboratoire de Biologie Parasitaire et Chimiotherápie, EP CNRS 1790, et Lab. de Chimie, Muséum National d'Histoire Naturelle, 61-63 rue buffon, 75231 Paris, France.
[3]Institut de Biologie et Institut Pasteur de Lille, 1 rue du Pr. Calmette, 59021 Lille, France.
[4]Lab. de Pesquisa em Doença de Chagas, Universidade de Brasília, CP 04436, 70919-970 Brasília, Brazil. E-mail: jsantana@unb.br

Advances in the biochemical and molecular biological knowledge of parasitic protozoa have shown that proteases play key roles in several aspects of their metabolism, differentiation, and infection of hosts. Taking advantage of a substrate containing the collagen motif "GPLGP" we have characterized a *Trypanosoma cruzi*-secreted 80 kDa prolyl-endopeptidase (Tc80) with specificity for native collagen. Its features suggest a role on *T. cruzi*-host cell infection.

To evaluate the possible role of the Tc80 activity on trypomastigotes entry into host cells we used available inhibitors of prolyl-endopeptidases, a specific Tc80 inhibitor that has been discovered by screening of a peptide combinatorial library, and others that have been synthesized based on the LGP sequence. Their specificity were assayed by comparing K_is for Tc80 and other *T. cruzi* proteases (oligopeptidase B or Tc120, cruzipain and cathepsin B-like) as well as for the human prolyl-endopeptidase, trypsin and chemotrypsin. The experiments consisted of the incubation of 5X10^5 trypomastigotes in DMEM containing 10% fetal calf serum with different concentrations of inhibitors during 1 h at 37 ºC. The treated parasites were used to infect 5X10^4 rat myoblasts during 3 h at 37 ºC. The cultures were then washed 4 times with the culture medium and incubated for 72 h, following fixation and staining. The controls consisted of untreated parasites in the presence of the highest concentration of DMSO used to solubilize the inhibitors. In other experiments, host cells were infected and then treated with inhibitors.

Those specific inhibitors with the highest affinity for the Tc80 arrested trypomastigotes entry into host cells but not amastigotes and epimastigotes growth. The K_is correlate well with the concentrations of inhibitors necessary to produce 50% inhibition of infection. These data suggest that the Tc80 plays an important role on *T. cruzi*-host cell infection and may represent a good target for chemotherapy of Chagas' disease.

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B-U-3 – AN ACIDIC VESICLE-TUBULAR NETWORK IS THE EARLY ENDOSOME OF *TRYPANOSOMA CRUZI*

Porto-Carreiro, I.;Miranda, K.; Sant'Anna, C.; De Souza, W.; Cunha-e-Silva, N.
Lab. Ultraestrutura Celular Hertha Meyer, Instituto de Biofísica Carlos Chagas Filho, UFRJ, Rio de Janeiro, Brasil.

The late events in *T. cruzi* epimastigotes endocytic pathway have already been described, with storage of nutrients in reservosomes been well documented (Soares and De Souza, 1991, Parasitol Res. 77: 461) Reservosomes were later characterized as late endosomes (Soares et al., 1992, J. Cell Sci, 102:157). The early events of protein uptake were less studied, however. Subpellicular microtubules preclude vesicle budding from plasma membrane, except for flagellar pocket membrane domain and the end of the cytostome. Data obtained from binding of fluorescein labeled or gold conjugated tracers pointed the cytostome as the site of entry. To follow the endocytic pathway from the cytostome, at the anterior region of the parasite, until the reservosomes, at the posterior end, we performed short time incubations with the same tracers. After two, five and fifteen-minute endocytosis, gold particles were found inside vesicles and tubules extending along the parasite, prior to fusing with reservosomes. Whole-unfixed cells that had uptaken gold-protein conjugates for fifteen minutes were washed in water diluted saline and dried onto electron microscope grids. Images obtained by selecting electrons with energy loss revealed long gold-filled tubules at the cell posterior end. The acidic nature of the early network was verified using acridine orange. Based on pH and protein uptake kinetics we propose that the vesicle-tubular network is the early endosome of *Trypanosoma cruzi* epimastigotes.

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B-U-4 – CHARACTERIZATION OF THE CELL ADHESION SITE AND THE 3F6-EPITOPE OF *TRYPANOSOMA CRUZI* METACYCLIC-STAGE SURFACE GLYCOPROTEIN GP82

Patricio M. Manque, Daniel Eichinger**, Maria A. Juliano#, Luiz Juliano#, Jorge E. Araya, Nobuko Yoshida
Departamento de Microbiologia, Imunologia e Parasitologia, # Departamento de Biofísica, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, S.P., Brasil, ** Department of Medical and Molecular Parasitology, New York University School of Medicine, New York, N.Y., USA

The insect-stage metacyclic trypomastigotes of *Trypanosoma cruzi* express the surface glycoprotein gp82 which has been implicated in mammalian cell invasion. By using recombinant proteins and synthetic peptides based on gp82, we have characterized the cell adhesion site of gp82 and identified the epitope for 3F6, a monoclonal antibody that inhibits parasite invasion. Recombinant protein J18, containing the full-length gp82 sequence (amino acids 1-516), bound to HeLa cells in a receptor-mediated manner, whereas the protein Del-4/8, lacking 65 amino acids (257-321) of the central domain was virtually devoid of binding activity. Two other proteins with shorter deletions, Del-4 (257-271) and Del-8 (293-321), retained their adhesive capacity but bound to HeLa cells to a significantly lesser degree than J18. The binding sites deleted in Del-4 and Del-8 contained a pair of acidic amino acids critical for cell adhesion. Thus, the protein Del-E/D, lacking two pairs of contiguous glutamic acid (259/260) and aspartic acid (303/304) residues, had negligible cell binding activity. None of a series of ten overlapping synthetic peptides spanning the gp82 central domain competed with the binding of J18, Del-4 or Del-8 proteins to HeLa cells. Inhibition of cell adhesion was achieved, however, with a 22-mer hybrid peptide p4/8 formed by two noncontiguous sequences (257-273 and 302-306) containing the acidic amino acid pairs. This peptide, generated by juxtaposition of sequences that are separated by a hydrophobic stretch in the linear molecule, may be mimicking a conformation-dependent cell binding site of gp82. Antibody competition experiments with the same set of 20-mer overlapping peptides mapped the 3F6-epitope to the gp82 sequence represented by peptide p3 (244-263), which has a partial overlap with the cell adhesion site.

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B-U-5 – PARTICIPATION OF IMMUNOGLOBULINS IN THE PATHOGENESIS OF INFLAMMATION IN LIVER IN VISCERAL LEISHMANIASIS

Mathias, R.and Goto, H. Instituto de Medicina Tropical de São Paulo, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil.

In visceral leishmaniasis interstitial inflammation occurs in liver, kidney and lung whose pathogenesis is not completely known. In kidney, liver and lung we have previously detected immunoglobulin deposits in visceral leishmaniasis in hamsters. However, some reports show that antigens are scarse or not detectable in the lesions; these data do not support the mechanism due to a deposition of immunocomplexes. Recently a novel mechanism of lesion due to internalization of IgG by endothelial cells has been described in Lupus nephritis. We have previously shown that IgG present in the sera from visceral leishmaniasis patients and from hamsters is internalized by endothelial cells in vitro. The aim of the present study is to deepen the study of the participation of immunoglobulin in the pathogenesis of visceral leishmaniasis in hamsters inoculated intraperitonially with $2x10^6$ amastigotes of *Leishmania (Leishmania) chagasi*. We analyzed the liver from hamsters with 30 days and 60 days of infection under transmission electron microscopy using immunodetection of immunoglobulins. Samples were incubated overnight with polyclonal anti-hamster gammaglobulin antibody and in a subsequent steps for one hour with protein A-gold (10nm). Eight to 10 micrographs were taken from each sample and morphometry done by counting the protein A-gold particles.

We detected an increased amount of immunoglobulin in the endothelial cells and in Disse space that was enlarged in infected animals compared with non-infected controls.

Immunodetection of immunoglobulins using protein A-gold (10 nm) in liver: morphometry. Number (mean) of protein A-gold particle /cm^2 after subtracting the background labeling.

Localization	Non-infected	30 days PI	60 days PI
Endothelial cell	0	0.10	0.23
Disse space	0.02	0.82	0.51
Sinusoid	0	0.22	0.10

We conclude that internalization of immunoglobulins by endothelial cells also occurs in visceral leishmaniasis 'in vivo' suggesting its participation in the pathogenesis in visceral leishmaniasis.

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B-U-6 – SURAMIN EFFECTS ON *TRYPANOSOMA CRUZI* ULTRASTRUCTURE

Ribeiro D.F., Meyer-Fernandes, J.R. & Souto-Padrón T.
Laboratório de Protozoologia I, Instituto de Biofísica Carlos Chagas Filho, CCS, Bloco I, UFRJ, Rio de Janeiro, RJ - tspadron@biof.ufrj.br

It has already been shown that when extracellular ATP interacts with purinergic receptors (P_2 receptors) on the surface of different cells, it is able to induce a wide diversity of effects on cellular physiology. However, the signal transduction induced by this nucleotide through its specific receptor can be interrupted by ecto-ATPases expressed on cell surface. Previous studies showed the presence of a Mg^{2+}-dependent ecto-ATPase associated to the plasma membrane of all the evolutive forms of the parasitic protozoan *Trypanosoma cruzi.* We had already shown that when tripomastigotes forms of *T. cruzi* has its Mg^{2+}-ATPase inhibited by Suramin, a potent antagonist to P_2 receptors, its ability to infect mouse resident macrophages is diminished by about 50% (Ribeiro et al., *Mem. Inst. Oswaldo Cruz*, Suppl. II, vol. 93, appendix, 1998). This data suggests a participation of the enzyme on parasite-host cell interaction. In the present study we analyze the effects of Suramin on epimastigotes growth and on the ultrastructure of all evolutive forms of *T. cruzi*. Epimastigote forms were grown in LIT medium containing 10% of fetal calf serum for 8 days. This parasites showed a strong decrease on its growth when 500µM of Suramin was added to the medium. Epimastigotes grown during 4 days in the presence of Suramin and analyzed by light microscopy showed a more elongated cell body when compared with control. Samples to be analyzed at the transmission electron microscope were fixed in 4% formaldeyde, 2.5% glutaraldeyde in 0.1 M Na cacodylate buffer pH 7.2 with 3.7% sucrose for 1 h at room temperature. Cells were then post-fixed in 1% O_sO_4 in Na cacodylate buffer containing 0.8 % potassium ferrocyanide and 5 mM calcium chloride for 2 h at room temperature, dehydrated in acetone and embedded in Epon. Thin sections were stained in uranyl acetate and lead citrate before being observed with a ZEISS 900 transmission electron microscope. After 40 minutes in the presence of Suramin epimastigote forms present several enlarged electron lucent membrane bound compartments. Besides being different form reservosomes, these compartments are probably related to the endocytic pathway of the parasite . Experiments to confirm this suspect are proceeding. The effects of the drug on amastigotes and tripomastigotes ultrastructure are also under investigation.

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B-U-7 – FATE OF IMMUNOGLOBULINS BOUND TO THE *TRYPANOSOMA CRUZI* SURFACE

Siqueira, P.L.A. , Coelho, R. & Souto-Padrón, T.
Laboratório de Protozoologia I, Instituto de Biofísica Carlos Chagas Filho, CCS, Bloco I, UFRJ, Rio de Janeiro, RJ
tspadron@biof.ufrj.br

Previous studies have described a receptor for immunoglobulin Fc (FcR) on the surface of *Trypanosoma cruzi* which function was related to the mechanism by which these parasite scapes from host immune response. Specific human and animal IgG antibodies against *T. cruzi* bound to trypomastigotes and induce mobility of surface antigens of live blood forms to form a cap in the anterior and posterior poles of the parasite in a process dependent of time, temperature and inhibited by sodium azide. Our group using primary polyclonal and monoclonal antibodies made several immunocytochemical studies analyzing the presence of specific antigens on different evolutive forms of the parasite. In all of them the incubation of pre-fixed parasites, only in the presence of a secondary antibody-gold complex, were made as control. In none of those controls binding of the secondary antibody was observed. An interesting approach is to analyze the binding and fate of non related immunoglobulins to the surface of live epimastigote, amastigote and trypomastigote forms of *T. cruzi*. Briefly all evolutive forms were incubated in the presence of different IgG-gold complexes such as human, goat and rabbit, using a pulse-chase protocol. Parasites were incubated for 15 minutes to 4 hours. Some samples were submitted to an incubation with BSA-gold complex during 4 hours followed by incubation in conjugate-free medium for 20 hour chase. In epimastigotes gold particles were observed inside the cytostome and reservosomes. Those located inside the reservosomes mainly after 60 minutes, are flocculated suggesting a proteolytic degradation of IgG. After the pulse chase experiment epimastigote presented many reservosome profiles labeled with flocculated gold particles. Comparing the endocytosis of BSA-gold complex with IgG-gold complex, at the same time, we noted that both complexes were co-localized and albeit the same dilution of them were used the number of IgG-bound particles is very high in comparison to the BSA-gold complex suggesting an internalization by receptor-mediated endocytosis. Incubation of trypomastigote in the presence of the same conditions as described above, showed that these forms shedded the immunogold complex from their surfaces after 15 minutes at the posterior and anterior end of the parasite. Prolonged incubation in the presence of gold-complex did not showed cell membrane labeling or any evidence of endocytosis. Amastigote forms did not shown any binding with IgG-gold complex in the several incubation time analyzed.

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B-U-8 – FURTHER EVIDENCE THAT SIALOADHESIN EXPERESSED ON MACROPHAGES DO PLAY A ROLE ON THE *TRYPANOSOMA CRUZI* INTERACTION

DaMatta, R.A.[1], Santos, C.L[1], Seabra, S.H.[1], Crocker, P.R.[3], de Souza, W[1&2]
[1]Laboratório de Biologia Celular e Tecidual, Centro de Biociências e Biotecnologia, UENF; [2]Laboratório de Ultraestrutura Celular Hertha Meyer, Instituto de Biofísica Carlos Chagas Filho, UFRJ, RJ, Brasil; [3]Department of Biochemistry, University of Dundee DD1 5HN, UK. renato@cbb.uenf.br

It has been shown that mouse peritoneal macrophages cultured with homologous serum (HS) express, on their surface, a sialoadhesin (Crocker and Gordon, *Immunology*, 65: 515-522, 1988). We have shown that cultured of macrophages with HS resulted in a higher association (double) after interaction with epimastigotes and trypomastigotes of *Trypanosoma cruzi* when compared to macrophages cultured with fetal bovine serum (DaMatta *et al*., *Men. Inst. Oswaldo Cruz, Suppl*. II, 93: 92, 1998). To determine if sialoadhesin expressed on the surface of these macrophages is involved in the higher association index, we used monoclonals antibodies (SER-4 and 3D6) to block the sialoadhesin receptor. Macrophages were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 2% HS (MøHS) or 2% fetal bovine serum (MøBS) for 48 hours. Interactions were performed for 2 hours with trypomastigotes and epimastigotes of *T. cruzi*. Macrophages were incubated with both monoclonal antibodies for 30 minutes (1µg/ml) at 37°C and trypomastigotes added. 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. After the interaction, cells were washed (to remove loosely-attached parasites), fixed with Bouin and stained with Giemsa. The survival of epimastigotes was also evaluated. Association index was determined by multiplying the percentage of infected macrophages by the mean number of parasites per cell. The association index for trypomastigote allowed to interact with MøBS was 7.7, it was 21.6 for MøHS, and 6.4 for MøHS treated with the monoclonals. MøHS and the MøBS destroyed epimastigotes, and no significant differences could be found between both macrophages. These results suggest that sialoadhesin is involved on the interaction of *T. cruzi* with MøHS.

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B-U-9 – BIOGENESIS OF RESERVOSOMES AND TRANSFERRIN UPTAKE DURING THE DIFFERENTIATION OF *TRYPANOSOMA CRUZI* TRYPOMASTIGOTES TO EPIMASTIGOTES IN LIT MEDIUM

Figueiredo, R.C.B.Q.[1], Rosa, D.S.[1], Gomes Y.M[2]., Nakazawa, M[2]. & Soares M.J.[3]
[1]Departamento de Patologia e Biologia Celular and [2]Departamento de Imunologia, CPqAM/FIOCRUZ, Av. Mores Rego S/N°, Campus da UFPE, Recife, PE. [3]Departamento de Ultra-estrutura e Biologia Celular, IOC/FIOCRUZ, Av. Brasil 4365, Manguinhos, Rio de Janeiro, RJ

Epimastigote forms of *Trypanosoma cruzi* present large membrane-bound organelles at their posterior end, named reservosomes. These organelles are absent in amastigote and trypomastigote forms. Previous studies have shown that proteins ingested by the parasites are accumulated in the reservosomes. Stereological studies demonstrated that these organelles gradually vanish during metacyclogenesis and it has been suggested that nutrients accumulated within the reservosomes are used as energy source for this process. Although these structures seem to play a pivotal role in *T. cruzi* endocytosis and metacyclogenesis processes, little is known about their biogenesis in epimastigote forms.

In this study we have incubated bloodstream and culture-derived *T. cruzi* trypomastigotes in LIT medium supplemented with gold-labelled transferrin (Tf-Au) in order to analyse, at the ultrastructural level, the occurrence of reservosomes and endocytosis during the differentiation process of trypomastigotes to epimastigotes.

Our results showed that the trypomastigote differentiation in LIT medium is a complex event. In the first 24 h bloodstream trypomastigotes have differentiated into amastigotes and intense cellular division were observed in the latter forms. With 48 h the amastigotes strongly adhered to each other and large cell clusters were observed. After 72 h of cultivation small reservosomes were detected close to the Golgi complex, in cells with characteristics between amastigotes and epimastigotes. After 96 h epimastigote rosettes were observed, with the release of parasites from the clusters borders. These same differentiation steps were observed when culture-derived trypomastigotes were used. However, the time required to complete differentiation by these cells was smaller than that required for bloodstream trypomastigotes. Tf-Au was observed only within the reservosomes of epimastigote forms. The absence of endocytic activity in both trypo- and amastigotes suggests that other energy source, such as glucose, might be used by these cells. Taken as a whole, these data suggest that endocytic activity is strongly related to reservosome biogenesis in *T. cruzi* epimastigote forms.

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B-U-10 – THE FATE OF SURFACE TRANSFERRIN RECEPTOR IN THE HEART MUSCLE CELL DURING ITS INTERACTION WITH *TRYPANOSOMA CRUZI*

Mendonça, RR, Guimarães, EV, Meirelles, MNL and Barbosa, HS
Lab. Ultra-estrutura Celular, Depto. Ultra-estrutura e Biologia Celular, IOC, FIOCRUZ, Rio de Janeiro, RJ, Brasil

The study of molecules involved in the interaction of *Trypanosoma cruzi* with the heart muscle cell has demonstrated that glycoconjugates participate in the stages of cellular recognition and interiorization of parasite as well as composition of the parasitophorous vacuole (Barbosa and Meirelles, 1992. Parasitol. Res. 70: 404-409; Barbosa and Meirelles, 1993. J. Submicrosc. Cytol. Pathol. 25: 47-51;. Meirelles et al, 1997. Scanning 19: 201-202; Soeiro et al, 1999. Cell Struct. Funct. 24:139-149).

We are studying the ultrastructural characterization of the participation of the transferrin receptor in the membrane of heart muscle cell during the adhesion and invasion phases of *T. cruzi* with host cell.

Primary cultures of heart muscle cells were incubated previously with the complex transferrin-colloidal gold for 30 min at 4°C and rinsed in Ringer solution and then infected with metacyclic forms of *T. cruzi* (Dm 28C clone) for periods of 30 min at 18 hours. The cultures were fixed and processed as routine for transmission electron microscopy.

The results here obtained indicate that: during the adhesion step *T.cruzi*-host cell, the marker for transferrin receptor, was not found mediating this adhesion; receptors present on the surface of the heart cell, were endocytosed and located in vesicles in the cytoplasm of the cell. The visualization of the endocytosis of the transferrin receptor and the absence of the marker in the parasitophorous vacuole membrane, indicate different routes of the receptor endocytosis and of the parasite interiorization in heart muscle cells. We observed the co-location of two metacyclic forms of the parasite in only one parasitophorous vacuole as we have already previously demonstrated (Barbosa et al 1993. J. Submicroc. Cytol. Pathol. 25; 613-615). After 15 h of interaction parasite-cell, the presence of the marker was found inside of the vacuole suggesting the fusion of endocytic vesicles with the vacuole containing the parasite.

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B-U-11 – JUNCTIONAL, CORBULAR AND PARALLEL SARCOPLASMIC RETICULUM DISTRIBUTION PATTERN IN *TRYPANOSOMA CRUZI* INFECTED AND NON INFECTED CARDIOMYOCYTES

Silva, D.T.; Soares, R.; Mello, T.G.; Baetas, W.C.; Nogueira, A.R. & Meirelles, M.N.L.
Lab. de Ultra-estrutura Celular, Instituto Oswaldo Cruz, FIOCRUZ, Av. Brasil 4365, 21045-900, RJ, Brasil

Primary cultures of cardiomyocytes *Trypanosoma cruzi* infected and non-infected were used to study the structure and function of endoplasmic reticulum/sarcoplasmic reticulum (ER/SR) by confocal laser microscopy, enzymatic treatments and ultrastructural cytochemistry. Studies of serial tomographies obtained with the fluorescent dye $DiOC_6(3)$ and by the confocal laser microscopy (CLM) showed that the reactive ER/SR profiles in non-infected and 72hs infected cells were intracellularly located at a space ranging from 2.5 to 6.5μm and 1.5 to 7.5μm, respectively, indicating a larger thickness in infected cells than in non-infected cells due to the presence of the intracellular parasites. It was not observed morphological differences between non-infected and 24hs infected cells. Intracellular calcium stock sites detected by the Oschman technique, revealed the parallel, sub-sarcolemmal and the corbular SR located as a network in the cytoplasm, near the sarcolemma or surrounding and within the myofibrils, respectively. Glucose6Pase distribution showed the same pattern in the cardiomycytes. These reactions were drastically reduced in *T. cruzi* infected cells that exhibited rare profiles in their cytoplasm, although intracellular parasites displayed positive reaction in the nuclear membrane, plasma membrane, flagelar pocket, cis Golgi cisternae and in rare cytoplasmatic membrane profiles. Even employing different methodologies, our results always point to a strong association between the host cell ER/SR profiles and the parasites besides an altered distribution pattern of cardiomyocytes ER/SR due to the presence of intracellular parasites. It is possible that the close proximity between the host cell ER/SR and the parasites may facilitate metabolic changes necessary for growth and differentiation of this intracellular protozoa.

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B-U-12 – GLYCOINOSITOLPHOSPHOLIPIDS FROM *TRYPANOSOMA CRUZI*: PROBABLE ROLE IN GROWTH AND DIFFERENTIATION OF THE PARASITE

Carreira JCA; Thomaz N & Jansen AM
Laboratório de Biologia de Tripanosomatídeos, Departamento de Protozoologia - Instituto Oswaldo Cruz - FIOCRUZ.

One of the major surface glycoconjugates of *Trypanosoma cruzi* are the Glycoinositolphospholipids (GIPLs). GIPLs presents a wide structural variation according to the parasite strain. They were correlated with down-regulation of T-lymphocyte activation and with transduction of distinct signals of the J774 macrophage cell line dependent on the GIPL domain involved. GIPLs also play a role in the adhesion of *T. cruzi* epimastigotes (Y strain) on the gut epithelium of *Rhodnius prolixus.* To analyze the biological significance of those molecules derived from other *T. cruzi* isolates, we studied purified GIPLs from, respectively, G-645 and C-48 strains (isolated from marsupials). The *in vitro* biological parameters evaluated were adhesion, proliferation and metacyclogenesis. We utilized the clone DM 28c of *T. cruzi* followed up by the methodology of metacyclogenesis in TAU 3AAG: after the stress phase in TAU (2 h), the parasites were inoculated in Leighton tubes with 1.5 ml of TAU 3AAG medium with a glass cover slip pre-adsorbed with purified GIPLs from G-645 or C-48 strains. The cultures were quantified after 24, 48, 72 and 96 hours.

A direct correlation between GIPLs from G-645 strain presence and parasite proliferation was observed. Differentiation in presence of G-645 GIPLs was significantly reduced, while no effect was observed with GIPLs from C-48. Parasites adhered preferentially to areas on the slides coated with GIPLs from G-645 but not with C-48. Similar results were observed with integral parasites from the same strains previously fixed with paraphormaldehyde and tested under the same conditions, suggesting a specificity of this phenomenon. Our results suggest that GIPLs are molecules important for adhesion, differentiation and proliferation of *T. cruzi*. We believe that GIPLs could have some role on the definition of the composition of some *T. cruzi* populations in a given habitat.

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B-U-13 – *TRYPANOSOMA CRUZI* PARASITOPHOROUS VACUOLE DESTINATION INSIDE VERO CELLS ACUTELY OR PERSISTENTLY INFECTED WITH *COXIELLA BURNETII*

Andreoli, W.K., Rabinovitch, M & Mortara, R. A.
Disciplina de Parasitologia, Escola Paulista de Medicina, UNIFESP, Rua Botucatu 862, 6º andar, CEP-04023-062, São Paulo, SP, Brasil.

Metacyclic or blood trypomastigotes from *T. cruzi* invade a wide variety of cells and forms a parasitophorous vacuole essentialy by lysosome recruitment. *Coxiella burnetii* is an obligate intracellular pathogen that causes Q fever. Once inside cells these bacteria live within large acidified vacuoles with lysosomal markers. Previous studies shows that *T. cruzi* can live and multiply within *C. burnetii* vacuoles. In the present work, we have attempted to demonstrate the destination of metacyclic and cell derived trypomastigotes parasitophorous vacuoles inside Vero cells, acutely or persistently infected with *C. burnetii* vacuoles. For these experiments, we first incubated Vero cells (10^4 cells/500ul per well) on coverslips with 10 µl aliquotes of medium containing *C. burnetii*. This innoculum was sufficient to cause large vacuole formation after 3 days of incubation and we defined this as acute *C. burnetii* infection. We have also established Vero cells persistently infected with *C. burnetii* defined these as those that has already one more trypsin subculturing. Coverslips were incubated with cell-derived or metacyclic trypomastigotes forms from *T. cruzi* (CL strain, 10^8parasites/10^6cells) for 3, 4, 6, and 24 hours. The proportion of parasitophorous vacuole fusion was determined using an epifluorescence microscope, counting parasites inside Vero cells labeled with rabbit serum against *T. cruzi* (FITC), human anti-LAMP (lysosomal protein) monoclonal antibodies and DAPI.

Our results indicate that acute *C. burnetii* infection promotes trypomastigote invasion in a higher degree than persistent infection (3 times higher). The peak of parasitophorous vacuole (LAMP labeled) formation is reached 4 hours after invasion with both trypomastigote forms. Fusion of metacylcics trypomastigotes parasitophorous vacuole is determined by colocalization of parasite within *C. burnetii* vacuoles. There is 30% of colocalization 3 hours after invasion increasing after 6 hours (60% of colocalization). Cell derived trypomastigotes maintained 30% of colocalization even 6 hours after infection.

