POSTERS

Biologia Celular – Cell Biology

BC01 - PROTEOMIC CHARACTERIZATION OF SOLUBLE SECRETED PROTEINS OF *Leishmania (Viannia) braziliensis*

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Introduction and objectives: *Leishmania (Viannia) braziliensis*, a protozoan parasite widespread in the New World, is considered the major etiological agent of American Cutaneous Leishmaniasis. Soluble proteins secreted by this intracellular parasite play important roles during interaction with host cells. In this study, a proteomic approach was carried out to identify soluble proteins secreted by *L. (V.) braziliensis*. Results and Conclusions: Using two-dimensional electrophoresis and MALDI-TOF-TOF mass spectrometry, thirty five proteins were identified in the conditioned medium collected from cultures of promastigotes of *L. (V.) braziliensis*. Several identified proteins are involved in immunosuppression, intracellular survival and signal transduction. Cytochemical and immunoflourescense analysis of secretion pathways of this parasite using confocal microscopy and transmission electron microscopy revealed the existence of proteins present in vesicular structures of exocytic pathways, indicating a vesicle-based secretion system in this parasite.

This work was supported by CNPq-FIOCRUZ.

BC02 - Transcriptomic signatures of alterations in a myoblast cell line infected with four strains of *Trypanosoma cruzi*

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We analyzed the transcriptomic changes in the myoblastic cell line L_6E_9 72 hour after infection with four strains of the parasite *Trypanosoma cruzi*: Brazil (*T. cruzi* type I), Y, CL and Tulahuen (TC II strains) compared to uninfected control cells. Expression of 6289 fully annotated unigenes was adequately quantified with 27k oligonucleotide arrays in each of the 20 RNA samples, including four separate cultures of parental and strains infection. Tulahuen strain was the most disruptive to the host transcriptome, with 17% significantly regulated genes, while in Y strain only 6% of the genes were regulated. Although the significantly regulated genes in the infected cells were largely different among the four strains, the expression ratios with respect to the parental cells were significantly proportional to each other. These results indicate that *T. cruzi* elicit similar pathways responses but with strain-dependent amplitude in the host cells during initial times after *in vivo* infection.

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BC03 - MORPHOLOGY AND MOLECULAR PHYLOGENY OF *Hemicycliostyla sphagni* STOKES, 1886 (CILIOPHORA: STICHOTRICHIA) FROM THE NORTHERN BRAZIL

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Hemicycliostyla sphagni, type of *Hemicycliostyla*, is a poorly known urostyloid ciliate recorded from freshwater habitats, and of which systematic position is doubtful, therefore in need of reinvestigation. We reassessed the morphology of this species based on a strain collected from mosses growing at an artificial freshwater stream in the campus of UFPA (Belém - PA). For the first time, divisional morphogenesis and 18S-rDNA data were provided for this organism, thus a redefinition of *Hemicycliostyla* is proposed and its phylogenetic affinities hypothesized from molecular analyses. Cells were studied *in vivo* and from protargol slides for morphologic and morphogenetic characterization. A 782bp gene fragment was

obtained and aligned in ClustalX along with 26 sequences from the literature. Parsimony and neighbor-joining analyses were conduced in PAUP*. Midpoint rooting method was adopted and nodal support assessed through 1000 *bootstrap* pseudo-replicates. *Hemicycliostyla* was redefined as: Retroextendia with flexible body; bicoronal frontal cirral pattern; multiple left and right marginal cirral rows. Presence/absence of transverse cirri variable; frontoterminal cirri present; caudal cirri absent. During division, the proximal adoral membranelles of the proter reorganize at the level of the cytostome. Cirri I/1 are produced by undulating membranes primordia; buccal cirri and rear corona degenerate and join the proter's ventral primordia. Parental midventral ciliature participates in the formation of frontoventral primordia of both dividers, partially crossing midventral complex level. Marginal rows and dorsal kineties replicate through within-row development, forming each two primordia (one for each divider). The phylogenetic analyses outputted trees of equal topologies, where *H. sphagni* is adelphotaxon of *Pseudourostyla franzi* (96% of similarity; *bootstrap* = 100%). Additionally, both species were found to share great morphologic similarity, corroborating their proximity. However, morphogenesis investigation in *P. franzi* is necessary to elucidate important taxonomic features used for discerning genera (e.g. replication manner of marginal rows) within urostyloids. Financial support: CAPES/CNPq/FAPERJ.

BC04 - REDISCOVERY OF THE RARE MARINE PLANKTONIC CILIATE *Hemicycliostyla marina* KAHL, 1932 (CILIOPHORA: STICHOTRICHIA)

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Hemicycliostyla Stokes, 1886, is a little known group of urostyloid stichotrichs composed of four valid species, all in urgent need of reassessment in order to improve this genus systematics. So far, the only record of *H. marina* was its original description by the remarkable protistologist Alfred Kahl, who obtained it from plankton samples of the Atlantic Ocean, collected between Iceland and Greenland in 1932. Recently, we found a population of a stichotrich ciliate that fitted the diagnosis of this species. The cells were collected from the sea coast of São Sebastião (SP, Brazil) during nocturnal plankton samplings, studied *in vivo* under DIC, after protargol-impregnation and SEM. *Hemicycliostyla marina* was redefined as: stichotrich cells measuring about 140x75µm *in vivo*; only slight flexible and contractile; fusiform outline; spiraled and slight dorsoventrally flattened; clear rusty colored at low magnification, with cytoplasm showing numerous optically empty vacuoles and lipid droplets; orange cortical granules present. Adoral zone of membranelles occupying about half cell length; large and deep oral cavity, with paroral membrane crescent-shaped and endoral almost straight, intersecting optically at their proximal region. Frontoventral ciliature organized in a frontal multicorona; two right marginal cirral rows; about 11 transverse and one longitudinal marginal rows; two long and one short ventral row; a triangular postperistomial field of about 14 transverse thigmotactic cirri rows; distinct transverse and frontoterminal cirri absent. Dorsal ciliature composed of three kineties. Macronucleus subdivided in approximately 70 nodules. This organism's cirral pattern is likely unique amongst known stichotrichs and sufficiently discrepant from congeners to suggest the erection of a new genus. The presence of a thigmotactic field has been so far described in the urostyloid *Thigmokeronopsis*. However, *H. marina* is herein considered *incertae sedis* in Stichotrichia until further investigation on morphogenesis can sustain reliable homologies to properly hypothesize its affinities. Financial support: CAPES/CNPq/FAPERJ.

BC05 - THE PERITRICHOUS CILIATE *Rhabdostyla* (CILIOPHORA, PERITRICHIA) ATTACHED TO THE LIMNIC OLIGOCHAETE *Limnodrilus hoffmeisteri* (OLIGOCHAETA, TUBIFICIDAE) IN AN URBAN STREAM

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Peritrich ciliated protozoa live as epibionts of a variety of species of aquatic metazoans, including various groups of invertebrates and vertebrates and play an important ecological role in freshwater ecosystems. The objective of the present study was to analyze the site preference and spatial and temporal occurrence of the *Rhabdostyla* sp. colonizing *Limnodrilus hoffmeisteri* in an urban stream in southeastern Brazil. Eleven collections were carried out at intervals of 30 days, at five sampling stations along the São Pedro stream (Rio

Paraibuna watershed), located in Juiz de Fora, Minas Gerais, Brazil. Sampling stations 1 and 2 are located in a rural area and receive a low sewage load, and 3, 4 and 5 are located in a heavily populated urban area and receive high sewage loads. Of the 2500 oligochaetes analyzed 132 (5.28%) were colonized by *Rhabdostyla* sp. Three patterns of site preference of the ciliates on the 132 oligochaetes were observed: concentrated in the posterior region (40.15%, n=53), dispersed in the posterior region (53.03%, n=70) and dispersed along the length of the host body (6.82%, n=9). The total abundance of *L. hoffmeisteri* over the 11 months of the study varied among the sampling stations, being greater at station 5 (3547.72 ± 3790.21), 4 (576.09 ± 618.15), 2 (437.00 ± 312.35) and 3 (322.36 ± 190.49). The prevalences of infestation also varied among the sampling stations, with higher values obtained at station 4 (4.68 ± 4.52) than at stations 5 (2.03 ± 1.88), 3 (0.24 ± 0.49) and 2 (0.08 ± 0.29). As much location of *Rhabdostyla* sp. predominantly on the posterior region of *L. hoffmeisteri as more* concentration of ciliates on urban area may be related to ecological needs of the ciliates: food availability, an oxygenated locale with less friction and host abundance. This study was supported by FAPEMIG.

BC06 - SPATIAL AND TEMPORAL PATTERNS IN THE OCCURRENCE OF *Rhabdostyla chironomi* KAHL, 1935 (CILIOPHORA, PERITRICHIA) ATTACHED TO CHIRONOMID LARVAE (DÍPTERA, CHIRONOMIDAE) IN A URBAN STREAM

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Epibiosis is a facultative association of two organisms: the epibiont, which colonizes the surface of live substrates, and the basibiont, which hosts the epibionts. A few studies related patterns of spatial and temporal distribution of epibiont ciliates, most of tem are morphological and taxonomical studies. The aim of this study was to analyze the spatial and temporal occurrence of prevalence, abundance and mean intensity of *Rhabdostyla chironomi* attached to chironomid larvae of genus *Chironomus decorus* group and investigate the factors that could be responsible for this variation. Were analyzed the distribution of the epibiotic relationship between *R. chironomi* and chironomid larvae during 12 collets on intervals of approximately 30 days from May 2005, to April 2006, at five sampling stations along the São Pedro stream (Rio Paraibuna watershed), located in the southeastern urban region of Juiz de Fora, Minas Gerais, Brazil. Sampling stations 1 and 2 are located in a rural area and receive a low sewage load. Sampling stations 3, 4, and 5 are located in a heavily populated urban area and receive high sewage loads. The epibiotic relationship were observed only at the sampling stations located at urban area (3, 4 and 5). The ciliates was recorded in 19.15% (3964) of the hosts analyzed (23542), with mean abundance of 0.950 (± 0.740) and mean intensity of 4.533 (± 1.530). The occurrence of epibiont ciliate attached to chironomid larvae showed spatial and temporal heterogeneity. The abundance of hosts, the bacterial density and the rain correlated with prevalence, abundance and intensity of infestation, what suggest that these factors contributed for spatial and temporal heterogeneity of this epibiotic relationship. This work was supported by FAPEMIG.

BC07 - INVENTORY OF TINTINNIDS (CILIOPHORA: CHOREOTRICHIDA) FROM THE COAST OF SÃO SEBASTIÃO – SP, BRAZIL

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Tintinnids are abundant components of marine microbial loop, grazing nano- and picoplankton organisms. Many species of this group exhibit substantial loricae polymorphism and are still little known, demanding studies from modern microscopy techniques. From August of 2006 to May of 2008, a survey of planktonic ciliates from the São Sebastião stream, located in the coast of the State of São Paulo, Brazil, was made in three sampling areas (23° 47' 59.5" S, 45° 23' 2.4"W; 23° 50' 4.2"S, 45° 25' 19.9"W; 23° 51' 3.4"S, 45° 26' 35.7"W) at different dephts, using a 25µm plankton mesh. The tintinnids were investigated *in vivo* using DIC microscopy, and after protargol-impregnation and scanning electron microscopy. A total of 78 species were recorded, distributed amongst the genera *Ascampbeliella, Amphorides, Codonellopsis, Cymatocylis, Dadayiella, Eutintinnus, Epicancella, Favella, Helicostomella, Leprotintinnus, Metacylis, Tintinnidium,*

Tintinnopsis, Ormosella, Salpingella, Steenstrupiella and *Stenosemella* Most of the records include new data on morphology and composition of the loricae, live cells, ciliature pattern, nuclear apparatus, morphogenesis and ecologic aspects. The results confirm the loricae polymorphism, which was largely observed in *Dadayiella* spp., *Favella ehrenbergii*, but also present in other species, like *Tintinnopsis beroidea* and *T. tocantinensis*. Financial support: CNPq/FAPERJ/FAPESP.

BC08 - A QUANTITATIVE ANALISIS OF THE EPIBIOTIC RELATIONSHIP BETWEEN *Rhabdostyla chironomi* KAHL, 1933 ATTACHED TO CHIRONOMID LARVAE (DIPTERA, CHIRONOMIDAE) IN A URBAN LOTIC SISTEM

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The peritrich ciliates of genus Rhabdostyla, were registered as epibionts of aquatic invertebrates such as crustaceans, dipterans and annelids. R. chironomi was described above ventral tubules of dipterans larvae and was registered once after description on ventral tubules of larvae of genus Chironomus in a urban stream. The objective for this work was to evaluate the prevalence, abundance and intensity of infestation of R, chironomi on chironomid larvae. Was done a collect in five sampling stations on Paraibuna River, located in the urban region of Juiz de Fora, Minas Gerais, Brazil. The sampling station 1 was located in the rural area and sampling stations 2,304 and 5 was located in urban area. The epibiotic relationship was observed in larvae collected at sampling stations 2, 4 and 5, however in sampling station 2 there were observed only one larvae infested, in larvae collected at sampling station 3 there were no epibionts and at the sampling station 1 no larvae was found. In the present study, R. chironomi was found at ventral tubules of the larvae and at cephalic region being the first record of R. chironomi outside the ventral tubules of chironomid larvae. The prevalence, abundance and intensity of infestation was respectively of 16%, 4.47 e 0.74 on larvae from sampling station 4 and 66%, 9.01 e 5.98 on larvae from sampling station 5. The chemical-physical parameters like conductivity, oxygen, phosphorus, nitrogen, pH and temperature changed among each sampling station, what indicate a change in the pollution of the river, being bigger at sampling station 4 and 5, where were found the biggest values of prevalence, abundance and intensity of infestation. The protozoan epibionts are a representative group in aquatic ecosystems, therefore, is necessary more studies that approach this ecologic relationship.

BC09 - MORPHOLOGY OF A BRAZILIAN STRAIN OF Trichodina heterodentata (CILIOPHORA: TRICHODINIDAE) INFESTING TADPOLES OF Rhinella pombali (ANURA: BUFONIDAE) WITH NOTES ON GEOGRAPHIC DISTRIBUTION AND SPECIFICITY

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Data on trichodinid ciliates in Brazil are scarce and generally do not use the modern silver-impregnation technique, which is essential to the taxonomy of the group. The present study reports not only on the first occurrence of Trichodina heterodentata in Brazil but also on the first record of this trichodinid infecting tadpoles of the species *Rhinella pombali*. This study also constitutes a second report on *T. heterodentata* from tadpoles. In September 2007, tadpoles were collected from a stream in a small farm in the agricultural area of the city of Juiz de Fora, southeastern Brazil. The ciliates found on the tadpoles' bodies and tails were submitted to techniques such as silver impregnation and scanning electron microscopy. Our biometric data of *T. heterodentata* population infecting *Rhinella pombali* were compared to other five South-African populations of *T. heterodentata* infecting tadpoles of *Xenopus laevis laevis*. The body diameter ranged from 49.8 ± 5.3 (38.9-60.0) µm, whilst denticle number ranged from 19-24.The biometric data and the shape of denticles of the studied population correspond to those of other authors. The most striking difference, however, between the population of the present study and others worldwide refers to the shape of denticles. In comparison with other previously recorded populations elsewhere in the world, the population studied here exhibited a much finer ray. Record of *T. heterodentata* on a variety of fish species and

tadpoles around the world surely confirm its importance in the study of parasitism, especially in the scope of introduced species. The present study shows that *T. heterodentata* can be found in a broader number of hosts and countries worldwide, thus, providing new insights into the discussion on the low host specificity and wide geographical distribution of the species.

BC10 - MORPHOLOGY OF *Tintinnopsis mortensenii* SCHMIDT, 1901 (CILIOPHORA: CHOREOTRICHIDA) FROM THE COAST OF SÃO PAULO, BRAZIL

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Tintinnids are a group of planktonic loricate ciliates which are important elements of the marine microbial loop. The characterization of most species is, however, based solely on the loricae morphology, which may exhibit substantial polymorphism. We contribute to the knowledge of *Tintinnopsis mortensenii* by presenting new data on the lorica, and for the first time, observations on the ciliature and divisional morphogenesis of this organism. Cells were sampled from the São Sebastião stream (SP) using 25µm plankton mesh, studied *in vivo* under DIC, after protargol impregnation and scanning electron microscopy. The ciliates presented campanulate loricae (length ≈ 75µm; oral and aboral diameter ≈ 85µm and 51µm respectively); composed of agglutinated homogeneous platelets with 2-4 conspicuous annuli extending from the middle of collar region to the nuchal constriction. Cells were *ca*. 60µm long, of rough turbinate outline, with peristomial rim slight campanulate, flexible and contractile, with a stalk that extends equivalent of the cell length. On average, cilature composed of 1 buccal and 13 collar membranelles, being 4 elongated into the eccentric buccal cavity, and 20 somatic kineties displayed in heterogeneous groups; usually four macronuclear nodules. Stomatogenesis of the opisthe occurred through the hypoapokinetal process. The oral primordium grows in a pouch at the middle of the cell equator, below the lateral and left kinety fields. Data on congeners that show campanulate loricae (e.g. *T. butschli*, *T. campanula*) is necessary to further improve the systematics of this group. Financial support: CAPES/CNPq/FAPERJ/FAPESP.

BC11 - QUANTITATIVE APPROACH OF THE INFRAPOPULATIONS OF *Trichodina heterodentata* (CILIOPHORA: TRICHODINIDAE) INFESTING TADPOLES OF *Rhinella pombali* (ANURA: BUFONIDAE) FROM JUIZ DE FORA, MINAS GERAIS

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Trichodinids include the ciliophorans with complex denticles in the adhesive disc. They may occur as either parasites or symbionts in a wide range of invertebrate and vertebrate hosts from both the aquatic and terrestrial environments. Researchers in Brazil begin to show an increasing interest in trichodinid ciliates mainly because of the damage they can bring to fish farming. Thus, much of the researches conducted in the country pursue both the prevention and control of trichodinid ciliates. In Brazil, trichodinids are reported to occur both in marine and freshwater mollusks. There is, however, a single record of trichodinid ectoparasites from anurans, in which *Trichodina steini* was found on *Bufo ictericus* tadpoles. In September 2007, eighty tadpoles collected from a stream in the farm in the agricultural area of the city of Juiz de Fora, southeastern Brazil, were analysed to study the infrapopulations of *Trichodina heterodentata* (Peritrichia, Trichodinidae). The ciliates found on the tadpoles bodies and tails were submitted to techniques such as silver impregnation and scanning electron microscopy. This thichodinid was recorded in all hosts analysed, with prevalence 100% and mean intensity 695.14 (75-1425). The infrapopulations of T. *heterodentata* showed the uniform pattern of distribution (0.286 index of discrepancy) and positive correlation between the host's weight and the number of parasites (Spearman test $p<0,005$). The distribuition pattern uniform showed by the trichodinids in the present work can be related with aggregative behavior commonly exhibited by larvae of toads. Tadpoles of the genus *Rhinella* form aggregations under natural conditions. Recent studies demonstrate that the presence of

T. heterodentata in more than 50 species of fish from 16 families and in tadpoles from four species, strongly indicates that this is a truly well adapted species with low host specificity. Low host specificity of these ciliates certainly explains its wide geographical distribution.

BC12 - A NEW SPECIES OF *Deviata* (CILIOPHORA: SPIROTRICHEA) FROM AN ACTIVATED SLUDGE SEWAGE TREATMENT PLANT IN RIO DE JANEIRO CITY, RJ

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The *Deviata* genus includes only four species, *D. abbrevescens*, *D. bacilliformis*, *D. estevesi* and *D. rositae*. The aim of this study was to describe the morphology of *Deviata* sp. nov., found in samples of raw sewage from an activated sludge sewage treatment plant in Rio de Janeiro, Brazil. The specimens were studied *in vivo* under phase contrast and after protargol-impregnation. The organisms were found to have flexible body, generally elongated and slightly elliptical. Size *in vivo* about 104 x 43 µm. Contractile vacuole spheroid and located at mid-body of organisms, adjacent to left margin. Size of impregnated specimens: 107.2 ± 24.2 x 43.9 ± 8.1 (n = 45). Adoral zone of membranelles (AZM) composed of 18-31 membranelles. There were three anterior frontal cirri, with 1-3 cirri behind the rightmost frontal cirrus; one buccal cirrus; four long cirral rows right of the AZM, four rows left of it and two dorsal kineties. The long cirral row 1 ended in the middle-body of the ventral surface. Transverse cirri were absent. The nuclear apparatus was usually composed of four elliptical macronuclear nodules and 2-4 micronuclei. The macronucleus is usually located below of the AZM and close to left margin. Stomatogenesis of the opisthe developed parakinetal to the cirri of row R1. Divisional morphogenesis occurred similar to that of *D. abbrevescens*, including the peculiar development mode of ventral primordia V and VI. Based on these results, we regarded this species as new to science. The main features that distinguished it from the congeners were the number of macronuclear nodules, the frontoventral cirral pattern and the cell dimensions. Hence, this species is supposed to be closer related to *D. bacilliformis* and *D. abbrevescens* than to the other two known species. This study also consists of the second record of *Deviata* in Brazil. Financial support: CNPq/FAPERJ.

BC13 - TAXONOMIC SURVEY OF CILIATES (PROTISTA: CILIOPHORA) FROM AN ACTIVATED SLUDGE SEWAGE TREATMENT PLANT IN RIO DE JANEIRO CITY, RJ.

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The use of ciliates protist to evaluate the quality of treated sewage has become a growing practice and this organisms have been studied extensively mainly from an ecological point of view. The purpose of this work was to provide a taxonomic survey on ciliates present in the Penha Sewage Treatment Plant (ETEP), Rio de Janeiro, Brazil, through a morphological study. Forty ciliate species were identified in samples of raw sewage and activated sludge were collected in the primary settling tanks, in the aeration tank and in the secondary settling tanks. The following species were characterized mophometrically: *Amphileptus punctatus*, *Aspidisca cicada*, *Blepharisma sinuosum*, *Colpoda inflata*, *Cyclidium* sp., *Deviata estevesi*, *Deviata* sp. nov., *Dexiostoma campylum*, *Didinium nasutum*, *Engelmanniella mobilis*, *Epysthilis plicatilis*, *Euplotes aediculatus*, *Euplotes* sp., *Glaucoma scintillans*, *Gonostomum affine*, *Kahliella* sp., *Lagynus elegans*, *Loxodes striatus*, *Loxophylum australe*, *Metopus contortus*, *Opercularia* sp., *Oxytricha* sp.1, *Oxytricha* sp.2, *Plagiopyla nasuta*, *Paramecium aurelia*, *Podophrya fixa*, *Prorodon ovum*, *Pseudoblepharisma* sp., *Pseudourostyla nova*, *Spathidium anguilla*, *S. deforme*, *Spirostomum minus*, *S. teres*, *Sterkiella cavicola*, *Stylonynchia pustulata*, *Thruricola* sp., *Tokophrya* sp., *T. quadripartita*, *Urocentrum turbo* and *Vorticella* sp. The morphology and infraciliature were described using live observation, silver impregnation and electromicrographic images of the specimens. From these, *P. aurelia*, *S. teres*, *E. aediculatus*, *A. cicada* and *Vorticella* sp. were the most frequent species with percentage of occurrence equal to 97.0%, 81.8%, 75.8%, 60.6%, 57.6%. The species of genus *Deviata*, *D. nasutum*, *E. mobilis*, *L. australe, P. nova*, *G. affine* and *S. cavicola* were

recorded for the first time in activated sludge plant. The results expand the knowledge on the richness of ciliates species present in sewage treatment plants, and provide new data useful for identification of ciliates potentially useful as pollution bioindicators. This work was financially supported by CNPq and FAPERJ.

BC14 - OCCURRENCE AND MORPHOLOGY OF TWO SPECIES of *Metadinium* Awerinzew & Mutafowa, 1914 (Entodiniomorphida, Ophryoscolecidae) in sheep OF THE SEMI-ARID in PERNAMBUCO, BRAZIL.

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In Brazil, studies about morphology of the rumen protozoa ciliates of domestic animals are scarce, mainly in sheep hosts. The genus *Metadinium* Awerinzew & Mutafowa, 1914 includes nowadays 13 species registered in several hosts. This study is aimed to make the first record of the *Metadinium spp.* in sheep in Brazil. It also intended to characterize morphologically two species of the genus *Metadinium* identified as *Metadinium* cf. *ossiculi* e *Metadinium* cf. *esalqum* in half-breed sheep Santa Inês maintained in regime of semi-extensive creation in natural pastures in caatinga. Biometric analyses (μm) were carried out in 30 individuals of each species. The population of the *Metadinium* cf. *ossiculi* studied shows 94,44±15.6 μm (76-156) of total length; 56,10±10,6μm (47-89) of total width; 61,45±12,25μm of total length of the plates; 21,07±5,55μm of total width of the plates. Presence of a caudal lobe in posterior extremity of the body and macronucleus rod-shaped with an anterior extremity rounded. The population of the *Metadinium* cf. *esalqum* studied has 94,60±26,8μm (65-186) of total length; 63,20±17,65μm (46-120) of total width; 74,30±22,1μm of total length of the plates; 29,50±10,4μm of total width of the plates; caudal lobe absente and F-shaped inverted macronucleus. Both species presented similarity to the body length and the width, but they differed in terms of biometrics and morphology of the skeletal plates and also in terms of the relationship of the morphology of the macronucleus. The biometric of the skeletal plate was suggested as a new character to be studied in order to enlarge the character diagnoses of the species of the genus *Metadinium*, taxon this that needs taxonomic revision and re-descriptions of species using modern techniques in ciliatology. The specific determination is fundamentally important for the knowledge concerning the paper these protozoa play in the organism of the host.

BC15 - PROTOZOAN CILIATE EPIBIONTS ON THE FRESHWATER APPLE SNAIL *Pomacea figulina* (SPIX, 1827) (GASTROPODA, AMPULLARIIDAE) IN AN URBAN STREAM OF SOUTHEAST BRAZIL

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Historically, epibiosis was understood as a commensal relationship between two or more organisms. However, some studies have shown that epibionts can cause deleterious effects to their hosts. The objective of this study was to record and analyze the prevalence of ciliated protozoa associated with prosobranchian snails of the species *Pomacea figulina* (Spix, 1827) collected from an urban stream in southeastern Brazil. Four collections were carried out between December, 2005, and March, 2006, from which 23 snails and 10 "empty" shells were obtained. The shells and opercula were scraped over Petri dishes and the ciliates were observed using bright field and phase contrast microscopy. We recorded seven species of ciliates on *P. figulina*: *Carchesium polypinum*, *Vorticella microstoma*-complex, *Vorticella campanula*, *Epistylis plicatilis*, *Epistylis* sp., *Opercularia* sp. and *Hypophrya fasciculata*. Of the 23 snails analyzed, 82.60% (n=19) were infected with at least on species of ciliate. No ciliates were found on the "empty" shells and opercula. Regarding the prevelance of infestation on *P. figulina*, the ciliate species were classified according to their status within the taxocenoses into central species: *C. polypinum* (78,26%), secondary species: *V. microstoma*-complex (47,82%), *V. campanula* (39,16%), and satellite species: *E. plicatilis* (30,43%), *Opercularia* sp.

(17,39%), *Epistylis* sp. (8,70%), *H. fasciculata* (8,70%). Among the species of ciliates recorded on the shells of *P. figulina*, *C. polypinum*, *V. microstoma*-complex, *V. campanula* and *E. plicatilis* showed no substrate specificity and have previously been recorded living on algae, geophytes, and root-floating leaved plants, and colonizing diverse groups of aquatic invertebrates. The species *H. fasciculata* have been recorded only on gastropod snails of the species *Lymnea attenuata* e *Physa osculans* and on *P. figulina* in the present study. The results are discussed in terms of ecological aspects involved in this association.

BC16 - MORPHOLOGY OF THE "FLAGSHIP" CILIATE *Neobursaridium gigas* BALECH, 1941 (CILIOPHORA, PENICULIDA) FROM BRAZIL, WITH NOTES ON ITS RESTING CYST AND GEOGRAPHIC DISTRIBUTION

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Neobursaridium gigas is a "flagship" ciliate with Gondwanan geographic distribution, recorded so far from Argentina, Benin, Brazil, Central African Republic, Congo, Gabon, India, Tchad, Thailand and Uganda. Flagship ciliates have restricted distribution and display distinct, conspicuous morphology which makes them easily recognizable in the microscopic world. The present study records the occurrence of *N. gigas* in an urban freshwater stream from the city of Juiz de Fora, (MG-Brazil), and contributes to the morphology and biogeography of this ciliate. The organisms were cultivated to maximize the number of analyzed individuals, which were then studied *in vivo*, after protargol-impregnation and scanning electron microscopy. The individuals of *N. gigas* measured approximately 395 (280-510) x 205 (150-300) µm, with ellipsoid outline, truncated at the anterior edge. Somatic ciliature comprised about 400 kineties; buccal apparatus exhibited a complex deep oral cavity, in which the oral ciliature showed two large *peniculi* and one *quadrulus*. Important features of this ciliate were the presence of 8–12 long sinuous collector ducts associated to the two large contractile vacuoles, and numerous trichocysts. The nuclear apparatus was formed by a large (140–260 µm long) halter-shaped macronucleus and 2–12 micronuclei. This species was found to produce spheroid resting cysts that measured approximately 180–200 µm in cross section and displayed a thick reticulated wall, surrounded exteriorly by a thin membrane. In spite of some protists having likely ubiquitous geographic distribution, modern morphology and molecular studies indicate that a considerable percentage of protists, including ciliates, are distributed in restrict patterns. Unfortunately, the alpha-taxonomy of these organisms faces a worldwide decrease in both interest from financial agencies and number of specialists. Significant improvement on the knowledge of micro-organismal biogeography, ecology and evolution is expected from the investigation of regions of high biodiversity, like Brazilian biomes. Financial support: CAPES/CNPq/FAPEMIG/FAPERJ.

BC17 - Canine Visceral Leishmaniasis in Florianópolis Santa Catarina, Southern Brazil: Detection of an imported case.

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Visceral leishmaniasis (VL) caused by *Leishmania chagasi* is an endemic parasitic disease in Brazil, affecting humans in all regions except the southern States. Since dogs may play an essential role in the parasite transmission cycle, transit of these animals represents a danger of spreading the disease to non-endemic areas. Recently, a family from Florianópolis went to summer holidays at Campo Grande City, Mato Grosso State, carrying an adult male poodle. After a two months period from their return, the dog showed apathy, revealed spleenmegaly and fever, being attended in a veterinary clinic. Indirect immunoflurescence revealed a positive reaction (1:160) for *Leishmania* in two distinct assays. Moreover, parasitological search on bone marrow, spleen and liver showed several macrophages containing *Leishmania* amastigotes. Culture of spleen and liver macerates in Schneider's insect medium were also positive. DNA from culture-derived promastigotes was used in a PCR assay directed to the kDNA mini-circles aiming specific characterization. DNA from *L.*

braziliensis (2904), *L. amazonensis* (575) and *L. chagasi* (PP75) were used as controls. Polyacrylamide gel electrophoresis revealed the expected *Leishmania*-specific ~120bp product for the isolated strain as well as for the control strains which were blotted onto nylon membranes for a *Southern blot* assay. Probes consisted of *L. amazonensis*, *L. braziliensis* and *L. chagasi*. PCR-amplified products were used and confirmed the identity of the isolated sample as *L. chagasi.* The present study demonstrates, for the first time, the occurrence of an *L. chagasi*-infected dog in Santa Catarina State, pointing out the possible introduction of VL, reinforcing the needs for better epidemiological surveillance for this disease in dogs.

Supported by CNPq and Fapesc.

BC18 - Morphobiological characterization of *Trypanosoma cruzi* strains isolated from chagasic schoolchildren of Jequitinhonha Valley, MG, Brazil.

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The objective was to assess morphobiologically six *T.cruzi* strains isolated from children seropositive for *T.cruzi* previously genetically characterized as *T.cruzi* II, subgroup 2b, before (strains 795, 806, 817, 829 and 855 A) and after treatment (strain 855 B).Eight females Swiss mice, 28 to 30 days old were intraperitoneally inoculated with 10,000 blood trypomastigotes. Infectivity, mortality, pre-patent period (p.p.p), patent period (p.p.), maximum of parasitemia, day of peak of parasitemia, curve of parasitemia and morphology of the parasites in the peripheral blood were considered. The infectivity of all strains was higher since 99.5% of the mice presented patent parasitemia, except one animal infected with 829 strain that showed additionally a long p.p.p. (17 days) in contrast to the other strains in witch the p.p.p. was 7 (806) and 9 (795, 855A and B, 817) days respectively. The strain 829 showed also the lowest p.p. (22 days) in comparison with the others (30 to 60 days). The day of peak of parasitemia for the strains 806 and 855A was the 18th after infection, 22th for 855B, 24th for the 817, and 26th for the 795 and 829. Strain 806 presented the lowest p.p.p. (8 days), higher parasitemia and was the only sample that causes mortality in 62,5% of the mice being the more virulent. Predominance of large or stout bloodstream forms was observed what can be associated with the delay of the parasitemia peak since these forms are less infective, take long time for cell invasion and consequently to begin the intracellular multiplication. Thus, we verified that the *T. cruzi* strains belonging to the same genetic group were in the majority of low virulence. Histopathology and the susceptibility to benznidazole are in progress what may give better orientation for clinical and therapeutic management of the chagasic individuals of this endemic area.

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BC19 - DIFFERENCES OF CELL SURFACE LABEL DISTRIBUTION AND REDISTRIBUTION PATTERNS BETWEEN *TRYPANOSOMA CRUZI* TRYPOMASTIGOTES FROM DIFFERENT STRAINS.