We concluded from these experiments that there are different patterns of parasitophorous vacuole fusion of the different *T. cruzi* invasive forms and that there is lysosome recruitment of by these forms in Vero cells with large acidified *C. burnetii* vacuoles. We observed no significant differences in parasitophorous vacuole fusion between acute or persistent infection with *C. burnetii*.

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B-U-14 – PARASITE-HOST CELL INTERACTIONS IN THE REACTIVATION OF CHRONIC *TRYPANOSOMA CRUZI* INFECTION IN ANIMALS SUBMITTED TO CHEMICAL IMMUNOSUPPRESION

Taniwaki, N.N. *, Silva, S.**, Fernandes, A.B. ** & Mortara, R.A.**
* Seção de Microscopia Eletrônica, Instituto Adolfo Lutz, São Paulo, SP, Brazil
** Departmento de Microbiologia, Imunologia e Parasitologia, Universidade Federal de São Paulo – Escola Paulista de Medicina, SP, Brazil

An experimental model of chronic Chagas' disease is being developed to gather information about the distribution of *Trypanosoma cruzi* epitopes during the intracellular life cycle of parasite in cardiac tissue after reactivation of the disease, aiming at a better understanding of the reactivation process that occurs in humans.

Calomys callosus were infected with 4000 trypomastigotes of the Y strain of *T. cruzi*. Parasitemia was determined periodically by a direct microscopic procedure, after acute phase, the animals were kept for three months. Cyclophosphamide (200 mg per kg) was administered for immunossuppression in four doses, once a week. When parasitemia increases were observed, animals were sacrificed, hearts were removed, fixed and embedded in paraffin; 5-8 µm thick sections were processed for confocal immunofluorescence.

Monoclonal antibodies (MAB) raised against *T. cruzi* forms were used based on the previously described reactivities: MAB 2C2, reacts with a carbohydrate epitope in Ssp-4, a major surface glycoprotein of amastigotes while MABs 1D9, 2B7, 3B9, 4B9 recognize epitopes on Ssp-4 different from MAB 2C2. Samples were also routinely double labeled with DAPI to visualize parasites' kinetoplasts and nuclei.

MAB 2C2 showed a fluorescence pattern homogeneously distributed over the amastigote surface; MAB 4B9 and 3B9 presented the same distribution observed in MAB 2C2. Very weak or negative labeling was observed with MAB 1D9 and 2B7, respectively. MAB 3B2 reacts with a noncarbohydrate epitope of flagellated forms and MAB 4B5 which also detects a noncarbohydrate epitope, stained flagellar pocket in elongated amastigotes and parasite's surface in dividing forms. Active proliferation was evidenced by 3B2 and 4B5 staining and we could also demonstrate the distribution of the amastigote epitopes within the host cell.

Supported by FAPESP, CAPES and CNPq

B-U-15 – DESTRUCTION OF NITRERGIC NEURONS OF THE HEART MOUSE IN THE ACUTE PHASE OF EXPERIMENTAL *TRYPANOSOMA CRUZI* INFECTION

Maifrino,L.B.M.; Liberti, E. & De-Souza, R.R.
Laboratório de Estudos do Sistema Nervoso Autônomo, Departamento de Anatomia, Instituto de Ciências Biomédicas, USP. Instituto Dante Pazzanese de Cardiologia , São Paulo, SP.

The objective of the present work was to study the cardiac plexus of mice acutelly infected with the Y strain of *Trypanosoma cruzi* by means of a histochemical method for NADPH-diaphorase, on whole mount preparations of the atria. The NADPH-diaphorase histochemical technique detect the presence of nitric oxide (NO), a putative neurotransmitter substance in the plexus. NO is sinthesized from L-arginine by the enzyme nitric oxide sinthase (NOS). Evidence has been provided that neuronal NOS is identical to nicotinamide adenine dinucleotide phosphate diaphorase (NADPHd) in brain and peripheral nervous tissues. Therefore NADPHd-histochemistry is used to label NOS- containing neurons in the nervous system. Male isogenic mice of 1 month (n=5) non infected and acutelly infected by the Y strain of *T. cruzi* were used in this study. The animals were killed with ether. The thorax was opened, the heart removed, the atria separated from the ventricles and dissected in order to remove excess of fat and connective tissue from external surface. The preparations were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS), Ph 7.2, at 4°C for 30 min. After 2x10 min rinses in PBS at room temperature the atria were incubated in the following solution for the demonstration of NADPHd for 60 min at 37°C, in the dark and with continuous agitation: b-NADPH reduced form (Sigma) 0,1 mg/ml, nitroblue tetrazolium (Sigma) 0.5 mg/ml in PBS containing 0.2% Triton X-100. The pieces were mounted as laminar preparations in glicerol. Cardiac neuron numbers were counted under the microscope. The mean ± S.D. were then calculated. The NADPHd-positive neurons were identified by the formazan deposits filling the cell bodies and processes, and their absence in the cell nuclei.

A population of NADPHd- stained neurons bodies was observed predominantly in the ganglia of the plexus over the muscular layer of the atria, dorsal to the muscle itself, in the connective tissue of the subepicardium. There were also many stained axons throughout the plexus and in the cardiac muscle layer. The numbers of NADPHd-positive neurons in the non-infected and in the infected mice were 57 ± 17 and 27 ± 7 stained neurons. There was a significant difference in the neuron number between the two groups of animals. Therefore, the number of neurons were decreased by 33% in the chagasic animals in comparison to the non-infected mice. These results suggest that the *T. cruzi* infection affects the population of nitrergic cardiac neurons and implies that the production and release of NO are affected and the potency of its action upon cardiac function consequently reduced.

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B-U-16 – VIRULENCE PATTERNS AND HISTOPATHOLOGY OF DIFFERENT *TRYPANOSOMA CRUZI* GENETIC GROUPS IN BALB/C MICE

Toledo MJO, Tafuri WL[*], Teodoro LF[**], Santos ACC[**], Lopes AM[**],Veloso VM[***], Carneiro CM[**], Bahia MT[***], Lana M[**]
Departamento de Análises Clínicas, Universidade Estadual de Maringá, Maringá, PR, [*]Núcleo de Pesquisas em Ciências Biológicas (NUPEB), [**]Departamento de Análises Clínicas, Escola de Farmácia, [***]Departamento de Ciências Biológicas, ICEB, Universidade Federal de Ouro Preto (UFOP), Ouro Preto, MG, Brasil

Fifteen laboratorial clones obtained by micromanipulation, pertaining to 3 distinct *Trypanosoma cruzi* genetic groups 19/20 (8 clones), 39 (3 clones) and 32 (4 clones), characterized by 22 *loci* enzimatic and RAPD (Tibayrenc and Ayala, 1988; Tibayrenc et al, 1993) were studied in Balb/c mice.

Groups of 10 mice were infected through intraperitoneal route with 10^4 blood trypomastigotes and the parasitemia (fresh blood examination and hemoculture), infectivity, mortality and histopathology analysed.

Parasitemia was significantly different among the 3 genetic groups and the decreasing number of trypomastigotes/ 0,1 ml of blood were: 7,895 to 695,000 for the genetic (group 19/20) > 4,500 to 217,800 (group 39) > 800 to 12,400 (group 32). Positivity of hemoculture was higher in the acute phase (AP) than during the chronic phase (CP) only for the genetic group 39 [83,33% (AP) and 30% (CP) respectively]. Among the other genetic groups, independent of the phase of the infection, the rates of positivity were similar (88,88% to 100%). The percentage of infectivity was very similar 95,68 and 97,5%) respectively for the genetic groups 19/20 and 39 and higher than the genetic group 32 (83,01%). The mortality rates were low and similar among the 3 genetic groups in both phases of infection. During the AP tissue parasitism was in general discreet or absent although amastigotes were relatively more easily detected in mice infected with clones of the genetic group 19/20 (3 out of 8 clones) than genetic group 32 (1 out of 4 clones) and 39 (0 out of 3 clones). Inflammatory process during AP was discreet to accentuated and present in heart, skeletal and smooth muscle (group 19/20), heart and smooth muscle (group 39) and smooth musculature (group 32) and always more discreet in CP.

Genetic group 19/20, more genetically distant of group 32 showed extreme values of parasitemia and group 39 situated in intermediary genetic position showed also intermediary number of blood trypomastigotes. However this parameter is not correlated with all measures of virulence.

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B-U-17 – FIRST EVIDENCE OF ESOPHAGUS ELECTOFUNCTIONAL ALTERATIONS IN HAMSTERS INFECTED WITH *T. CRUZI* IN CHAGAS' DISEASE CHRONICAL AND ACUTE PHASES

Almeida, R.S.; Pereira, S.; Silva, A.A.; Crema, E.; Ramirez, L.E.; Lages-Silva, E.
Disciplinas de Parasitologia e Cirurgia do Aparelho Digestivo; Departamentos de Ciências Biológicas e Cirurgia da Faculdade de Medicina do Triângulo Mineiro-FMTM, Uberaba, MG.

The electromanometry of the esophagus inferior sphincter (EIS) analyses the neuromuscular function of the esophagus precociously detecting functional alterations, even before anatomical alterations. Experimentally, the hamster reproduces the acute and chronical phases in Chagas'disease. In this animal the digestive form of the infection presents histopathological similarities to the human infection, as well as developing of megas. In this work the techniques of electromanometry was adapted to hamsters and a probe was standardized so as to be adjusted to the anatomical conditions of the animals. The use of this probe possibilitated to measure, with reproductibility, the pressure levels in the EIS in hamsters, both in normal and infected one with *T. cruzi*. Four groups with ten to eleven hamsters were used. One as control group, two in the acute phase (30 days), infected with VIC and JG strains, and one in the chronical phase (10 months), infected with VIC strain. The ideal probe was 45cm length, 1,22cm of external diameter. It was constituted of three catheters, each one having an orifice in its distal portion, disposed at 120º from one to another (two in the extremity and the third, 2cm above these ones). The electromanometry was performed with an infusion pump — "MK II Manometric Perfusion Pump"— , a polygraf — "PC Polygraf HR" — and with the "Polygram for Windows 2.02 program". Ketamine hydrochoride (10mg/kg) was used as anesthetic. We observed that the pressure value, normal to the EIS, vary from 10.3 to 46.8mmHg (mean of 32.55mmHg). In the acute phase the animals infected with JG strain showed values between 17 and 116.9mmHg (mean of 26.4mmHg), and, with VIC strain, between 13.6 and 145.4mmHg (mean of 36.8mmHg). In the chronical phase, the pressure has oscillated from 27.8 to 81.4mmHg (mean of 36.8mmHg). According the preliminary data, we concluded that the hamster, either in acute or chronical phase of Chagas'disease, presented functional alterations of EIE pressure, since the sphincter hypertonicity was detected with pressure levels two-three times higher than normal values in 20% of the animals in acute phase (3/15), and in 9% of chronical phase (1/11) with the two strains analysed.

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B-U-18 – MORPHOFUNCTIONAL ALTERATIONS IN THE KIDNEYS OF HAMSTERS (MESOCRICETUS AURATUS) INFECTED AND REINFECTED WITH *TRYPANOSSOMA CRUZI*

Cabrine, M. S., Lara, V.C., Chapadeiro, E., Matos, A., Gomes, R.A.S., Santos, V.M., Ramírez, L.E.
Discipline of parasitology – Department of Biologic Science of Triângulo Mineiro Medical School, Uberaba, Minas Gerais, Brazil.

In experimental studies with hamsters, we have observed that one of the most frequent Kidney alterations during the chronic stage of the Chagas'disease is nephrosclerosis or glomerulosclerosis with or without amiloidosis. These findings have not been reported in the literature. Considering these facts, we decided to study the morphofunctional alterations in the kidney of hamsters infected and reinfected with *Trypanosoma cruzi*.

We studied 120 young male hamsters, that were divided in six groups of 20 animals. The first group (G0) was the control one. In groups G1 to G5, 2 x 10^3 *T.cruzi* blood forms of VIC strain were inoculated one to five times in intervals of 45 days. The survivals were killed ten months after the first inoculation and submitted to autopsy. The kidney was fixed in 10% formalin, included in paraffin 4µ slices were stained with hematoxilin and eosin. The blood was drawn and the serum was properly stored for biochemical studies (urea, creatinine, sodium, albumine, total proteins and globulins) using comercial kits.

The average and mean standard deviation for the groups with one to five (G1 to G5) infections and for the control group were: urea, mg/dL (G1=119,81 ± 35,56; G2=66,97 ± 5,33; G3=97,81 ± 43,64; G4=165,15 ± 36,68; G5=80,85 ± 42,29 e G0=55,75 ± 8,9), creatinine, mg/dL (G1=0,74 ± 0,05; G2=0,47 ± 0,04; G3=0,41 ± 0,12; G4=0,20 ± 0,11 e G0=0,38 ± 0,04), total proteins, g/dL (G1=6,22 ± 0,93; G3=6,98 ± 0,65; G4= 6,51 ± 0,51 e G0=6,15 ± 0,82), albumin, g/dL (G1=1,88 ± 0,45; G2=2,15 ± 1,16; G3= 2,26 ± 0,26; G4=2,18 ± 0,41 e G0=2,40 ± 0,20), globulins, g/dL (G1=4,34 ± 0,49; G3=4,72 ± 0,45; G4=4,33 ± 0,25 e G0=3,75 ± 0,81). The collected blood was insufficient for the measurement of total protein and globulins in group 2, and creatinine, total proteins, albumine and globulins in group 5. The histopathological analysis confirmed the findings of nephrosclerosis or glomerulosclerosis, with or without renal amyloidosis in the following percentages: nephrosclerosis (G1=50,0; G2=12,5, G3=40,0; G4=14,3 e G5=50,0), glomerulosclerosis (G1=16,6; G2=25,0; G3=0; G4=28,6; G5=50,0), amyloidosis (G1=83,3; G2=25,0; G3=50,0; G4=42,9; G5=100,0). These alterations were not present in the control group.

These findings suggest that important kidney lesions, in different stages of Chagas'disease, can cause chronic renal failure and death in these animals. The high incidence of amyloidosis in the infected and reinfected groups compared to the absence of amyloidosis in the control one suggest that *Trypanosoma cruzi* may cause secondary renal amyloidosis by cronic antigenic stimuli. Our findings suggest that the reinfection is not necessary for kidney lesions but can make them more important.

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B-U-19 – ALTERATIONS OF THE ENTERIC NERVOUS SYSTEM IN CHAGAS'DISEASE REVEALED BY WHOLE-MOUNT IMMUNOHISTOCHEMISTRY

Moraes, S.R.A ., Coutinho, H.B., Robalinho, T., Miranda, J, Galindo, L.C.M., Dias, E.S.F.
Laboratório de Patologia do CpqAM/FIOCRUZ – Cidade Universitária, UFPE, Recife, PE.

The primary function of the nerve supporting cells is to permit the cell bodies and processes of neurons to be arranged and maintained in a proper order. Besides recent studies suggest that these cells also act as neurotrophic factores. The nerve supporting cells have not been more investigated in Chagas'disease. So the aim of this study has been to perform a nerve supporting cells lesions within the myenteric plexus of the intestinal wall by means of whole-mount immunohistochemistry during Chagas'disease.

Segments of colon of the Swiss mice infected with the Y strain of *Trypanosoma cruzi* were submitted immunohistochemical procedures using antibodies against glial protein (protein S100).

In control mice there was strong staining of nerve fibers by S-100 in myenteric plexus. Examination of the myenteric plexus in Chagas' disease S-100 immunoreactivity nerve plexus was markedly thinner than those of control case. Because supporting nerve cells are essential in the maintenance of basic physiological functions of neurons, this study suggest that their reduction in myenteric plexus during Chagas'disease may be an important factor in disorders of muscle wall.

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B-U-20 – MICROVASCULAR ALTERATIONS DURING THE CLEARANCE OF EPIMASTIGOTE FORMS OF *TRYPANOSOMA CRUZI.* AN INTRAVITAL MICROSCOPY STUDY

[1] Umekita , L. F., [1] Lopes-Ferreira, M. V., [2] Fontes Piazza, R. M., [4] Umezawa, E. S., [4] Nascimento, M. S., [3] Farsky, S. H. P. & [1] Mota, I.
Lab. Imunopatologia (1), Lab. Microbiologia (2) e Lab. Imunoquímica (3) do Instituto Butantan, Av. Vital Brasil 1500, São Paulo, SP e Lab. Protozoologia (4), Instituto de Medicina Tropical-USP, Av. Dr. Enéas de Carvalho Aguiar 470, São Paulo, SP.

The basic mechanism of non-infectivity of epimastigote forms of *T. cruzi* is supposed to be due to lysis mediated by activation of the alternative pathway of the complement. However, in murine model lysis is not the only mechanism that prevent the infection by these forms of the parasite since they are non-infective in mice whose serum does not lyse them. In the present study, we investigated the microcirculatory alterations during the clearance of epimastigote forms of *T. cruzi*. Direct microscopy examination of the microcirculatory events of the exposed mouse cremaster muscle was done, before and after intravenous inoculation of labelled and non-labelled parasites, using intravital microscopy. We observed circulating clumps, possibly of parasites and platelets, immediately after parasite inoculation and its deposition to some sites of the endothelium of venules and capillaries resulting in the interruption of the microcirculation in some of these vessels probably by obstruction by the clumps. The retention on the capillaries, although intense, could only be observed when labeled parasites were injected. The mobility of epimastigotes in the clumps indicate that parasites were alive in the lumen of vessels. The interaction between parasites, platelets and endothelial cells may be crucial for initiation of the inflammatory reactions and may indicate one of the pathways through which mice are able to eliminate these parasite forms. These observations are certainly very important and are consistent with our previous observation that epimastigotes are not lysed by complement activation but are phagocytosed and destroyed by cells through a mechanism dependent of platelets, component C3 of the complement and distinct population of cells (Umekita et al., J. Parasitol., 84: 1190-1195, 1998).

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B-U-21 – *TRYPANOSOMA CRUZI* - BIOLOGICAL AND HISTOPATHOLOGICAL VARIABILITY OF POPULATIONS ISOLATED FROM VERTEBRATE HOSTS AFTER 2 AND 7 YEARS OF INFECTION

Veloso, V.M.[1]; Mendes, D.V.M.[1]; Andrade, E.M.[1]; Alves, C.F.[1]; Carneiro, C.M.[1]; Lana, M.[2]; Tafuri, W.L.[1]; Chiari, E.[3]; Bahia, M.T.[1]
[1] Departamento de Ciências Biológicas, UFOP; [2] Departamento de Análises Clínicas, UFOP; [3] Departamento de Parasitologia, UFMG

T. cruzi presents a considerable biological and biochemical variability, as verified by various experimental studies. Clonal structure has been proposed to explain these differences (Tibayrenc *et al.*, 1988). The passage of the multiclonal population of *T. cruzi* through the vertebrate host may cause the elimination of some clones due to their inability to compete and propagate in the host environment (Macedo & Pena, 1988). To verify this phenomenon, comparative studies were performed using Be-78 strain and four isolates from dogs infected with this strain seven (Be-78-A, B and C) and two (Be-78-D) years ago. Biologic parameters (infectivity, parasitemia, mortality and histopathology) were comparatively evaluated in Swiss mice inoculated through the intraperitoneal route with 5000 blood trypomastigotes of the 1th, 5th, 10th, 15th , 20th and 25th passages in mice of the isolates as well as the original Be-78 strain. Parasitemia was evaluated daily according to Brener (1962). Histopathological studies were carried out during the peak of parasitemia (acute phase) and 120th day of infection (chronic phase). Necropsy was complete with systematic collection of the brain, heart 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. Parasitism and inflammation were not observed in the brain. There was no differences in tissue tropism between Be-78 strain and the isolates (Be-78 A, B, C, and D). All lesions observed during acute and chronic phase were more intense in animals infected with Be-78C isolate, compared to Be-78 strain. The Be-78 A and B isolates caused lesions with intermediate intensity, similar to Be-78 strain. All animals infected with Be-78D showed more discreete lesions than animals infected with Be-78 strain. The data agree with parasitologic results displayed in the following table. These results suggest that *T. cruzi* undergoes changes and/or selection throughout the infection in vertebrate hosts. This selection could depend on the host-parasite relationship. Further biochemical and genetic characterization of the four populations will be done.

strain	pre-patent period (day after inoculation)	peak of parasitemia (trypomastigotes /0.1 ml de blood)	mortality
Be-78	5º	80249	0%
Be-78A	10º	40608	3.03%
Be-78B	8º	47694	3.33%
Be-78C	6º	168910	13.8%
Be-78D	9º	27944	0%

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B-U-22 – ECOLOGY OF *TRYPANOSOMA CRUZI* TRANSMISSION CYCLE IN THE SYLVATIC ENVIRONMENT: STUDY OF INFECTION IN *LEONTOPITHECUS ROSALIA* AND *CALLITHRIX JACCHUS* (PRIMATA: CALLITHRICHIDAE)

Lisboa, CV; Verona , CE*; Ruiz-Miranda , C*; Ivo, A & Jansen, AM.
Departamento de Protozoologia, Laboratório de Biologia de Tripanosomatídeos – IOC/FIOCRUZ; *Laboratório de Ciências Ambientais, Universidade Estadual Norte Fluminense – UENF.

Previous studies on the ecology of the *Trypanosoma cruzi* transmission in different ecosystems fragments, have shown that distinct and simultaneous transmission cycles may occur. It's worthy enphasizing that the barriers which separate these cycles are not determined by the niche or by the forest layer that the reservoir or vectors occupy. These findings show that the wild transmission cycle of the *T. cruzi* is much more complex than it has been being described and also they call attention to the mistakes that may be caused by epidemiological conclusions based on secondary data. The studies at the Poço das Antas Biological Reserve, Silva Jardim, put the question very clearly: in this fragment of the Rain Forest, the opossum (*Didelphis marsupialis*), widely considered as the most important reservoir for the *T. cruzi* was the mammal that presented the lowest prevalence, even lower than the one of the rodents, animals that are not normally considered to be reservoirs. In this area, the principal reservoir for the *T.cruzi* is the golden lion tamarin (*Leontopithecus rosalia*). This specie shows high infection taxes (50%) and still, is infected with a subpopulation of the parasite associated to human cases of the Chagas' disease, *T. cruzi* II. Golden lion tamarins infected by *T.cruzi* have also been observed in the farms that surround the reserve and in one of them we found a particularly intriguing situation: in the Rio Vermelho farm, where many groups of golden lion tamarins live associated with *Callithrix jacchus*), the infection of the *T. cruzi* has been observed in six specimen of the later specie and in none specimen of the *L. rosalia*. It's known that *C. jacchus* live in sympatric with the golden lion and behaviour data show that these animals share, in some cases, almost half of their time together. Is it that some behaviour patterns of these animals are influencing the transmission and maintenance of the parasite in this area? What are the associations between these two sypatrical species? These aspects should be taken into account not only in relation to *T.cruzi*, but also in relation to other parasitic diseases in general, principally in species that are subdued to any type of handling.

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B-U-23 – EXPERIMENTAL INFECTION IN OPOSSUMS (*DIDELPHIS ALBIVENTRIS*) BY DIFFERENT STRAINS OF *TRYPANOSOMA CRUZI*

Braz, A.A, Borges, F.Z., Matos, A., Lages-Silva, E., Ramirez, L.E.
Laboratório de Parasitologia, Departamento de Ciências Biológicas, Faculdade de Medicina do Triângulo Mineiro - FMTM, Uberaba, MG.

The opossum is considered as one of the most important wild animals in the epidemiology of Chagas' disease. Recent studies have shown that the opossum is able to shelter two different cycles of *T. cruzi*: one intracellular common to all the reservoirs and another extracellular in the lumen of its anal glands. The aims of this work are to observe the parasitological behavior of various *T. cruzi* strains in the opossum (*D. albiventris*), experimentally infected, in both the acute and chronic phase, as well as to verify the presence of extracellular cycle in the anal glands.

Twenty-five opossums (*D. albiventris*), kept in the FMTM laboratory, and proved negative for *T. cruzi* or *T. rangeli*, were used. These animals were infected by intraperitoneal via with different strains and inocula of *T. cruzi*: Y, Vicentina strains (isolated from a patient with the cardio-digestive form), and GO02 (isolated from the opossum). During the acute and chronic phases, parasitological tests were performed: fresh examination (Brenner, 1962), microhematocrit, hemoculture, xenodiagnosis (*Rhodnius prolixus*), anal gland contents fresh test. Later, the animals were sacrificed. Besides, the immunosuppression in eight animals was carried out.

The analysis of the results obtained in the acute phase of the infection showed: 6 (40%) of the animals inoculated with Y strain showed positive through xenodiagnosis and 3 (20%) also positive by microhematocrit; the animals inoculated with Vicentina and GO02 strains were negative according to all the parasitological methods used. All the results obtained in the chronic phase and during the immunosuppression were proved negative by the already mentioned methods. At the postmortem macroscopic examination, no animal presented significant alterations. After this study, we concluded that nor direct quantitative relation among different inocula and parasitemia found neither extracellular cycle in the lumen of anal glands occurred. The small number of animals proved as positive during the acute phase, as well as the absence of parasitemia during the chronic phase and after immunosuppression may indicate: a) less susceptibility of the opossum to the strains kept in laboratory; b) low index of infection through intraperitoneal via in opossums, even in those inoculated with strain isolated from the same species; c) *D. albiventris* species may be able to abort the infection through mechanisms which must be more studied.

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B-U-24 – BIOLOGICAL BEHAVIOUR OF SAMPLES OF *TRYPANOSOMA CRUZI* ISOLATED FROM HUMAN CASES, VECTORS AND WILD MAMMALS IN THE BRAZILIAN AMAZON

Araujo, IB[1]; Junqueira, ACV[1]; Paiva, DD[3]; Pacheco, RS[2]; Sá, VRF[3]; Gonçalves, AQ[1] & Coura, JR[1]
1- Dept. Medicina Tropical, 2- Dept. Bioquímica e Biologia Molecular, 3- Dept. Patologia e Laboratórios, Instituto Oswaldo Cruz - FIOCRUZ, Universidade Estadual do Rio de Janeiro - UERJ

Although the Chagas' disease in the Brazilian Amazon has always been considered as to be a sylvatic enzootic, cases of human infection by *T. cruzi* has been reported (Shaw et al., 1969 Rev. Saúde públ. 3: 153-157; Valente & Valente, 1993 Rev. Soc. Bras. Med. Trop. 26: 68-70). However, little is known about the biology of the parasite populations in that region. Aiming to learn the biological characteristics of trypanosomas, isolated from human, vectors and reservoir host in the middle and high region of Rio Negro, State of Amazonas, Brazil, groups of male albino mice weighting 7-12g were inoculated i.p. with 5 to 6 x 10^3 and 9 to 10 x 10^3 blood trypomastigotes. The parameters to evaluate the biological behaviour of the samples studied were the following: infectivity, parasitemic curve, morphology, virulence and patogenicity. The results showed that out of the 12 samples studied, 11 were infecting for mice while only 1 was not infective for experimental animal. Out of the 11 infecting samples, 9 presented patent parasitaemia and 2 produced subpatent parasitaemia in mice. However, 4 out of the isolates that displayed patent parasitaemia demonstrated difficulty of maintenance in mice as their parasitaemic levels were always very low. In relation to virulence, only 2 (22.2 %) of the 9 samples than showed detectable parasitaemia were of high virulence, whereas 1 (11.1 %) was of medium and 6 (66.7 %) presented low virulence. Neither of the virulent samples were isolated from human infection. This finding corroborates recents data with isolates from the State of Rio Grande do Sul (Fernandes et al., 1997 Mem. Inst. Oswaldo Cruz 92: 343-351). The morfological study of bloodstream trypomastigotes from 3 *T. cruzi* samples showed a predominance of broad forms and a low percentage of slender forms. Preliminary results of the histopatological investigate have shown the absence of alterations pathological of Chagas' disease and amastigotes in the human isolates. In contrast, was detected in one isolate from *R. brethesi* the presence of amastigotes with skeletal muscle parasitism and cardiac and also the presence of lesions. Interestingly, these data support results about virulence that show a high percentage of mortality in samples isolated from vectors. In addition, the isoenzymatic study also showed a high heterogeneity in the T. cruzi populations,in particular of the Z1, and correlation of some isolates related to the Z1 with high virulence and presence of muscle tissue lesions.