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Spontaneous and ligand dependent capping and shedding were described in vitro in T. cruzi trypomastigote and amastigote forms. Shedding was observed to be an energy-,temperature- and time-dependent phenomena and involves a plasma membrane vesiculation, the action of proteases or the solubilization of GPI-membrane anchored components like Ssp4. In the present study we report the behavior of CF- and Con-A-binding sites on the surface of living trypomastigotes from the Y and CL strains. Tissue culture-derived trypomastigotes (Y strain and CL Brener clone) were obtained from the supernatants of infected LLC-MK_2 cells and incubated under different conditions in the presence of 10 ug/ml CF and Con A or Con A-FITC conjugate. In the presence of both ligands, the number of vesicles budding from different regions of the parasite surface increases although shedding of amorphous material could be also observed by scanning electron microscopy. The localization of CF- and Con A-binding sites were determined by

transmission electron microscopy. Mobility of CF-binding sites was induced at 4C in Y trypomastigotes whereas in CL Brener parasites no redistribution of CF was observed. The shedding dynamics of Con A-binding sites in both samples were also analyzed by flow cytometry. Although both trypomastigotes shed Con A binding sites at 4C the process is more intense in Y parasites. No redistribution of Con A binding sites were observed in CL Brener trypomastigotes at 4 or 37C. Incubation of parasites in the presence of CF and Con A protected them from the lytic effects of anti alpha-Gal antibody and interfere in the antibody labeling pattern. The way Y and Cl Brener trypomastigotes modulate their surface components and the consequences to the survival and parasite-host cell interaction will be discussed.

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BC20 - FURTHER STUDIES ON THE ULTRASTRUCTURE OF TOXOPLASMA GONDII CYSTS

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To analyze the ultrastructural aspects of cysts of *Toxoplasma gondii* and achieve a better comprehension of the biology of this important life stage, cysts from brain of mice chronically infected with the Me49 strain, were analyzed by different techniques: scanning electron microscopy and quick freeze followed by deep etching. For all procedures cyst were isolated with Dextran 20%. Metal replicas revealed that the cyst wall was formed by a rigid compact layer, localized in the most outer part of the structure. Beneath, there was another layer in contact with the cyst matrix, three times thicker than the former, presenting a spongy-like arrangement, which will be referred as inner layer. The wall also presented numerous vesicles of different sizes within and in connection with the inner layer. Quick freeze/ deep-etched cysts also showed the presence of large irregular vesicles of approximately 300 nm in the matrix and in the vicinity of cyst wall. In the same replicas we were able to observe bradyzoites presenting pore-like structures in their surface and a network interconnecting the parasites, formed by small vesicles and tubules of similar dimensions of the intravacuolar network found in tachyzoites' parasitophorous vacuoles. Cysts observed by scanning electron microscopy revealed that the outer compact layer had a very rough aspect. These data strongly suggest that cyst wall of *T. gondii* is not homogeneous and that the intravacuolar network persists during the differentiation process. This work was supported by CNPq and Faperj.

BC21 - ULTRASTRUCTURAL AND BIOCHEMICAL ALTERATIONS INDUCED BY A NOVEL NAPHTHOPTEROCARPANQUINONE IN *LEISHMANIA AMAZONENSIS*

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Quinones have been studied for antitumoral, molluscicidal, anti-inflammatory, antimicrobial, and trypanocidal activities. In studies of antileishmanial synthetic molecules, designed by combination of naphthoquinone and pterocarpan moieties, we previously demonstrated the activity of LQB118. This compound presented *in vitro* and *in vivo* activity, without significant toxicity to macrophages and animals, in the therapeutic range, and did not affect the nitric oxide production by infected macrophages. These results demonstrated the selective antileishmanial activity of LQB118. In the present study, we investigated the mode of action of LBQ118. To evaluate the effects on the ultrastructure of *L. amazonensis*, promastigotes cultured in Schneider medium and incubated or not with 2,5μM and 10μM of LBQ118 were prepared for analysis by transmission electron microscopy. Our results showed that treatment with 2,5μM of LBQ118 affected mainly the parasite golgi complex and induced the formation of vacuoles in the cytoplasm. The most drastic changes, including cell death (apoptosis), were observed when the parasites were incubated with 10μM of LBQ118. Morphological alterations comprised mitochondrial damage, disarrangement of the endoplasmatic reticulum, golgi complex and nuclear envelope,

compaction of the chromatin, rarefaction of the cytoplasm and increase of the number of lipid bodies and autophagic vacuoles. A disarrangement and vesiculation of the flagellar pocket was observed. This structure showed intense membrane shedding and formation of membrane blebs. Once several alterations in membranes were observed, we investigated the parasite lipid composition. Promastigotes were incubated with 1,25μM, 2,5μM and 10μM LQB118 for 24h. Lipids were extracted and submitted to thin-layer chromatography. We observed a strong band reduction with concomitant arisen of two other bands, directly related to LQB118 concentration, indicating an inhibition of the one end point product, with accumulation of precursors. Altogether, these results indicate that LQB118 induces an overall damage in parasite, probably by affecting the lipid membrane composition. FAPERJ, CNPq

BC22 - CELL DAMAGE BY *LEISHMANIA AMAZONENSIS* LEISHPORIN: AN ATOMIC FORCE MICROSCOPY STUDY

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Leishporin is a pore-forming protein produced by species of the Genus *Leishmania*. Because it is optimally active at acidic pH (5,5) and at 37□C, we have postulated that it may act in the mammalian host, being involved in phagolysosome and plasma membrane rupture, crucial steps for parasite survival and infection recrudescence. In previous works, we showed that leishporin does not need proteins or carbohydrates as receptors to lyse cells; target membranes lipids are sufficient for cytolysin binding and membrane rupture. In the present work we have studied the damage caused on target membranes by leishporin using the Atomic Force Microscopy tapping-mode technique. We have used erythrocytes and liposomes as membrane models, both highly susceptible to leishporin activity. After hemolytic or lipolytic assays we analyzed the damage caused by promastigote extracts in both membrane surfaces. The images obtained showed pore-like-shaped forms in both models. The circular structures measured 40-50 nm of radius and 4-8 nm of depth, the latter being sufficient to span lipid bilayer. This work provided the first visual evidence of leishporin activity. Support: CAPES, CNPq, FAPEMIG, PRONEX, WHO.

BC23 - EFFECT OF HYDROXYUREA ON THE *Trypanosoma rangeli* DIFFERENTIATION *IN VITRO*

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The possibility of obtaining large amounts of infective trypanosomes *in vitro* was a major breakthrough on *Trypanosoma rangeli* research. However, the precise mechanisms and factors involved on this phenomenon are not fully understood. In this study, the effect of hydroxyurea (HU) on induction of *T. rangeli* differentiation *in vitro* was assessed. For that, epimastigote forms of *T. rangeli* Choachi strain into the exponential growth phase in LIT medium were cultivated in triplicates in the presence of 20mM HU for 24 hours at 27°C. After this period, the parasites were washed twice in PBS and cultivated in the DMEM medium supplemented with 0,05% of L-Glutamine. Differentiation was assessed at 0, 2, 4 and 6 days of incubation by counting the number of trypomastigotes among 200 randomly chosen parasites in Giemsa stained smears. Analysis on the 6th day of incubation showed a differentiation rate of >95% for HU-induced parasites while non-HU induced parasites (control) presented a differentiation rate of 75%. The obtained results point out that HU is an important inducer of *T. rangeli* metacyclogenesis *in vitro*, suggesting that HU-induced parasites may synchronize metacyclogenesis due blocking of DNA replication since HU act primarily as an inhibitor of ribonucleotide reductase and, consequently, reduces the deoxyribonucleotide levels. By enhancing the *in vitro* metacyclogenesis rate, large amounts of *T. rangeli* trypomastigotes can be obtained for *in vivo* and *in vitro* studies on the cellular and molecular biology of this parasite. Furthermore, this approach provides means to address and characterize molecules of the parasite, which are relevant to biological processes.

Supported by CNPq, CAPES and UFSC.

BC24 - SITE PREFERENCE OF *RHABDOSTYLA CHIRONOMI* KAHL, 1935 (CILIOPHORA, PERITRICHIA) ATTACHED TO CHIRONOMIDAE LARVAE: A SEM STUDY

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Some epibiont ciliates are particularly attached to certain parts of their hosts that must be related with food habit as much of the epibiont as the host and it can reflect characteristics from population dynamics of epibiont that are going to colonize the substrate. The aim of this study was to analyze the site preferences of the epibiont ciliate *Rhabdostyla chironomi* attached to Chironomid larvae. Twelve collections were carried out at intervals of approximately 30 days from May 2005, to April 2006, at five sampling stations along the São Pedro stream (Rio Paraibuna watershed), located in the southeastern urban region of Juiz de Fora, Minas Gerais, Brazil. Were investigated the cephalic region, 12 body segment, ventral and anal tubules tubes from each larvae, to quantify the occurrence of the peritrich ciliates in each appendix. Some epibiont ciliates observed *in vivo* (bright field microscope) were fixed to carry out the scanning electron microscope procedure. Were colleted 23542 larvae, those 3964 (16.8%) were infested by *R. chironomi.* All the ciliates found were located at the ventral tubules of the larvae what suggest a site preference of this epibionts for this appendix. The localization of these ciliates in ventral tubules may be related to the ventilation behavior shown by chironomids. These larvae are apneustics and breathe the oxygen diluted in water through the body surface, mainly through the ventral and anal tubules. Furthermore, they generate ventilation flows by moving their posterior end expansions (tubules) or through a swimming behavior, which are means that favor respiratory exchanges. This work was supported by "FAPEMIG".

BC25 - MODELING THE THREE-DIMENSIONAL ULTRASTRUCTURE OF *TRYPANOSOMA CRUZI* DURING METACYCLOGENESIS

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Very little is known about *Trypanosome cruzi* ultrastructural changes occurring during the differentiation of epimastigotes to metacyclic trypomastigotes. Here we aimed at providing accurate information about the morphology of the parasite over the differentiation process, known as metacyclogenesis. For this purpose, parasites were fixed in a glutaraldehyde and formaldehyde mixture, post-fixed in osmium tetroxide with potassium ferrocyanide for membrane contrast enhancement, dehydrated in an ethanol series, and embedded in Epon resin. Ultrathin serial sections of parasites were obtained, examined and photographed in the transmission electron microscope. Reconstruct software was used for alignment and assembly of serial sections for producing a representative three-dimensional model. Subsequently, 3D model of selected structures was rendered with Blender 3D modeling software. A progressive reallocation of the nucleus relative to the flagellar pocket, as well as large change in the chromatin and organelle organization was observed providing new insights about the cellular reorganization taking place during the metacyclogenesis.

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BC26 - STRUCTURAL ORGANIZATION OF PFR OF *Trypanosoma cruzi*

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Many advances in Atomic Force Microscopy (AFM) have been accomplished in last years. Acquisition of information on the elastic and adhesive properties, at high resolution, by analyzing phase AFM signals, has made the AFM an attractive tool

to the study of flexible and structurally modulated cellular components. In a recent work, our group has used the AFM to examine the ultrastructure of *Trypanosoma cruzi* and, additional information on the organization of the sub-structure of the flagellum was obtained. The paraflagellar rod (PFR), a filamentous structure present in some kinetoplastid flagella, is involved in the motility of these protozoa. Its importance to *Trypanosoma brucei* was demonstrated by the silencing of genes involved in the assembly of this structure, which led to partial or total impairment of the motility. Intense biochemical and structural characterization of PFR has been performed. However, the inner organization of this structure is poorly understood. In this work, we compared transmission electron microscopy images of quick frozen, freeze-fractured, deep-etched and rotary shadowed replicas, with AFM images of epimastigote forms of *T. cruzi* to obtain information of the sub-structure of the PFR. Analysis of *T. cruzi* flagellum by both deep-etching and AFM height images demonstrated similar and equivalent structures. Overlay of phase and topographic AFM images showed that the organization of PFR filaments is modulated by its bending states. Such overlay images provided a better view of these filaments and the participation of them on flagellum bending. Altogether, these results suggest that the structural aspect of the PFR is modulated by flagellar beating.

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BC27 - *Trypanosoma cruzi* infective and proliferative forms use distinct mechanisms to inhibit DNA replication

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Trypanosoma cruzi alternates between infective and proliferative forms during its life cycle. It has been well established that infective forms are not able to replicate, but the molecular bases that warrant the non-replicative status of these forms remain to be determined. In eukaryotic cells DNA replication initiates with the assembly of the pre-replication machinery at multiple sites along the chromosomes. This assembly occurs at G1 phase of the cell cycle through the sequential binding of the heterohexamer Orc_{1-6}, the Cdc6 and Cdt1 molecules and finally the heterohexamer MCM_{2-7}, whose helicase activity is essential for replication. To prevent replicated origins becoming ready to replicate again during or after S phase, eukaryotic cells need to downregulate the pre-replication machinery assembly at non G1 cell cycle stages. Cdc6 degradation and DNA-Orc interaction inhibition are some described ways to get DNA replication blockage. The genomic database of *T. cruzi* showed that differently from other eukaryotes trypanosomes do not contain sequences in their genome that could codify for Orcs or Cdc6. Unlike, these parasites contain one open reading frame homologous to Orc_1 and Cdc6. Here we show that *T. cruzi* Orc/Cdc6 presents an AAA+ domain, a typical characteristic of some members of pre-replication machinery, and replaces yeast Cdc6 as observed in phenotypic complementation assay. Orc/Cdc6 is expressed during the entire cell cycle of epimastigote forms. Extraction of soluble proteins followed by DNAse digestion visualized by western blotting and immunofluorescence assays showed that most of Orc/Cdc6 is bound to chromatin in epimastigotes. In contrast, although Orc/Cdc6 localizes at nucleus of trypomastigote cells, it was readily extracted as a soluble protein from this form, suggesting that Orc/Cdc6 interacts weakly with DNA in this non-replicative stage.

Supported by FAPESP

BC28 - ORC/CDC6 AND PCNA REMAIN CONSTRAINED AT NUCLEAR PERIPHERY DURING LATE G1/S PHASES OF THE TRYPANOSOMA CRUZI'S CELL CYCLE

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In *Trypanosoma cruzi*, the agent of Chagas disease, replication sites localize at nuclear periphery, where chromosomes remain constrained during the S phase of the cell cycle. In order to analyze the dynamics of molecules involved in replication, we followed Orc/Cdc6 as marker for the pre-replication machine, and PCNA

as marker for the replication machine during the cell cycle of *T. cruzi*. We found that both molecules present two patterns of nuclei distribution: a periphery pattern, where molecules are constrained at nuclear periphery and a dispersed pattern where molecules are dispersed through the nuclear space. Fifth percent (50%) of G1/S cells present Orc/Cdc6 and PCNA dispersed into nuclear space, while the other half of G1/S cells present these molecules constrained at nuclear periphery. In the other stages of the cell cycle these molecules are dispersed through the entire intranuclear space. Double-labeling assay showed that in G1/S cells there are Orc/Cdc6 at nuclear periphery while PCNA is dispersed. Also, we found cells with both Orc/Cdc6 and PCNA constrained at nuclear periphery. Moreover, the co-localization of Orc/Cdc6 and PCNA was observed just at nuclear periphery. These data allowed us to conclude that the replication of DNA at nuclear periphery is not due to localization of replications factors at nuclear periphery; instead it depends on the movement of these factors to the appropriated sites. Moreover, we concluded that the pre-replication factor Orc/Cdc6 goes to nuclear periphery before PCNA replication factor. Finally, the co-localization of Orc/Cdc6 and PCNA at nuclear periphery shows that replication also begins at this region, which point to a very confined region of replication in the nucleus of *T. cruzi*.

Supported by FAPESP

BC29 - DISTRIBUTION OF HISTONE H4 POST-TRANSLATIONAL MODIFICATIONS IN THE NUCLEUS OF *TRYPANOSOMA CRUZI*

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Histones N-termini are targets of post-translation modifications (PTMs) at their different residues. These modifications are thought to play crucial roles in chromatin structure, gene regulation control and DNA repair. In *Trypanosoma cruzi,* the agent of Chagas´ disease, histones are highly divergent compared to most eukaryotes. We have previously reported that histone H4 is acetylated at lysines 4, 10 and 14 in *T .cruzi*. Using specifics antibodies against each acetylation, we show here that each one of the histone H4 acetylation present distinct nuclear localization. H4K4 localizes in dense chromatin, preferentially at the nuclear periphery whereas K10 and K14 are present in non-dense chromatin regions. Acetylation in H4K4 is decreased in trypomastigotes forms. H4K14 is 2-fold increased during G2/M, whereas no acetylation changes of H4K4 and H4K10 were detected. Acetylations at H4K10 and H4K14 co-localize with transcriptional sites and RNA polymerase II labeling. Gamma radiation increased acetylations of histone H4K10 and H4K14 and decreased K4 acetylation, but no changes were observed for hydrogen peroxide treatment or ultraviolet exposure. For better understanding the role of these acetylations, we started the characterization of histone acetyltransferases (HAT) present in *T. cruzi* data base. In this way, we could be able to identify which HAT is responsible for each acetylation and the effects of their super expression for the biological process in *T. cruzi.*

Supported by FAPESP and CNPq.

BC30 - DIVISION SYNCHRONY BETWEEN THE SYMBIOTIC BACTERIUM, THE NUCLEUS AND THE KINETOPLAST OF TRYPANOSOMATIDS

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Trypanosomatids present typical structures, many of them are single-copy ones, as the nucleus, the flagellum and the mitochondrion, this last comprising the kinetoplast that is constituted by a network of circular DNA molecules. Some monoxenic trypanosomatids present a single endosymbiotic bacterium in the cytoplasm. This endosymbiont co-evolves through a mutualistic relationship with the host protozoan, constituting a valuable model to understand the origin of organelles, such as the mitochondrion. The cell cycle of trypanosomatids involves a co-ordinated replication and segregation of the nucleus, the kinetoplast and the flagellum. In trypanosomatids that harbor an endosymbiotic bacterium, such as

Blastocrithidia culicis and *Crithidia deanei*, this process is more complex, since the symbiont divides synchronically with the host protozoa. In this work we describe the morphological events that occur during endosymbiont-bearing trypanosomatids cell cycle, in particular the chronological division of the symbiotic bacterium, regarding other protozoan host structures, as the nucleus, the kinetoplast and the flagellum. The endosymbiont was found associated with the host cell nucleus, as observed by immunolabeling and by transmission electron microscopy. In most of the cells the bacterium is found as an elongated and constricted form, but single round bacterium forms are also detected by immunofluorescence microscopy. Importantly, protozoan with two endosymbionts, in the constricted, or round shape were found to contain one nucleus and one kinetoplast, while no cells with a single symbiont were detected in protozoa with two nuclei, or kinetoplasts. Therefore, the endosymbiont divides before the kinetoplast and the nucleus. Then, each endosymbiont is delivered to each daughter protozoan cell during cytokinesis, suggesting that the symbiont division is linked by the host protozoan cell cycle.

Supported by CNPq and FAPERJ.

BC31 - A TOR-RELATED LIKE KINASE IS ASSOCIATED WITH ACIDOCALCISOMES IN *T. BRUCEI*

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TOR (Target of Rapamycin) are evolutionary conserved protein kinases that coordinate information from energy levels, mitogenic signals and nutrient sufficiency to control cellular growth. Studies with mammalian and yeast cells signaling pathways showed that nutrient starvation inhibits TOR activity, which results in G1 cell cycle arrest, and triggers a stress response program leading to a blockade of translation initiation. The same stress response can be observed in cells treated with rapamycin, an imunosupressant drug, which binds to FKBP12 prolyl-isomerase forming a complex with the TOR kinase. By searching *T. brucei* genomic database there are four candidates for TOR related kinases. We have previously showed by immunofluorescence that one of them (GeneBank XP_844230.1), known as Tb TOR1 Rel is found in foci in all cytoplasm. Here we demonstrated that these foci are acidocalcisomes, which are organelles responsible to control the osmotic and intracellular pH. RNA interference of TbTOR1 in *T. brucei* procyclic forms showed a slow growth phenotype with a distinct morphology (big cells, zooids, multinucleate and multiflagellate cells) and an increased amount of cells between S and G2 stages of the cell cycle. Studies about the function of acidocalcisomes in these cells are underway.

Supported by FAPESP and CNPq

BC32 - INTRACELLULAR SORTING OF PHOSPHOGLUCOMUTASE TO GLYCOSOMES IN *Trypanosoma cruzi*

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Glycosomes are specialized peroxisome-like microbodies that compartmentalize β-oxidation of fatty acids, biosynthesis of ether lipids and glycolysis in trypanosomatids. Glycosomal enzymes are synthetized in the cytoplasm and then transferred to glycosomes by mechanisms similar to those described to the import of peroxisomal proteins. These molecules bind to carrier proteins called peroxins via two classic targeting motifs: PTS-1 and PTS-2, located at the COOH and at the amino terminal of the target enzyme, respectively. In a few exceptions, the protein is directed to glycosomes by an internal PTS (I-PTS). In *Trypanosoma cruzi*, the production of glucose-1-phosphate, required to the formation of UDP-galactose, is dependent on the activity of phosphoglucomutase (PGM). Hexokinase, the enzyme responsable for the generation of the PGM substrate, glucose-6-phosphate is located in glycosomes. In previous studies, we showed that *T. cruzi* PGM (TcPGM) is located inside glycosomes in all life stages of the parasite. However, there are no classical PTS1 or PTS2 motifs in TcPGM, suggesting the presence of an alternative domain that could mediate its transport to glycosomes. In this work, we produced genetically modified *T. cruzi* cell lines expressing different domains of TcPGM fused to the green

fluorescent protein (GFP). The sub-cellular location of the TcPGM-GFP fusion proteins revealed that the region ranging from amino acids 260 to 380 of TcPGM is sufficient to target GFP to glycosomes. To confirm the presence of an I-PTS domain in TcPGM, a new construct containing GFP fused to a TcPGM gene fragment comprising residues 260-380 was generated, and transfected into *T. cruzi* epimastigote forms. Western blot and immunofluorescence analyses using anti-TcPGM and anti-aldolase are underway. Since glycosomes are crucial organelles for the survival of trypanosomatids, the molecular mechanisms of protein import to trypanosomal glycosomes are attractive targets for the development of novel anti-parasitic drugs.

Supporteb by CNPq and FAPERJ

BC33 - Ultrastructural and biochemical characterization of Trypanosoma cruzi reservosome lipid inclusions

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Epimastigotes from *Trypanosoma cruzi* possess a remarkable ability to ingest different classes of macromolecules from medium, via cytostome or flagellar pocket, and accumulate them inside reservosomes until digestion. Reservosomes are acidic lysosome related organelles, which also store proteases, such as cruzipain and serine carboxypeptidase. They present an ATP binding cassette, TcABCA1, involved in cholesterol homeostasis, and store high amounts of neutral lipids, such as cholesteryl esters and cholesterol, probably as a result of low density lipoproteins (LDL) particles endocytosis (reviewed in Cunha-e-Silva et al., Parasitol. Res.99:325, 2006).

Recently, our group have shown that inside reservosomes stored lipids can be observed as spherical lipid droplets or as rectangular inclusions surrounded by a phospholipid monolayer (Sant'Anna et al, Microsc Res Tech 2008 *in press*), whose ultrastructural aspect is akin to that of lipid inclusions found in foam cells (Bobryshev 2006). Epimastigotes were cultivated in LIT medium supplemented with low (1%) or high (20-50%) fetal calf serum. Electron microscopy analysis of thin sections of low serum epimastigotes showed reservosome fusion and devoid of lipid particles, while abundant sword-shaped lipid profiles were observed crossing the organelle in epimastigotes from high serum cultures. Furthermore, when high serum epimastigotes were incubated for three hours in serum free medium lipid inclusions disappeared, suggesting that the parasite is able to metabolize these peculiar shaped lipids.

We have isolated lipid inclusions from purified reservosome fractions. Electron microscopy showed an enriched fraction of both rectangular and spherical lipid inclusions. HPTLC first analysis revealed 36,8% cholesteryl esters, 13,8% cholesterol, 15,2% fatty acids, 17,8% triacylglycerols, 8% non determined lipid and 9,3% phospholipids. Nile Red stained intensely either reservosomes in situ or the highly concentrated lipid fraction. Mass spectrometry of isolated lipid fraction is in course, aiming to determine lipid composition and identify proteins associated to these structures.

Supported by: CNPq, PRONEX, FAPERJ

BC34 - MOUSE PERITONEAL MACROPHAGE MICROBICIDAL ACTION REDUCTION DUE TO LIPID BODY PRESENCE AND ASSOCIATION OF THIS ORGANEL WITH *TOXOPLASMA GONDII*

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Introduction: Although lipid bodies (LB) are organelles present in several cell types, their function are not completely understood. It has been suggested that LB are structural markers of cells involved in inflammatory process responsible for prostaglandin E_2 (PGE_2) generation. However, it is not known how the presence of this organelle alters macrophage microbicidal capacity, since PGE_2 can change activation of these cells. **Objective:** Here we analyze how the presence of LB alters the microbicidal action of mouse peritoneal macrophages (Mo), and if this possible alteration is related to the association of this organelle to *T. gondii*. **Results:** Mo cultured with homologous serum presented LB, produced less

nitric oxide and more PGE_2 and was less efficient to control *T. gondii* growth when compared to Mo cultured with fetal bovine serum. The association of vacuole containing *T. gondii* with LB was observed by optical and transmission electron microscopy, indicating that the parasite may benefit from this lipid source, justifying its greater growth in Mo cultured with homologous serum. **Conclusion:** The presence of LB turned Mo cultured with homologous serum less microbicidal, possibly by the deactivation of the cell as a consequence of PGE_2 generation and/or lipidic supplement availability.

Keywords: Macrophage, Lipid Body, *Toxoplasma gondii*, microbicidal action.
Supported by: CAPES, CNPq, FAPERJ

BC35 - EVIDENCE THAT PHOSPHOLIPASE A2 ACTIVITY IS REQUIRED IN THE VESICULAR TRANSPORT IN *Leishmania amazonensis* PROMASTIGOTES.

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The endocytic pathway in Trypanosomatidae presents a singular architecture. The morphological and functional aspect of the distinct compartments differs from that previously described for mammalian cells. In the present study, we use the irreversible inhibitor of Ca(2+)-independent PLA2 (iPLA2), Bromoenol lactone (BEL), to analyze the effect on the ultrastructure of the endocytic/exocytic compartments, the effect on the secretory and endocytic activities of *Leishmania amazonensis*. Parasites grown in Schneider medium for 72h were incubated for 24 hours in the absence or the presence of 2.5 µM BEL and analyzed by transmission electron microscopy, cytochemical localization of acid phosphatase activity, flow cytometry. BEL promoted a significant change in the ultrastructure of Golgi, revealed by an increased number of vesicles near the Trans-Golgi Network (TGN) and at the flagellar pocket region and an increase in the volume of the multivesicular tubules (MVT). Cytochemical detection of phosphatase acid activity in control parasites was observed on cell and flagellar membranes, inside of the flagellar pocket and in the multivesicular tubules. After BEL treatment, labeling on the cell membrane and inside the flagellar pocket was reduced and the phosphatase activity was concentrated inside the enlarged MVT suggesting an inhibition of exocytic activity. Actually, BEL reduced in 16% the total exocytic activity of promastigotes. No changes were observed in the surface expression of Gp63 and LPG as analysed by flow cytometry and immunofluorescence although a concentration of GP63 labeling in intracellular compartments had been observed. The quantitative analysis of endocytosis and the immunocytochemical detection of antigens on the influence of BEL are in course. Here we provide evidence that membrane-bound receptors and soluble cargo in promastigotes of *L. amazonensis* are segregated to different routes some of them under the influence of iPLA2 activity.

Supported by CNPq, CAPES, FAPERJ, Pronex

BC36 - Group II Trans-sialidases are localized in the plasma membrane of *Trypanosoma rangeli* epimastigotes.

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Trypanosoma rangeli, an American nonpathogenic parasite for humans, is not very well-known in many biochemical, immunologic and cellular biology aspects. However, this protozoan infects human in many areas of Center and South-America where *T. cruzi* is endemic, and they share a wide range of triatomine vectors and vertebrate hosts. The lack of appropriate specific diagnostic procedures and the absence of clinical manifestations have been responsible for the underestimate of the infection for *T. rangeli*. Recently we have described that *T. rangeli* possesses genes of the group II of the Trans-sialidase superfamily (TrGP), collectively known in *T. cruzi* as gp85, and it has been implicated in the invasion and infectivity of the host cells. In contrast, since *T. rangeli* does not enter into

mammalian cells, gp85-like proteins may play a different role. To analyze the expression and cellular localization of these proteins in epimastigote forms of a Venezuelan *T. rangeli* isolated we generated polyclonal antibodies against a recombinant peptide of 235 AA (~25 KDa) from the N-terminal region of a TrGP member (GenBank: AF426022). Western blots using sera directed against GPI-anchored proteins from plasma membrane of *T. rangeli* epimastigotes detect specifically the recombinant protein. Immunofluorescence analysis revealed TrGP expression in permeabilized epimastigotes at surface localization. Thus we confirmed that TrGP Trans-siliadase group II members are surface proteins bound to the membrane by a GPI anchor. More experiments are in progress to evaluate the expression of TrGP in other *T. rangeli* stages.

Keywords: *Trypanosoma rangeli*, Trans-sialidase, surface proteins.

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BC37 - ANALYSIS OF FIBRONECTIN PARTICIPATION IN DERMAL FIBROBLAST-*LEISHMANIA (LEISHMANIA) AMAZONENSIS* INTERACTION

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Leishmania species ability to invade and survive within host cells involves the interaction of molecules at parasite and host cell surface. In the present study the participation of fibronectin (FN) was analyzed *in vitro* in dermal fibroblast-*Leishmania (L.) amazonensis* interaction. Dermal fibroblast primary cultures were obtained from mice embryos skin by the fragmentation and dissociation. For ultrastructural analysis of fibronectin at fibroblast surface was used anti-fibronectin antibody, followed by antibody IgG-peroxidase and was revealed with diaminobenzidine. Using transmission electro-microscope was observed electron-dense staining at the surface of fibroblast, showing the participation of this molecule and its receptor in the focal contact where cell adheres to substrate. For analysis of fibronectin participation in the interaction fibroblast-*Leishmania* we used two approaches: infected fibroblasts maintained in Eagle / BSA, or Eagle / BSA with human plasma fibronectin during the infection. For detection of fibronectin in fibroblast culture by immunofluorescence polyclonal antibody anti-fibronectin and secondary antibody conjugated to FITC was used. Analysis by light microscopy and immunofluorescence, cultures maintained in medium supplemented with fibronectin during the interaction showed a greater percentage of fibroblasts with parasites adhered than the cultures which received only Eagle /BSA. In cultures supplemented with fibronectin during infection was observed with 24h post-infection, a percentage of 38,34% and, with 48h post-infection, 15,57%. Cells cultured with just Eagle/BSA was observed with 24h post-infection a percentage of 2,54%, and with 48h post-infection, 0,36%. Despite the significant difference in percentage of fibroblasts with parasites adhered, we have not observed a significant difference in internalization. Our results indicate the participation of fibronectin in *Leishmania*-fibroblasts adhesion process. This is a pioneering approaching and perspectives are open to investigate the role of dermal fibroblasts as host cells in the first and last stages of *Leishmania* infection, and study of the participation of receptor-ligand complexes in this interaction.

BC38 - Cysteine protease inhibitors reduce *Trypanosoma cruzi* adhesion to an insect host model

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Cruzipain is the main lysosomal cysteine protease of *Trypanosoma cruzi*, the causative agent of human Chagas' disease. Studies with synthetic irreversible cysteine protease inhibitors provided the first line of evidence that *T. cruzi* infectivity and

intracellular growth in mammalian cells depend on the activity of cruzipain. L-leucylamido-(4-guanidino)butane (E-64) is a novel type of tight-binding inhibitor of papain-like cysteine proteases. Although the role of *T.cruzi* cysteine proteases cruzipain in mammalian infection has been widely investigated, their role in parasite interaction with the vector has been overlooked. Here, we analyzed the effect of the pre-treatment *T.cruzi* with E-64 on the parasite adhesion to *Aedes aegypti* midgut. This insect has been used routinely as a model for trypanosomatids-vector interaction. Our data reveals that E-64 in the concentration of 10μM and 50μM , reduced the rate of adhesion the insect midgut nearly in 60%, when compared to the control. Therefore, our results suggest that there is a possible involvement of cysteine proteases in the interaction between *T.cruzi* and the invertebrate host.

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BC39 - ARF-6 GTPASE IS ASSOCIATED WITH VERO CELL INVASION BY *TRYPANOSOMA CRUZI* EXTRACELLULAR AMASTIGOTES

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Extracellular amastigotes (EA) cell invasion have shown to promptly aggregate actin filaments by attaching to HeLa cells dorsal surface microvilli. Microvillus aggregation was followed by the formation of cup-like structures underneath the parasite that resemble the pedestals formed during *Escherichia coli* attachment. Besides the formation of actin-rich cups on the surface of HeLa cells, remarkable responses of the cell membrane were also observed upon invasion of Vero cells by EA. In this fibroblastic cell line, devoid of surface microvilli, protrusive lamellae that formed at the sites of amastigote invasion were markedly similar to the membrane expansions observed during *Shigella flexneri* attaching. Amastigote invasion of either HeLa or Vero cells required functional actin microfilaments, once cytochalasin D treatment always inhibited invasion.
ADP-ribosylation factor-6 (ARF-6) belongs to the ARF family of small GTP-binding proteins. ARF-6 regulates membrane trafficking and the actin cytoskeleton at the plasma membrane. It is also implicated in the formation of actin-rich membrane protrusions and ruffles. Recently, it was demonstrated that *Chlamydia caviae*, that penetrated host epithelial cell by inducing cytoskeleton and membrane rearrangements similar to that observed during EA cell invasion, induced a sharp and transient activation of the endogenous ARF-6, which was also required for efficient uptake of the pathogen. Here, we described for the first time that ARF-6 is recruited to the parasitophorous vacuole of invading EA from G and CL strains into Vero cells transfected with a plasmid to express ARF-6 in fusion with a HA tag. Also, we have observed that transfection of Vero cells with ARF-6 RNAi showed a significant inhibition of cell invasion and intracellular multiplication of EA from both strains. We are currently addressing the activation of endogenous ARF-6 and the activation of ARF-6 downstream effectors, such as, phospholipase D (PLD) and phsophatidylinositol 4-phosphate 5-kinase (PIP 5-kinase) during EA cell invasion. Financial support: FAPESP, CAPES, CNPq.