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B-U-25 – *CALOMYS CALLOSUS* INFECTED WITH *TRYPANOSOMA CRUZI*: STUDIES OF A LONG TERM INFECTION

Lourenço, A.M., Levy, A.M.A.,Prado Jr. J.C., Leal, R.C. & Kloetzel, J.K.
Laboratório de Xenodiagnóstico do Instituto de Cardiologia Dante Pazzanesi, Av.Dr. Dante Pazzanesi, 500 - cep 04012-180, São Paulo - SP.

The wild rodent *Calomys callosus* known by its relative resistance to Chagas' disease normally shows scarce tissue damage and innaparent parasitemia. Like other rodents, *T.cruzi* infection in *C.callosus* seems to be gender dependent. The objectives of this work is to investigate the development of a long term infection and its control in *C.callosus* infected with *T.cruzi*. Male and female *C.callosus* divided in four groups of 6 animals each, were infected with $4x10^6$ blood trypomastigotes of the Y strain of *T.cruzi*. On 45^{th} and 60^{th} days after inocule, blood was colected from retro-orbital plexus for complement mediated lysis. Two days later , animals were anesthesized with uretana and submitted to xenodiagnosis using 20 third stage nymphs of *T. infestans*. After 48 hours, half of the animals of this group were randomly choosen and sacrified. Blood was collected, and a group of sensitive strain of mice A/Sn, male, aged 30 days old and weighing 22-25g were infected. The remaining 50% were sacrified. From these animals, blood, heart, thymus and spleen were collected and submitted to culture in LIT medium. A/Sn group displayed aproximatelly 75% of positivity for both subinocules from animals of 45 and 60 days. Infected A/Sn group either with blood from male or female infected *C.callosus* showed positive results. A significant difference was observed between inocules from females with 45 days (100% of positivity) and 60 days (44% of positivity). Hemocultures from male and female chronic infected animals were positive. Significant differences were seen with lytic antibody percentage, with negative results on 45^{th} day after inocule compared with 100% of positivity on 60^{th} day. However, xenodiagnosis was positive for both sexes, on 45 and 60^{th} days after infection. With organs culture, gender differences were observed: Male heart culture showed 100% of positivity when compared to females (80%). For thymus both male and female displayed similar results (10% of positivity), while spleen cultures were always negative for both sexes. It seems that steroid gonadal hormones play an important role together with the immune system, rendering to infected female *C.callosus* an enhanced resistance, lower parasitemic levels and tissue damage, as well as higher lytic antibody percentages, but not sufficient to eliminate the parasite and to reach a self cure in Chagas' disease. We think that, the fact of *C.callosus* controls not only *T.cruzi* infection but many others pathogens, sophisticated mechanisms must be involved that enable this animal species to be considered an ideal natural reservoir for this and several different diseases.

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B-U-26 – PALEOPARASITOLOGY OF CHAGAS DISEASE: ISOLATION OF *TRYPANOSOMA CRUZI* DNA IN 2,000-YEAR-OLD MUMMIFIED HUMAN TISSUES FROM CHILE

Britto, C.[1], Fernandes, O.[1,2], Cardoso, M. A.[1], Araujo, A.[3], Morel, C.[1] & Ferreira, L. F.[3]
[1]Fundação Oswaldo Cruz, RJ, Brasil - Instituto Oswaldo Cruz, Departamentos de Bioquímica e Biologia Molecular & Medicina Tropical; [2]Universidade do Estado do Rio de Janeiro, RJ, Brasil; [3]Fundação Oswaldo Cruz, RJ, Brasil, Escola Nacional de Saúde Pública

By studying the pathology of skeletons and mummies, paleopathology and paleoepidemiology provide inferences about disease patterns in the past, contributing to the knowledge of diseases in prehistoric populations. Evidences have been described for the presence of prehistoric Chagas disease, raised by the findings of pathological alterations (megasyndromes) in ancient Chilean mummies dated from 2,470 to 1,400 years B.P. (before present) (Rothhammer *et al*.,1985, *Am.J.Phys.Anthropol.* 68: 495-498). Immunochemistry, electron microscopy and molecular biology have also documented the putative presence of *Trypanosoma cruzi* in mummified human tissues (Fornaciari *et al.*, 1992, *Lancet*, 339: 128-129; Guhl *et al.*, 1997, *Lancet*, 349: 1370; Guhl *et al.*, 1999, *Am.J.Phys.Anthropol.*, 108: 401-407). In our study, a segment of DNA, unique to the kinetoplast of *T. cruzi*, was successful identified in mummified human tissues dated from 2,000 to about 550 years B.P., from the coastal areas of the Atacama desert of northern Chile. Archaeological materials were sampled from bodies stored at the Museo Arqueologico de San Pedro de Atacama, and parasite specific DNA was recovered using the polymerase chain reaction (PCR) methodology. Following rehydration of the desiccated human tissue fragments, nucleic acid was isolated and amplification of the conserved region of the minicircle molecule of *T. cruzi* was achieved in four of the six samples tested. Amplified products (120 bp = conserved region, and 475 bp = multimer composed by one conserved region + one variable region + another conserved one) corresponding to genetic fragments of the parasite were revealed by hybridization experiments with *T. cruzi* specific molecular probe. The method enables the identification of Chagas disease in ancient Chilean bodies, in the absence of recognizable anatomic pathological changes. This represents a preliminary study of a much more extensive investigation designed to identify the antiquity, demography, and specially the paleoepidemiology of Chagas disease (American trypanosomiasis) in the New World.

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B-U-27 – PRELIMINARY BIOLOGICAL CHARACTERIZATION OF HUMAN ISOLATES OF *TRYPANOSOMA CRUZI* FROM THREE AREAS IN MINAS GERAIS

Devera R. & Coura J.R.
Department of Tropical Medicine, Oswaldo Cruz Institute, FIOCRUZ. Rio de Janeiro, RJ-Brasil.

The characterization of *Trypanosoma cruzi* strains is important for the determination of the possible role of these different strains in the development of different clinical forms, the diverse epidemiological patterns, as well as the different cure rates after treatment observed in Chagas' disease. The objective of the present investigation was to study in albino mice the biological behavior of *T. cruzi* strains from chronic chagasic patients from three different Minas Gerais areas (Pains, Iguatama and Berilo). The parasites were isolated by xenodiagnosis and inoculated intraperitoneally into mice for maintenance and adaptation. For the study of the biological behavior of the strains, 7 to 13 male mice weighing 10-15 g were infected with 1 x 10^3 blood trypomastigotes for the assessment of infectivity, parasitemia, morphology of the blood trypomastigotes, and virulence and distribution of the parasites in tissues. Eleven *T. cruzi* strains were characterized (2 from Pains, 2 from Iguatama and 7 from Berilo). The prepatent period ranged from 6 to 19, 5 to 19 and 6 to 16 days, respectively, for the Pains, Iguatama and Berilo strains. Although strains from the three areas presented a similar prepatent period, the highest parasitemia levels were observed in the Berilo strains, although the parasitemia peak varied widely from 16 to 44 days. We emphasize peaks of less than 300 parasites/5 µl blood in the two Pains strains. The mean values were higher than 1000 parasites in only 27.3% of the samples. Wide-shaped parasites predominated in all samples studied during the acute phase of infection. The cumulative death rate was very low, ranging from 0 to 11.1%. In conclusion, these preliminary results reveal that the *T. cruzi* strains from the areas studied are of low virulence, with a higher frequency of strains causing low parasitemia and predominance of wide forms (type 2).

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B-U-28 – DIFFERENT PATTERNS OF AGGLUTINATION AGAINST THREE STRAINS/CLONES OF *TRYPANOSOMA CRUZI* IN HAEMOLYMPH AND CROP EXTRACT OF FIVE TRIATOMINE'S SPECIES

Carvalho- Moreira, C. J[1]., Gombarovits, M.E.C.[1], Santos, L.C[1].; Garcia, E.S. [2]; Coura, J.R.[1]; Azambuja, P.[2] & Mello, C.B.[3]
[1] Department of Tropical Medicine, Oswaldo Cruz Institute, FIOCRUZ; [2] Department of Biochemistry and Molecular Biology, Oswaldo Cruz Institute; FIOCRUZ; [3] Department of General Biology, Universidade Federal Fluminense.

The correlation between the lectin activity from haemolymph and crop of *Rhodnius prolixus* and the success or failure of different strains of *Trypanosoma cruzi* in these tissues infected "in vivo" was recently described. Concomitantly, an haemolymph galactose-binding protein of *R. prolixus* was purified, by affinity chromatography. In the present study, the lectin activity of haemolymph supernatant and crop extract was investigated in five Triatomine's species: *Rhodnius neglectus*, *Rhodnius brethesi*, *Panstrongylus megistus*, *Triatoma infestans* and *Dipetalogaster maximus*, against three strains/clones of *Trypanosoma cruzi*: Y, isolated from a Chagas' disease human case; AM 4167, isolated from *R. brethesi* collected in the Brazilian Amazon basing; and clone DM28c, isolated from marsupial (*Didelphis marsupialis*). The insects haemolymph showed different titers (1/32– 1/4096) and patterns of agglutination (big clusters, small clusters, networks, tenuous agglutination, and lysis of parasites). The haemolymph lectin activity of *P. megistus* demonstrated the highest titer against the culture forms of the *T. cruzi* strains and showed a characteristic type of agglutination- the formation of big clusters. The haemolymph supernatant of *R. brethesi* agglutinated *T. cruzi* AM 4167 and DM28c forming a network and immobilising the parasites. The same pattern of agglutination was verified with haemolymph supernatant of *R. neglectus* against *T.cruzi* AM 4167. Preliminary results showed that the haemolymph proteins from five species of Triatomines had different migration profiles in the electrophoresis-gels (SDS-PAGE).

In the experiments with the crop extracts, it was observed lower titers, varying from 1/4 to 1/128 and was not detectable the formation of networks in the agglutination tests. In some cases lysis and immobilisation of parasites were verified in the incubations with crop extract. At this moment we are carrying out some experiment to demonstrate that this agglutination activities against the parasites is involved with its growth and development into the digestive tract of these Triatomine's species.

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B-U-29 – ROLE OF GALβ1→3GAL IN THE INTERACTION OF *LEISHMANIA. (L.) AMAZONENSIS* AMASTIGOTES WITH MACROPHAGES

[1]Tanaka, A.K., [2]Gorin P.A.J., [1]Straus, A.H. & [1]Takahashi, H.K.
[1]Department of Biochemistry, Universidade Federal de São Paulo, 04023-900, São Paulo, SP, Brasil, and
[2]Department of Biochemistry, Universidade Federal do Paraná, Curitiba, PR, Brazil.

Mouse monoclonal antibody (ST-3) produced against *L. (L.) amazonensis* amastigote glycosphingolipids (GSLs) showed to inhibit significantly the *L. (L.) amazonensis* infectivity in culture of peritoneal macrophages (Straus et al. 1993, J. Biol. Chem. 268, 13723). The smallest GSLs antigen recognized by ST-3 was characterized as a β-Gal-globotriaosylceramide (Galβ1→3Galα1→4Galβ1→4Glcβ1→1Cer). Since the monoclonal antibody (MoAb) ST-3 reactivity is abolished after treatment with β-galactosidase it was assumed correctly that the terminal residue of β-D-galactopiranose was essential for the ST-3 reactivity. In order to establish with accuracy the fine epitope structure recognized by ST-3, inhibition assays using different glycosides were carried out. Among the different glycosides tested only Galβ1→3Gal at 100 mM concentration was able to significantly inhibit the ST-3 binding to β-Gal-globotriaosylceramide by solid-phase radioimmunoassay (about 70%). Lactose or methyl β-D-galactopiranoside did not show any inhibitory effect, indicating that the sub terminal galactose residue is an important structural feature for the binding of ST-3 to Gal-globoside, as well as other antigens recognized by this monoclonal. In order to confirm that the structure Galβ1→3Galpresent in *Leishmania* is recognized by specific receptor of macrophages, inhibition of binding assays of amastigotes to peritoneal macrophages culture were performed in presence of different glycosides. Disaccharide Galβ1→3Gal at 1 mM inhibited 34% of the binding, whereas lactose at same concentration did not inhibit the binding. These results indicate that besides the terminal β-D-galactopiranose, the glycosidic linkage, and perhaps the subtermimal sugar residue are important features for the parasite/macrophage interaction. The effect of this disaccharide in the infectivity and the putative macrophage receptor for the glycosphingolipids carrying terminal Galβ1→3Gal structure are currently under investigation.

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B-U-30 – ECTO-PHOSPHATASE ACTIVITY OF *LEISHMANIA AMAZONENSIS* ISOLATES FROM HUMAN PATIENTS WITH DISTINCT CLINICAL MANIFESTATIONS

Martiny A[1], Silveira FT[2], Simões AS[1], Meyer-Fernandes JR[3], Vannier-Santos MA[1]*
[1] Laboratório de Biologia Celular Parasitária, Instituto de Biofísica Carlos Chagas Filho and [3] Laboratório de Bioquímica Celular, Departamento de Bioquímica Médica, UFRJ, Rio de Janeiro, RJ; [2] Laboratório de Leishmanioses, Instituto Evandro Chagas, Belém, PA. * corresponding author: vannier@biof.ufrj.br

Leishmania amazonensis is the main cause of anergic diffuse cutaneous leishmaniasis (DCL) in Northern Brazil. The disease is characterized by non-ulcerated chronic nodules which resemble those of lepromatous leprosy, that can affect most of the body surface, being generally unresponsive to current chemotherapy. WHO estimates that 30% of *L. amazonensis*-infected people will develop DCL. Host immune defects leading to a specific anergy to leishmanial antigens were ascribed as the primary cause of the disease. Nevertheless, some authors believe that this condition may be due to the parasite. In this regard, it is suggested that factors other than cytokines are implicated in the inability of DCL patients to mount an anti-*Leishmania* immune response (Bomfim *et al*., 1996) and parasites isolated from simple cutaneous (CL) or DCL cases present antigenic diversity (Leon *et al*., 1990; 1992).We have recently found that amastigotes of a *L. amazonensis* strain isolated from a human DCL case are able to circumvent protein tyrosine kinase activity by dephosphorylation of macrophage proteins, including Erk1 MAP kinase (Martiny *et al*., 1999). Dephosphorylation rendered macrophages permissive to infection and was correlated to decreased nitric oxide release (Martiny *et al*., 1996). This phenomenon was associated to a parasite-derived ecto-phosphatase activity. Here we attempted to compare ecto-PPase activities from three geographically distinct clinical isolates from DCL (Pará, Maranhão and Bahia states) and CL (Pará) due to *L. amazonensis*. LC patients were successfully treated with current antimoniate therapy and presented no recidiva. DCL cases were unresponsive to all drugs so far tested, with the exception of one patient that presented an intermediate manifestation and was cured (Silveira, personal observation). Promastigotes from the isolate obtained from this patient displayed 2-fold less ecto-PPase activity as compared to the unresponsive DCL-causing strains. LC parasites also presented less PPase activity, but all were highly sensitive to the vanadium derivatives sodium orthovanadate, mpV(pic) and pbV(phen), known tyrosine phosphatase inhibitors. The optimum pH of the enzyme was 5.0, but it was still 50% active at physiological pH, which can render this enzyme active under different environmental conditions. The phosphotyrosine protein profile of the macrophage J774A.1 cell line as analyzed by Western blot revealed that the borderline DCL and LC strains induced a slightly different pattern, which may indicate different abilities in inducing an effective immune response.

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B-U-31 – EFFECTS OF A PUTRESCINE ANALOGUE IN *LEISHMANIA AMAZONENSIS*

Valentim C[1], Duque-de-Mello M[1,2] , Abril AR[1], Yarlett N[3], Vannier-Santos MA[1]
[1] Laboratório de Biologia Celular Parasitária, Instituto de Biofísica Carlos Chagas Filho, UFRJ, Rio de Janeiro, RJ; [2] Laboratório de Entomologia Médica, Centro de Pesquisas Rene Rachou, Fundação Oswaldo Cruz, Belo Horizonte, MG; [3] Haskins Laboratories, Pace University, New York, NY.

Polyamines are low molecular weight organic cations that play pivotal roles in cell growth and differentiation. Impairment of polyamine biosynthesis and/or transport were shown to be valuable tools for cancer as well as African sleeping sickness chemotherapy. Polyamine analogues can interfere with major cellular functions by inhibiting macromolecules synthesis. Synthesis of polyamines in most cells is initiated by ornithine decarboxilase (ODC), which catalizes the conversion of ornithine to putrescine. In aerobic organisms conversion is accomplished from extracellular ornithine, and production of spermidine and spermine by addition of aminopropyl groups from decarboxylated *S*-adenosylmethionine (dc-AdoMet). Although some parasitic protozoa do not produce spermine, *Leishmania* amastigotes synthesize it in small quantities.

Here we employed 1,4-diamino-2-butanone (DAB), a putrescine analogue, to evaluate the role of this polyamine in *Leishmania amazonensis* sub-cellular organization. Promastigotes grown in the presence of 20mM DAB for 24hs presented remarkable ultrastructural alteraions. Many DAB-treated promastigotes presented endoplasmic reticulum cisternae enveloping portions of the cytoplasm as well as vacuoles containing myelin-like figures indicating that putrescine depletion may trigger autophagy. The parasite membranous system disorganization including the flagellar pocket area suggests that polyamines may regulate membrane fusion in this cell. DAB-treated promastigotes also presented swollen mitochondria with altered matrix appearance, possibly indicating an anti-oxidant role for polyamines in the *Leishmania* redox organelle. In this regard, we have recently shown that DAB leads to destruction of *T. foetus* hydrogenosomes (Reis *et al*., 1999).

The first-line chemotherapy of leishmaniasis is based on the use of antimonial compounds that are frequently associated to toxic side-effects and treatment failure. The search for new chemotherapic drugs are needed and polyamine metabolism poses promising candidates. The bis(benzyl)polyamine analogue MDL27695 presents significant *in vivo* activity against *L. donovani* amastigotes (Bauman *et al*., 1990). Also, the observation that DAB can affect energetic organelles unrelated parasites seems to indicate a conserved mechanism of action of this compound and raises the possibility of developing a broad specificity antiparasitic drug.

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B-U-32 – LIPOPHOSPHOGLYCAN TRAFFIC AND DEGRADATION IN *LEISHMANIA*-INFECTED MACROPHAGES

Duque-de-Mello M[1], Ho JL[2], Vannier-Santos MA[3], Pimenta PFP[1]
[1] Laboratório de Entomologia Médica, Centro de Pesquisas Rene Rachou, FIOCRUZ, Belo Horizonte, MG; [2] Department of Medicine, Division of International Medicine and Infectious Diseases, The New York Hospital-Cornell Medical Center. New York, NY; [3] Laboratório de Biologia Celular Parasitária, Instituto de Biofísica Carlos Chagas Filho, UFRJ, Rio de Janeiro, RJ

Lipophosphoglycan (LPG) is the major glycoconjugate present on the surface of *Leishmania* promastigotes. In *L. major* the LPG forms a dense glycocalix which covers the entire parasite cell surface. The expression of this molecule is developmentally-regulated and modified during metacyclogenesis. This phenomenon appears to be a key determinant of the parasite invasion into macrophage and survival in vertebrate and invertebrate environments.

In this study, we used the monoclonal antibody 79.3 against the LPG to observe the ultrastructural distribution and possible modifications of the molecule during the first steps of interaction with the macrophage. Our results showed that LPG molecules were, after the parasite internalization, present in the parasitophorous vacuole membrane and in the cytoplasmic vesicles surrounding the parasites. This aspect is suggestive of the redistribution of LPG molecules inside the infected macrophages. In order to determine whether the LPG molecules remained intact within the host cells, we performed Western blots of *L. major*-infected macrophage cell line J774A.1 (60 min and 24 hours post-infection) as well as either cell type. Using the same antibody against LPG, parasite samples displayed the expected broad band of 110-50 kDa. In lysates of macrophages infected for 24 hrs, but not for 1 hr, an extra band of 21 kDa was detected presumably indicating LPG degradation within the host cell. These fragments can be spread inside the macrophage, and also incorporated on its cellular membrane. LPG molecules incorporated into the macrophage plasma membrane could alter the antigenic properties of the host cell. This could result in directing the T-cell responses, and therefore influence the outcome of disease. Further studies are required to the better understand the role of LPG fragments in the leishmanial infection.

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B-U-33 – EFFECT OF PLATELET-ACTIVATING FACTOR ON THE INTERACTION OF *LEISHMANIA AMAZONENSIS* WITH PERITONEAL MOUSE MACROPHAGES: INFLUENCE OF MODULATORS OF PROTEIN KINASE C

[1]Rosa, M.S.S; [1]Lopes, A.H.C.S; [2]Pereira, A.F; [1, 2]Vieira, R.B. and [1, 3]Dutra, P.M.L.
[1]Instituto de Microbiologia Prof. Paulo de Góes, Bloco I, CCS, [2]Departamento de Análises Clín. e Toxicológicas, Bloco A2, Faculdade de Farmácia, Cidade Universitária - UFRJ; [3]Depto. de Patologia e Laboratórios, Faculdade de Ciências Médicas, UERJ, Rio de Janeiro, R.J., Brasil.

Parasites of the genus *Leishmania* are the causative agents of leishmaniasis, which can be fatal, affecting 12 million people worldwide. These parasites are transmitted to their mammalian hosts by the bite of an insect vector, the phlebotomine sandfly. During their life cycles these trypanosomatids face several different environmental conditions, as they infect both invertebrate vectors and vertebrates. In the vertebrate hosts, these protozoa need to escape the host mechanisms of defense. Platelet-activating factor (PAF) is a potent lipid autacoid which exerts a wide range of biological activities, including cellular differentiation, inflammation and allergy. PAF is synthesized and released from a variety of stimulated cells, including macrophages. All PAF effects described to date occur through specific receptor on the cell surface and signal transduction pathways, that induce activation of protein kinase C. In recent reports, we have demonstrated that 10^{-6} M PAF triggers the process of cell differentiation of *Herpetomonas muscarum muscarum* and of *Trypanosoma cruzi*, as well as modulates the ecto-phosphatase activity of these protozoan. Here we analyzed the effects of 10^{-6}M and 10^{-9}M PAF, in the presence or in the absence of modulators of protein kinase C (20 ng / ml TPA or 50 ng / ml sphingosine) in the interaction of *Leishmania amazonensis* promastigotes with tioglicolate-elicited peritoneal mouse macrophages *in vitro*. These assays were also performed in the presence or in the absence of an immunogenic *L. amazonensis* cytoplasmic fraction. This fraction was prepared by a combination of conventional cell fractionation procedures. In order to obtain this fraction, *L. amazonensis* promastigotes were grown in BHI medium containing 10% fetal calf serum. The number of infected macrophages, as well as the number of parasites within each macrophage was evaluated after 24 hours of infection. Our results indicate that the percentage of infected macrophages was higher when the parasites were treated with PAF prior to the infection. Also, in this case, the number of parasites in the infected macrophages was higher than when the macrophages were infected with non-treated parasites. On the other hand, when the macrophages were pretreated with PAF, a severe inhibition of the infection was observed. We could also observe an inhibition of this infection when both the parasites and the macrophages were pretreated with PAF. This inhibitory effect in the interaction between *L. amazonensis* and mouse macrophages was even more significant when either the macrophages or the parasites were pretreated with the *L. amazonensis* cytoplasmic fraction. We here demonstrate that sphingosine and TPA influence the interaction between *L. amazonensis* and mouse macrophages, both in the presence and in the absence of PAF.

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B-U-34 – A QUANTITATIVE ANALYSIS OF THE PARAMETERS FOR STANDARD GROWTH OF *LEISHMANIA* PROMASTIGOTES *IN VITRO*

Dórea, R.C.C., Oliveira, O.R. & Oliva Neto, P.
Departamento de Ciências Biológicas, Faculdade de Ciências e Letras, Universidade Estadual Paulista - Campus de Assis, Av. D. Antonio, 2100, 19800-000, Assis, SP.

Leishmania parasites exist in two forms, as extracellular flagellated promastigotes in the alimentary tract of the fly vector and as obligatory intracellular amastigotes within the phagolysosome vacuoles of mammalian macrophages. At all life stages, *Leishmania* spp. are exposed to growth conditions that differ significantly between vector and host in temperature, pH, nutrients and serum components. Taking into account the biology of natural infections, culturing of *Leishmania* parasites in vitro has benefited from new medium formulations and additional supplements which are increasingly being investigated. While some of these represent an economical alternative for the standard systems of parasite cultivation, their comparison with conventional media has been done in a rather empirical way. In the present work, the growth dynamics of *L.major* (MRHO/SU/59/NEAL/P) and *L.braziliensis* (MHOM/BR/ 97/SCP1) was studied comparatively in order to determine the specific growth rate, the duplication time (DT) and the number of generations (G) within the first 48 hours of culturing. Promastigotes were maintained by weekly subculture (28ºC) in liquid monophasic media consisting of BHI or RPMI 1640 supplemented with human urine (HU, 2% v/v) and heat-inactivated fetal calf serum (FCS, 10% or 20%, v/v). The cultures were seeded in 10-ml volumes of 25 cm^2 or 75 cm^2 Sigma tissue culture flasks and monitored at 6 h interval up to 48 h and daily from 72-168 h. Growth parameters were calculated using a polynomial regression software (TBL curve) and Instat was used for the statistical analysis. Logarithmic growth phase of both species usually ended at 48 h and was compatible with a straight line. The maximun growth rate of *L.major* (μ= 7.5 day^{-1}) was found to be higher than that of *L.braziliensis* (μ= 1.9 day^{-1}). In contrast, no significant differences were found in the growth rates of *L.braziliensis* cultures started with inoculum sizes varying from 5 x 10^5 up to 5 x 10^7 parasites per ml. The DT (3.6 h) and G (13.3 generations in 48 h) of the lower inoculum did correlate with higher cell yields at the end of logarithmic-phase *L.braziliensis* cultures. To study further the variables that could affect the in vitro growth of *Leishmania*, promastigotes were cultured in 10ml-volumes of medium supplemented with 10% (v/v) or 20% (v/v) of FCS using flasks of 25 cm^2 or 75 cm^2. The inoculum size found to be optimum (5 x 10^5 parasites/ml) was used throughout this study. In *L.braziliensis* cultures the rate of cell division and the number of generations achieved before stationary phase were significantly higher in 75 cm^2 flasks in the presence of 20% FCS. The same effects were observed with *L.major* cultures, even though to a lesser extent. This quantitative analysis of *Leishmania* growth can be useful for several applications, including immunomodulation with growth inhibitors for therapeuthic purposes.