BC40 - AMASTIN, A *TRYPANOSOMA CRUZI* AMASTIGOTE SPECIFIC PROTEIN, IS INVOLVED IN HOST CELL INVASION AND INTRACELLULAR MULTIPLICATION.

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Extracellular amastigotes (EA) forms of *Trypanosoma cruzi* from G strain (*T. cruzi* I) show high infectivity towards host cells *in vitro* when compared to the traditionally more infectious CL strain (*T. cruzi* II). We have performed a DNA microarray from EA from G and CL strain, in order to identify components that would modulate infectivity of these strains. It was identified a 21 kDa protein that upregulates cell invasion by EA from G strain. On the other hand, we have not investigated the mechanisms beneath the low infection rate of EA from CL strain. The possibility of negative modulators was considered once previous studies showed that poorly infectious

metacyclic trypomastigotes express high levels of gp90, a stage-specific surface glycoprotein that modulates negatively cell invasion. We observed in the microarray data that amastin, a stage specific surface protein, was 21 times more expressed in EA from CL strain. In order to study the putative involvement of amastin in cell invasion, we used amastin transfected clone of CL-Brener strain expressing high levels of the protein. Preliminary results showed that EA from parasites expressing high levels of amastin had a significant decrease in cell invasion when compared to the wild-type clone. Furthermore, when the number of intracellular amastigotes was assessed 48 hours after the invasion, it was significantly higher in cells infected with parasites expressing high levels of the protein. These results indicated that amastin downregutates cell invasion in the same way as it upregulates intracellular parasite multiplication. Financial support: CNPq, CAPES, FAPESP

BC41 - Describing Membrane Dynamics in Cardiomyocyte Cell Invasion

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The infection of mammalian cells by *Trypanosoma cruzi* utilises a rearrangement of the target cell's membrane and cytoskeleton to drive the invasion process. Contact between the trypanosome and host cell results in molecular interactions between the surface molecules of both, but there is debate as to the exact roles of the various molecules in driving these rearrangements. We are exploring membrane dynamics using the lipophilic fluor laurdan, which incorporates into the membrane and fluoresces differentially depending on the degree of phospholipid packing. This technique has demonstrated an increased packing in the organised trypanosome flagellum, and by comparison a low level of packing over the non-infected mammalian cell. At the parasite synapse it is believed membrane packing associated with lipid raft proteins enables the recruitment of the cytoskeleton to aid invasion. Use of laurdan to image membranes of live cells, combined with fluorescent chimaeras of cytoskeletal components, is allowing us to examine the interaction between the various components of the parasite synapse. By breaking down the study of the synapse into single invasion factors, we are gaining a better understanding of the roles of each. We have looked at two different types of *T. cruzi* invasion factors, transialidase and the predominant cysteine protease cruzipain, using beads coated with the protein or their trans-expression in the related but non-invasive *T. rangeli*. Our results so far indicate that the invasion factors increase the packing of the membrane around the contact point, along with microtubule rearrangement to the synapse. Cell lines usually used in these studies are typically non-physiological in nature, and so we are also using a human stem-cell line differentiated into cardiomyocytes. These homogenous populations of physiologically relevant cells can provide answers more closely related to the pathology in mammals caused by the parasite.

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BC42 - USE OF DINASORE TO BETTER ANALYZE THE PARTICIPATION OF DINAMIN DURING *TRYPANOSMA CRUZI* ENTRY'S PROCESS.

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Trypanosoma cruzi is an intracellular parasite that as others intracellular pathogens target specific proteins of the host cell vesicular transport machinery. Modulation of host cell processes in response to a parasite results in a generation of unique phagosomes. In mammalian cells, several molecules have been identified that selectively regulate the formation of endocytic transport vesicles and the fusion of such vesicles with appropriate acceptor membranes. Among these, the GTPase dynamin plays an important role in clathrin-mediated endocytosis and recently it was

shown that dynamin can also play some role during phagocytosis. Wilkowsky *et al* (2002) suggested that dynamin is a force generating molecule whose function could be crucial to the initial invasion steps by which trypomastigote forms of *T. cruzi* enter in non phagocytic cells. Macia *et al* (2006) used a compound named dinasore that has the ability to block the GTPase activity of dinamin. Dinasore acts as a potent inhibitor of endocytic pathways by blocking coated vesicle formation within seconds after dinasore addition. Here, we investigated if there is a dynamin participation in the *T.cruzi*'s entry process in phagocytic and non phagocytic cells. For these peritoneal macrophages and LLC-MK_2 cells were treated with crescent concentrations of dinasore before the interaction with trypomastigotes, amastigotes or epimastigotes. We could observe that in both cells types the parasite's internalization was drastic diminished when we used a 100µM of dinasore. The *T.cruzi*'s adhesion index in both cell types was not altered. By scanning electron microscopy, we observed that the entry's process is blocked in different stages in both peritoneal macrophages and LLC-MK_2. In LLC-MK_2 this process is blocked earlier when compared with peritoneal macrophages. In LLC-MK_2 trypomastigote forms were observed linked to cellular filopodia, while in peritoneal macrophages they were surrounded (not completely) by the macrophage plasma membrane.

Support: CNPq/DECIT-MS, CAPES and FAPERJ

BC43 - INVASION AND INFECTIVITY OF *TOXOPLASMA GONDII* TACHYZOITES EGRESSED AT 2 HOURS POST-INFECTION

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Egress and invasion are important events in *Toxoplasma gondii* life cycle, and both of them share some characteristics as the dependence of calcium. Calcium ionophore has been used to artificially trigger release of the parasites from infected cells. We investigated if parasites egressed at 2 hours post-infection (hpi) under stimulation by A21387 calcium ionophore were able to invade and establish infection in new host cells. Interaction of these parasites with confluent LLC-MK2 cells monolayer fixed after 24hpi was compared to similar monolayers infected with peritoneal tachyzoites. Although the dynamics of parasite egress in both times is similar, as observed by electron microscopy, the behaviour of egressed parasites resulted quite different. Peritoneal tachyzoites normally infected the permissible monolayers, while 2hpi ionophore treated parasites were able to invade, but were not capable of establishing the infection. Using acridine orange we observed parasites from ionophore induced early egress inside acidic vacuoles. These data indicate that one or more factors essential to the establishment of the infection could be absent early after *T. gondii* entry in the host cell. Support: CAPES.

BC44 - ARF-6 GTPASE IS RECRUTED TO THE PARASITOPHOROUS VACUOLE OF TAQUIZOITES FROM *TOXOPLASMA GONDII* AND IS ASSOCIATED TO VERO CELL INVASION

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Toxoplasma gondii infect a variety of different cell types in a range of different hosts. Unlike phagocytic uptake or entry of bacterial pathogens, host cell invasion by *T. gondii* occurs by active penetration of the host cell, a process classically described as independent of host actin polymerization. The parasitophorous vacuole has been shown to resist fusion with endocytic and exocytic pathways of the host cell. Thus, the vacuoloar membrane was traditionally considered devoid of host cell marker, so far. ADP-ribosylation factor-6 (ARF-6) belongs to the ARF family of small GTP-binding proteins. ARF-6 regulates membrane trafficking and the actin cytoskeleton at the plasma membrane. It is also implicated in the formation of actin-rich membrane protrusions and ruffles. Here, we have argued if ARF-6 would be recruited to the parasitophorous vacuole of taquizoites from *T. gondii* RH strain and also if it would play role in the parasite cell invasion. Our results showed for the first time that ARF-6 is recruited to the parasitophorous vacuole of invading taquizoites from *T. gondii* into Vero cells transfected with a plasmid to express ARF-6 in fusion with a HA tag. Also, we have observed that transfection of Vero cells with ARF-6 RNAi showed a significant

inhibition of cell invasion by taquizoites from *T. gondii*. Besides, we treated Vero cells with cytocalasin D previous to invasion and observed that it reduced taquizoites internalization. Thus, contradicting the traditional literature. We are currently addressing the activation of endogenous ARF-6 and the activation of ARF-6 downstream effectors, such as, phospholipase D (PLD) and phsophatidylinositol 4-phosphate 5-kinase (PIP 5-kinase) during *T. gondii* cell invasion. Financial support: FAPESP, CAPES, CNPq.

BC45 - ULTRASTRUCTURAL ASPECTS OF SECRETORY ORGANELLES OF *TOXOPLASMA GONDII* DURING HOST CELL INVASION

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Toxoplasma gondii must invade a host cell for survival and replication. Successive active invasion depends on the apical complex that includes specialized secretory organelles (rhoptries and micronemes) and cytoskeletal structures (polar rings and conoid). *T. gondii* invasion is a quick, dynamic process resulting from the sequential secretion of micronemes and rhoptries. We have followed the initial steps of this process by multiple approaches that included transmission and field emission scanning electron microscopy, 3-D reconstruction from serial sections, selective detection of basic proteins. Our experimental model employed the RH strain of *T. gondii* obtained from 48 hour infected mice. Mouse peritoneal macrophages and monolayers of LLC-MK2 were used as host cells in a 10:1 parasite-host cell ratio. Interaction was for 15 min at 4^{o}C min and 3min at 37 ^{0}C. Invading parasites had the conoid extruded. Transmission electron microscopy showed in some instances empty rhoptries, and small membrane bound vesicles inside the conoid. Basic proteins, detected by Phosphotungtic acid, were present in the neck of rhoptries and in micronemes, as well as dense granules. 3-D reconstruction from serial sections showed that the host cell membrane made contact with the tachyzoite leaving a space between the conoid and the cell surface. FE-SEM showed a ruffling of the host cell membrane at the point of entrance. These observations indicate that active invasion by the parasite does not exclude host cell membrane reorganization. Active endocytosis by host cells was also observed.

Supported by: Conselho Nacional de Desenvolvimento Científico e Tecnológico- CNPQ and Fundação Carlos Chagas Filho de Apoio à Pesquisa do Rio de Janeiro- FAPERJ

BC46 - COMPARATIVE STUDY OF CELL INVASION MECHANISMS OF *TRYPANOSOMA DIONISII* AND *TRYPANOSOMA CRUZI* STRAINS OF GENETICALLY DIVERGENT LINEAGES

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Trypanosoma dionisii, isolated from bats, is phylogenetically related to *Trypanosoma cruzi*, the agent of Chagas' disease. Although it is known that *T. dionisii* can invade cultured mammalian cells, there is no information on the mechanisms involved in that process. Here we aimed at clarifying the issue by performing a comparative analysis of metacyclic forms of *T. dionisii* and two *T. cruzi* strains (G and CL), which belong to different phylogenetic groups and induce distinct signaling pathways to enter target cells. Invasion assays using HeLa cells showed that, similarly to G strain but in contrast to CL strain, *T. dionisii* invaded cells poorly in the presence of serum. In PBS^{++} (PBS supplemented with Ca^{2+}, Mg^{2+} and K^{+}), the rate of invasion of *T. dionisii* and G strain metacyclic forms was y increased. The signals induced in *T. dionisii* during invasion, in PBS^{++}, were similar to those of CL strain, with activation of protein tyrosine kinase and phosphoinositide 3 kinase. Western blot analysis using antibodies against CL strain metacyclic forms revealed a protein of ~82 kDa in *T. dionisii*, which may be related to the invasion-promoting surface molecule gp82 of CL strain. On the other hand, anti-*T. dionisii* antisera failed to recognize *T. cruzi*. Co-infection with *T. dionisii* and enteroinvasive *Escherichia coli* (EIEC), a strategy used to assess the involvement of host cell actin cytoskeleton, resulted in reduced HeLa cell invasion, a pattern similar to that of CL strain. Experiments to determine the signaling cascades triggered in the host cell during *T. dionisii* invasion are in progress. Supported by FAPESP.

BC47 - *T.CRUZI*-INFECTED AND INTERFERON-γ-ACTIVATED PERIPHERIC GLIAL CELLS PRODUCE NITRIC OXIDE *IN VITRO* SIMILARLY TO MACROPHAGES

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INTRODUCTION AND OBJECTIVES Neuronal lesions and peripheral denervation in Chagas' disease are related to local inflammation; however, the pathogenic mechanisms are still unclear. We have previously demonstrated that iNOS-derived NO production by *T.cruzi*-infected and interferon-γ (IFN-γ) activated macrophages is the major molecule involved in *in vitro* neuronal lesions. However, the role of NO production from *T.cruzi*-infected and IFN-γ activated glial cells on neuron damage has yet to be studied, specially considering its potential participation in many neurodegenerative diseases. The objective of this work was to establish primary peripheric glial cell cultures in order to access their parasitism and NO production after *T.cruzi* infection and IFN-γ activation in comparison to macrophages. We suggest a role for glial cells in chagasic neuronal lesions. RESULTS Flat and irregular shaped glial cells formed a confluent monolayer culture and showed GFAP-imunnopositivity, a glial cell marker. *T.cruzi*-infected and IFN-γ activated glial cell cultures showed a greater quantity of parasitized cells than macrophage cultures under the same conditions. iNOS immunopositivity was present in both cultures, but in lower intensity in glial cell cultures. Furthermore, *T.cruzi*-infected and IFN-γ activated glial cell cultures showed high NO production in comparison to controls, but no difference was detected between glial cell or macrophage cultures under the same conditions. Interestingly, in LPS and IFN-γ activated glial cell cultures the nitrite production did not differ from controls and were lower than the NO production by macrophages after LPS and IFN-γ activation. CONCLUSIONS Since glial cells are capable of producing NO after *in vitro T.cruzi* infection and IFN-γ activation, we could suggest a role for these cells in the pathogenesis underlying Chagas´ disease neuropathology. We could also imply that iNOS activation and regulation mechanisms in glial cells may differ from the ones in macrophages depending on the stimulus.

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BC48 - EFFECTS OF BLOCKADE OF ENDOTHELIN RECEPTORS ETA AND ETB ON THE CYTOKINE AND NITRIC OXIDE SECRETION PATTERN IN MACROPHAGES INFECTED WITH *TRYPANOSOMA CRUZI*

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Endothelin is involved in the vascular dysfunction and cardiomyopathy observed in Chagas' disease. Previous studies in our laboratory suggest that expression of endothelin could be related to a cascade of events involved in the initial control of the infection. We have also demonstrated that treatment with endothelin receptors antagonist affects both the inflammatory process and parasitism in heart and skeletal muscle of rats infected with *T .cruzi*. Now we are interested in the effects of endothelin on macrophages, key cells in the control of parasitism and maintenance of vascular tonus. Rat peritoneal macrophages were treated with bosentan and/or Endothelin and then infected with Y strain of *T. cruzi*. The infection rate and the number of intracellular parasites after 24 hours of infection were evaluated, as well as the secretion of cytokines and nitric oxide. Endothelin treatment reduces the production of nitric oxide (NO), tumor necrosis factor (TNF-α) and interleukin-10 (IL-10). The proportion of modulatory/inflammatory cytokines were significantly altered by the treatment, inducing macrophages inflammatory profile. Our findings suggest that Endothelin may have a role in the control of host response to *T. cruzi* infection. This work was supported by CNPq and FAPEMIG.

BC49 - INTERACTION BETWEEN *TRYPANOSOMA CRUZI* AND PERITONEAL MACROPHAGES: MORPHOLOGICAL ANALYSIS AND INVOLVEMENT OF PI3 KINASE.

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Trypanosoma cruzi, an intracellular protozoan parasite, is the etiological agents of Chagas' disease. The trypomastigote form of *T.cruzi* is infective and invades diferent host cell types. How trypomastigote forms interact with the host cell and signaling to gain entry and survival in is not completely understood, but evidence suggests that *T.cruzi* interacts with different signaling systems of the host cell. Todorov *et al* (2000) and Vieira et al (2002) showed that *T.cruzi* infection activates host PI3 kinase (PI3K), since the PI3K inhibitor wortmannin strongly blocked *T. cruzi* infection. Using scanning electron microscopy, we observed the interaction between Wortmanin or LY294002 non treated and treated macrophages and trypomastigotes of *T.cruzi*. We show important morphological differences between control and drug-treated cells. While epimastigotes utilize the anterior region of the body to entry into macrophages, trypomastigotes penetrate using both parts of the body, but in most cases the posterior region was involved. In interaction with epimastigotes, we always observed that part of the parasite's body is recovered with a tubular structure originated from the macrophage's plasma membrane or with a coiling structure of host cell plasma membrane origin. Such a process is very rare with trypomastigotes. We used Lucifer Yellow to label macrophage's lysosomes and wortmanin as PI3K inhibitors. Inhibition of PI3K reduced by 50% the fusion of macrophage's lysosomes with the site of parasite internalization. Treatment of the macrophages with PI3K inhibitors also interfered with the process of trypomastigote internalization. Under control conditions about 30% of the parasites enter through the anterior region while after inhibition of PI3K this percentage increased to 65%. Inhibition of PI3K also interfered with the process of invasion. Internalization where a tubular structure formed around the parasite, as described for epimastigotes, was also seen during invasion of trypomastigote into drug-treated macrophages. Supported by CNPq, Faperj and CAPES.