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B-U-35 – *LEISHMANIA* METACYCLOGENESIS: PRELIMINARY RESULTS OF A FLOW CYTOMETRIC STUDY

Cysne-Finkelstein, L.(1), Santiago, M.A.(1), Bertho, A.L.(1), Coutinho, S.G.(1) & Leon, L.L.(2)
Laboratório de Imunidade Celular e Humoral em Protozooses (1), Laboratório de Bioquímica de Tripanosomatídeos, Instituto Oswaldo Cruz (2), Pavilhão Carlos Chagas, FIOCRUZ, Rio de Janeiro, RJ.

Leishmania parasites during their developmental transition from non infective logarithmic form to the infective metacyclic stationary phase undergo some changes on their surface coats. Studies have shown that peanut agglutinin (PNA) fail to agglutinate metacyclic promastigotes in stationary cultures of *Leishmania major* and this loss of ability to agglutinate the parasites can be used as the basis for separation of infective promastigotes (PNA-) and non infective forms (PNA+) (Sacks et al., 1985). On the other hand, flow cytometry has been extensively used to investigate expression of surface molecules at a single-cell level. In this context, fluorochrome-conjugated lectins, which can bind to specific surface sugars, have been used on several studies for characterization and purification of different parasite developmental stages (Palma et al., 1997). We adapted a flow cytometric protocol to evaluate the binding properties between lectin and *L.major* (LV39) promastigotes at different developmental stages. In order to validate this technique, the identification of non infective and infective forms was done by comparing with *in vitro* unlabelled PNA agglutination. Parasites were cultured in Schneider's Insect Medium with 10% fetal calf serum for 7 days. After different time points, promastigotes were harvested by centrifugation at 2000g for 10 minutes, washed twice in PBS, adjusted to 2 x 10^7 parasites/ml and stained with PNA-FITC for 30 minutes at 4ºC. Then, parasites were centrifuged twice at 2000g for 10 minutes and resuspended in PBS for flow cytometric analysis. According to the difference in PNA-FITC staining we were able to evaluate the percentages of parasites in log phase (PNA+) or stationary phase (PNA-) at each day of culture. Our results suggest that the highest percentage of metacyclic form was found at the fifth day of growth. A dose curve response was also done with different PNA-FITC concentrations (1-50 ug/ml) to determine the best amount, which avoid cell agglutination. We found that 10 ug/ml was the best PNA-FITC concentration because it did not promote cell agglutination and also provided a good discrimination between the negative and positive cells in the flow cytometric analysis. FITC-PNA binding was inhibited by using D-galactose (0.2M) or unlabelled PNA as a competition assay for determining the specific binding of lectin to the promastigote surface. The latter was chosen as a negative control. This work reports our preliminary results using flow cytometry in the study of *L. major* metacyclogenesis. This methodology showed to be accurate, rapid and feasible and can be used as a powerful tool in the study of this phenomenon. Studies are presently undertaken using different subcultures and *Leishmania* species.

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B-U-36 – DETERMINATION OF INSULIN-LIKE GROWTH FACTOR (IGF-I) LEVELS IN CHILDREN WITH AMERICAN VISCERAL LEISHMANIASIS FROM AN ENDEMIC AREA OF SÃO LUÍS, MARANHÃO

Gomes, C.M.C.[1]; Matta, V.L.R.[2]; Caldas, A.J.M.[1]; Costa, J.M.[1]; Bracco, O.L.[3]; Corbett, C.E.P.[2]
[1]Depto. de Patologia da UFMA; [2]Laboratório de Patologia de Moléstias Infecciosas (LIM-50), Depto. Patologia da FMUSP, São Paulo, S.P., Fone Fax: 011-881-7799, E-mail: ccorbett@usp.br. Brazil; [3]Laboratório de Endocrinologia Celular e Molecular (LIM-25) da FMUSP .

Insulin-like growth factor I (IGF-I) is an evolutionary well-conserved polypeptide with a molecular mass of 7.5 kDa. Functionally, IGF-I is an important endocrine growth and differentiation factor in inflammation and immune activation. Most cell types have the ability to produce IGF-I although the main site of production is the liver. This factor is detectable in circulation (soluble form) and associated to IGF-I-binding proteins in tissues.

Previous studies have demonstrated that IGF-I induces both *Leishmania* promastigote and amastigote *in vitro* proliferation (Gomes et al, 1997, Acta Tropica 64: 225-8; Gomes et al. 1998, J. Euk.Microbiol. 45: 352-5). Moreover, *in vivo* studies showed that IGF-I increases the phagocytosis, the number of intracellular parasites and the lesion size in mice infected with *L (L.) amazonensis* (Goto et al. 1998, Proc. Natl. Acad. Sci. USA 95: 13211-16).

As the experimental studies have demonstrated an important role of IGF-I in the pathogenesis of leishmaniasis, the present study aims to determine the serum levels of IGF-I in children with visceral leishmaniasis.

The serum levels were determined by immunoradiometric assay (IRMA) in 15 children (0 to 5 years old), with clinical history of active disease varying from 2 months to one year, from an endemic area of São Luís-MA. They were classified as eutrophic or distrophic according to anthropometric parameters.

In the eutrophic group, 70% showed normal IGF-I levels while 30% presented low levels.

Children with moderated or severe malnutrition showed low levels of IGF-I.

Further studies will be necessary to better define the IGF-I serum level as a possible biological marker in American visceral leishmaniasis.

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B-U-37 – SUBCELLULAR LOCALIZATION OF A *LEISHAMNIA AMAZONENSIS* SERINE PROTEINASE

Morgado-Diaz, J[1]., Alves C.R[1]., Soares M[2], Corte-Real S[2].and Giovanni-De-Simone S.G.[1,3]
[1]Departamento de Bioquímica e Biologia Molecular; [2]Departamento de Ultraestrutura e Biologia Celular, Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, RJ, Brasil; [3]Departamento de Biologia Celular e Molecular, Instituto de Biologia, UFF.

Among the proteases reported so far in *Leishmania* species, the cysteine and metallo-type enzymes have been the more studied, nevertheless in relation to serine type peptidases little or nothing is known. In the present work we report the subcellular localization of a serine proteinase from *Leishmania amazonensis* promastigote forms. Using cell fractionation we were able to obtain four representative fractions which were used to evaluate protease activity. Serine protease activity was evaluated during the cell fractionation procedure using α-N-p-tosyl-L-arginine methyl ester (TAME) as substrate and phenyl-methyl sulphonyl fluoride (PMSF) and TPCK as inhibitors. The enzymatic activity was localized mainly in a membranous vesicular fraction (specific activity 6.5-fold enriched in relation to whole homogenate) followed by a plasma membrane fraction (2.0-fold). Immunolocalization studies using a polyclonal antibody raised against the purified serine proteinase (anti-SP) revealed that it was present on the cell surface, as well as, in membranous cytoplasmic vesicles of the parasite. SDS-PAGE using co-polymerized gelatine showed a single hydrolyzing activity with 68 kDa in all fractions. On the other hand, a band with identical molecular weight was also recognized, in immunoblots, by the antibody anti-SP with higher immunoreactivity in the membranous vesicular fraction.

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B-U-38 – ISOLATION AND CHARACTERIZATION OF VIRUS-LIKE PARTICLES FROM *LEISHMANIA (V.) GUYANENSIS*

Anacleto, C., Campos, J. S., Camargos, E. R. S.*, Moreira, E. S. A.
Laboratório de Biologia de Microrganismos, * Departamento de Morfologia, Instituto de Ciências Biológicas, Bloco C4-191, Universidade Federal de Minas Gerais, Belo Horizonte, MG.

Protozoa-infecting viruses were first described in the 1960's but only more recently they have been isolated and characterized in *Leishmania*. These viruses belong to the *Totiviridae* family and are known as "*Leishmania* RNA virus" or LRV. They have been isolated from 12 *Leishmania* cell lines from the *braziliensis* complex, mostly from the New World. It had been suggested that theses viruses may be involved in the pathogenicity of some *Leishmania* cell lines. Here we report the isolation of virus-like particles in drug-sensitive and drug-resistant *Leishmania (V.) guyanensis* cell lines. Molecular analyses, including PFGE, have shown the presence of a 6.7 kb nucleic acid fragment in both lines. Susceptibility to Mung-bean nuclease and resistance to different restriction endonucleases and RNAse-free DNAse suggest that the particles consist of double strand RNA (dsRNA). Negative stainning and ultra-structure studies have indicated that the approximate diameter of the particle is 70 nm. No direct correlation between resistance and the presence of the virus can be made at this point. This type of study is important as viruses can be used as a molecular tool for tasks including transfection of exogenous genes and they can also be models for gene regulation in tripanosomatids.

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B-U-39 – LEISHMANIAL INFECTION ENHANCES ATP-INDUCED PERMEABILIZATION OF MACROPHAGES

Torres-Santos, E.C.; Mantuano, M.B.; Oliveira, S.A.B.; Persechini, P.M.; Coutinho-Silva, R. & Rossi-Bergmann, B.
Instituto de Biofísica, Universidade Federal do Rio Janeiro. E-mail: ects@biof.ufrj.br

In addition to its well-known intracellular properties, ATP is able to trigger events in several lymphohematopoietic cells by binding to purinergic receptors on the membrane, such as P2Z/P2X7. The most dramatic event triggered by macrophage exposure to extracellular ATP and P2Z/P2X7 activation is the opening of large membrane pores permeable to molecules with molecular mass up to 900 D, which is easily monitored by dye entrance. Although the physiological role of P2Z/P2X7 receptor is not clear, it has been implicated in several events like superoxide generation, enhancement of NO synthase expression and killing of intracellular mycobacteria. In this work, we studied the sensitivity of leishmania-infected macrophages to extracellular ATP. For *in vitro* experiments, peritoneal macrophages were infected with *Leishmania amazonensis* for 4 h. At the end of the culture, the medium was removed and the cells were incubated for 10 minutes at 37º C with ATP in the presence of the fluorescent dye lucifer yellow. The cells were then exaustively washed with pre-warmed PBS and lysed by freezing in distilled water. The fluorescence in the lysate was measured using a Fluoroskan II apparatus. In presence of 500 to 4000 µM of extracellular ATP, the infected cells were in average 30 % more permeable to the dye than the uninfected ones To evaluate these observations *in vivo*, female BALB/c mice were infected with *L. donovani* amastigotes by intravenous route. After 17 - 23 days, the spleen cells were obtained and incubated in suspension with several concentrations of ATP and ethidium bromide (EB) in PBS at 37º C for 10 minutes. The permeability to EB was measured by flow citometry. We found that with 500 µM ATP, 59 % of cells from infected animals were permeable as opposed to 24 % in the cells from uninfected animals. Furthermore, in the infected animals the responsiveness to ATP was much higher. The threshold of responsiveness of cells from uninfected animals was 30 µM whereas it was 8 µM for cells from infected animals, suggesting that not only more cells were expressing P2Z, but also that those cells were expressing more receptors. These results suggest that ATP may be used as a new adjuvant in the treatment of leishmaniasis by selectively enabling the entrance of small drugs through membrane pores in infected macrophages. CAPES, PRONEX.

B-U-40 – USE OF FLUORESCENT LEISHMANIA FOR FASTER QUANTITATION OF PARASITE GROWTH IN VITRO AND *IN VIVO*

Rossi-Bergmann, B; Lenglet, A; Bezerra-Santos, CR; Costa-Pinto, D* and Traub-Czeko, YM*
Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro.*Departamento de Bioquímica e Biologia Molecular, Fundação Oswaldo Cruz-RJ, Brazil. e-mail: bbergman@biof.ufrj.br

The methods normally used for the evaluation of intracellular leishmania growth are either very fastidious and subjective (Giemsa-stained cultures), unreproducible (transformation and expansion as promastigotes), or non-representative (axenic amastigotes). We are presently developing a relatively fast and quantitative method where green fluorescent protein (GFP) - transfected parasites are quantitated by fluormetry.

Promastigotes of *Leishmania amazonensis* were transfected by electroporation with the gene fragment coding for the C-terminal extension of cysteine proteinase fused to the reporter GFP in the pXG-'GFP+ vector developed by S. Beverly et al. The transfected promastigotes showed a very bright cytoplasmic fluorescence which could be easily quantitated by a microplate reader fluormeter. To investigate whether the plasmid was stable in the amastigote stage, 10^6 mouse peritoneal macrophages were infected with GFP-promastigotes for 1h, washed and cultured for 65h, after which time the cells were full with bright parasites. The cultures were lysed with dH2O and transferred to a black microplate for fluorescence measurement, giving a significantly higher fluorescence (662 ± 5 mUF) as compared to uninfected cultures (156 ± 10 mUF). To assess whether the intracellular parasites were viable and not just phagocytosed promastigotes, parallel infected macrophage cultures were placed at 28º C. After 5 days the cultures were full with bright promastigotes, indicating that the plasmid was stable in both promastigote and amastigote forms of the parasite.

To investigate whether fluorescent parasites can also be used to measure parasite load in the lesions, BALB/c mice were infected in the footpad with 4 x 10^6 GFP-promastigotes. Four weeks later the animals were killed, the feet teased and crushed against a steel mesh and the cell suspensions serially diluted in black microplates with PBS. The results showed a significantly higher fluorescence in the infected as compared to uninfected footpads. Again, bright promastigotes could be recovered from the tissue lysates cultured at 28ºC.

These preliminary results suggest that GFP-promastigotes may be successfully used in the development of a new method for in vitro and in vivo quantitation of parasite load, which can be applied in faster drug screening and pre-clinical sudies and other situations where measurent of parasite growth is necessary. We are presently evaluating its superiority in comparison to the more conventional methods.

B-U-41 – STUDY OF MAST CELL POPULATIONS IN LESIONS FROM PATIENTS WITH AMERICAN TEGUMENTARY LEISHMANIASIS (ATL)

Oliveira MP[1], Oliveira-Neto MP[2], Surrage A[3], Coronha ML[3], Butterfield JH[4] & Pirmez C[1].
1.Dept. Biochemistry & Molecular Biology, 2. Evandro Chagas Hospital, 3. Dept. Fisiology & Pharmacodynamic – FIOCRUZ, RJ, 4. Mayo Clinic, MN

Mast cells have been implicated in diseases involving chronic inflammation such as worm or experimental *Leishmania* infection. The aim of this study was to investigate the role of these cells in human ATL. Mast cells were characterized by a double immunostaining technique using the monoclonal antibodies anti-triptase (Dako) and anti-chymase (Chemicon), anti-IgE (Pharmingen) and anti-histamine (Sigma). Two distinct populations of mast cells, described as MC^{TC} (triptase$^+$/chymase$^+$) and MC^T (triptase$^+$), were quantified in normal skin and in lesions from patients with localized cutaneous leishmaniasis (LCL), mucocutaneous leishmaniasis (MCL) and mucosal leishmaniasis (ML). As described by many authors, we observed that in normal skin the majority of mast cell belonged to M^{TC} population. The number of MC^T (8,78 ± 7,4/mm^2) was significantly increased in lesions from LCL patients, particuraly those presentig less than three months of duration (13 cases) (p=0,033). Such difference was not noted in the other forms of the disease. We also observed immunoreactivity for histamine and IgE in chymase positive cells of cutaneous and mucosal lesions. Mast cell activation was examined using the cell line HMC-1 cultured with procyclic and metacyclic promastigotes total antigen of *L. braziliensis* ($2x10^7$ parasites/ml), stem cell factor (100ng/ml) and substance P (30μM) at 37ºC during different times. Reactions were blocked by the addition of ice-cold buffer. Histamine content was determined in cell pellet and culture supernatant by spectrofluorimetric method using o-ophtalaldehyde. The histamine levels observed in supernatant of HMC-1 cultured with metacyclic antigen during five (45,04 ± 28,1 ng/ml) and 15 minutes (44,7 ± 27,5) was significantly higher than controls (5,6 ± 1,8 ng/ml) (p=0,003). Procyclic antigen also induced significant histamine release after five (9,1 ± 3,3 ng/ml) and 15 minutes (11,2 ± 4) of culture (p=0,009). The modified ratio of MC^{TC} and MC^T in lesions from LCL patients indicate a possible role of mast cell in regulation of the specific immune response. The local production of histamine and the presence of cell surface-bound IgE in mast cells can indicate that the activation of these cells in lesions occurs by an immunological mechanism. Furthermore, the release of histamine after addition of *Leishmania* antigen in HMC-1 cultures suggests that the degranulation of mast cells in inflammatory site is likely to be also associated with nonimmunological mechanisms.

Suppoted by FAPERJ

B-U-42 – CHARACTERIZATION OF ACTIVITIES FROM RABBIT RETICULOCYTE LYSATE AND KINETOPLASTID PROTOZOANS EXTRACTS INVOLVED IN SPECIFIC RECOGNITION OF THE *LEISHMANIA* SPLICED LEADER SEQUENCE

de Melo Neto, O. P.$^+$, Gomes, F. C. and Standart, N.*
Centro de Pesquisas Aggeu Magalhães, Fundação Oswaldo Cruz, Av. Moraes Rego s/n, Caixa Postal 7472, Recife PE 50670-420. * Depto. of Bochemistry, University of Cambridge, 80 Tennis Court Road, Cambridge UK CB2 1GA$^+$*e-mail*: opmn@cpqam.fiocruz.br

All mRNAs in Trypanosomes and other kinetoplastid protozoans are generated through *trans*-splicing. A 39nt 5' terminal exon (the spliced leader, SL) and its unique cap4 structure is transferred from a SL RNA precursor to the 5'end of the pre-mRNAs. The two independently transcribed RNA sequences are joined and the policistronic primary transcript is therefore processed generating the mature monocistronic mRNAs. As a result, the same short, capped, non-coding leader sequence is present at the 5'end of each mRNA. Very little is known about the role of the SL sequence in the mature mRNA metabolism. Our group has been investigating the possibility that the SL sequence might influence the translation of parasite RNAs. We have previously obtained in vitro transcribed reporter RNAs which varied as to the presence or absence of the SL sequence and shown that surprisingly mRNAs containing this sequence were translated more efficiently in a mammalian in vitro system, the rabbit reticulocyte lysate (RRL). This effect was independent of the Cap or any methylations in the SL sequence and suggests that the machinery required for recognizing the SL signal is evolutionary widespread and conserved. Here we describe results obtained with the use of Band-Shift assays of ^{32}P labelled RNAs containing the *Leishmania* SL sequence incubated with either the RRL or parasite extracts competent for protein synthesis. We have observed that both systems contain an activity capable of specifically binding to the wild type spliced leader sequence. This binding can be inhibited by 5 mM Heparin and excess ribosomal RNA, poly-G and to a lesser extent poly-U. Poly-A or poly-C does not seem to affect the binding even when present in more than 100 fold excess. The RNA structure seems to be important for the recognition by the binding activity since denaturing/renaturing of the RNA probe enhances binding. Mutations that interferes with the secondary structure of the RNA, as ascertained by predictions obtained through the program "mfold", also prevent the shift seen in our assays. These results are consistent with a stem loop structure formed by the SL RNA being required for the binding by proteins in both RRL and parasite extracts. The obsevation that antisense SL RNA, which folds into a similar structure, also produces the shift corroborates this hypothesis. We are now trying to see if a correlation between structure, band-shift activity and translational stimulation can be observed.

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B-U-43 – APOPTOSIS IN INFLAMMATORY CELLS IN EXPERIMENTAL CUTANEOUS LEISHMANIASIS IN MICE

Lindoso, J.A.L. & Goto, H. Instituto de Medicina Tropical de São Paulo - Depto. Med. Preventiva - FMUSP, Instituto de Infectologia Emílio Ribas - São Paulo- Brasil

The balance between the mechanisms of protection and susceptibility determines the evolution of *Leishmania* infection. However, the pathogenetic mechanisms are not completely known. We have previously shown the presence of apoptosis in inflammatory cells in the liver and the spleen of hamsters with visceral leishmaniasis that could be responsible for immunossupression and then for the progression of disease (Lindoso et al. 1998 *Mem. Inst. Oswaldo Cruz 93* Suppl: II). In addition, in experimental Chagas' disease *T. cruzi* antigen-driven T cell apoptosis had been shown (Lopes et al. 1995 *J. Immunol., 154*, 744). On the other hand, it has been described that infection of macrophages by *Leishmania donovani* protects these cells from the death in apoptosis. In this work we evaluated the apoptosis in the skin lesion in cutaneous leishmaniasis in susceptible (BALB/c) and resistant (C57BL/6) mouse strains. Mice were injected subcutaneouly with 10^7 stationary phase promastigotes of *Leishmania (L.) amazonensis*. We evaluated the inflammatory infiltrate and the presence of apoptosis in cells in the skin lesion at 20, 40, 80 and 130 days post-infection. As negative control we used footpad of mice injected with sterile PBS. For the detection of apoptosis we used the TUNEL method using sections of tissues treated with DNAse as a positive control. In BALB/c, the inflammation progresses to a process characterized by vacuoleted macrophages harboring increasing number of parasites from 40 days onwards. In C57Bl/6 the infiltrate is characterized by lymphocytes and activated macrophages containing few parasites. In BALB/c mice, there is an initial increase in apoptosis of vacuolated macrophages until 80 days post-infection with reduction at 160 days post-infection. In C57BL/6 mice apoptosis in macrophages was not present in the initial phase but it is present in lesser degree compared with BALB/c mice from 80 days onward in areas where parasites were still present. Apoptosis in lymphocytes was not seen in BALB/c mice but we observed an increase in apoptosis in lymphocytes from 40 days onwards in C57BL/6 mice. In susceptible and resistant mouse strains apoptosis affects lymphocytes and macrophages in different ways. These data suggest that the apopotosis can be an important factor underlying the control or progression of disease.

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B-U-44 – VASCULAR PERMEABILITY CHANGES DURING VISCERAL LEISHMANIASIS (VL) IN HAMSTERS

Svensjö, E., Mathias, R., Gidlund, M., Rocha e Silva, M. & Goto, H.
[1]Divisão de Experimentação, Instituto do Coração do Hospital das Clínicas, [2]Instituto de Medicina Tropical de São Paulo - Dep. Medicina Preventiva, - [3]Dep. Patologia, Faculdade de Medicina da USP, São Paulo, Brasil.

The pathogenesis of interstitial inflammation present in VL is not completely known. In the inflammatory process vascular permeability changes represent a crucial step induced by different factors including vasoactive substances and oxygen free radicals. In VL *Leishmania* parasites proliferate within macrophages and during the development of the disease oxidized products are generated (Lindoso et al. - Mem. Inst. Oswaldo Cruz 90(Suppl): 104, 1995) that can alter vascular response to different stimuli during infection.

To evaluate vascular permeability changes during VL, hamsters were injected intraperitoneally either with medium (control) or with purified *Leishmania chagasi* amastigotes and the microcirculation of the cheek pouch was studied at 15, 30, 45 and 60 days post-infection by intravital fluorescent microscopy. FITC-dextran was used as a plasma marker. Vascular permeability increase (plasma leakage) was induced by local application of histamine $5 \cdot 10^{-6}$ M and *tert*-butylhydroperoxide (TBOOH) and the number of sites with extravasation of plasma at 5 min after local application was measured. Parasite burden was evaluated in the spleen and the liver from each animal. Control hamsters responded normally to histamine and TBOOH stimulations with reversible increases in number of leaky sites in postcapillary venules with only minor variation during the experimental period. Infected hamsters showed no difference in response at 15 days post-infection but there was a 50% increased response to TBOOH ($p<0.05$) at 30 days of infection which then turned into a 70% reduced response on day 60 in comparison. Parasite burden increased gradually during the observation period.

Conclusion: Visceral leishmaniasis in hamsters resulted in temporary variations in vascular permeability as induced with an oxidant (TBOOH) which might be related to the phase of the parasite induced inflammatory process.

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B-U-45 – CANINE VISCERAL LEISHMANIASIS: A HISTOPATHOLOGICAL REPORT ON AN UNUSUALLY SEVERE CASE FROM JOÃO PESSOA, BRAZIL

Tafuri, W.L.[1]; Oliveira, M.R.[2]; Melo, M.N.[3]; Tafuri, Wg. L. [1]
1-Departmento de Patologia Geral, Instituto de Ciencias Biológicas (ICB), Universidade Federal de Minas Gerais (UFMG); Belo Horizonte, MG, Brazil. 2-. Departamento de Biologia Molecular do Centro de Ciências Exatas e da Natureza da Universidade Federal da Paraíba (UFPB), João Pessoa, Pb, Brasil. 3- Departamento de Parasitologia, ICB/UFMG; Belo Horizonte, MG, Brazil

Canine Visceral Leishmaniasis (CVL) in Brazil has been occurred in many capitals. In this case, we have reported a remarkable histopathological picture of the canine disease. A mongrel symptomatic dog was provided by of the City Hall of João Pessoa (Zoonosis Department), Paraíba, Brazil. Sorological tests (IFAT and CRF) were positive. The animal was sacrificed with thiopenthal sodic. Liver, lungs, kidneys, skin, abdominal lymph nodes and spleen were collected and fixed in formalin 10% buffered solution for histopathology. Fragments of liver showed a severe leishmaniotic chronic granulomatous inflammatory reaction with innumerous intralobular granulomas with variable size and volume. Hypertrophy and hyperplasia of kupffer cells loaded with amastigotes, an intense congestion of the sinusoids vessels and a moderate inflammatory mononuclear cells focus around portal space with hyaline trombones of the vessels were observed. The presence of amastigotes could be determined into macrophage granulomas, kupffer cells and even inside of hepatocytes. Kidney fragments showed a diffuse membranoproliferative glomerulonephritis in the most part of the glomerulus. Cellularity of the glomerular tuft was increased by proliferation of endothelial, epithelial, or mesangial cells. Intravascular accumulations of neutrophils and monocytes were accompanied the cellular proliferation. Glomerular capillary walls were thickened due to the thickening of the basement membrane and the parietal and visceral endothelial and epithelial swelling (PAS-positive). Thickening of Bowman's capsule occurred due to the hyperplasia of parietal epithelial cells, thickening of the basement membrane and periglomerular fibroses. In fact, we observed some glomerulus showing tuft atrophy in consequence of scarring. Nonglomerular alterations included tubular proteinuria and an intense and diffuse chronic interstitial inflammation accompanied for the tubulointersticial damage and hypertrophy and hyperplasia of the monolayer medium of the arterioles intertubulares. Lungs tissue fragments showed an intense chronic interstitial pneumonitis. The basic lesion was the intralveolar accumulation of various mononuclear cells (mostly macrophages) and interstitial thickening by accumulations of macrophages, lymphoid cells and fibrous tissue. However, the interstitial pneumonia showed in different phases, the exudative phase and the proliferative phase. The intralobular septa thickening were constituted by leukocytes, or by fibroblasts and collagen, respectively. Hyperplasia of smooth muscle was remarkable observed in all intralobular septa walls. Arteries and arterioles showed a spectrum of pathological alterations that is not reported in the most part of the cases. The endothelial and the media vessel layer were deep damage associated to hyalin thrombosis or progressive fibroblastic proliferation in some of them. These alterations could be seen occluding the lumen of the vessels. Inflammatory focus in the subpleural region was characterized by focal accumulations of foamy macrophages. Spleens tissue sections showed a diffuse chronic inflammatory reaction in the capsule, subcapsule region, trabecular system and red pulp. The capsule and the trabecular system were thicker and the trabecular vessels were dilated and congested. The red pulp showed profound modifications due to the marginal macrophages proliferation and the granulomatous inflammatory reaction. Macrophages were organized in granulomas and they were loaded with parasites and brown crystals (hemossiderin). The Malpighi follicles were depleted formed by only some lymphocytes around the central arteriole. An intense parasitism was observed in the thickness of the capsule, subcapsular and perifollicular region (marginal zone). The same picture we observed in the lymph nodes. Interestingly, skin sections of nose, ears and abdominal lymph nodes showed absent of inflammatory response and amastigotes. The spectrum of lesions observed in this case was complex and we intend to isolate and determine the virulence of the species of *Leishmania* involved.

B-U-46 – KINETICS OF THE EXPERIMENTAL INFLAMMATORY REACTION INDUCED BY *LEISHMANIA MAJOR* DURING THE IMPLANTING OF PARAFFIN-TABLETS IN MICE

Tafuri, Wg.L.[1] ; Melo, M.N.[2]; Paiva, M.C.[1].; Tafuri, W.L.[1]
[1]Departamento de Patologia Geral and [2] Departamento de Parasitologia, ICB, Universidade Federal de Minas Gerais, Belo Horizonte, MG, 31270-901.

In Leishmaniasis macrophages have been incriminated to be the host cell and at the same time an effector cell exerting an important role in the host cellular immune response. *Leishmania* in the host tissues provokes the appearance of bone marrow-derived blood monocytes. These cells may be critical to the cell-mediated immune response. In this work we observed how the inflammatory response induced by *Leishmania major* could be modulated in the presence of one inflammatory agent: the paraffin-tablets. This inflammatory model provokes a continued recruitment of the monocytes to the site of inflammation. Balb/c and C57BL/6 mice had their back shaved and a skin incision were carried out for the implanting paraffin-tablets. The paraffin tablets (220,0mg x 1,0cm x 0.5cm) were subcutaneously implanted in the dorsal region of mice. Then, mice received an injection of 1 x 10^6 promastigotes of *L. major*/0.1 ml subcutaneously at the same anatomical region. Mice infected with promastigotes of *L. major* without paraffin-tablets were used as control. Then, mice were sacrificed 15, 21, 30 and 45 days after the implanting and experimental infection. Fragments of tissue skin and an inflammatory capsule were collected for histopathology. After the 15th day, the microscopical analysis of skin of both groups of animals in which the paraffin-tablets were implanted showed a delicate granulation tissue (fibroblasts, small blood vessels and leukocytes) that became mature by day 21, 30 and 45 days with giant cell formation. Balb/c mice inoculated with *L. major* only showed a chronic inflammatory reaction constituted by a cellular exudate with lymphocytes, macrophages loaded with intracellular amastigotes and rare neutrophils during all the time points. However, Balb/c mice inoculated with *L. major* after the paraffin-tablets implanting, the inflammatory reaction and the parasite burden was more severe. The same fact was observed in C57BL/6 mice excepted about the parasite burden. After the 30 and 45th day the parasite burden was very lower. However, there was an intense chronic inflammatory reaction characterized by fibroblast proliferation and collagen production. These results from C57BL/6 mice were very important because as they are resistant to *Leishmania* we understand that these mice could be considered susceptible to *L. major* at least during the 15-21th days after infection. So far, based on our results we have evidence that monocytes-macrophages are acting more as host cell than being effector cells during the *L. major* infection in BALB/c and C57BL/6 mice considering this inflammatory experimental model. In according of these results we are going to determine the immunological pattern in Balb/c and C57BL/6 mice during the *L. major* infection modulated by this inflammatory inert agent: the paraffin-tablets.

B-U-47 – CLINICAL, HISTOPATHOLOGICAL AND IMMUNOPARASITOLOGICAL ASPECTS OF SPLEENS OF DOGS NATURALLY INFECTED WITH *LEISHMANIA (LEISHMANIA) CHAGASI*

Chiarelli, I.M.de[1] ; Paiva, M.C.[1]; Andrade, H.M.[2]; Costa-Val, A.P [3] ; Carneiro, C.M.[4]; A; Tafuri, Wg.L.[1]
1- Departamento de Patologia Geral and 2- Departamento de Parasitologia, ICB, UFMG and 3- Departamento de Clínica e Cirurgia Veterinária, Escola de Veterinária, UFMG, Belo Horizonte, 31270-901, MG, Brasil 4- Departamento de Análises Clínicas, Escola de Farmácia, UFOP, Ouro Preto, MG, Brasil.

In the New World the canine visceral leishmaniasis is caused by *Leishmania (Leishmania) chagasi*. In the last decades, a spreading of this disease has been associated with urbanization of large cities, such as Belo Horizonte, Brazil. Eighteen mongrel dogs, obtained from the City Hall of Belo Horizonte were previously classified as asymptomatic, oligosymptomatic and symptomatic animals (weakness, cutaneous lesions, alopecia, and clinical anemia were the most common symptoms). The dogs were killed with an overdose of thiophental sodic and samples of blood obtained for serological studies (indirect immunofluorescence test-IFAT). During necropsy, spleens were weighed and samples collected for Giemsa-smears ("imprints"). The slides (smears) were observed under a light microscope using a 100x objective to determine the spleen parasite burden L.D.U. (*Leishmania Donovan Units*). Other spleens fragments were obtained and fixed in formalin (10% and buffered) for histopathological and immunohistochemistry studies. Hematoxilin-eosin stain, Gomory and Silver stain to characterize and collagen fibers and a streptoavidin-peroxidase immuno-stain to detect amastigotes of *Leishmania* were carried out. Microscopically, the hypertrophy and hyperplasia of the white pulp and the hypertrophy and hyperplasia of the macrophages of the red pulp were the main alterations observed in all infected animals. In some cases we observed granulomas formation in red pulp. Immunolabelled amastigotes of *Leishmania* were found in all animals in the cytoplasm of macrophages of the red pulp, capsule and the granulomas. Morphometrical analysis was carried out using a Zeiss Imaging Processing Software (KS300). In the spleen paraffin sections of all animals we analyzed number and size of the white pulp, collagen deposition and the density of parasites immunolabelled. The parasite burden (L.D.U.) and IFAT titers did not correlate with the number or size of the white pulp and neither with the density of the parasitism tissue or with the clinical aspects. However, the collagen deposition was higher in the symptomatic groups. In fact, the reticular fibers were deeply thickened in the capsule and red pulp and they were aligned in various directions. Some were thicker than others forming a compact network in certain points. This study suggests that clinical aspects of canine visceral leishmaniasis do not necessarily indicate absence of lesions or parasites in the spleen. Future studies will be aimed to continue this study considering the clinical and immunopathological features of the disease.

Financial Support: FAPEMIG, Prpq-UFMG

B-U-48 – CLINICAL, HISTOPATHOLOGICAL AND IMMUNOPARASITOLOGICAL ASPECTS OF LIVER OF DOGS NATURALLY INFECTED WITH *LEISHMANIA (LEISHMANIA) CHAGASI*

Paiva, M.C.[1]; Chiarelli, I.M.de[1]; Andrade, H.M.[2]; Costa-Val, A.P.[3]; Carneiro, C.M.[4]; A; Tafuri, Wg.L.[1]
1- Departamento de Patologia Geral and 2- Departamento de Parasitologia, ICB, UFMG; 3- Departamento de Clínica e Cirurgia Veterinária, Escola de Veterinária, UFMG, Belo Horizonte, MG. 4- Departamento de Análises Clínicas, Escola de Farmácia, UFOP, Ouro Preto, MG, Brasil.

American Visceral Leishmaniasis (AVL) is a zoonosis and dogs represent the main domestic reservoir. In the last decades, a spreading of this disease has been associated with urbanization of large cities, such as Belo Horizonte, Brazil. In this study we have tried to make correlation among clinical, histopathological and immunoparasitological features of the disease in dogs involving more aspects of the pathogenesis of the disease. Eighteen mongrel dogs, obtained from the City Hall of Belo Horizonte were previously classified as asymptomatic, oligosymptomatic and symptomatic animals (weakness, cutaneous lesions, alopecia, and clinical anemia were the most common symptoms). The dogs were killed with an overdose of thionembutal and samples of blood obtained for serological studies (indirect immunofluorescence test-IFAT) and liver function testing (TGO, TGP, Alkaline Phosphatase). During necropsy, livers were weighed and samples collected for Giemsa-smears ("imprints"). The slides (smears) were observed under a light microscope using a 100x objective to determine the liver parasite burden L.D.U. (*Leishmania Donovan Units*). Other samples obtained from the same livers were fixed in formalin (10%, pH 7.0) for histopathological and immunohistochemistry studies. Hematoxilin-eosin, Gomory and Silver stain for collagen fibers and a streptoavidin-peroxidase method to detect amastigotes of *Leishmania* were carried out. Microscopically, intralobular and periportal hepatic granulomas were the main lesion observed in all infected dogs. The granulomas were rarely confluent and found mainly in the sinusoid lumen. Macrophages, parasited or not, were the main cell type but there were also some epithelioid cells, a small number of lymphocytes and rare neutrophils. Amastigotes of *Leishmania* immunolabelled were found in all animals in the cytoplasm of kupffer cells and in the cytoplasm of the granuloma macrophages. Morphometrical analysis was carried out using a Zeiss Imaging Processing Software (KS300). Student's t test was carried out for statistical analysis. In the liver paraffin sections of all animals we analyzed number and size of the granulomas, collagen deposition and the density of parasites immunolabelled. The parasite burden (L.D.U.) and IFAT titers did not correlate with the number or size of granulomas and neither with the density of the parasitism tissue or with the clinical aspects. Liver function tests were normal in all animals. This study suggests that clinical aspects of canine visceral leishmaniasis do not necessarily indicate absence of lesions or parasites in the liver. Future studies will be aimed to compare the clinical and immunopathological aspects of the disease.

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B-U-49 – CHRONIC INTERSTITIAL PNEUMONITIS IN DOGS NATURALLY INFECTED WITH *LEISHMANIA (LEISHMANIA) CHAGASI* IN BELO HORIZONTE, MINAS GERAIS

Gonçalves, R.[1]; Paiva, M.C [2] ; IM Chiarelli [2] ; Andrade, H.M[3]; Tafuri, Wg. L [2]
1- Depto de Ciências Exatas e Biológicas (DCBI), UFOP, Ouro Preto, MG; 2- Depto de Patologia, ICB, UFMG, Belo Horizonte, MG; 3- Depto de Parasitologia, ICB, UFMG, Belo Horizonte, MG

American Visceral Leishmaniasis (AVL) is a zoonosis and dogs represent the principal domestic reservoir of the disease. In the New World the etiological agent of the disease is *Leishmania (Leishmania) chagasi* which is transmitted by the phlebotomine *Lutzomyia (Lutzomyia) longipalpis*. In Brazil the disease is endemic and the pathology of the disease in dogs was described since Deane, 1950. Later on, many works have been done showing the prevalence at the disease in many states of Brazil. Interstitial Pneumonitis ha ve been reported in dogs naturally infected (George *et alii* 1976; Duarte *et alii* 1986) and experimentally infected (Genaro,1993). These lesions are always strict to Leishmania infections. However, the pathogenesis of this disease in lungs is not clear. The aim of this study is to make correlation among clinical, histopathological and immunoparasitological features of this pathology. Eighteen mongrel dogs, obtained from the City Hall of Belo Horizonte were previously classified as asymptomatic, oligosymptomatic and symptomatic animals (weakness, cutaneous lesions, alopecia, and clinical anemia were the most common symptoms). Eight control dogs were used as controls and they were divided in two groups: group I with five animals from City Hall of Belo Horizonte (serum negative to Leishmania), group II with three from the UFOP animal facility. They were provided with water and chow "ad libitum" and they were vaccinated against distemper, parvovirus, leptospirosis, parainfluenza, and infection hepatitis (Masteguard-Univet) and treated with antihelmintc drugs. The dogs were killed with an overdose of thionembutal sodic and samples of blood obtained for serological studies (indirect immunofluorescence test-IFAT). At the necropsy lungs samples were obtained and immediately fixed in formalin (10%, pH 7.0) for histopathological and immunohistochemistry studies. Hematoxilin-eosin, Gomory and Silver stain for collagen fibers and a streptoavidin-peroxidase method to detect amastigotes of *Leishmania* were carried out. Morphometrical analysis was carried out using a Zeiss Imaging Processing Software (KS300) to analyze the collagen deposition. Student's t test was carried out for statistical analysis. The central feature observed in the fragments of lungs was the chronic interstitial pneumonia. The basic lesion was the intraalveolar accumulation of various mononuclear cells (mostly macrophages) and interstitial thickening by accumulations of lymphoid cells and fibrous tissue. However, the interstitial pneumonia showed in different phases, the exudative phase and the proliferative phase. Histologically, the intralobular septa thickening were constituted by leukocytes, or by fibroblasts and collagen, respectively. Hyperplasia of smooth muscle was remarkable observed in all intralobular septa walls and very well elucidate by Gomori and silver stain. In some case we observed inflammatory focus in the subpleural region characterized by focal accumulations of foamy macrophages. Areas of emphysema were recognized as abnormal enlargement of airspaces distal to terminal bronchioles with evidence destruction of their walls. Some blood vessel showed their subendothelial thickened due the subendothelial deposits of immune complexes (PAS positive). Parasites immunolabelled were rare and absent in most part of the cases.. The IFAT titers did not correlate with the density of the parasitism tissue or with the clinical aspects. However, the collagen deposition was higher in all the infected animals (p£0,05). In fact, the reticular fibers were deeply thickened in the interstitial. These collagen alterations seem not be correlated with the parasitism considering our immuhistochemical results. Besides, there was no correlation with the clinical and serological results (IFAT titers) of the disease. We have considered that the pathogenesis of the interstitial pneumonitis is more an immunological disorder than a infectious disease.

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B-U-50 – USE OF FLUORESCENT LEISHMANIA FOR FASTER QUANTITATION OF PARASITE GROWTH *IN VITRO* AND *IN VIVO*

Rossi-Bergmann, B; Lenglet, A; Bezerra-Santos, CR; Costa-Pinto, D* and Traub-Czeko, YM*
Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro.*Departamento de Bioquímica e Biologia Molecular, Fundação Oswaldo Cruz-RJ, Brazil. e-mail: bbergman@biof.ufrj.br

The methods normally used for the evaluation of intracellular leishmania growth are either very fastidious and subjective (Giemsa-stained cultures), unreproducible (transformation and expansion as promastigotes), or non-representative (axenic amastigotes). We are presently developing a relatively fast and quantitative method where green fluorescent protein (GFP) - transfected parasites are quantitated by fluormetry.

Promastigotes of *Leishmania amazonensis* were transfected by electroporation with the gene fragment coding for the C-terminal extension of cysteine proteinase fused to the reporter GFP in the pXG-'GFP+ vector developed by S. Beverly et al. The transfected promastigotes showed a very bright cytoplasmic fluorescence which could be easily quantitated by a microplate reader fluormeter. To investigate whether the plasmid was stable in the amastigote stage, 10^6 mouse peritoneal macrophages were infected with GFP-promastigotes for 1h, washed and cultured for 65h, after which time the cells were full with bright parasites. The cultures were lysed with dH2O and transferred to a black microplate for fluorescence measurement, giving a significantly higher fluorescence (662 ± 5 mUF) as compared to uninfected cultures (156 ± 10 mUF). To assess whether the intracellular parasites were viable and not just phagocytosed promastigotes, parallel infected macrophage cultures were placed at 28º C. After 5 days the cultures were full with bright promastigotes, indicating that the plasmid was stable in both promastigote and amastigote forms of the parasite.

To investigate whether fluorescent parasites can also be used to measure parasite load in the lesions, BALB/c mice were infected in the footpad with 4 x 10^6 GFP-promastigotes. Four weeks later the animals were killed, the feet teased and crushed against a steel mesh and the cell suspensions serially diluted in black microplates with PBS. The results showed a significantly higher fluorescence in the infected as compared to uninfected footpads. Again, bright promastigotes could be recovered from the tissue lysates cultured at 28ºC.

These preliminary results suggest that GFP-promastigotes may be successfully used in the development of a new method for in vitro and in vivo quantitation of parasite load, which can be applied in faster drug screening and pre-clinical sudies and other situations where measurent of parasite growth is necessary. We are presently evaluating its superiority in comparison to the more conventional methods.

B-U-51 – SITUATION OF DOGS IN ENDEMIC AREA OF AMERICAN TEGUMENTAR LEISHMANIASIS

Arraes, S.M.A.A.; Zanzarini. P.D. & Rodrigues, M. *
Laboratório de Imunologia Clínica, Laboratório de Ensino e Pesquisa em Análises Clínicas (LEPAC), Departamento de Análises Clínicas (DAC), Universidade Estadual de Maringá (UEM) - Paraná; *Fundação Nacional de Saúde - Maringá

American Tegumentar Leishmaniais (ATL) is endemic in the north of the State of Paraná. In the years from 1991 to 1994, 23,3% of cases of ATL are notified for "15th Regional de Saúde" happened at places to the margins of the river Ivaí (Teodoro et al, *An. Bras. Dermatol*., 72: 124-36, 1997). This work was accomplished together with the Fundação Nacional de Saúde, in the year of 1997, with the objective of evaluating the paper of dogs in the epidemiology of ATL.

For that, 33 dogs were studied in small farms located to the margins of the river Ivaí, in Doutor Camargo city, northwest of Paraná. Samples of blood were collected for the research of antibodies anti-*Leishmania* by the reaction of Indirect Imunofluorescence (IF) and biopsy of lesion for microscopy in 5 animals.

They were considered positive the samples with larger title or the same to 40 for IF - *Leishmania* and Chagas' Disease. The results were:

IF - *Leishmania*	< 20	20	40	80	160	320	Total
Number	2	3	7	9	9	3	33
%	6,06	9,09	21,21	27,27	27,27	9,09	100
Pos. Microscopy				1	1	1	
IF - Chagas'Dis.	18	10	3*	1†	0	1#	33

*One of this dogs has positive microscopy and title 160 for IF - *Leishmania*, other presented title 80 and other 40 for IF - *Leishmania*; †This animal presented characteristic lesion and title 160 for IF - *Leishmania;* #This animal presented positive microscopy and title 320 for IF - *Leishmania*

These results suggest that the dogs can have important paper in the epidemiology of ATL. Additional studies should be accomplished in the area to deepen the knowledge about the participation of these animals in the chain of transmission of ATL.

LEPAC/DAC/UEM, FNS

B-U-52 – EXPERIMENTAL INFECTIONS OF THE OPOSSUM *DIDELPHIS MARSUPIALIS* (MARSUPIALIA: DIDELPHIDAE) WITH TWO DISTINCT *LEISHMANIA* SPECIES

De Oliveira[1], V. L., Baptista, C., Moreira, C. F. S., Ribeiro, R. V[2]., Grimaldi Jr. G. & Franco, A.M.R.
[1]Setor de Veterinária, Fundação Rio-Zôo, [2]Lab. Histologia, Universidade Federal Rural do Rio de Janeiro, Lab. Leishmaniose, Instituto Oswaldo Cruz, Rio de Janeiro, RJ

Leishmaniasis is endemic in many areas of tropical and subtropical America, where the disease in this region is basically a zoonosis; humans are only an incidental host in the life cycle of the various human pathogenic parasites species (Grimaldi & Tesh, 1993). Species of *Didelphis* (opossum) captured in sylvan areas of Neotropics have been found infected with leishmanial parasites, including *L. amazonensis, L. braziliensis, L. guyanensis, L. chagasi* and *L. forattini* (Grimaldi et al. 1989; Yoshida et al., 1993). The developmental biology of the various *Leishmania* species in their natural reservoir (vertebrate) hosts is not well studied, but infection is often assymptomatic, with little pathology (Lainson 1988). In this pilot study, 9 laboratory-reared opossums (*Didelphis marsupialis*) were infected with either *L.* (*L.*) *amazonensis* or *L.* (*L.*) *chagasi* to determine the susceptibility and the course of cutaneous or visceral leishmaniasis in this mamalian species. Animals infected intradermally with *L. amazonensis* did not developed conspicuos skin lesions at the sites (extremities and nose) of inoculation. However, animals were susceptible to both leishmanial parasites, since the inoculated dose (6×10^7 promastigotes/animal) employed consistently resulted in infection. Postmortem culturing examinations (two months post-inoculation) of tissue samples (from skin, spleen, liver, lympho nodes) allowed the demonstration of visceralized subclinical infection in all animals. The immunologic features during infection [delayed type hypersensitivity (DTH) responses to leishmanin antigens; levels of antibodies) were assesed, but no parasite-specific responses were detected in this preliminary study. In addition, postmortem histological examinations of paraffin sections (prepared from several tissue samples and stained with hematoxylin-eosin) are under investigation for studying the parasite-host interactions *in vivo*.

B-U-53 – *CERDOCYON THOUS* (L.) (CARNIVORA: CANIDAE) NATURALLY INFECTED WITH *LEISHMANIA (LEISHMANIA) CHAGASI* (CUNHA & CHAGAS,1937) IN THE STATE OF MINAS GERAIS, SOUTHEAST BRAZIL

Silva, E. S. *; Pirmez, C.**; Gontijo,C. M. F.*; Fernandes, O. ***& Brazil, R. P.*
*Laboratório Leishmanioses, Centro de Pesquisas René Rachou-FIOCRUZ, Av. Augusto de Lima 1715, Belo Horizonte, MG 30190-002, Brazil. **Lab. Imunopatologia - Depart. Bioquímica e Biologia Molecular and ***Depart. Medicina Tropical - IOC/FIOCRUZ.

Visceral leishmaniasis (VL) is an important public health problem in many areas of South and Central America. More than 90% of the cases of VL reported in the Americas were found in Brazil. Minas Gerais State is an endemic area for visceral leishmaniasis where domestic dogs are incriminated as reservoir of the disease in rural and periurban areas. During field work in an endemic area of visceral leishmaniasis in the Jequitinhonha region (16° 30' S 41° 0' W), north of Minas Gerais State, Brazil one fox recently hit by a car were captured. Cranial measurements, body proportions and pelage colour were consistent with the crab-eating fox, *Cerdocyon thous* (Courtenay *et al*. 1996). The animal were externally examined, autopsied and part of the material from a small lesion in the nose, spleen and liver were inoculated in NNN blood- agar medium. Another part of the material processed for histopatologic analysis after fixation in buffered formalin and paraffin embedding. Histopathologic analysis of the material from nose lesion showed the presence of residual perivascular mononuclear imframatory infiltrate with the presence of a small number of parasites. Positive results for PCR were obtained in material from lesion, spleen and liver. All positive materials were indentified as belonging to the subgenus *Leishmania* by positive hybridization signal of amplified products with *L. chagasi*. For the first time is demonstrated a natural infection a fox, *Cerdocyon thous* in the State of Minas Gerais in the sotheast region of Brazil. This seems to confirm the role of wild canids in the epidemiology of visceral leishmaniasis in Brazil as shown in several ecologial region of the country (Lainson et al., 1969; Silveira et al., 1982; Mello et al., 1988).

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B-U-54 – STUDY OF *LEISHMANIA* INFECTION IN RODENTS DURING AN OUTBREAK OF AMERICAN TEGUMENTARY LEISHMANIASIS IN AN ENDEMIC AREA OF RIO DE JANEIRO, BRAZIL

Soares V.B.[1], Carvalho R.W.[4], Fernandes O.[3], Pirmez C.[2] & Sabroza P.C.
[1]Escola Nacional de Saúde Pública, FIOCRUZ
[2]Dept. Biochemistry & Molecular Biology, [3]Dept. Tropical Medicine, Instituto Oswaldo Cruz, FIOCRUZ
[4]Dept. Entomology, FIOCRUZ.

The main goal of this study was to investigate if rodents present in the surroundings of the houses could be infected with *L. (V.) braziliensis*. The rationale was that the probability to find infected rodents would be dependent on the timing of capture and examination, which should be during the human transmission period. A monitoring system for new cases was organized in the state of Rio de Janeiro, based on the notification of leishmaniasis to the National Health Foundation. All the entries were analyzed month by month, and cases were plotted in a detailed municipality map, allowing the distribution of the locations with a high and/or ascending incidence. The municipality of Paraty, located in the South of the State, and particularly the locality of Saco do Mamanguá, was chosen because of the increasing incidence of the disease in the human population: 1.10% in 1995, 2.54% in 1996, and 4.36 in 1997. The locality is mainly a resort place, with isolated houses used only during weekends. Around these houses, there are small communities, inhabited by people who had fishing as main activity. The distribution of the cases was heterogeneous, and one of the communities (Ponta da Romana), which is inhabited by 71 persons, presented 27 cases of leishmaniasis. In this particular community, a total of 35 rodents were captured indoors or near the surroundings of the houses in 12 days: 21 *Oligoryzomys nigripes*; five *Rhipidomys mastacalis*, two *Akodon cursor*, two *Proechimys iheringi*, two *Rattus rattus*, one *Oryzomys raytticeps*, one *Akodon arviculoides* and one *Nectomys squamipes olivaceus*. Animals were sacrificed and three fragments of the skin were collected (tail, ear and snout), one from skin lesion when present, and peripheral blood. Genomic DNA was extracted from each sample using a kit (Pharmacia), and analyzed by PCR using oligonucleotides that anneal to the conserved region of the minicircle kDNA molecule. Although some samples showed the expected 120 bp band on agarose gel electrophoresis, only one sample, from *Rhipidomys mastacalis* was confirmed by hybridization as belonging to the *Viannia* subgenus. This result, although preliminary, suggests that rodents might represent an amplification factor in areas where the transmission is peridomiciliary.