BC50 - INTERACTION OF MURINE MACROPHAGES WITH SUBPOPULATION OF *Toxoplasma gondii* THAT EXPOSE OR NO PHOSPHATIDILSERINE

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Phosphatidylserine (PS) exposure is a main event that indicates apoptosis. This exposure is fundamental for the Transforming Growth Factor-beta1 (TGF-b1) signaling that induced an anti-inflammatory response during phagocytosis of apoptotic cells. Some protozoan parasites expose PS, inhibiting macrophage inflammatory activity by mimicking the uptake of apoptotic cells. Toxoplasmosis is a worldwide disease caused by *Toxoplasma gondii*. Activated macrophages control *T. gondii* growth by nitric oxide (NO) production. However, *T. gondii* active invasion inhibits NO production, allowing parasite persistence. Our group showed that the mechanism used by *T. gondii* to inhibit NO production persisting in activated macrophages is similar to what Leishmania uses depending on PS exposure. In this work were realized interactions with isolated population of *T. gondii* and murine macrophages for analysis of penetration mechanism and survival of parasite. For this, *T. gondii* PS+ and PS- subpopulation were separated with annexin V conjugated magnetic beads. After the isolation interactions were realized for 1, 24 and 48 hours with macrophages. Results by Scanning Electron Microscopy shown that PS+ population of *T. gondii* invade macrophages by active penetration, but PS- population invades by a phagocytic mechanisms. The infection index and multiplication parameters shown that *T. gondii* PS+ as PS-, were slow when compared with the total population of parasite. The results suggest that PS+ and PS- population of *T. gondii* do invade macrophages with different forms and the growth of parasite depend on the total population.

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BC 51 - SECRETED *TOXOPLASMA GONDII* PROTEINS ALTERS MACROPHAGE YEAST PHAGOCYTOSIS

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Toxoplasma gondii is a protozoan parasite of the phylum apicomplexa, that causes toxoplasmosis. This disease can be congenital or acquired and affects about one billion people around the world. The protozoan is able to invade and multiply in cells of the immune system such as macrophages. During the invasion by *T. gondii* various secreted proteins are necessary for penetration, survival and multiplication. To determine whether these proteins may modulate the phagocytic capacity of macrophages, these cells were cultured with conditioned media by *T. gondii* obtained from the peritoneal cavity of mice. It was observed that the conditioned media inhibited phagocytosis of yeast. However, the active factor was from the peritoneal cavity of mice that were adsorbed at the parasite surface. Thus, to determine whether the phagocytic modulator factor has parasite components, a conditioned medium of parasites obtained from infected VERO cells culture was tested. Parasite concentrations of 3x107 or 1x108 parasites/ml obtained from cultures of VERO and from the peritoneal cavity of mice (control) were used to condition DMEM for 24 hours. After macrophage cultures, modulation of yeast phagocytosis was evaluated. Preliminary results indicate that the conditioned medium by parasites obtained from cell cultures also reduce the phagocytic capacity of macrophages. This result indicates that conditioned medium is a good way to assess factors secreted by *T. gondii*.
Supported by: FAPERJ, CNPq

BC52 - PROTEOMIC SCREENING OF MOLECULES EXPRESSED ON *LEISHMANIA MAJOR*- OR *LEISHMANIA AMAZONENSIS*-INFECTED MACROPHAGES

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CBA/J mice are resistant to *L. major* and susceptible to *L. amazonensis* infection. Also, CBA/J macrophages control *L. major* infection and are permissive to *L. amazonensis*, suggesting an important role for macrophages on determine *Leishmania* infection outcome. Next, using short oligonucleotide microarray analyses we showed that *L. amazonensis*- and *L. major*-infected macrophages express different sets of genes related to the early host cell-*Leishmania* interaction and to the immune-inflammatory response. Microarray analysis does not provide information about several important modulations occurring during the infection, which is only seen at proteome level. With a proteomic approach we compare 6 and 24 hours of *L. major* and *L. amazonensis* infection on CBA/J macrophages. Protein extracts were obtained to characterize peptides by LC-MS/MS in a MudPIT approach. Only 62 proteins were exclusive of *Leishmania* infection. However, 162 proteins were modulated on macrophages infected by *L. amazonensis* in comparison to *L. major*-infected cells. These proteins were ranked from A to C based on the quality of differences on the expression during *Leishmania* infection. Sixteen proteins were ranked as class A, 48 as class B and 98 as class C. The class A proteins are involved in phagocytosis, cytoskeleton rearrangement, oxidative stress metabolites production and apoptosis. Current western-blot analyses are now been performed to corroborate the differences on protein expression observed in proteomic studies. Taken together, the data point out to a pivotal role for the parasite on determining the subsequent immune response and course of infection. The identification of protective or susceptible markers of infection could help design of new therapeutic drugs against leishmaniasis. Financial support: CAPES & FAPESB

BC53 - INDUCTION OF AUTOPHAGY INFLUENCE THE INITIAL CONTACT BETWEEN *LEISHMANIA AMAZONENSIS* AND MACROPHAGE

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Autophagy is essential for regulation of cell growth, maintenance of homeostasis and energy generation during cell stress. The capacity of autophagy to interfere on intracellular survival of pathogenic microorganisms has been described. It was recently demonstrated that autophagy may contribute to control of bacterial infections. In addition, few studies evaluated the autophagic phenomenon in protozoa infection. It was demonstrated that *L. mexicana* can acquire nutrients by microautophagy. Since *L. amazonensis* is the same complex of *L. mexicana*, our hypothesis is that autophagy contributes to establishment of *L. amazonensis*-infection. This study aims to analyze the influence of autophagy in CBA/J mouse macrophage infected by *L. amazonensis in vitro*. Macrophages were infected by *L. amazonensis* or exposed to yeast, previously or after autophagy induction. The previous induction of autophagy was performed by nutrient deprivation or by rapamycin treatment. Both methods reduced the macrophage phagocytic capacity. This effect is independent on macrophage or mouse strain origin. In addition, blockage of autophagyc stimulus partially recovers the macrophage phagocytic capacity. These data suggest there is a communication between autophagyc and phagocytic pathways. Furthermore, there was an enhancement in zymosan-FITC binding to phalloidin-labelled cells, previously subjected to nutrient deprivation at 4^{o}C. These data indicate that phagocytosis inhibition is a direct consequence of autophagy and occurs in a downstream step after particle binding. All together, these data show that the reduction on phagocytic capacity of macrophages due to a previously autophagyc induction is a general mechanism of macrophage cell biology. Moreover, the induction of autophagy after infection contributes to a discrete reduction in the percentage of *L. amazonensis*-infected CBA/J macrophages. The data herein suggest the role of autophagy in reducing the macrophage phagocytic capacity and the intracellular *L. amazonensis* survival. More studies are necessary to understand the uncommon molecular mechanisms that link phagocytosis and the autophagyc process.

BC54 - HISTOPATHOLOGICAL ASPECTS OF EAR MODEL OF *L. MAJOR* INFECTION

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Experimental murine *Leishmania major* infection implied the Th1/Th2 balance as determinants of resistance or susceptibility to the parasite. A model using low parasite dose into ear skin has been established but still not systematically studied. We describe histopathological aspects of lesions in susceptible (BALB/c) and resistant (C57BL/6 and IL-4 deficient – IL-4 KO) mouse strains along 12 weeks of infection with *L. major* by microscopy and immunohistochemical methods. Significant differences in size of lesions were observed among lineages. IL-4 KO showed very discrete lesions along the experimental period. Histomorphometric confrontation was done between BALB/c and C57BL/6. Moderate infiltrations of mononuclear and polymorphonuclear leukocytes were distributed similarly in the strains from 6 hours to 1 week of infection. After 4,5 weeks mononuclear monocytes were the predominant cells type observed. Morphometrical studies did not indicate differences in the cellular composition among lineages. Mast cells inside the lesions exhibited extensive degranulation from 6 to 24 hours p.i, but there were no differences in mast cells numbers/mm² over time among lineages. Parasites were not detected by immunohistochemistry at the site of inoculation in any lineage before 4,5 weeks and persisted in C57BL/6 up to 12 weeks, even after healing, concomitantly with high levels of expression of i-NOS. Parasitized macrophages were less frequent in C57BL/6 than in BALB/c. The parasite number/mm² increased in BALB/c along the examined period. In contrast, the expression of iNOS increased in C57BL/6. Histopathological patterns of tissue response are characteristic of each lineage and the size of lesions varied drastically among lineages. Mast cells do not influence the differential response to low dose *L. major* infection. Considering the IL-4 KO resistance to development of lesions in this model, we can

suggest that susceptibility is partially determined by IL-4 although a role for IL-10 has yet to be investigated.

Support: FAPEMIG(CBB-1048/05), CAPES, CNPq.

BC55 - REACTIVE OXYGEN INTERMEDIATES PRODUCTION DURING INNATE IMMUNE RESPONSE OF CBA/J MICE MACROPHAGES INFECTED BY *LEISHMANIA MAJOR* OR *LEISHMANIA AMAZONENSIS*

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CBA/J macrophages control *L. major* infection and are permissive to *L. amazonensis*, indicating an important role for macrophages on determination of *Leishmania* infection outcome. It is well known that some *Leishmania* spp. are destroyed by reactive oxygen intermediates (ROI) inside macrophages. *Leishmania* could also inhibit ROI production by those cells. Our proteomics analyses of CBA/J macrophages infected by *L. major* compared to *L. amazonensis* identified higher expression of catalase and anti-oxidative enzymes, such as glutathione-S-transferase, in response to *L. major*. We hypothesized that although CBA/J macrophages liberate ROI in response to either *L. major* or *L. amazonensis* infection, only *L. amazonensis* is resistant to these radicals. Using a high sensitive approach based on photon counts of lucigenin/luminol-enhanced chemiluminescence, we detected superoxide and hydrogen peroxide production during phagocytosis of *L. major* or *L. amazonensis*. It was observed that *L. major* induced a higher superoxide production when compared to *L. amazonensis*-infected cells. In addition, *L. major* also induces higher levels of hydrogen peroxide production by CBA/J macrophages when compared to *L. amazonensis*-infected cells. These results suggest that CBA/J macrophages control *L. major* infection by a mechanism dependent on ROI production by host cell. Current experiments goals to evaluate the mechanisms related to ROI effects on the reduction of *L. major* parasitism inside macrophages and the mechanism of *L. amazonensis* resistance to CBA/J macrophages' killing mechanisms. Financial support: CAPES & FAPESB ET64/2004; 5742/2006.

BC56 - INCREASED *IN SITU* EXPRESSION OF IL-17 AND IL-6 IS ASSOCIATED WITH THE DEVELOPMENT OF MUCOSAL LEISHMANIASIS

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Human infection with *Leishmania braziliensis* may lead to cutaneous (CL) or mucosal (ML) clinical forms. Previous studies have shown that ML is associated to an exacerbated inflammatory response, possibly due to a deficient expression of the IL-10 receptor. A number of recent studies have shown that Th17 cells play critical role in the pathogenesis of autoimmune and inflammatory diseases. Our hypothesis is that IL-17 expression is associated with the intense inflammatory response observed in human leishmaniasis. Thus, we determined the expression of IL-17 and IL-6, an inflammatory cytokine associated to the development of TH17 cells, in biopsies from CL and ML patients, using confocal microscopy. Our analysis showed a higher number of IL-6+ cells in lesions from ML as compared to CL. Not only the frequency of IL-6+ cells was higher but also the intensity of expression of this cytokine was increased in ML lesions. Although the expression of IL-17 was similar among ML and CL lesions, the frequency of expression of this cytokine was high in CL and ML lesions: approximately 40% of total the cells and 70% of the CD4+ T cells expressed IL-17. Further analysis showed that while in ML lesions $CD4^+$ cells are responsible for 76% of the total IL-17 expression, in CL lesions this population contributes to approximately 65% of the IL-17 production. Also, the intensity of expression of IL-17 was higher in ML as compared to CL lesions. Interestingly, a statistically significant positive correlation was seen between total the IL-17+ cells and the intensity of the inflammatory infiltrate in lesions from CL but not ML patients. This data show that TH17 cells are present in CL and ML lesions and that a greater expression of IL-6 and IL-17 is associated to ML, suggesting a role for these cytokines in the pathogenesis of human leishmaniasis.

Financial support: CAPES, CNPq, TDR/WHO

BC57 - INFLUENCE OF INHIBITORS OF SERINE PEPTIDASES OF *Leishmania major* IN THE EARLY STAGE OF INFECTION.

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Bacterial ecotins are potent competitive inhibitors of Clan PA, family S1 serine peptidases such as trypsin, cathepsin G and neutrophil elastase (NE). Three ecotin-like gens, ISP1, ISP2 and ISP3, were identified in the genome the *Leishmania major*. However, no Clan PA serine peptidases are encoded in the Leishmania genome, which raises the possibility that the inhibitors modulate the activity of host enzymes. *L. major ISP2* and *ISP3* double null mutants (*Δisp2/3*) were generated by homologous recombination and these parasites were used to analyse different parameters of the host-parasite interaction. *Δisp2/3* mutants were internalized by mice peritoneal macrophages more efficiently than wild type, a phenotype that was reversed by the re-expression of both genes in the mutant. The uptake of *Δisp2/3* resulted from the up-regulation of the phagocytic activity of macrophages by a mechanism dependent on serine peptidase activity and mediated by the complement type 3 receptor (CR3). The amastigotes does not divided up to 48h, but after this period cellular growth was reinstated. *Δisp2/3* mutants were more infective in BALB/c in the three first days of infections, but infection progressed was similar to the wild type. Intracellular growth in the macrophages by *Δisp2/3* was equivalent to wild type levels upon addition of aprotinin, recombinant ISP2 (rISP2), suggesting that defective intracellular growth results from the lack of serine peptidase inhibition during parasite uptake. The ability of the transgenic parasites to cause lesions in susceptible mice was analysed by injection of promastigotes in the footpad. We observed that lesions provoked by *Δisp2/3* developed similarly to those caused by wild type during 40 days, but were significantly reduced thereafter. Collectively, our data suggest that ISPs play an important role in early stages of infection of the mammalian host.

BC58 - Apoptotic metacyclic promastigotes are present in the sand fly gut and play a central role in the infectivity of Leishmania amazonensis parasites.

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Introduction: In amastigotes of *Leishmania (L) amazonensis* (La), phosphatidylserine (PS) exposure and its consequent recognition by macrophages mediates parasite internalization and modulates macrophage inflammatory activity. This mechanism has been quoted as apoptotic mimicry since those parasites are viable and do not display other morphological markers of apoptosis. We evaluate whether metacyclic promastigotes, which are infective for the mammalian host, employ this mechanism of macrophage deactivation and infection.

Results: Metacyclic promastigote forms, which differentiate in the midgut of the sand fly vector and initiate the disease in mammalian hosts, comprise two clear subpopulations: PS^{pos} and PS^{neg} parasites. Both phenotypes can be found in axenic cultures and in promastigotes purified from sand flies. Similarly to what happens with amastigote infection, PS^{pos} metacyclic promastigotes are capable of down modulating the microbicidal activity of the macrophage. They are indispensable, but not sufficient, for the establishment of an efficient infection *in vitro* and *in vivo* due to their capacity to establish a cooperative relationship with PS^{neg} metacyclics, which are the truly infective forms. Different from amastigotes, PS^{pos} metacyclic forms display several markers of apoptotic death such as DNA nucleosomal degradation, positive TUNEL staining and characteristic morphological features. The ultrastructure of PS^{pos} parasites in the foregut of infected sandflies or shows several signs of death. Addition of caspase inhibitors to the culture reduces PS exposure by those parasites,

indicating a role for parasite proteases in the induction of apoptotic death.

Conclusion: Our results demonstrate that although PS exposure by amastigotes and metacyclic promastigotes are both able to deactivate macrophage leishmanicidal capacity and thus playing a role in the establishment of infection, PS^{pos} metacyclics are undergoing apoptotic death instead of apopototic mimicry as described for amastigotes.

Financial Support: CAPES, INCa/MS, FAPERJ

BC59 - CANINE EXPERIMENTAL INFECTION WITH BE-62 TRYPANOSOMA CRUZI STRAIN: A PRELIMINARY HISTOLOGICAL STUDY OF LYMPH NODES OF DOGS DURING THE ACUTE AND CHRONIC PHASES.