B-U-55 – EPIDEMIOLOGICAL SURVEYS BASED ON MONTENEGRO SKIN TEST CONFIRM INCREASING BURDEN OF CUTANEOUS LEISHMANIASIS IN PERNAMBUCO, BRAZIL

Brandão-Filho, S.P.[1], Campbell-Lendrum, D.[2], Brito, M.E.F.[1], Shaw, J.J.[3] & Davies, C.R.[2]
[1]Instituto Aggeu Magalhães, Fundação Oswaldo Cruz, Av. Moraes Rego, Campus UFPE, Recife-PE, CEP 50670-420, Brazil; [2]London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK; [3]Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo-SP, Brazil.

Health service records for North-East Brazil suggest a consistent rise in cases of cutaneous leishmaniasis (CL) due to *Leishmania* (*Viannia*) *braziliensis* over the last decade. Most published surveys were either cross-sectional in design or were limited to the investigation of new clinical cases. In this study we describe the results of a prospective survey of both *L. braziliensis* infection and CL disease in three neighbouring villages in 'Zona da Mata' of Pernambuco. The principal aims were to verify the apparent increase in incidence over recent years, to identify the demographic risk factors for infection in order to indicate whether *L. braziliensis* transmission in Pernambuco typically takes place inside or away from the domestic environment and to describe the characteristic clinical outcome of *L. braziliensis* infection. Prospective, cross-sectional and retrospective epidemiological surveys of infection based on positive Montenegro Skin Test response and/or clinical symptoms confirmed a high current force of infection (0.092/year), and an approximately 10-fold increase in transmission during the last 10 years. Cross-sectional analysis indicated that the incidence rate amongst children (aged 15 years or less) was lower than that amongst adult immigrants exposed for similar time periods, but there was no apparent difference in transmission rate according to gender. Coupled with the known behaviour of the predominant local sandfly vector, *Lutzomyia whitmani*, this suggests that most people are infected outside their houses, rather than either indoors or while visiting remnant rainforest. The estimated proportion of infections which lead to cutaneous lesions (0.81 - 0.87) is relatively high for this *L. braziliensis* area. However, an unusually low proportion of clinical infections (0.002) apparently lead to metastasis.

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B-U-56 – 3-D RECONSTRUCTION OF CULTURE DERIVED MATURE CYSTS OF *LEPTOMONAS WALLACEI* N. SP. ISOLATED FROM *ONCOPELTUS FASCIATUS* (HEMIPTERA: LYGAEIDAE)

Romeiro A, De Souza W & Attias M
Laboratório de Ultraestrutura Celular Hertha Meyer, Instituto de Biofísica Carlos Chagas Filho, Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro. 21949-900, Ilha do Fundão-RJ BRAZIL

Cysts are forms of dormancy of some organisms when in adverse environmental conditions. *Blastocrithidia* and *Leptomonas* are the only genera of the Trypanosomatidae family that produce cysts during their life cycle. Encystation involves a set of morphological and physiological stages of differentiation until maturation of the cyst. *L. wallacei* n. sp. (Romeiro *et al*., 1997) promastigotes (parasite active form) isolated from *O. fasciatus* shows either in the digestive tract of the insect or in culture medium small ovoid forms adhered to its anterior portion, close to the opening of the flagellar pocket. In the present work we determined, by 3-D reconstruction ultrathin serial sections the morphology and organization of organelles in culture derived mature cysts of *L. wallacei* n. sp. A 3-D model was generated in a Silicon Graphics workstation with the SYNU software (Hessler *et al*., Microscopy: the key research tool. March 73-82; 1987). Mature cysts showed an electrodense cell coat, an external membrane and a homogeneous subpellicular region (HSR) limited by a double negatively stained membrane (endoplasmic reticulum like structure) that clearly separates it from the very densely packed cytoplasm. The nucleus was anterior and the nuclear chromatin as the k-DNA fibrils were more condensed than in typical promastigotes. A small flagellar pocket was present but no flagellum was observed. An intracellular system of tubular membranes (MS) of unknown nature was evident. There was no sign of polymerized tubulin in the cyst. The single mitochondrion was spread between the flagellar pocket and the nucleus and a few thin cristae could be observed, besides the very compact K-DNA. The table below compares the relative volume of the nucleus and mitochondria to the whole cell of promastigotes of *Herpetomonas roitmani*, and *Crithidia deanei* to cystic forms of *L. wallacei:*

Cell Type	Cell volume	Mitochondrion	Nucleus	$V_{mitochondrion}/V_{cell}$	$V_{nucleus}/V_{cell}$
L. wallacei (cyst)	85 mm^3	13.62 mm^3	2.98 mm^3	16%	3.5%
C. deanei	99 mm^3	10.57 mm^3	4.23 mm^3	10.6%	4.2%
H. roitmani	142 mm^3	16.23 mm^3	9.20 mm^3	11%	6.4%

From it we can conclude that *L. wallacei* cysts are a little smaller than the very small promastigotes of *C. deanei*, when compared to "typical" *H. roitmani* promastigote cells. However, nucleus is very reduced in cysts, while mitochondrion has a volume that compares to the promastigotes. Considering that cysts are dormant metabolically slow cells, it has to be considered if this mitochondrion, poorer on cristae than the ones of promastigotes, are active. These data show that 3-D reconstruction technique can besides providing nice images, be a valuable tool on stereological and comparative studies.

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B-U-57 – COCULTURE OF *PHYTOMONAS SP.* AND *AEDES ALBOPICTIUS* CELLS, ATCC CCL 126

Keller, D.G. [A] , da Cunha , M. [A], de Souza ,W. [B] and Miguens, F.C. [A]
A – Laboratório de Biologia Celular e Tecidual, Centro de Biociências e Biotecnologia, Universidade Estadual do Norte Fluminense; B – Laboratório de Ultraestrutura Celular Hertha Meyer, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro.

The occurrence of trypanosomatids in plants has been reported since the beginning of this century. The genus *Phytomonas* include all trypanosomatids found in the floem and laticifer tubes of plants. They have also been described in fruits and seeds. *Phytomonas* from laticifers tubes had been cultivated in axenic medium. However, there are often alterations in parasites morphology. None of the research attempts to cultivate the parasites obtained from sieve tubes elements of *Elaeis guineensis* and *Cocos nucifera* were successful. In this work we report the isolation, coculture cultivation and morphological analysis of *Phytomonas sp.* found in latex of *Chamaesyce thymifolia,* as a basis for isolation and culture of *Phytomonas staheli* form palm plants. ATCC 126 cell line from *Aedes albopictius* larvae were cultivated in culture flask containing 5 ml of Mitsuhashi and Maramorosch (MM) insect medium supplemented with 10% fetal bovine serum without antibiotics and maintained at 28°C. *Chamaesyce thymifolia* were collected in the northern region of Rio de Janeiro State. Plants were brushed with iodinated alcohol and leaves were cut with a sterilized razor blade in a laminar flow hood. The latex drops were collected with a sterilized Pasteur pipette and diluted in 500 µl of phosphate buffered saline. After that, protozoa were transferred to culture flasks containing a monolayer of the insect cell line. Alternatively, protozoa and insect cells were put simultaneously into culture flasks. These cultures have been established for 6 months and subcultivated approximately 40 times. Cultures were observed every day under phase contrast illumination to check protozoa growth and morphology. *Phytomonas sp.* from laticifer tubes of *C. thymifolia* present an elongated and twisted promastigote form with a long flagella when maintained in coculture. On the other hand, protozoa change their typical form presenting no twisted parasites and often small flagella in MM medium without ATCC 126 cell line. No contamination were observed. Samples were processed for transmission and scanning electron microscopy. Observation of the cultures of *Phytomonas sp.* by scanning electron microscopy showed that most of parasites are elongated, twisted and the body length measuring about 12 µm. The length of flagella was approximately 12 mm. Transmission electron microscopy of protozoa showed that it has all the structures typical of the *Trypanosomatidae* family. In conclusion, we believe that coculture of *Phytomonas*/ ATCC 126 cell line is a good method for protozoa culture because the ultrastructure and form of the protozoa did not change.

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B-U-58 – EFFECT OF *GINKGO BILOBA* EXTRACTS ON TRYPANOSOMATIDS GROWTH AND MORPHOLOGY

Venturelli, S.M.*, Faria-e-Silva, P.M.*, Soares, M..J.** & Fiorini, J.E.***
*Lab. de Microbiologia, Escola de Farmácia e Odontologia de Alfenas (EFOA), Alfenas–MG. ** Depto. de Ultraestrutura e Biologia Celular, Instituto Oswaldo Cruz/FIOCRUZ, Rio de Janeiro-RJ. ***Lab. de Biologia e Fisiologia de Microrganismos, Universidade de Alfenas–UNIFENAS, Alfenas-MG.

Ginkgo biloba extracts are natural compounds that have been widely used in the alternative medicine for treatment of a wide range of disturbs, such as Alzheimer's disease and diabetes. Several *Ginkgo* properties have been described, including inhibition of the monoamine oxidase and a neuroprotective effect. Furthermore, possible relationships between *Ginkgo* extracts and the platelet-activating factor (PAF) have been suggested (Smith PF, Maclennan K & Darlington CL. *J. Ethnopharmacol.* 50(3): 131-139, 1996). Aim of our work was to verify the possible effects of *G. biloba* extracts on the cell growth and differentiation of monogenetic trypanosomatids. *Herpetomonas samuelpessoai* and *Herpetomonas* sp. were used for the experiments, which were carried out at 28ºC in Roitman's chemically defined medium. *G. biloba* extracts were obtained in a Soxhlet apparatus at 50ºC for 2 hours. Dehydrated *G. biloba* leaves fragments (20 g) were diluted in 400 ml of 70% ethanol. The alcohol was evaporated at 50ºC in a rotary evaporator, the extract was sterilized by membrane filtration and then aseptically added to the medium at different concentrations (4, 20, 40, 60, 80 and 100 mg/ml). Cell growth was determined using a Neubauer chamber, with cell countings performed at 24-hour intervals. Smears stained by the Panótico method were observed by light microscopy and used to determine the percent of pro-, para-, and opisthomastigote forms in the cultures. Our results showed that *G. biloba* extracts inhibited growth of *Herpetomonas* sp. at concentrations higher than 20 mg/ml. For *H. samuelpessoai*, cell growth inhibition was observed at concentrations higher than 40 mg/ml. No effect was observed on the cell differentiation of both *Herpetomonas* species used in this work. However, in *H. samuelpessoai* several alterations on the cell morphology could be noted at concentrations that inhibited cell growth (60 and 80 mg/ml). Such alterations are probably related to the cell division process, as cells containing three/four nuclei could be observed. Cytoplasmic expansions, perhaps representing an unsuccessful process of cell division, were frequently found. Ultrastructural analysis by transmission electron microscopy showed cells with homogeneous nuclei and absence of nucleoli.

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B-U-59 – MORPHOLOGICAL AND MOLECULAR DIFFERENTIATION OF *LEPTOMONAS WALLACEI* N.SP. IN THE INTESTINAL TRACT OF ITS NATURAL HOST *ONCOPELTUS FASCIATUS* (HEMIPTERA, LYGAEIDAE)

Romeiro A[1], Saraiva E[2], De Souza W[1], and Attias M[1]
1- Laboratório de Ultraestrutura Celular Hertha Meyer. Instituto de Biofísica U.F.R.J. 21949-900 Rio de Janeiro, BRAZIL 2- Departamento de Imunologia. Instituto de Microbiologia U.F.R.J. Rio de Janeiro, Brazil

Leptomonas wallacei n. sp. infects the 3rd and 4th ventricles of the midgut and the whole hindgut of its host *Oncopeltus fasciatus*. In the midgut ventricles, the parasite adheres mainly to the perimicrovillar membranes that cover the intestinal epithelium. In the hindgut, the adhesion occurs only in the rectal pads due to the wax layers of the epicuticule and the parasites are often observed free in the lumen.

There are some differences in cell morphology between parasites from the midgut and hindgut of the insect. In order to check the significance of this morphological diversity between intestinal compartments, we measured, at the scanning electron microscope, the length of cell body and flagellum of the populations of each segment. In parallel, the surface carbohydrates of these parasites were analyzed by a set of 11 lectins conjugated to fluorescein (FITC). The mean size of parasites in midgut is larger than in hindgut. While the cell body of 88% of the midgut population measured from 10 to 20μm, only 68% of hingut individuals belonged to this interval. This difference between the populations was statistically significant (*t* test, $p > 0.05$). The flagellum length was very variable in both populations. In the midgut parasites, the flagellum of 80% of the individuals were in the range of 20 to 40 mm. On the other hand, in the hindgut parasites, the longest flagellum did not exceed 26 mm, and in the majority of the individuals (84%) the length was in the range of 10 to 25 mm. The total length (cell body + flagellum), and parasite thickness were not correlated and the differences observed were not statistically significant (*t* test, $p < 0.01$).

The table below summarizes the labeling with fluorescein tagged lectins.

	ConA	LPA	WGA	GS I	HPA	PNA	BPA	GS II	MPA	SBA
Midgut	+	+	+	-	-	+	-	-	-	-
Hindgut	+	+	+	+	+	+	-	-	-	-
Culture	+	+	+	+	+	-	-	-	+	+

These results show that *L. wallacei* not only reproduces, but also differentiates along the intestinal tract of its natural host, expressing α-Gal, and α-GalNAc residues as it moves towards the hindgut. Besides it, culture forms, not necessarily repeat the surface carbohydrate distribution found in the host.

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B-U-60 – DNA AND RNA DISTRIBUTION IN THE NUCLEUS OF TRYPANOSOMATIDS: A CYTOCHEMICAL AND IMMUNOCYTOCHEMICAL STUDY

[1]Motta, M.C.M.; [1]De Souza, W. & [2]Thiry, M. [1]Laboratório de Ultraestrutura Celular Hertha Meyer, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro; [2]Laboratory of Cellular and Tissular Biology, University of Liège, Liège, Belgium.

Many studies about the ultrastructure of trypanosomatids have been published, however litlle attention has been given to the nucleus. Subjected to morphological changes according to the species, the nucleus also presents modifications in the chromatin condensation and distribution during the cell cycle. Chromosomes are never seen during the division process and this low level of DNA compactation is related to its weak association with histones, possibly reflecting modifications in gene expression which can be exploited in attempts to control the trypanosomiasis. In the present work, in order to characterize the DNA and RNA distribution in the nucleus, the Terminal deoxynucleotidyl Transferase (TdT) immunogold technique combined to the acetylation method and anti-RNA antibodies were applied to different species of trypanosomatids, some of them harbouring an endosymbiotic bacterium in the cytoplasm. When the TdT technique was applied, an intense labeling was seen over the condensed chromatin associated to the nuclear envelope and also over the electron lucid nucleolar central region. The application of the acetylation method allowed further the identification of two distinct components in the peripheral region of the nucleolus: an inner zone composed of fibrils and an outer zone formed by granules. When applied to thin sections, monoclonal antibodies to RNA were seen in association to the nuclear pores and also over the interchromatin space, but the condensed chromatin found close to the nuclear envelope was devoid of gold particles. In contrast to DNA labeling, gold particles associated to anti-RNA antibodies were preferentially detected over the fibrillar and the granular parts. In mitotic cells, the nucleolar components maintained a central location, but they did not constitute a ponctual, individualized structure. Small electron-dense bodies (arrows), which probably correspond to the prenucleolar bodies (PNBs), were seen randomically distributed throughout the nuclear central region at the end of the mitotic process. During the cytokinesis, the nucleolus was already reorganized. Generally, the nucleolus consists of five main components: the fibrillar centre, the dense fibrillar component, the granular component, the interstices and the nucleolus-associated chromatin. However, these ultrastructural features is characteristic of mammalian cells. In previous reports, the nucleolus in trypanosomatids was described as a homogenous structure showing no indication of different structural compartments. In the present work, we clearly show that the nucleolus of trypanosomatids is not seen as an amorphous body but comprises two distinct parts: a centrally-located and fibrillar zone surrounded with a granular zone. The fibrillar zone includes sometimes a chromatin-containing interstice.

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B-U-61 – EVALUATION OF *IN VITRO* METACYCLOGENESIS OF *TRYPANOSOMA RANGELI* IN DIFFERENT CULTURE MEDIA

Emmanuelle Machado, P.; Eger-Mangrich, I.; Grisard, E.C. & Steindel, M.
Departamento de Microbiologia e Parasitologia - CCB, Universidade Federal de Santa Catarina, Caixa postal 476, Florianópolis, SC – Brazil, 88040-900, e-mail: ccb1mst@ccb.ufsc.br

Nitrophorin is one of the major components present in salivary glands of *Rhodnius* sp. triatomines. Linked to nitric oxide, these hemeproteins are crucial for the blood sucking process acting as anticoagulant. A decrease of this protein amount in *T. rangeli*-infected triatomines was observed by different authors. However, it is not clear if the parasite presence and division within the salivary glands of the triatomine bug, which is a condition for anterior transmission, have influence on this reduction or even in the protein synthesis. Thus, the aim of our work is to verify if these biomolecules have influence in *T. rangeli* metacyclogenesis *in vitro.* This metacyclogenesis was initially evaluated with *T. rangeli* SC-58 and Choachi strains in three different culture media (TC-100, Grace and DMEM) supplemented with 10% of fetal bovine serum at 28ºC. Parasites in the exponencial growth phase in LIT medium were washed twice in PBS, pH 7.4, resuspended to a concentration of 5 x 10^6 parasites/ml in 1ml of each media prepared in three different pH (6.0, 7.0 and 8.0). These experiments were performed in triplicates in 24 well plates, revealing that *T. rangeli* metacyclogenesis was, despite the pH, higher in DMEM medium. Based on these results, both *T. rangeli* strains were cultivated in triplicates in the same three different pH of DMEM medium supplemented with crude extract of *Rhodnius prolixus* salivary glands correspondent to 1, 2, 4 and 8 glands. Metacyclogenesis rate was microscopically evaluated at 0, 3 and 6 days by counting metacyclic trypomastigotes in Giemsa stained smears. In comparison with the control group, an increased metacyclogenesis rate was observed in *T. rangeli* Choachi strain cultivated in both pH 6.0 and 7.0 at 6 days in the presence of salivary gland extract equivalent to 2 glands ($p<0.05$). For *T. rangeli* SC-58 strain, no significative difference in the metacyclogenesis rate was observed between parasites cultivated in the presence of salivary gland extract from the control groups. These results revealed the absence of correlation between the amount of crude salivary gland extract in the media and *T. rangeli* metacyclogenesis rate *in vitro*.

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B-U-62 – ACTION OF HUMAN SERA AND VENEZA ZONATA (HEMIPTERA, COREIDAE) HAEMOLYMPH ON CULTURE FORMS OF TRYPANOSOMATIDS

Baccan,G.B[1].;Bernardo,B.S[1].;Girotto,E[1].;,Souza,W[2], Attias,M[2];Jankevicius,J.V[1]. & Itow Jankevicius,S.[1].
[1]Laboratorio de Biologia de Tripanosomatideos, Departamento de Microbiologia, Centro de Ciencias Biologicas, Universidade Estadual de Londrina; [2]Laboratorio de Ultraestrutura Celular Hertha Meyer, Instituto de Biofisica Carlos Chagas Filho, CCS, Bloco G, Subsolo, Cidade Universitaria, UFRJ, Rio de Janeiro, RJ

The interaction of Trypanosomatids with phytophagous hemipterans can define the localization of the flagellates in the insects (digestive tract and/or salivary glands) and classify the trypanosomatids as monoxenous (parasites of insects) or heteroxenous (involving parasites of insects and plants). The action of human sera and Veneza zonata haemolymph was analyzed on 3 groups of Trypanosomatids. The first group was composed by the trypanosomatids Phytomonas serpens, P.mcgheei and the strain 714 TD, isolated from digestive tract of V.zonata, all of them establishing infection in the salivary glands of the insect V. zonata, and transmitting infection to plants. The second group (Leishmania (L.) amazonensis) represents trypanosomatids that could not established an infection in V. zonata. The third group represents the strain 563 TD, a trypanosomatid isolated from the digestive tract of the phytophagous hemipteran Piezodorus guildini, pathogenic to the V. zonata.

The experiments with human sera used 30mL of undiluted normal sera mixed with 30mL of culture forms of trypanosomatids (10^6 cells/mL) in Eppendorf tubes, incubated at 25°C and aliquots examined after periods of time, directly and stained with May-Grunwald-Giemsa. Almost immediately after the contact with human serum, the flagellates suffers strong agglutination. Groups 1 and 3 showed flagella-flagella, body-body and body-flagella interactions, whereas group 2 (Leishmania (L.) amazonensis) showed a faster flagellar agglutination.

With Veneza zonata haemolymph, collected with automatic pipette (30mL) from a leg of V. zonata ,was processed as with serum samples. The results of group 1 showed a recognition of protozoa by haemocytes in 5 minutes and at 35 minutes it was seen several nodules, without agglutination and in 1 hour, several culture forms presents round forms. L. (L.) amazonensis seemed to be more sensitive than isolates from group 1. The flagellates adhered to haemocytes at time 0, with much more intense agglutination and at time 10 min., it was observed large rosettes of flagellates and at time 30 minutes, including haemocytes. The group 3 showed no significant differences from group 1. The morphologic alteration is studied by electronic microscopy and fenoloxidase biochemistry.

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B-U-63 – THE ROLE OF LIPOPHORIN IN PHOSPHOLIPID TRANSFER TO *TRYPANOSOMA RANGELI* IN *RHODNIUS PROLIXUS* HEMOLYMPH

[1, 2]Leite-Lopes FA, [1]Golodne DM, [2]Lopes AHCS and [1]Atella GC
[1]Deptº de Bioquímica Médica, ICB/CCS; [2]Instituto de Microbiologia Prof. Paulo de Góes, CCS, UFRJ, Cidade Universitária, Rio de Janeiro, CEP 21941-900, RJ, Brasil.

Trypanosoma rangeli is a hemoflagellate that employs a wide variety of American mammalian hosts and hematophagous hemipterous insects in its life cycle, including *Rhodnius prolixus* (Reduviidae, Triatominae). Once the insect is infected, the parasites pass through the gut epithelium and traverse to the hemolymph where they can obtain resources for their metabolism. The hemolymph lipophorin is a major haemolymphatic lipoprotein in insects, which carries and distributes many lipid classes among the tissues involved in lipid absorption, storage and utilization.

In this work we investigated a possible role of lipophorin in lipid delivery to *Trypanosoma rangeli*. When 32P-lipophorin was incubated in the presence of *Trypanosoma rangeli*, the radioactivity was transferred to the parasites. The amount of radioactivity transferred to *T. rangeli* increased with time up to 120 minutes. After lipid extraction, the radioactivity was found only in the lipid moiety. Analysis of this fraction by thin-layer chromatography followed by autoradiography showed that phosphatidylethanolamine and phosphatidylcholine were the major phospholipids transferred. These data suggest that *T. rangeli* is capable of receiveing lipids from lipophorin; whether this is a specific transfer or an endocytic process remains to be investigated.

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B-U-64 – ULTRASTRUCTURAL STUDY OF THE GUT OF *RHODNIUS PROLIXUS* AFTER IRRADIATION (CS 137) AND INFECTION WITH *TRYPANOSOMA RANGELI*

Graciano, G.L., Gomes, SAO; Garcia, ES; Azambuja, P., Nogueira-Higino, N.F.S., De Souza, W.
Laboratório de Biologia celular e Tecidual - Centro de Biociêcia e Biotecnologia – UENF.

Trypanosoma rangeli is a parasite of man, domestic and wild animals, and triatomine insects in America. In contrast to *T. cruzi*, it is harmless to the mammalian host, but a strong pathogen for the vector (D'Alessandro & Saraiva, 1992). An initial study performed by Eloi Garcia and co-workers, showed that insects irradiated and then infected with *T. rangeli* decreased the time of hemocel invasion by the parasite (data not published). The objective of this work is to verify the ultrastructure of the gut of these insects using Transmission Electron Microscopy experiments.

The surface of epithelial gut cells of *Rhodnius prolixus* insects, irradiated with 400 rads and 12 days after a blood meal, as seen by light and electron microscopy, were recovered by regularly distributed microvilli, witch were covered with a low amount of extracellular membranes. The nucleus on most of these cells ("dark cells") was located in the medium basal region, and was elongated, regular shaped and with one or two evident nucleolus. These cells had in the cytoplasm, a great quantity of highly electron dense granules, and vacuoles. A small group of cells showed a pale cytoplasm ("pale cells") and a central rounded nucleus with a irregular shape. Inside these cells there were few iron containing inclusions and a lot of lightly electron dense granules. The basal region of the cells, darks and pales, showed discrete invaginations and a large amount of mitochodria.

In the insects that were infected with *T. rangeli* (H14 strain), after a 400 rads irradiation, two very distinct types of cells were seen. The first, showed an homogenous cytoplasm with a great quantity of lightly electron dense granules, and a few iron granules. The microvilli were regularly distributed among the surface of these cells. The nucleus was elongated, regular shaped, and was located in the medium basal region, with a large number of cisternae of the endoplasmatic reticulum arround the nucleus' axis. The second type showed a more "empty" cytoplasm, just as if the cell had suffered a kind of extraction process. It also showed a large amount of irregularly distributed ER cisterns. The surface of these cells showed regions with no microvilli and the nucleus usually had an irregular shape. The extracellular membranes appeared in a very distinct way, they seem to be compacted. In transversal sections of the gut wall, they were compacted, resembling a net. Some rounded shaped cells, with an irregular spherical nucleus, poor in iron inclusions, but having a few lightly electron dense granules and apical microvilli, were also observed.

In the gut of the insects that were infected with *T. rangeli*, after a 1200 rads irradiation, showed a large number of epithelial cells with an emptiness cytoplasm. The cytoplasmatic inclusions were very rare. The nucleus was rounded shaped and localized in the center of the cell, the nucleolus was rarely seen. The surface of these cells, in some regions, had no microvilli, and where these structures were found, the extracellular layers were compacted, resembling a net. The basal region of these cells, showed discrete invaginations and a large amount of mitochondria.

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B-U-65 – INTERACTION OF A TRYPANOSOMATID WITH A FLY (*CHRYSOMYA ALBICEPS*) MIDGUT: FURTHER STRUCTURAL OBSERVATIONS

Borges, R.A.*, Cavalcanti D.****, Faria-e-Silva, P.M.*, Fiorini, J.E.**, Nascimento, S. B. *** , Motta, M.C.M.**** and de Souza W.****
*Lab. de Microbiologia, Esc. Farm. e Odontologia de Alfenas – EFOA, Alfenas - MG
**Lab. de Fisiologia dos Protistas, Universidade de Alfenas – UNIFENAS, Alfenas - MG
*** Lab. de Entomologia, IBCCF – UFRJ, Rio de Janeiro – RJ
**** Lab. Ultraestrutura Celular Hertha Meyer, IBCCF – UFRJ, Rio de Janeiro – RJ

Trypanosomatids are widespread in the nature, being found as parasites of a large variety of hosts. Most of the monogenetic protozoa which belong to this family are mainly found in the orders Diptera and Hemiptera. The isolation and characterization of new trypanosomatids using several modern approaches have been described in the last decades, however few investigations have focused on the host-parasite association. Thus, some questions remain unclear as the influence of these flagellates in the life cycle of the insects and on the other hand, the development of some stage-specific forms of trypanosomatids (e.g. opisthomastigotes in *Herpetomonas* genus) during the course of natural infections. In the present work we describe the preliminary results of a study involving trypanosomatids and naturally infected flies of the specie *Chrysomya albiceps*. Five insects were captured in Alfenas, Minas Gerais, and examined in order to verify the presence of flagellates. Fresh preparations observed in optical microscopy showed that three of them were parasited. The flagellates were found near the Malphighian tubules (but not into the tubules), either free in the gut lumen or attached to a net of membranes (peritrophic membrane). Histological preparations stained by hematoxilin-eosin revealed that the membrane-attached flagellates appear as great clusters containing over than fifty cells. Using transmission electron microscopy, protozoa were seen attached to the peritrophic membrane by the cell body, some of them presented bacterium-like endosymbiont in the cytoplasm. We are presently aiming to colonize the flies to better characterize this host-parasite interaction.