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Trypanosoma cruzi (Kinetoplastida: Trypanosomatidae), the agent of Chagas disease, is a widely distributed trypanosomatid. It has been detected in more than 100 mammalian species, belonging to eight orders, which are spread through all phytogeographic regions of the Neotropics. Domestic canines in South and Central America are considered an important reservoir in the urban Chagas disease transmission cycle, and serve as surveillance sentinels for human infection. The general objective of the present study is analyze the lymph nodes histological alterations during acute and chronic phases of dogs experimentally infected with Be-62 *T. cruzi* strain. Ten no infected and twenty nine dogs infected with 2000 metacyclic trypomastigotes by conjunctival route were evaluated. In the infected group, twenty were euthanized on acute phase and nine in the chronic phase. After necropsy, lymph nodes were collected and fixed in buffer formalin and processed for histopathological analyses. Four micrometers paraffin sections were obtained, H&E stained and analyzed by optical microscopy. The presence or absence of thickness and capsule inflammation, the cortical follicular hyperplasia, the hypertrophy and hyperplasia of macrophages of the medullary cords and sinus, edema, congestion, hemosiderosis and hemorrhagic process were evaluated. No differences were observed between the acute and chronic phase tissue damage. However the hypertrophy and hyperplasia of macrophages of the medullar area and the cortical follicular hyperplasia was higher in infected animals than no-infected ones. The general histopathological picture of all lymph nodes showed a diffuse inflammatory reaction. The observed cellular exudate was composed mainly by plasmocytes, macrophages and lymphocytes and easily observed the medullar cords and the medullar sinus and peripheral sinus subjacent to the capsule. We are looking forward to carry out a morphometrical and parasitological studies to correlates the observed alterations and clinical, immunological and parasitological aspects of the Chagas disease.

Sponsors: FAPEMIG, CNPq and UFOP

BC60 - *Trypanosoma cruzi* release plasma membrane-derived vesicles (PMVs) to avoid lysis by the complement system.

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Trypanosoma cruzi, the agent of Chagas's disease, has to survive the attack of the complement system to infect the host cells and cause the disease. The complement system when triggered by these parasites initiates a cascade resulting in the deposition of a membrane attack complex (MAC) on the parasite surface leading to lysis. The complement system can be activated by the classical pathway, when immunoglobulins bind on the parasite surface; the lectin pathway, when serum lectins (MBL and ficolins) bind on the parasite surface; and the alternative pathway, when C3 factor binds on the parasite surface. *T. cruzi* metacyclic forms avoid the complement lysis by expressing molecules that prevent MAC formation; for example, CRP (Complement Regulatory Protein) and DAF (Decay Accelarating Factor) that dissociate C3 and C4, and CRIT (Complement C2 Receptor Inhibitor Trispaning) that binds C2. We have detected that concentrations of 20% and 40% NHS (Normal Human Serum) induce *T. cruzi* metacyclic forms to

release PMVs. Kinetic studies of PMV release show that parasites start to vesiculate after 10 minutes when incubated with NHS 20% at 37°C. The vesicles have sizes between 100-500 nm as detected by electron microscopy. The complement factors DAF and CRIT were detected on the surface of the vesicles by flow cytometry. Vesicles released by NHS stimuli have the complement factors MBL, H-ficolins, L- ficolins and C2 associated with the surface. The results indicate that *T. cruzi* metacyclic forms vesiculate to eliminate complement factors deposited on the surface as a late mechanism to avoid the lysis.

BC61 - *Trypanosoma cruzi* infection alters cardiac caveolin-3 expression

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Caveolae are plasma membrane invaginations enriched in cholesterol, sphingolipids, and the marker protein caveolin (cav). Caveolae and cavs are implicated in transcytosis of macromolecules, cholesterol transport and signal transduction. Knock-out mice for cav-3 (muscle specific) develop progressive cardiomyopathy with ventricular wall thickening and increased levels of the extracellular signal-regulated kinase (ERK 1/2), which is involved in cardiac remodeling. Infection with *Trypanosoma cruzi* leads to acute myocarditis and chronic cardiomyopathy. Acutely infected mice displayed decreased mRNA levels of cardiac caveolins and increased mRNA levels of ERK. Interestingly, *T. cruzi* decreases phosphorylated ERK (pERK) in human fibroblasts, but the infection of endothelial and smooth muscle cells increases pERK expression. Since cardiac myocytes are important target of *T. cruzi* in the *in vivo* infection, we studied the effect of the infection in caveolin-3 and pERK expression in cultured cardiac myocytes and hearts of chronically infected mice. Infected cultures displayed reduced caveolae and caveolin-3 abundance at 72hr post infection (hpi) as revealed by electron microscopy and confocal microscopy. Western blot analyses revealed unaltered cav-3 levels in initial times of infection and decreased (50%) protein levels after 48-72hpi. pERK expression remained unaltered during the *in vitro* infection. We observed progressive decrease in cav-3 levels in the *in vivo* infection, resulting in 50% decrease after 60 and 90 days of infection. Our data indicate that infected cardiac myocytes do not contribute to increased pERK observed in the *in vivo* infection. Moreover, the decreased expression of muscle-specific caveolin-3 induced by *T. cruzi* may be involved in the development of the chagasic cardiomyopathy.

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BC62 - Inactive *trans*-sialidase compromises T cell migration to heart of *Trypanosoma cruzi* infected mice

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trans-Sialidase from *Trypanosoma cruzi* is the product of a multigene family encoding both active and inactive proteins. We have demonstrated that despite lacking enzymatic activity due to a single mutation, Tyr342→His, the inactive *trans*-sialidase (iTS) behaves as a lectin, binding to sialic acid with identical specificity to that of its active analogue (aTS). aTS have been implicated in the virulence of *T. cruzi*, however, no study has demonstrated the role of iTS during *T. cruzi* infection. Here we studied the effect of iTS on T cell homing during *T. cruzi* infection. Mice were treated with iTS and infected with blood trypomastigotes from Y strain. Parasitemias were evaluated at days 6-10 post-infection (pi) and the hearts examined at day 15 pi. We have observed that iTS inhibits T cell adhesion to activated endothelial cells *in vitro*, besides iTS reduces the number of $CD4^+$ and $CD8^+$ T cell subsets in the cardiac tissue from *T. cruzi*-infected mice. In agreement, there was a significant reduction in creatine kinase activity in the sera of mice from iTS group with a concurrent delay in the life time of infected mice treated with iTS when compared to infected mice treated with PBS. Together, these results indicate that, inactive members from TS family are involved in the recruitment of circulating leukocytes to target sites and play a major role in the inflammatory response during *T. cruzi* infection, contributing to the pathogenesis of chagasic

disease. Supported by CNPq, FAPERJ, The Millennium Institute for Vaccine Development and Technology (420067/2005-1).

BC63 - EVALUATION OF TISSUE EOSINOPHILIA IN MICE IMMUNIZED WITH A *Trypanosoma cruzi-like* STRAIN AND INFECTED WITH *Trypanosoma cruzi*

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The granular components of eosinophils are cytotoxic to *Trypanosoma cruzi* trypomastigotes and amastigotes and to several cell types of the organism, revealing either their role in the parasite elimination or in the production of tissue lesions. We aimed to evaluate factors responsible for the tissue eosinophilia increase in mice immunized with a *T. cruzi-like* strain and infected with *T. cruzi* strains. Non-isogenic mice were divided into 16 groups that received from zero to three inoculations of *T. cruzi-like* RM1 strain (isolated from bat and not able to infect mice), in the presence or absence of adjuvant, followed by challenge with VIC or JG strains of *T. cruzi*. Uni- and multivariate associations were performed between the severity of tissue eosinophilia and the immunization strategies induced by the *T. cruzi-like* strain (adjuvant, reinoculations and parasites), likewise the peak of parasitaemia and the severity of inflammatory process. Regarding the univariate analysis, the tissue eosinophilia was higher in immunized animals, without significant differences. Nevertheless, there was a significant association between the tissue eosinophilia and the number of inoculations with *T. cruzi-like* strain. The presence of eosinophils was higher in mice which received adjuvant and in those infected with JG strain. In addiction, there was an augmentation of muscular lesions (myositis) accompanied by a greater tissue eosinophilia and a positive correlation among the eosinophilia intensity and parasitaemia peak. The multivariate analysis showed that the following variables, peak of parasitaemia, number of inoculations with *T. cruzi-like* strain and myositis severity, were decisive as a predictive factor of tissue eosinophilia, in comparison with adjuvant and challenged *T. cruzi* strains. Tissue eosinophilia proved to be an important parameter in the pathogenesis of experimental Chagas disease and might be related to reinfections, parasite multiplication ability and severity of inflammatory process. **Financial support:** CAPES, CNPq, UFTM.

BC64 - HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL PATTERNS OF GENITAL ORGANS OF NATURALLY *LEISHMANIA INFANTUM* INFECTED BITCHES

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The canine visceral leishmaniasis is a zoonotic and systemic disease caused by the protozoan *Leishmania infantum*. During infection, various host organs are affected including: liver, spleen, bone marrow and skin. Recently parasites were detected in testicles, epididymis and prepuce. The aim of this study is examine the presence of amastigote forms of *Leishmania infantum* and the histopathological changes in the genital system of bitches with canine visceral leishmaniasis. Thirteen female dogs in diestrus/anestrus with positive parasitological test by bone marrow and/or lymph nodes cytology were selected. Samples from cervix, vulva, vagina, uterus, uterine tubes, and ovaries were processed for histological analysis and immunodetection of *Leishmania* sp. It were observed a discreet to moderate nonspecific chronic folicular cervicitis (23%), vulvovanitis (7.7%) and salpingitis (7.7%). Amastigotes were not visualized in the sections. Base on our results, we suggested a possible tissue resistance of female genital tract to *Leishmania* sp. Infection at these fases of estrous cycle.
Supported by: PROBIC/PUC Minas.

BC65 - HIF-1ALPHA: HYPOXIA-INDUCIBLE FACTOR OR LEISHMANIA-INDUCIBLE FACTOR?

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Hypoxia is the low oxygen partial pressure (pO_2) present in diseased tissues. Hypoxia-inducible factor-1α (HIF-1α) is expressed in response to hypoxia and has been demonstrated in a variety of cells such as tumor cells and tumor-associated macrophages. Increasing evidence indicates tha HIF-1α can be upregulated by nonhypoxic stimuli such as growth factors, cytokines, nitric oxide and lipopolysaccharides. In this study we evaluated HIF-1α expression in the cutaneous lesions of BALB/c mice during *Leishmania amazonensis* infection and in mouse macrophages parasitized *in vitro*. Using the hypoxia marker pimonidazole, we also evaluated the presence of hypoxia in the lesions and infected macrophages *in vitro*. In mice, during the early stages of infection (3 and 20 days), HIF-1α is downregulated and pimonidazole staining is negative. In contrast, HIF-1α is overexpressed in the later stages of infection (70 and 150 days), when the size lesion is maximal, parasite burden is enormous and massive numbers of recruited macrophages and ulcers are observed. The upregulation of HIF-1α is related to hypoxia in the lesions, as indicated by pimonidazole adducts. In mouse macrophages parasitized in vitro during 24 h, although HIF-1α staining is positive mainly in the nucleus, pimonidazole staining is negative. Taken together, these results demonstrated that infection with *L. amazonensis* did not lead to a substancial decrease in oxygen concentration within macrophages. Hypoxia is a consequence of several characteristics of leishmania lesions at later stages, such as microcicurlation impairment, proliferating amastigotes, secondary bacterial infection and increased metabolic demand. We suggest that during first hours of infection macrophages express HIF-1α independently of hypoxia, as a host cell response to the presence of the parasite. When the infection is established HIF-1α is downregulated in the lesions. The leishmanial lesions become a hypoxic microenvironment with time, which is the later stimulus to HIF-1α activation.

Supported by FAPESP, CNPq.

BC66 - EXPRESSION OF HYPOXIA-INDUCIBLE FACTOR-1α IN THE RUMP LESIONS OF BALB/c MICE INFECTED WITH *Leishmania amazonensis*.

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Hypoxia-inducible transcription factor-1 (HIF-1α) is a major regulator of energy homeostasis and cellular adaptation to low oxygen stress (hypoxia). It has been recently demonstrated in macrophages from healing wounds and in alveolar macrophage-derived cell line stimulated by the tumor necrosis factor, nitric oxide or lipopolysaccharides. Several characteristics of leishmanial lesions in human and in animal models, such as microcirculation impairment, metabolic demand for leukocyte infiltration into infected tissues, parasite proliferation, and secondary bacterial infection are strong indications of a hypoxic microenvironment that may play a role in the outcome of infection. Recently our group reported that HIF-1α is expressed in the footpad lesions of BALB/c mice during *Leishmania amazonensis* infection. HIF-1α was expressed only in the later stages of infection and mainly in the cytoplasm and around parasites inside the parasitophorous vacuoles of macrophages. Although many works has evaluated footpad lesions induced by *Leishmania*, rump lesions had many similar characteristics to human cutaneous leishmaniasis. In this work we have performed analyses of HIF-1α expression by immunohistochemistry in rump lesions of BALB/c mice. These lesions are different from footpad lesions because there is no massive numbers of vacuolated and heavily parasitized macrophages. Histological analyses revealed that in the early stage of infection, at 6 and 10 weeks, the epidermis and glandular structures in rump lesions are conserved. In this time the dermis contained leishmania amastigotes, intense inflammatory cellular infiltrate with infected macrophages and have no HIF-1α immunostaining. In the later stages of infection by 16 and 20 weeks, the lesions had an extensive cellular infiltrate with massive numbers of recruited mononuclear cells, and positive HIF-1α-infected macrophages throughout the lesion. These data suggest that expression of HIF-1α protein is time-dependent in cutaneous murine leishmaniasis when ulcerative and necrotic areas with bacterial contamination beginning were observed. Supported by CAPES and FAPESP.

BC67 - ACTIVATION OF APOPTOSIS CASCADE BY PAC-1 ON PROMASTIGOTES OF *LEISHMANIA (LEISHMANIA) AMAZONENSIS*

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Putt and collaborators (Nature Chem. Biol. 2006) reported the property of PAC-1 to transform procaspase-3 into active caspase-3, the main executioner of apoptotic death. PAC-1 acts by disabling an Asp-Asp-Asp (DDD) sequence, which protects procaspase-3 from autocatalytic maturation or activation by upstream caspases. Analyzing sequences of metacaspases from different *Leishmania* spp, we noticed that DDD sequences are conserved, in this molecule, in all the studied species. Metacaspases are present in plants, fungi and protozoa, bearing the predicted secondary structure and the catalytic dyad histidine/cysteine characteristic of caspases. These findings prompted a search for the role of metacaspases in apoptotic death of unicellular parasites, which in spite of all the efforts, still remains unclear. A role in leishmanial cell-cycle control has been described. In leishmanial metacaspases, DDD lies in position 178-180, close to IPLD-169 which constitutes a putative caspase substrate according to the CasPredictor model. An Asp28, which in procaspase-3 defines the cleavage site that removes a short prodomain from the large subunit, is also present. This observation prompted us to look for the effect of PAC-1 on promastigotes of *Leishmania (L.) amazonensis.* PAC-1 induces a dose-dependent inhibition of cell growth along with inhibition of mitochondrial transmembrane potential ($\Delta\psi$) and induction of PS exposure. These two phenotypes are characteristic of apoptotic death; together with cell cycle arrest they can be interpreted as resulting from metacaspase activation. The physicochemical nature of metacaspase activation is still unknown and the prerequisite of proteolytic maturation for its activity is controversial. PAC-1 can be a useful tool to address those issues. Furthermore, the absence of metacaspases in mammals warrants the development of strategies for the use of PAC-1 as an anti-parasitic drug. Support: CNPq.

BC68 - DIFFERENT WAYS OF CELL DEATH INDUCED IN *Trypanosoma cruzi* BY CHEMOTHERAPIC AGENTS: AN ULTRASTRUCTURAL STUDY

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Electron microscopy proved to be a reliable and essential tool to determine morphological alterations and target organelles in the investigation of new drugs for Chagas disease. In this review, we focused on the evaluation of *Trypanosoma cruzi* death induced by different agents, i.e. lysophospholipids analogues, naphthoquinones and derivatives, cytoskeletal inhibitors and natural products. Apoptosis-like presents as morphological characteristics DNA fragmentation, membrane blebing and apoptotic bodies formation. Autophagy involves autophagosome formation, with the appearance of membranes surrounding organelles and cytosolic structures. Necrosis directs to the loss of osmotic balance, an increase of cytoplasmic vacuolization, and plasma membrane disruption. Mitochondrion appears as a central checkpoint in both apoptosis and necrosis. Our ultrastructural evidences of the *T. cruzi* treated with the different classes of compounds point to dramatic mitochondrial alterations and similar autophagic phenotypes. Lysophospholipid analogues interfere in the lipid biosynthesis in epimastigotes, altering the content of both phospholipids and sterols, and consequently the physical properties of the membrane. Naphthoquinones derivatives led to a strong DNA fragmentation in trypomastigotes and to cysteine proteases release from reservosomes to cytosol in epimastigotes, starting a proteolytic process which results in parasite death. The susceptibility of reservosomes was also observed in parasites treated with propolis, suggesting impairment of lipid metabolism, compromising membrane fluidity and leading to lysis. The cytoskeletal agents blocked mitosis of epimastigotes, arresting cell cycle, impairing the parasite proliferation. The variety of drug stimuli converge to the same pathway of death suggests

an intense cross-talking between the three types of PCD in the protozoa.

Financial support: PAPES/Fiocruz, CNPq and FAPERJ.