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B-U-66 – *IN VITRO* INTERACTION OF *SCHIZOTRYPANUM* TRYPANOSOMES ISOLATED FROM BATS WITH MACROPHAGES AND VERO CELLS: PRELIMINARY DATA

Dambrós, B.; Eger-Mangrich, I., Grisard, E. C. & Steindel, M.
Departamento de Microbiologia e Parasitologia, Universidade Federal de Santa Catarina, Caixa postal 476, Trindade, Florianópolis, SC – Brazil, 88040-900, e-mail: ccb1mst@ccb.ufsc.br

Trypanosomes from the *Schizotrypanum* subgenus were isolated from *Eptesicus* sp. bats in Santa Catarina State. Previous analysis by different methods revealed that these strains are distinct from the other bat trypanosome species tested. In this work, the *in vitro* interaction of these bat trypanosomes with mouse peritoneal macrophages (Mϕ) and Vero cells was analyzed. Interaction assays were performed on glass coverslips using culture parasites from strains M504 and M519. For this purpouse, 120µl of each cell suspension were cultivated in DMEM medium, pH 7.4, supplemented with 5% fetal bovine serum at 37ºC at 5% CO_2 atmosphere. After 2 hours of interaction with Mϕ and 4 hours with Vero cells, using a 10:1 parasite/cell ratio, non-adherent parasites were removed by PBS washing. *T. cruzi* Y strain parasites were used as control in these assays. Cells and parasites were then incubated in DMEM for 24, 48 and 96 hours. After these interaction periods, coverslips were removed, briefly washed in a saline solution, fixed in Bouin and stained by Giemsa. Cell infection rates were analyzed by microscopical examination of 500 randomly choosen cells, counting the number of infected cells and the total number of parasites per infected cell. Preliminary results revealed that 24, 72 and 96 hours after interaction with Vero cells, infection rates of 1.6%, 1.7% and 1.6%, presenting an average of 1.22, 18.95 and 35.92 parasites/cell, respectively. After 96 h of infection, trypomastigote forms were observed in Vero cells culture supernatant. The interaction with Mf revealed at 0 and 24 hours, 3.9% and 0.45% of infection rate and an average of 1.2 and 1.1 parasites/cell, respectively. Neither intracellular parasites nor trypomastigotes were observed in Mϕ cultures after 48 hours of interaction. Up to now, bat trypanosomes of M504 and M519 strains, despite their low infectivity, are able to invade Vero cells and differentiate to trypomastigote forms in 96 hours. However, these trypanosomes can infect but does not multiply in Mϕ.

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B-U-67 – KINETICS OF THE NITRIC OXIDE PRODUCTION INHIBITION ON MOUSE MACROPHAGES AFTER THE INTERACTION WITH *TOXOPLASMA GONDII*

Seabra, S.H.[1]; De Souza, W.[1,2] and DaMatta, R.A.[1]
1- UENF, CBB, Laboratório de Biologia Celular e Tecidual; 2- UFRJ, IBCCF, Laboratório de Ultraestrutura Celular Hertha Meyer.

It has been shown that *Toxoplasma gondii* inhibits nitric oxide (NO) production by *in vitro* activated mouse macrophages derived from blood monocytes (MØNO) and peritoneal macrophages (MØ) (Seabra, S.H. *et al*., Mem. Inst. Oswaldo Cruz, Suppl. II, 93:285, 1998). Trying to understand the kinetics of the NO production inhibition on MØNO and MØ caused by *T. gondii*, interactions took place with different a) parasites/MØ ratios and b) time of interactions. MØNO were obtained by blood monocytes purification on a Percoll cushion and cultivated for 6 days in Dulbecco's Modified Eagle's Medium (DMEM) containing 5% inactivated fetal bovine serum (FBS) supplemented with macrophage colony-stimulating factor (10% of L929 conditioned medium - M-CSF). MØ were cultured for 24 hours in DMEM containing 5% FBS. One day before or after the interactions, macrophages were activated with interferon-γ (IFN) and Lipopolysaccharide (LPS). Tachyzoites of the RH strain, were obtained by peritoneal washes of infected mice. Before the interaction, macrophages were washed with DMEM and infected with a 10 to 1 parasites/MØNO ratio or 5, 10, 25, 50 and 100 to 1 parasites/MØ ratio. After 2 hours of interaction macrophages were washed with DMEM and new medium containing FBS, supplemented with IFN, LPS and M-CSF was added. MØ were activated with IFN and LPS 24 hours before the interaction or right after the interaction. After 3, 6, 9, 12, 18, 24 and 48 hours of *T. gondii* interaction with MØNO activated 24 hours before the interaction or 12, 24, 30, 36, 42 and 48 hours of the interaction with MØNO activated right after the interaction, supernatants were collected and assayed with the Griess reagent for the presence of NO_2^-. After 24 hours of interaction with MØ, the supernatants were collected and NO assayed. The results show that MØNO activated 24 hours before the interaction produce NO with 3 hours of interaction. However, MØNO activated right after the interaction produce NO with 12 hours of interaction. NO production by MØNO was inhibited right at the beginning of the interaction (3 hours) with the tachyzoites. Significant differences were observed in the NO production by MØ that interacted with 5, 10 and 25 parasites per MØ ratio when compared with non infected MØ. These results suggest that *T. gondii* is capable of NO production inhibition in the beginning of the interaction with MØNO; and a 5 to 1 parasites/MØ ratio is sufficient to inhibit the NO production in MØ.

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B-U-68 – ULTRASTRUCTURAL EFFECTS OF PACLITAXEL ON INTRACELLULAR *TOXOPLASMA GONDII*

Passos,CA[1] & Melo,EJT[1]
1. Laboratório de Biologia Celular e Tecidual, CBB, Universidade Estadual do Norte Fluminense, Campos-RJ.28015-620

Toxoplasma gondii is an intracellular parasite widely distributed among the vertebrate animals. Tachyzoites of *T.gondii* proliferates within parasitophorous vacuole until the lysis of the host cell. Paclitaxel (taxol) induces tubulin polymerization, resulting in the formation of unstable and nonfunctional microtubules and has been found to inhibit the growth of intracellular *Toxoplasma gondii*. In the present study we decided to analyze the ultrastructural effect of paclitaxel on intracellular parasite.

Vero cells were cultivated in Linbro tissue plates that contained a sterile coverslip (3-5 10^5/well) and maintained at 37°C overnight. The cultures were infected with tachyzoites of *T. gondii* and incubated for 24 hours. After that time, 0.16mM of paclitaxel was added to cultures for 24 and 48 hours. Later on the untreated control and treated cultures were washed with PBS, fixed with Bouin solution, and stained with Giemsa. The cells were observed with a Zeiss AXIOPLAN photomicroscope equipped with 40x objective. For ultrastructural analysis the cells were fixed in solution containing 1-% glutaraldehyde, 4% paraformaldehyde, 5mM $CaCl_2$ and 5% sucrose in 0.1M cacodylate buffer, pH 7.2. The cultures were postfixed for 1h in solution containing 1% OsO_4 in 0.1% cacodylate buffer, at room temperature. The cells were rinsed with cacodylate buffer, dehydrated in acetone and embedded in Epon. Ultrathin sections were examined using Zeiss 900 Electron Microscope.

Infected host cells were incubated with paclitaxel for 24 and 48 hours. After the treatment the number of uninfected host cells increased while the number of infected host cells decreased. Tachyzoites within parasitophorous vacuole were disorganized and some time disrupted when the cultures were treated with paclitaxel for 24 and 48hs. These results suggested that paclitaxel has a drastic action on the structure of intravacuolar tachyzoites.

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B-U-69 – ANALYSIS OF VIABILITY AND INFECTIVITY OF *TOXOPLASMA GONDII* PARASITES MAINTAINED UNDER AXENIC CONDITIONS

Belote, DF, Jesus, AMC, Barbosa, HS
Lab. Ultra-estrutura Celular, Depto. Ultra-estrutura e Biologia Celular, Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, RJ, Brasil

Aiming to study the biology and to test the action of drugs on *T. gondii*, we have designed experiments to evaluate the resistance and the infection capacity of parasites that have been maintained *in vitro*. In the literature few reports exist on the attempts of axenic maintenance of *T. gondii*. Most studies have been done employing mice infection or cell cultures. Some of our approaches required at least the *in vitro* maintenance of the parasites for periods up to 48 hours. We have established some experimental cultive protocols: a) in PBS; b) in PBS + 10% fetal calf serum; c) in PBS + 20% fetal calf serum. All experiments were tested in 3 different temperatures: 4°C, 28°C and 37°C. Each experiment was made at least in duplicate and 10^7 parasites/ml were maintained in Eppendorf tubes. The parasites were collected after 1, 3, 5, 24, 48 and 72 h, quantified in Neubauer chamber and observed by phase contrast microscopy to evaluate the mobility and other general aspects.

Our results demonstrated that the number of parasites during the whole course of the experiments remained constant in all experimental conditions, indicating that the parasites did not die nor multiplicate. In order to analyze the infectivity of *T. gondii* under the different tested variable conditions, parasites obtained after 72 hours of each experimental condition were inoculated in two mice and a parasitemia control was made 3 days post-inoculation. Our preliminary results demonstrated that infection occurred at similar levels independent of the temperature and it was only positive in those groups where the fetal calf serum was added to the PBS. In addition, maintenance of the parasites for 1 week in the above different mentioned experimental conditions showed that there was a loss in 20% in the number of parasites. Infectivity to mice was also dependent of serum presence in PBS, without interference on the cultivation temperature.

These data will help to study with safety the effect of drugs on *T. gondii.*

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B-U-70 – PRELIMINARY OBSERVATIONS ON THE 3-D ORGANIZATION OF THE PARASITOPHOROUS VACUOLE OF *T. GONDII* INFECTED VERO CELLS

Attias M [1], Ferreira S[2] , De Souza W [1]
1- Laboratório de Ultraestrutura Celular, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro. 21949-900 Rio de Janeiro, RJ. BRAZIL 2- Lab. de Biologia Celular e Tecidual, Universidade Estadual do Norte Fluminense

Toxoplasma is an important opportunistic pathogen of humans. Its life cycle include a short extracellular stage for the active penetration of host cells where, inside a parasitophorous vacuole (PV), they escape the immune system survey, and multiply by endodiogeny, a particular type of asexual reproduction where each individual generates two daughter cells. The PV membrane is devoid of host cell integral proteins; however, lipids of both parasite and host cell plasma membrane seem to be present. Meanwhile it multiplies, forming intra-vacuolar rosettes, the parasite creates inside the vacuole a complex network of tubular membranes. We have made observations at the ultra-structural level on Vero cells after 1 and 12 hours of infection with *T. gondii* tachyzoites. These cells have been fixed and processed for electron microscopy as a monolayer. Serial sections of this monolayer were obtained in order to get 3-D information about the internal organization of the PV. Also, a random thick (1 μm) section of an infected cell was used for electron tomography with the same objective. At this stage (12 hours) most of the infected Vero cells contained PVs, with 2 tachyzoites in each. Both tachyzoites had their apical portions oriented to the same side, which we will refer as anterior portion of the vacuole, and their longitudinal axis was parallel to the lateral spreading of the host cell. At the EM level, elements of the tubular network were seen both fused to the PV membrane, and budding from all over the parasite cell body, suggesting a connection of the parasite surface with the cytoplasm of the host cell. From the PV membrane projections are formed towards the vacuolar matrix, establishing contact with the tubules. Dense granules exocytosis seem to be closely associated with the generation of both the tubules, and the PV membrane. These discharges seem to result in the formation of lamellated structures that will result in new tubules or in the growth of the PV membrane. The posterior portion of the parasites, and the vacuole was completely distinct, both by the appearance of vacuolar membranous profiles, and by the apparent lack of cellular organization of this part of the parasites, suggesting that this may not only be a site for discharge of dense granules, as proposed by several authors, but also a residual body resulting from the endodiogenic division. Both serial sections and tomographic generated volumes, suggest that the PV growth is also dependent from fusion of host cell vesicles from Golgi origin.

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B-U-71 – ASSOCIATION OF LIPIDS INCLUSIONS DURING *TOXOPLASMA GONDII*-SKELETAL MUSCLE CELLS INTERACTION

[1]Andrade, E.F., [2]Carvalho, L. & [1]Barbosa, H.S.
[1] Lab. Ultra-estrutura Celular, Depto. Ultra-estrutura e Biologia Celular, IOC, FIOCRUZ; Lab. Cultura de Células, IB, UERJ, Rio de Janeiro, RJ, Brasil

Toxoplasma gondii, a coccidian protozoan agent of toxoplasmosis, is able to infect and to replicate within different eukaryotic cells. Human infection with *T. gondii* is an important cause of morbility and mortality. In host with intact immunity, infection is usually benign, owing to a rapid, effective immune response that forces encystation of the tachyzoites in brain and muscle cells. Little attention has been given to the interaction of *T. gondii* with skeletal muscle cells (SMC) although it is one of the cells involved in the chronic phase of toxoplasmosis. Our proposal in this study is to analyze intracellular events of *T. gondii* during the course of its infection with SMC.

SMC were obtained from thigh muscles of 18-days-old mouse embryos. The tissue dissociated was resuspended in Dulbecco's modified Eagle medium supplemented with horse serum, fetal calf serum and chick embryo extract. The cells were infected with tachyzoite forms of *Toxoplasma gondii* (RH strain) maintained by intraperitoneal passages in Swiss mice. The cell cultures were fixed after 30 min to 24h of interaction parasite-host cell and processed as routine for transmission electron microscopy.

The ultrastructural analysis showed that the parasite intracellular was always located surround by a membrane in a typical parasitophorous vacuole (PV). Mitochondria and endoplasmic reticulum were found associated with the membrane of the PV. Lipids inclusions of the host cell were associated with the membrane of the vacuole integrated into the vacuolar space in contact with the membrane of the parasite and sometimes in communication between two vacuoles. Those data suggest the possibility that lipids of the host cell can be transferred to parasitophorous vacuole membrane, can act in the fluidity of this membrane or to the intravacuolar parasite, during its interaction with skeletal muscle cell.

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B-U-72 – SURVEY ON *TOXOPLASMA GONDII* INFECTION IN OUTDOOR PIG PRODUCTION SYSTEM FROM SOUTHERN REGION OF BRAZIL

Silva , R. A.M. S[1], Bonassi, C. A.[2], Vidal, C. E. S[1] , Mores, N.[1] & Dalla-Costa, O. A.[1], Dubey, J.P.[3]
1-EMBRAPA/Centro Nacional de Pesquisa de Suinos e Aves, BR 153, km 110, Vila Tamanduá, CEP: 89700-00 Concórdia, SC, Brasil.
2-Universidade do Contestado, Curso de Ciências Biológicas, Bairro Salete, Parque de Exposições, CEP: 89700-00 Concórdia, SC, Brasil.
3-Parasite Biology and Epidemiology Laboratory, Livestock and Poultry Science Institute, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland 20705-2350, USA.

Toxoplama gondii is a protozoan parasite that is common in domestic livestock. The definitive host for the parasite is the cat, which sheds *T gondii* oocysts in its feces. *T. gondii* infection is a potencial public health problem, because the infection can be transmitted to human beings. Among human beings, immunocompromised individuals and fetuses have the greatest risk for developing clinical toxoplasmosis. Serum samples from 115 pigs of 13 of outdoor pig production systems from Santa Catarina and Rio Grande do Sul States, Brazil were examined for cross reacting antibodies to *T. gondii* by the use of the modified agglutination test (MAT). Antibodies to *T. gondii* were found in 86.08% of 115 pigs. The antibody titers were 1:50 (4 pig), 1:100 (7 pigs), 1:200 (11 pigs), 1:400 (8 pigs), 1:800 (8 pigs), 1:1600 (8 pigs) and 1:3200 (64 pigs) by the MAT. This is the first report on *T. gondii* infection in outdoor pig production system in Brazil. According to Assadi-Rad et al. (*Vet Parasitol*, 57: 289-97, 1995) sows kept outdoors at any time were 23 times more likely to be seropositive than those kept indoors. The consequences of *T. gondii* infection in swine may be similar to those in humans. First time infection in pregnant sows may cause stillbirth or abortion (Dubey, Vet. Parasitol., 19:181-223, 1986). Also, infection in younger animals may be fatal. Sows that are soropositive for *T. gondii* may have aborted at one time or another (Assadi-Rad et al., 1995). Probably the fecal contamination of the environment by cats or by consumption of infected rodents may be the most significant sources of toxoplasmosis for outdoor-reared pigs.The economic impact of *T. gondii*, as well as its public health importance need to be assessed.

B-U-73 – SURVEY ON PORCINE TOXOPLASMOSIS FROM SWINE FARMS IN SOUTHERN REGION OF BRAZIL

Silva, R. A. M. S.1, Barato, P.2, Bonassi, C. A.2, Vidal, C. E. S.1, Mores, N.1 & Dalla-Costa, O.A.1, Dubey, J.P.3
1-EMBRAPA/Centro Nacional de Pesquisa de Suinos e Aves, Br 153, km 110, Vila Tamanduá, CEP: 89700-00 Concórdia, SC, Brasil.
2-Universidade do Contestado, Curso de Ciências Biológicas, Bairro Salete, Parque de Exposições, CEP: 89700-00 Concórdia, SC, Brasil.
3-Parasite Biology and Epidemiology Laboratory, Livestock and Poultry Science Institute, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland 20705-2350, USA.

Toxoplama gondii, a protozoan parasite that infect all species of mammals and birds. Felines, the definitive hosts, are the only animals that pass oocysts into environment. Pigs are commonly infected with *T. gondii* and pork is an important potential source of human infection with the organism.

Serum samples from 223 pigs of 65 farms pig from of Paraná (PR), Santa Catarina (SC) and Rio Grande do Sul (RS) states, Brazil were examined for cross reacting antibodies to *T. gondii* by the use of the modified agglutination test (MAT).Anti-*T. gondii* titers of > or =1:200 were found in 12.69% (8/63) of pigs from PR, 10.00% (10/100) of pigs from SC and in 26.66% (16/60) of pigs from R.S. Toxoplamosis in pigs has worldwide distribution and are numerous reports about infection in several countries. In Canada during 1991/1992, market-age pigs were sampled and the seroprevalences ranged from 3.5 to 13.2% (Gajadhar et al, *J Parasitol*. 84:759-63, 1998). Van Knapen et al (Veterinary Quartely, 17:87-91, 1995) in the Netherlands showed very low seroprevalences of *T. gondii* in finishing pigs (1.8%). *T. gondii* antibodies have been demonstrated in 3% of slaughter pigs in Denmark (Nielsen & Wegener, *Rev Sci Tech*, 16:513-24, 1997). In Brazil are numerous reports on surveys on porcine toxoplasmosis. A review study by Dubey (*Vet. Parasitol*., 19: 181-223,1986) reported founds in Brazil from 11% to 67%. Our results are an indicative that the situation is the same after 13 years of Dubey´s report. As yet, there are no interventions designed to reduce swine exposure in the region. Freezing to -20 degrees C, cooking to an internal temperature of 67 degrees C, or gamma irradiation (0.5 kGy) can kill tissue cysts in meat.

B-U-74 – SURVIVAL AND INFECTIVITY OF CYSTS *TOXOPLASMA GONDII* (ME-49 STRAIN) IN HOME MADE FRESH CHEESE PRODUCED WITH ARTIFICIALLY INFECTED MILK

Hiramoto, R.M.[1,2], Galisteo Jr., A.J.[1], Mayrbaurl-Borges, M.[1], Cardoso, R.P.A.[1], Macre, M.S.[1] & Andrade Jr., H.F.[1]
1- Lab.Protozoologia, IMTSP & 2- S.Radiobiologia IPEN/CNEN SP
Av.Dr.Enéas C. Aguiar 470 – 05403-000 - São Paulo, SP, BRAZIL. email: hfandrad@usp.br

Toxoplasmosis, caused by the protozoan *Toxoplasma gondii*, is a benign disease, with occasional eye involvement, affecting around 60% of Brazilian adult population. The infection is transmitted through the ingestion of food and water contaminated with oocysts of the feces of felines, definitive hosts, or raw meat containing tissue cysts in meat from warm blood animals, intermediate hosts. Milk dairy products were frequently home made in small farms of rural areas in Brazil, without milk pasteurization, being one of the most important protein sources in these areas. The raw milk was described as source of infection in man, however a study on the survival of the parasite in dairy products was never studied. We verify the survival and infectivity of *T.gondii* cysts in milk products, by artificially infection of pasteurized bovine milk with 10 viable cysts from ME49 strain *T.gondii* infected mice brain. Fresh cheese was produced by artisanal technique, using market reagents available to Brazilian farmers. These cheeses was offered individually to groups of four C57Bl/6j mice, fresh or stored in 4º C for 5, 10 and 20 days. The ingestion of the cheese was monitored, with mice follow-up by 5 weeks. Only one animal died in the group that ingested fresh cheese immediately after the production, in all other groups no mortality was observed. Control mice, orally infected with 10 cysts in milk, presented a 50% mortality in same period. After 5 weeks, mice were killed, with search of cysts of *T.gondii* in the brain, with serological survey of the infection. We found *T.gondii* cysts in brains of all mice from groups that received contaminated fresh cheese or stored during 5 or 10 days at 4º C. These data were confirmed through ELISA, with presence and progressive increase of specific IgG antibodies in these groups. Mice that received cheese stored for 20 days at 4º C neither presented nor brain cysts or seroconversion. Antibody specificity was also confirmed through Western blotting and histology of the brain of all of the infected mice. Our data prove that *T.gondii* survives the process of fresh cheese production, without significant loss in the viability and infectivity, until 10 days of storage in common refrigerator temperatures, demonstrating that milk dairy products could be an important source of human contamination, reinforcing pasteurization of all milk before any processing or ingestion.

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B-U-75 – HYDROXYUREA INDUCE DESTRUCTION OF INTRACELLULAR *TOXOPLASMA GONDII*

Mayerhoffer,RO[1] & Melo,EJT[1]
1. Laboratório de Biologia Celular e Tecidual, CBB, Universidade Estadual do Norte Fluminense; Campos-RJ.CEP:28015-620

Toxoplasma gondii is an intracellular parasite that proliferates within parasitophorous vacuole allowing the lysis of the host cells. During intracellular tachyzoites cycle there is no fusion of host cell lysosomes with parasite-containing vacuoles. We have demonstrated that hydroxyurea, a specific drug that arrest cell cycle in G1/S phase, stop the intracellular *Toxoplasma gondii* in different host cells. However, little know about the mechanism of infected host cells use to eliminate the intracellular parasite. In the present study we decided to analyze the mechanism of infected host cell use to eliminate the intracellular *Toxoplasma gondii* .

Vero cells were cultivated in Linbro tissue plates that contained a sterile coverslip (3-5 10^5/well) and maintained at 37°C overnight. The cultures were infected with tachyzoites of *T. gondii* and incubated for 24 hours. After that time, 4mM of hydroxyurea was added to cultures for 24 and 48 hours. Cultures were washed with PBS, incubated at 37°C for 2h in Lucifer Yellow dye, washed, and again incubated for 20 min with 199 medium. The cells were examined in a Confocal Laser Scan Microscope (CLSM), using a 488 and 543nm argon laser. For ultrastructural analyses the cells were fixed in solution containing 1-% glutaraldehyde, 4% paraformaldehyde, 5mM $CaCl_2$ and 5% sucrose in 0.1M cacodylate buffer, pH 7.2. For detection of acid phosphate activity, the cells were fixed for 30min at 4°C in 1% glutaraldehyde, rinsed and incubated in medium containing 1mM $CeCl_3$, 5% sucrose and 0.05M TRIS-maleate buffer(pH 5.0). The cells were postfixed for 1h in solution containing 1% OsO_4 in 0.1% cacodylate buffer, at room temperature. The cells were rinsed with cacodylate buffer, dehydrated in acetone and embedded in Epon. Ultrathin sections were examined using Zeiss 900 Electron Microscope.

In the infected host cells treated with hydroxyurea, and stained with Lucifer Yellow, the fluorescence indicative of acidic compartments was observed in the cytoplasm and inside of the parasitophorous vacuole staining the parasites. However, when the same cells were excited with wavelength of 543nm, only parasitophorous vacuole was observed. In the infected Vero cells treated with hydroxyurea, indicative reaction of the presence of enzyme hydrolytic inside parasitophorous vacuole was observed. These results suggested that infected host cell treated with hydroxyurea eliminated intracellular parasite by acidification of parasitophorous vacuole.

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B-U-76 – STRUCTURAL CHANGES DURING ENCYSTMENT OF *ACANTHAMOEBA POLYPHAGA*

Ferreira, G.A., Alfieri, S.C.& Milder, R.V.
Depto. de Parasitologia - Instituto de Ciências Biomédicas, USP. Av. Prof. Lineu Prestes 1374, CEP 05508-900, São Paulo, S. P., Brasil

Acanthamoeba are free living amoebae associated with chronic granulomatous encephalitis and keratitis. Encystment occurs naturally in adverse conditions and may be induced experimentally. Morphological events studies during the encystment process have been limited to very few species. Therefore, we are now studying this process in *Acanthamoeba polyphaga* (ATCC 30872) by optical and electron microscopy.

Trophozoites were grown either on coverslips or tissue culture dishes in PYG (proteose peptone, yeast extract, glucose) medium and were used at logarithmic growth phase. At the optical level, trophozoites present many spike-like projections, a well-defined nucleus with a central nucleolus and numerous vacuoles of variable sizes distributed through the whole cell. Ultrastructural study of this stage defined many characteristic morphological features, such as the locomotor pseudopodia and acanthopodia, presence of mitochondria, a well developed Golgi apparatus and a peculiar distribution of the rough endoplasmic reticulum. Digestive vacuoles are always filled with amorphous material and a very developed complex of small vesicles and tiny tubules are associated with the water expulsion vacuole.

To monitor the encystment process, log phase trophozoites were washed and resuspended in Neff's saline (100 mM KCl, 20 mM Trizma base, 8 mM $MgSO_4$, 4 mM $CaCl_2$, and 1 mM $NaHCO_3$; pH 8.5) and samples were taken at 2, 6, 12 and 24 hours. During the beginning of the encystment process large amounts of extruded cell debris can be seen along with cells already harboring a forming exocyst. Subsequently, cysts tend to form huge clumps; cytoplasm condenses, endo and exocyst wall are established and mitochondria display structural changes. Lipid droplets and autolysosomes increase in number and volume simultaneously with decrease of the digestive vacuoles and water expulsion vacuole.

Although general structure is similar to other studied acanthamoeba, some peculiar features are characteristic of *A polyphaga.*

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B-U-77 – *ENTAMOEBA DISPAR*: VIRULENCE CHARACTERIZATION

Fusrt, C. Gomes, M.A., Viana, J.C., Costa, A.O. & Silva, E.F.
Departamento de Parasitologia, ICB-UFMG. Av. Antônio Carlos, 6627, Belo Horizonte, MG – Brasil

One of the largest accomplishments in the field of *Entamoeba* research was the confirmation of *Entamoeba dispar* as species nonpathogenic, made responsible by the asymptomatics cases, previously attributed for the *Entamoeba histolytica*. As the two species are similar morphologically, some tools have been used to distinguish them, as isoenzyme patterns, inoculations in hamster liver and mouse caecum, phagocytosis of human erythrocytes, among others. In our studies, we isolated a strain of *E. dispar*, together with its bacterial flora, clonned and characterized through isoenzyme pattern, experimental inoculations and molecular biology techniques. The strain was isolated of an asymptomatic patient, with a nonreactive serum for *E. histolytica*. After obtaining four clones of the isolated we determined the isoenzyme patterns, being attributed to the isolated one and its clones the same pattern, type I. Was also observed different behaviours when the strains were inoculated in hamster liver and mouse caecum. In the inoculations in hamster liver, were inoculated 50.000 and 1.000.000 trophozoites. There was variation in the lesion production among the strains and the inoculations. The clones Wil1 ClA3, Wil1 ClC3, Wil1 ClD3, didn't produce lesions with the inoculation of 50.000 trophozoites, and the clone Wil1 ClB3 was capable to produce lesions, although with bacterial aspect. With the inoculation of 1×10^6 trophozoites, all the trophozoites presented lesions, varying the lesion degree. When we observed histologically the lesion of the strain Wil1 ClB3, we didn't observe invasion of the tissue by amebae. In the inoculations in mouse caecum, 500.000 trofozoítos were used and the results agreed with the inoculations in hamsters. The strains Wil1 ClC3 and Wil1 ClD3 were not capable to provoke any lesion. The strain Wil1 ClB3 agreeing with the results of the inoculation in hamster was capable to provoke lesions that varied from the degree I to IV. The strain Wil1 ClC3 provoked lesion in two animals, both of degree II. As demonstrated by the inoculations, the clones seem to have different virulence degrees, and the result agrees with both used tools (inoculations in hamster liver and in mouse caecum). We can still conclude that the bacterial flora possesses great importance in the determination of the virulence of the strain, since the histological exam didn't demonstrate presence of amebae in the liver.

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B-U-78 – AXENIZATION OF *ENTAMOEBA HISTOLYTICA* STRAINS

Silva, E.F., Viana, J.C., Pires, E.M. Ludgero, M.L. & Gomes, M.A.
Departamento de Parasitologia, ICB-UFMG, Av. Antônio Carlos, 6627, Belo Horizonte, MG, Brasil.

The different clinical forms observed in amebiasis are related to intrinsic factors of the host, to the medium where the amebae live and to the *E. histolytica* itself. The study of these factors can enlarge the knowledge about the disease pathogenicity. A lot was cleared about the virulence of the *E. histolytica* from the establishment by Diamond in 1968 of a medium for axenic growth of the amebae. However, *E. histolytica* adaptation to axenic cultivations was shown problematic and few axenic strains exist in the world.

In this work, we presented a new approach for obtaining axenic strains of this parasite. Positive feces for cysts were sterilized through treatment with the following solutions: HCl 2% in 4 °C for 1 week to 2 months, $HgCl_2$ 0,002% for 45 minutes, $KMnO_4$ 0,02% for 45 minutes and acriflavin 0,002% for 24 hours in 4 °C.

After this treatment, the sterile cysts were inoculated in medium for axenic growth of the cells containing *Crithidia fasciculata* in 37 °C. After 72 to 168 hours of the planting trophozoites began to be liberated from the cysts, multiplied and established the culture. After some weeks of association, *C. fasciculata* was eliminated gradually, being established the axenic culture. Six strains were axenizated with this methodology, being 2 isolated from symptomatic patients and four from asymptomatic ones. This method allowed the obtainment of trophozoites that didn't suffer adaptation pressures, offered mainly by the antibiotics, and for the passage of the polixenic medium for the axenic one.

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B-U-79 – TUBULIN HETEROGENEITY IN THE LOWER EUKARYOTE *TRITRICHOMONAS FOETUS*

Coutinho, L[+]; Ribeiro, K*; Benchimol, M[+]
* Dept. Anatomia da Universidade Federal do Rio de Janeiro, [+] Universidade Santa Úrsula, Rua Jornalista Orlando Dantas, 59

Microtubules are cylindrical, stiff polymers found in all eukaryotes. They are involved in a great variety of cellular processes including mitosis, flagella motility, and intracellular transport of vesicles and organelles. They may also play purely cytoskeletal roles determining the morphology of certain cells. The structural subunit of MT is the 100kDa protein tubulin. The tubulin molecule has long been known to form a heterodimer of two polypeptide chains designated a and b. Lately, another isoform, the g tubulin, was discovered on microtubule organizing centers. Because of the numerous functions in which MT are engaged, it has always been tempting to hypothesize that different functions may require the existence of multiple species of tubulins produced by post-translational modifications.

In the present work, tubulin heterogeneity was explored along the cell cycle of the primitive eukaryote *T. foetus* by immunolocalization at the electron and light microscopy level. Confocal laser microscopy was applied to yield fluorescence images of cells in interphase and during mitosis. On the other hand, immunocytochemistry on Unycril thin sections or on cryosections, in addition to the sandwich technique were employed to reveal the fine localization and detail of the labeling. We report the presence of a and b tubulin showing different staining patterns. Previous works by Viscogliosi and Brugerole (1986) described the absence of microtubule tirosination in trichomonads and demonstrated that the mitotic spindle was stained by antibodies against a-tubulins. Concerning post-translational modifications we could observe acetylation and tyrosination of flagella and axostyles. It is important to point out that acetylation was already reported previously but tyrosination is a novel information, which is controversial in literature and sheds light on evolutionary considerations.

The evolution of tubulin isotypes is intimately related with the evolution of the eukaryote cell. We report that the organization of this lower eukaryote's cytoskeleton differs from typical higher eukaryote cells. Exploration of isotype differences in this cell model may be a fruitful line of work for the study of the eukaryotic cytoskeleton evolution.

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B-U-80 – POLYAMINE ANALOGUES ARREST PROLIFERATION AND INDUCE AUTOPHAGY IN TRICHOMONADS

Santoro G.F.[1], Yarlett N.[2], Vannier-Santos M.A.[1]
[1] Laboratório de Biologia Celular Parasitária, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro; [2] Haskins Laboratories and Department of Physical Sciences, Pace University, New York. * corresponding author: vannier@biof.ufrj.br

Polyamines such as putrescine, spermine and spermidine are ubiquitous, low molecular weight, organic polycations that play important roles in proliferation and differentiation in most cell types. The trichomonad parasites *Trichomonas vaginalis* and *Tritrichomonas foetus* are, respectivelly, the causative agents of human and bovine trichomoniases. The former infects annually up to 250 million people worldwide whereas the latter is the cause of considerable economic losses in agriculture since it may lead to abortion in cattle. These parasites produce huge amounts of putrescine and uptake spermine from the hosts. Impairment of protozoa polyamine biosynthsesis and/or transport may comprise valuable targets for chemotherapy as reported for African sleeping sickness (Bacchi and Yarlett, 1995).

We have recently observed that 1,4-diamino-2-butanone (DAB), a putrescine analogue, inhibits the *in vitro* proliferation of *T. foetus* and leads to hydrogenosomal destruction (Reis *et al*., 1999). Here we extended these data to *T. vaginalis* and also employed the spermine analogue N1,N11-diethylnorspermine (DenSPM). Both polyamine analogues induced ultrastrutural alterations of the hydrogenosomes and large glycogen deposits in the cytoplasm, particularly surrounding these organelles. Many drug-treated cells presented endoplasmic reticulum cisternae enveloping portions of the cytoplasm or forming myelin-like figures, presumably as a result of autophagy induction. Micromolar concentrations of DENSPM remarkably inhibited parasite axenic growth of these trichomonads.

These data suggest that putrescine production and spermine internalization take part in the trichomonad parasite energetic metabolism and proliferation. Thus polyamine analogues may comprise useful tools for the rational drug design in the therapy of trichomonal infections.

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B-U-81 – A FURTHER CHARACTERIZATION OF CA^{2+} HOMEOSTASIS IN *TRITRICHOMONAS FOETUS*

Almeida, J.C.A.[1], Okorokov, L.A.[2] & De Souza, W.[1]
1-Laboratório de Ultraestrutura Celular Hertha Meyer, Instituto de Biofísica Carlos Chagas Filho, CCS,UFRJ, Cid. Universitária,21949-900 Rio de Janeiro, RJ; 2- Laboratório de Fisiologia e Bioquímica de Microorganismos, CBB, UENF, Av. Alberto Lamego 2000, 28015-620 Campos dos Goytacazes, RJ.

T. foetus is a microaerophilic protozoan from the urogenital tract of cattle that causes abortion and sterility. *T. foetus* is located in the bottom of the evolutionary tree of eukaryotes and presents such typical structures as the hydrogenosomes, ATP synthesizing organelles that produce molecular hydrogen. Recently it has been proposed (Martin, W. and Müller, M. 1998, Nature 392,37-41) that eukaryotes have arisen through symbiotic association of a hydrogen-dependent archaebacterium with an eubacterion that generated hydrogen as end product of his metabolism. From the evolutionary point of view *T. foetus* is therefore a microorganism of special and high interest. In this work we concentrated our attention on the analysis of Ca^{2+} homeostasis and determination of activities of different types of ATPases in various organelles of *T.foetus.*To this end a preparation of crude membranes isolated from the 24 h cells were submitted to subcellular fractionation in a sucrose density gradient. The resulting membrane fractions were analyzed for their capacity to take up $^{45}Ca^{2+}$under different conditions (presence or absence of ATP and different inhibitors) by a standard Millipore filtration technique. The activity of marker enzymes for the Golgi (GDPase, Kex2) and hydrogenosomes (Malate dehydrogenase) and ATPase activity sensitive to vanadate, nitrate or azide was also evaluated. Crude membranes show vanadate sensitive Ca^{2+} uptake which was resistant to protonofore FCCP. The main contribution in the Ca^{2+} uptake by all membranes is made by the Golgi or Golgi-like membranes. Indeed a huge peak of Ca^{2+} transport activity is definitely separated from the peak of hydrogenosomes, but comigrates with a peak of GDPase activity. An enrichment of membranes comigrating with MDH or with GDPase by hydrogenosomes or by the Golgi, correspondingly, is also demonstrated by routine transmission electron microscopy. An activity of the vacuolar type ATPase is found in several membrane fractions, however, activity of Ca^{2+}/H^{+} antiporter is absent. Data are in an agreement with a conclusion that Ca^{2+} homeostasis in this ancient microorganism is due to Ca^{2+}-ATPase(s), mainly due to enzyme of the Golgi.

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B-U-82 – ULTRASTRUCTURAL LOCALIZATION OF CP30 IN *TRICHOMONAS VAGINALIS*

Mendes, O.C.[1], Arroyo, R.[2] & Benchimol, M.[1,3].
[1]Laboratório de Ultraestrutura Celular-Universidade Santa Úrsula- Rio de Janeiro-Brazil. [2] CINVESTAV del IPN-Departamento de Patologia Experimental- Mexico.
[3] Instituto de Biofísica Carlos Chagas Filho-UFRJ, Rio de Janeiro, Brazil

Trichomonas vaginalis is the causative agent of human trichomoniasis, one of the most common parasitic infections in humans. They are flagellate protist which interact with the squamous epithelium lining the human urogenital cavities. Despite the high incidence, especially among women of all world societies, little is known about the pathogenesis of trichomoniasis and many questions remain open. The parasite does not penetrate the epithelium and is therefore considered as non-invasive. It adheres to the epithelial cells often forming interdigitagions (Rasmussen et al. 1986; Arroyo et al. 1993; Gonzalez-Robles et al. 1995), using at least four adhesins (Arroyo et al. 1992). Since trichomonad adherence to vaginal epithelial cells is a multifactorial process, also cysteine proteinases are needed (Arroyo and Alderete, 1989). About 23 cysteine proteinases (CPs) and two metallo-proteinases activities were detected in *T.vaginalis*. Two CPs termed CP30 and CP65 with affinity to the surface of host cells have been related to cytoadherence and cytotoxicity of the parasite, respectively. They seem to be preferentially located at the parasite surface (Arroyo and Alderete, 1995). Recently, we have demonstrated that the CP30 is involved in trichomonal cytoadherence and it is secreted *in vitro* and *in vivo* (manuscript under review). Therefore, it is important to localize precisely the CP30 in the cell. We have performed indirect immunofluorescence using antibodies against the CP30 and *T. vaginalis* fixed and permeabilized with 70% ethanol at -20 ^{o} C. Cryo-sections and Unicryl embedding cells were also used for labeling with gold-labeled antibodies for observation at EM level. We observed strong reaction on the cell surface of *T. vaginalis* with a patchy distribution. The flagella presented reaction, as well as intracellular vesicles, probably showing the pathway of the synthesis of this protein. Our next step is to determine the presence of this protein on the secretory pathway.

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B-U-83 – THE EFFECTS OF ANTI-HELMINTIC DRUGS AGAINST *GIARDIA LAMBLIA* TROPHOZOITES: A TRANSMISSION AND VIDEO-LIGHT MICROSCOPY STUDY

Campanati, L., Gadelha, R.A.P.& Monteiro-Leal, L.H.
Laboratory of Microscopy and Image Processing, Department of Histology and Embryology, State Universtity of Rio de Janeiro. Av. Prof. Manoel de Abreu, 48 - 3° andar. Maracanã - Rio de Janeiro CEP 20550-170.

Many drugs currently used in the treatment of giardiasis were first described to treat infections caused by intestinal helminthes. Most of these are known to cause severe undesirable side effects to the patient and because of their reversible nature, they must be administrated for several days. The benzimidazole are potent inhibitors of tubulin and their derivatives (benzimidazole carbamates) albendazole and mebendazole, have effective action against *Giardia lamblia* trophozoites. Metronidazole, a drug of the 5'-nitroimidazoles family, besides giardiasis, is used in the treatment of infections caused by other anaerobic protozoa such as *Tritrichomonas vaginalis*. In this work we studied the effects of thiabendazole, furazolidone, albendazole, mebendazole and benzimidazole by Transmission Electron Microscopy (TEM) and Video-Light Microscopy (VLM) in trophozoites of *Giardia*. All the drugs, except metronidazole that was dissolved in distilled water, were dissolved in DMSO. The growth curves showed different profiles to each drug, but all of them have significant action against *Giardia lamblia* growth. The effects observed by VLM include changes in the lateral flange, flagella beating pattern and frequency, adhesion pattern and general shape. By transmission electron microscopy, we observed modifications in the peripheral vesicles, cytoplasm and adhesive disk. In many works of drugs effects, the drugs were dissolved in dimethylformamide (DMF). We also tested the effects of these drugs dissolved in DMF, but the control cells presented strong modification in their shape, cytoplasm and others organelles. Supported by: CNPq, SR-2 UERJ and PRONEX

B-U-84 – ULTRASTRUCTURAL AND VIDEO-LIGHT MICROSCOPY STUDY OF THE EFFECTS OF PYRANTEL PAMOATE IN *GIARDIA LAMBLIA* TROPHOZOITES

Campanati, L., Gadelha, R.A.P. & Monteiro-Leal, L.H.
Laboratory of Microscopy and Image Processing, Department of Histology and Embryology, State Universtity of Rio de Janeiro. Av. Prof. Manoel de Abreu, 48 - 3° andar. Maracanã - Rio de Janeiro CEP 20550-170. E-mail: mleal@uerj.br

Giardia lamblia is a flagellate protozoan that causes giardiasis colonizing the small intestine of vertebrates. This intestinal disease is spread world wide affecting mainly developing countries and causing undernutrition and the failure to thrive syndrome in children. Many drugs used in the treatment of *Giardia lamblia* infection were first described to treat helminthic intestinal infections. The benzimidazole carbamates, for example, because of its anti-tubulin action, are one of the currently choices for treating giardiasis. Other drugs are the 5´-nitroimidazoles, such as metronidazole. Most of the currently used drugs causes strong and undesirable side effects to the patient. Few works were done concerning the use of pyrantel pamoate in the treatment of *Giardia*, and they report efficient results in the use of this drug in combination with other drugs. By means of transmission electron microscopy (TEM) and video-light microscopy (VLM), we described the effects of this drug in *Giardia lamblia* trophozoites. Pyrantel pamoate was dissolved in DMSO and diluted in the culture medium to for different concentrations. The cells general aspect was changed and modifications in the cytoplasm, lateral flange and peripheral vesicles were observed. The cytoplasm presented extracted and with big membranous structures, the lateral flange was also affected, appearing completely destroyed. The flagella structure was not changed, although its beating frequency was slowed. No modifications were observed in the adhesive disk by VLM, but when seen by TEM, some cells presented this organelle broken. The peripheral vesicles changed in shape and size and were not homogeneously distributed in the dorsal side of the cells.

Supported by: CNPq, SR-2 UERJ and PRONEX

B-U-85 – ULTRASTRUCTURAL STUDY OF *SPIRONUCLEUS BARKHANUS*

Lima Prates, N[1]., Horner, D[2]. Embley, M.[2] & Benchimol, M.[1,3]
[1,3]Laboratório de Ultraestrutura Celular- Universidade Santa Úrsula, Rio de Janeiro, RJ.
[1]Laboratório de Ultraestrutura Celular Hertha Meyer, Rio de janeiro, RJ
[2] Dept. Zoology, Natural History Museum, London, UK

The protist *Spironucleus barkhanus* was studied by scanning and transmission electron microscopy. These flagellates were obtained from infected Atlantic salmon, and had been isolated from muscles abscesses of diseased fish (Sterud *et al*., 1997). Cell culture was supplied by the ATCC, no 50377 and stored in liquid nitrogen with DMSO. Prior to ultrastrucutral studies, the cells were thawed and cultured in TYI-S- 33 medium at 5 - 12 º C. Cells were fixed at room temperature in 2.5% (v/v) glutaraldehyde in 0.1 M Na-cacodylate buffer (pH 7.2), and post-fixed in 1% OsO_4 in cacodylate buffer plus 5 mM $CaCl_2$ and 0.8% potassium ferricyanide (Hepler, 1980). Cells were washed, dehydrated in acetone and embedded in Epon. Thin sections were stained and observed in a Zeiss 900 electron microscope. For Scanning Electron Microscopy (SEM) the cells were collected by centrifugation at 1,500 *g* for 5 minutes and fixed in 2,5% glutaraldehyde in 0.1-M cacodylate buffer, pH 7.2, for 2 h at room temperature. After fixation, they were washed, adhered to poly-L-lysine-coated glass coverslips, post-fixed in 1% OsO_4, dehydrated in ethanol, critical point dried with CO_2, coated with gold, and observed in a Jeol 5800 electron microscope. We observed trophozoites presenting six flagella, at anterior-lateral region and two recurrent flagella as seen by SEM. In our study we found trophozoites ovoid in shape. Mean length was 6.8 mm (range: 5.4 – 9.4 μm), and mean width was 4.35 μm (range: 3.5 – 6 μm). Glycogen particles and rosettes are seen throughout the cytoplasm, as well several vesicles located mainly close the cell surface. Some vesicles are also seen below the nuclei. Several layers of rough endoplasmic reticulum are present following the recurrent flagella. No other membrane-bounded organelles were observed in this cell.

Supported by: FAPERJ, CNPq, PRONEX.

B-U-86 – THE ACTIVATED SLUDGE PROCESS, CONSISTS IN THE INDUCTION OF THE DEVELOPMENT OF A FLOKULATED MICROBIOLOGICAL CULTURE IN A ARENTED TANK FEED BY THE TO BE TREATED

Silva, S. B. A. da, Silva, T. C. R. da & Silva-Neto, I. D. da
Laboratório de Protistologia, Departamento de Zoologia, CCS, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Ilha do Fundão, Rio de Janeiro, CEP 21941-590.

The ciliates are important protozoans in the process of biological treatment of sewer and their presence in a great number of sewer processing plants is related to a production of a clear effluent of good quality due to their ability of feeding of bacterial and particles in suspension.

The primary objective of this study, is the morphological identification of the ciliatofauna present in the experimental station that is feed by the primary sewerage from the station of treatment of sewerage of CEDAE, localized at Penha neighborhood, Rio de Janeiro, correlated to the following parameters (CDO, total particles in suspension, volatile solids in suspension of the sludge, effluent, pH of affluent and effluent, oxygen dissolved in the sludge and temperature of the sludge).

Collections were realized during one year and the samples were analyzed at the laboratory. After the trial using micropipettes, under the stereoscopic microscope, some ciliates were used to *in vivo* observations, other were cultivated using mineral water and grains of wheat or rice.

The ciliates were prosecuted for silver impregnation (Protargol) as described by Dieckmann, 1995; Europ. J. Protistol. 31, 342-382 and dry silver as described by Klein, 1958; J. Protozool., 5 (2); 99-103. The silver protargol technique allows the observation of the somatic and bucal infraciliature and internal structures such as macro and micronuclei. The dry silver method makes the citoskeleton formed by the cortical plates evident. Using these techniques, species from the groups: Heterotrichida, Peritrichia, Hymenostomata Hypotrichia, Nassulida, Cyrtophorida e Suctoria. were identified. The species *Euplotes aediculatus, Gastronauta membranaceus, Glaucoma cintillis* will be shown with morphological details.

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B-U-87 – STUDY MORPHOLOGICAL, IN OPTICAL MICROSCOPY AND SCANING ELETRON MICROSCOPY AND BIOMETRY OF PROTOZOA *HOLOSTICHA SP.* (HYPOTRICHIDA: CILIOPHORA) FROM LAGOA RODRIGO DE FREITAS, RIO DE JANEIRO

Moreira, M.G.G. & Silva-Neto, I. D. da
Laboratório de Protistologia, Dep. de Zoologia, Instituto de Biologia, CCS, Universidade Federal do Rio de Janeiro, Ilha do Fundão, Rio de Janeiro, CEP. 21.941-590.

The Lagoa Rodrigo de Freitas is a tropical lagoon and located on the coast, between the longitudes 043º11'09'' / 043º13'03''W and the latitudes 22º57'02'' / 22º58'09''S on an urbanized area in the city of Rio de Janeiro. It is connected to the atlantic ocean through of the narrow stream and presents a low salinity, close to 5.4, and that presents an important biodiversity of protozoa ciliates.

The ciliate *Holosticha sp* collected in two sites of Lagoa Rodrigo de Freitas was cultivated in laboratory with several types of food and sterile water. It developed in cultures with rice and wheat grains, fragments of coconut and diatoms. It was copared with other species of the genus showing differences.

The ciliate was morphologically investigated through observation *in vivo*, silver protargol impregnation according to Dieckmann, J.,1995; Europ. J. Protistol. 31, 372-382 and through scaning eletron microscopy. The morphological characteristics are: a size of 205µm of length and 50mm of width *in vivo*, presence of colorless cortical granules, contractile vacuole on the left of the body, 15-30 macronuclei fragments, 60 membranelles in Adoral Zone, 2 paraoral kineties, 6 dorsal kineties, 3-5 frontal cirri, 2 frontoterminal cirri, 1 buccal cirri, 8-11 transverse cirri, 47-52 left marginal cirri and 45-50 right marginal cirri. The morphological characteristics are shown through schematic representation, optical photography, eletronics photomicrography and a biometrical table.

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B-U-88 – BIODIVERSITY OF SOIL CILIATES IN THE ATLANTIC FOREST, RIO DE JANEIRO

Custódio, F.L., Flinte, V., Matos, A.B.A. & Silva-Neto, I.D. da
Laboratório de Protistologia, Departamento de Zoologia, Instituto de Biologia (C.C.S.), Universidade Federal do Rio de Janeiro, Ilha do Fundão, CEP. 21941-590, Rio de Janeiro - Brasil.

The soil of the tropical forest has a wide diversity of microorganism of the most diversified taxonomic groups.

One of ours objectives is to assess soil ciliates biodiversity in Atlantic Forest. The ciliates have a very important rule in the maintenance of trofic chains of soil organisms, particularly in population control, since ciliates are amongst the most important micropredators, which includes bacteria, fungi, seaweeds, protozoans and even small metazoans such as nematodes and rotifers. Because these microorganisms are responsible for the return of nutrients to the macrobiodiversity, their importance is clearly established.

We propose to show how rich is the biodiversity of ciliates on the Atlantic Forest in Rio de Janeiro, not only in known species, but also in unclassified ones thus contributing to the knowledge of the group, with the aid of schematic drawings, photomicrographs and micrographys electron. We have found so far many species belonging to the groups Heterotrichia, Hypotrichia, Peritrichia, Colpodea, Gymnostomatida and Himenostomata. These groups are incredbly diversified and broad.

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B-U-89 – CILIATES IN THE CHAMBERS OF THE STOMACH OF BOVINES

Salvio, G.M.M. & D'AGOSTO, M.
Laboratório de Protozoologia, Pós Graduação em Ciências Biológicas, Instituto de Ciências Biológicas, Campus Universitário, UFJF, Juiz de Fora, MG.

The ruminants are characterized by intense presence of microorganisms in the stomach, responsible they are for the majority of nutrients digestion. The protozoas were the first identified microorganisms in rumen, but, their importance to the animals is not well cleared yet. The present work had as objective collecting and quantifying the genus of ciliates that occur in the rumen, reticulum, omasum and abomasum in bovines abated in Além Paraíba MG. There have been collected 45 ml of rumen, reticulum, omasum and abomasum content from 30 animals. At the moment of collecting, temperature and pH were measured and later samples were analyzed in laboratory. The results showed that the rumen and reticulum ambients are rather stable with an average temperature between 36 and 37º C and pH 6.5. The prevailing genus most found in the rumen were *Entodinium* (64.99%), *Isotricha* (6,95%), *Diplodinium* (5.74%), *Ostracodinium* (5.14%), *Eremoplastron* (4,53%), *Dasytricha* (3.84%) and some other seven genus which make the total of 8.72% remaining. In the reticulum the results were *Entodinium* (35.24%), *Isotricha* (23.41%), *Dasytricha* (15.35%) *Eodinium* (7.2%), *Eremoplastron* (5.03%), *Diplodinium* (4.33%), *Ostracodinium* (3.77%) and some other five genus making the total of 5.62% remaining. In the omasum there were found the same genus, even so in smaller amount that in the rumen and reticulum. In abomasum, there weren't found ciliates.

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