BC69 - Trypanosoma cruzi AUTOPHAGIC DEATH INDUCED BY NAPHTHOIMIDAZOLES DERIVED FROM β-LAPACHONE

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The flagellate protozoan *Trypanosoma cruzi* is the causative agent of Chagas' disease. Current clinical treatment of this disease is still unsatisfactory, being nifurtimox and benznidazole efficacy during the chronic phase controversial. Extensive efforts in developing new alternative drugs, which that interfere in metabolic pathways of the parasite. In this framework, our group investigates the potential trypanocidal effect of natural quinines and derivatives. As previously reported, among 60 compounds the most active were the naphthoimidazoles **N1**, **N2** and **N3** synthesized from β-lapachone, being the mitochondrion, reservosomes and DNA their main targets in *T. cruzi* (Menna-Barreto et al., 2005, 2007). Programmed cell death (PCD) is an important phenomenon in cell biology, participating of several central processes in multicellular organisms, through three pathways: apoptosis, autophagy and necrosis. In unicellular organisms, PCD have been studied in *Leishmania spp*., *Toxoplasma gondii* and *T. cruzi*. Here, we described autophagic cell death phenotypes induced by the naphthoimidazoles in *T. cruzi*. Our ultrastructural evidences revealed autophagic characteristics in treated epimastigotes and trypomastigotes, such as appearance of concentric membranar structures, multivesicular bodies and autophagosomes-like. These latter structures were commonly found surrounded by endoplasmic reticulum profiles. The treatment of both forms also induced a strong increase of the labeling with MDC, a known autophagic marker, and, consequently, of the percent of parasites containing autophagic vacuoles. The pre-incubation of *T. cruzi* with two autophagic inhibitors - WT and 3-MA - abolished the effect of naphthoimidazoles, reinforcing the induction of autophagic cell death by the naphthoimidazoles in *T. cruzi* and further experiments are needed to detail the molecular features involved in parasite death.

Financial support: PAPES/Fiocruz, CNPq and FAPERJ.

BC70 - *TRYPANOSOMA CRUZI* EPIMASTIGOTE FORMS AND CELLS VERO UNDER THE EFFECT OF TRIAZOLIC-DERIVATES

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Chagas disease, caused by protozoan *T. cruz*i, is an endemic health issue in Latin America. Research programs have been focused on the study of alternative natural and synthetic drugs for the treatment. Until now benzidazol is the only available therapeutic agent for Chagas. So in this present work the effects of new synthetic substances, triazolic derivates, were described on epimastogotes forms of *T. cruzi*. **Materials and Methods** 1-*T.cruzi* Dm28c epimastigotes were raised in BHI-medium. 2-Trypanocidal Assay - Stock solutions of Triazoldaal, Triazoldagal, Triazolmxi, Triazoldaf1 and Triazoldaf2 were prepared in DMSO and their effect at 50μM and 100μM was determined after quantification of active parasites on the 72 h and 144 h of the culturing, by counting in a Neubauer chamber using optical microscopy. DMSO 1% was used as negative control. 4- Cytotoxic on the Vero cells was described as reported by Ferreira VF *et al.,* 2006 (Bioorg Med Chem. 14(16): 5459-66). **Results and Conclusion –** All the substances were more active after 144h of culturing. With 50μM the best results were obtained with Triazoldagal (26,30% of alive cells) and Triazolmxi (2,00% of alive cells). With 100μM, best results were found with Triazolmxi (3,65% of alive cells) and Triazoldaf1 (10,49 %of

alive cells). We investigated the toxic effects of these molecules in 3 different concentrations on the Vero cells. Among the 3 substances with best results in *T. cruzi*, Triazoldagal and Triazolmxi were less citotoxic to the cells. This study can lead to the development of new anti-chagasic drugs **Supported by UFF, FAPERJ and CNPq.**

BC71 - TOWARDS THE SET UP OF DRUG SCREENING ASSAYS FOR CHAGAS' DISEASE USING ORGANOTELLURIC COMPOUNDS

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Most of the work related to the development of drugs to treat Chagas' Disease has used the*Trypanosoma cruzi* epimastigote form, which corresponds to the parasite found in the midgut of triatomine bugs. Only more recent studies trials have tested the drugs in mammalian cells infected with the parasite. This type of assays is too toilsome, and the amount of substances that can be tested simultaneously is limited. Inhibitors of cysteine proteases seem to be able to block *T. cruzi* cellular invasion and parasite growth. To setup a more efficient drug screening assay, the anti-parasite properties of the organic tellurium compounds were herein investigated. The compounds were used to inhibit cellular invasion and in intracellular proliferation in L6 culture cells originated of rat myoblasts, since they showed inhibitory activity for the *T. cruzi* cysteine proteases at low concentrations. Five compounds showing low cytotoxicity against L6 cells by MTT assay after 48 h incubation (until 50 µM) were selected. Among them, three significantly decreased the trypomastigotes cellular invasion and one retarded the intracellular replication at doses of 5 µM. Those results will allow (i) to setup an automated assay to detect intracellular parasites, in order to facilitate the screening of additional anti-*T. cruzi* drugs; (ii) to verify how effective are the organotelluric compounds in the treatment of infected animals; (iii) to check a possible synergic interaction of those compounds and benznidazole, a drug already used for Chagas' Disease treatment. Supported by CNPq and FAPESP.

BC72 - *GIARDIA LAMBLIA* LIFE CYCLE: A REPORT OF DRUG EFFECTS UNDER CELL DIFFERENTIATION

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The *Giardia lamblia* life cycle is characterized by two phases during cell differentiation: the encystation and excystation processes. During encystation the trophozoite forms a cyst, the resistant form. Once ingested, the cysts are stimulated to excystation in the stomach and the excysted trophozoites adhere to the epithelium. Our work here analyses the effects of four benzimidazole compounds during *Giardia* differentiation into cysts and evaluates the excystation efficiency of the water resistant cysts. Alterations in cell morphology, especially in the cytoskeleton were observed by light and electron microscopy. Albendazole (AB) showed the most significant results by inhibiting encystation of viable cysts and by the decreased capability of the cysts to excyst. The adhesive disk was the structure which was the most affected in the cysts obtained by treatment with AB. Although the other benzimidazoles showed some effect on encystation, they were not efficient enough to prevent the excystation process. Interestingly, even under albendazole the resistant cells differentiated on cysts. The infectivity test on cysts eliminated after treatment with different drugs has been little explored in the literature. Based on this argument and on our data, we suggest that the cysts eliminated during host treatment could be potentially infective. The prevention of the Giardia infection is a question of extreme importance mainly in underdeveloped countries, where poor sanitary conditions are related to high indices of Giardia infection. In our work, we present a report of the effects of benzimidazoles and resistance of Giardia during its most critical phases: its

differentiation processes. We evaluated in detail the interference of albendazole over the full Giardia life cycle. This report is related to the importance of keeping the environment free from infective cysts and on Giardia's drug resistance and differentiating abilities. This work was supported by CNPq, CAPES, SR2-UERJ, IOC/FIOCRUZ and FAPERJ.

BC73 - CHARACTERIZATION OF THE SURFACE CARBOHYDRATES OF *Crithidia deanei*: INFLUENCE OF THE SYMBIOTIC BACTERIUM

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Crithidia deanei is a monoxenic trypanosomatid that harbours an intracellular bacterium in a mutualistic relationship. Molecular studies of the ribosome RNA sequence shows that this endosymbiont presents a great similarity with bacteria from *Bordetella* genus. Previous studies showed that the endosymbiont influences the carbohydrate composition and the surface charge of the host trypanosomatid. Here we studied the surface carbohydrates of *C. deanei*, as well as, isolated symbionts obtained after protozoa fractioning. We also used the symbiont free *C. deanei* (cured or aposymbiotic strain) to investigate the endosymbiont influence in the protozoan surface carbohydrates. Thus, cells were labeled with lectins-FITC and analyzed by cytometry and fluorescence microscopy. Cells, fixed in 4% paraformaldeyde were incubated with different lectins in 1% PBS-BSA for 1h. Whole protozoa and isolated symbionts were submitted to SDS-PAGE and Western blot and incubated with several lectins. Such samples were also treated with 2% Triton X-114 in order to better analyse soluble and membrane proteins. Our results showed that the endosymbiont-bearing strain was labeled by Wheat germ agglutinin (WGA), *Griffonia simplicifolia*-II (GSII), which recognizes N-acetylglucosamine and *Helix pomatia* (HPA), which binds to N-acetylgalactosamine. The aposymbiotic strain was labeled by the same lectins and also by *Griffonia simplicifolia*-I (GSI), which is specific to N-acetylgalactosamine. Cytometry analysis revealed that the symbiont-containing strain presents higher amounts of N-acetylglucosamine when compared to the cured strain. The endosymbiont homogenate contains proteins that were recognized by GSII, WGA and Concanavalin A (Con A), a lectin which recognizes mannose residues. The endosymbiont soluble proteins were labeled with Con A, whereas the membrane fraction was recognized by GSII and WGA. Our next approach is to verify the effect of glycosilation inhibitors, such as tunicamycin, in protozoa and in the symbiont carbohydrate composition.

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Keywords: Carbohydrates; *Crithidia deanei*; Endosymbiosis; Lectins; Trypanosomatids

BC 74 - GASTRIC MUCIN-BINDING MOLECULES OF *TRYPANOSOMA CRUZI* METACYCLIC TRYPOMASTIGOTES IMPLICATED IN INFECTION

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T. cruzi infectivity in mice inoculated orally with metacyclic forms is associated with the ability to interact with gastric mucin, and to invade the subjacent epithelial cells, which is the portal of entry for systemic infection. Of the two gastric mucin-binding parasite molecules identified so far, one is the stage-specific surface molecule gp82 and the other is an as yet to be characterized protein of ~43 kDa. We used synthetic peptides based on the gp82 sequence to perform assays of inhibition of J18, the recombinant form of gp82, in binding to gastric mucin. Four peptides showed significant inhibitory effect. The two exhibiting the highest inhibitory activity (p7 and p10), along with two control peptides, were used in cell invasion assays, in absence or in the presence of gastric mucin. HeLa cell entry of metacyclic forms (CL strain) was inhibited by p7 and to a lesser extent by p10, in the presence of gastric mucin, whereas the control peptides showed no effect. Experiments

to determine the effect of p7 in oral infection in mice are under way. As regards the ~43 kDa protein, it appears to be expressed also in other T. cruzi developmental forms and possibly is not localized on the parasite surface. When metacyclic forms were incubated with HeLa cells in the presence of antibodies directed to this protein, an increase in the number of internalized parasites was observed. Attempts to purify this protein for further structural and functional studies are in progress.

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BC75 - *LEISHMANIA (V.) BRAZILIENSIS* STRAINS: IDENTIFICATION OF CARBOHIDRATE AND IMPORTANCE OF PHOSPHATIDYLSERINE RESIDUES IN PARASITE-MACROPHAGE INTERACTION

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Tegumentary leishmaniasis is a vectorborne disease caused by many species of Leishmania, mainly by *Leishmania (Viannia) braziliensis,* which presents a wide clinical spectrum, ranging from benign cutaneous leishmaniasis (CL) to more severe forms, such as mucosal leishmaniasis (ML). Studies suggest that the occurrence of those clinical forms depends on host immunological response and parasite virulence factors like phosphatidylserine (PS), surface carbohydrate (SC) and the Lipophosphoglycan (LPG). Our group demonstrated through flow cytometric a difference in the PS expression between ML and CL strains of *L. braziliensis* which CL strains expressed more PS indicating a high infectivity. Meanwhile, ML strain expressed less PS, indicating a high virulence, which characterizes this clinical form. This study has the objective to compare the SC expression among the two strains, through agglutination test, and PS on the infectivity of the parasite during host cell interaction through Annexin V assay. We observed that the ML strain presented a lower concentration of SC in logarithm phase (LP) than in stationary phase (SP). In the other hand, CL strain did not showed differences between these two phases, although this strain presented the expression of β-acetyl-galactosamine which has not been detected in ML strain. These differences may have relationship with the infectivity and virulence levels of the strains, once that all detected carbohydrates are presented in LPG molecule. The phagocytic index of CL strain demonstrated a decrease of 92.1% and 92.2% in SP and LP, respectively, when the PS binding sites were blocked by Annexin V. Whereas the ML strain presented decrease of 55.3% in LP and 89.7% SP, confirming the results obtained previously by flow cytometric. This study demonstrated the importance of the virulence factors identification on parasite surface to better understand the interaction mechanism between host cells and parasites and its relationship with different disease clinical forms.

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BC76 - MICROCULTURE FOR PRIMARY GROWTH OF *Leishmania* spp: A RAPID, SENSITIVE AND FEASIBLE METHOD IN THE DIAGNOSIS OF LEISHMANIASIS.

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Leishmaniases comprise a group of diseases caused by protozoan parasites of the *Leishmania* genus that include cutaneous, mucocutaneous and visceral forms. Wild canids and domestic dogs are the main reservoirs of the zoonotic american visceral leishmaniasis. In Brazil some places are endemic for both cutaneous and visceral leishmaniasis. In Bauru city, located in the center – west region of São Paulo State, since 2002 visceral leishmaniasis (VL) is a serious public health problem. The diagnostic of VL is made including traditional culture (TCM) that is labor intensive and has a poor sensitivity. The aim of this study was to evaluate the microculture method (MCM) in 96 well plates to primary growing of *Leishmania* spp as an alternative and complementary procedure to diagnose VL in dogs naturally infected by these parasites. The samples used were spleen aspirated of 58 dogs from Zoonozes Control Center from Bauru city. The samples were screening by DiaMed – IT LEISH test, subsequently cultivated in TCM and MCM. The TCM was realized in tubes containing 7ml of BAB (blood agar base), 2 ml of BHI (brain heart

infusion) with 20% FBS (fetal bovine serum) + gentamicin and 300µL of sample. The MCM was realized in 96 well sterile plates, each well containing 100µL of BAB, 50µL of BHI + 20% FBS + gentamicin and 20µL of sample. The present study revealed the MCM as an effective instrument for the diagnostic of the VL. The MCM revealed growth since 48 hours after inoculation. MCM showed 83.3% of sensibility and 81.3% of specificity while TCM 34.1% of sensibility and 92.9% of specificity. MCM presented 82.8% of agreement with DiaMed – IT LEISH test and the TCM 49.1%.

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BC77 - *TOXOPLASMA GONDII* INHIBITS THE *IN VITRO* MYOGENESIS OF SKELETAL MUSCLE CELLS

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The Apicomplexans, including *Toxoplasma gondii*, belongs to a group of parasitic protozoa that essentially lead obligate intracellular lifestyles. *Aims:* We employed mouse primary culture of skeletal muscle cells (SKMC) as a model for experimental toxoplasmosis studies. So, we decided to investigate the infective capacity of *T. gondii* tachyzoites in SKMC, the influence of the infection on the myogenesis and the possible role of the parasite on the modulation of family cadherin protein during this process. *Methods:* SKMC cultures were obtained from thigh muscles of 18-days-old mouse embryos. After 24-time-old and 4-day-old SKMC were allowed to interact with tachyzoites (1:1 parasite: host cell) for 24 h. Fixed with paraformaldeid (PFA) and subjected to tests of immunofluorescence or for quantitative analysis. *Results:* The analysis of 4-day-old SKMC infected with tachyzoites for 24 h showed that myotubes are 1.6-fold less infected than myoblasts. Our results showed that myoblasts were more infective than myotubes indicating differences in the ability of tachyzoites to invade the cell types presents in the culture during the differentiation stage. Cultures with 24-time-old infected for 24 hours presented an inhibition in the percentage of formation of myotubes of 75%. It was observed that the parasites are able to interfere molecularly the host cell, preventing membranes fusion and consequently affecting the myogenesis process. Infected myoblasts presented low expression of the cadherin and were incapable to form myotubes. Our results demonstrated that in low ratios of infection, with culture only presenting 43% (± 0.02) of infected cells, the parasited myoblasts did not fuse either with infected or non-infected cells. *Conclusions:* These data suggest that *T. gondii* is able to module negatively the cadherin expression interfering as with the myogenesis process. This work was supported by CAPES, CNPq, FAPERJ and IOC/FIOCRUZ

BC78 - THE ROLE PLAYED BY PMSF-SENSITIVE PROTEASES IN BASIC PROPERTIES OF *Acanthamoeba polyphaga*

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Acanthamoeba is a group of free-living amoebae, widely distributed in the environment, which occasionally can cause human infections including granulomatous amoebic encephalitis, osteomielitis and amoebic keratitis. It has been reported that *Acanthamoeba polyphaga* secretes serine, cysteine and metallo proteases which in turn, play important roles in tissue invasion and immune evasion. Most of these enzymes are able to degrade extracellular matrix (ECM) components, such as collagen I and elastin, as well as immunoglobulins and plasminogen. Most of the *Acanthamoeba* proteases involved in both cytoadhesion and cytotoxicity exerted by the parasite belong to the serine family. Furthermore, amoeba proteases induce cytopathic effects in monolayers formed by mammalian cells. In this work, experiments were designed to investigate the role played by proteases, particularly the serine ones, in each one of encystment/excystment, and cell cycle regulation. Phenylmethylsulfonyl fluoride (PMSF), a serine proteases inhibitor, was used throughout. Zymographic assays were carried out by using amoebic conditioned medium treated or not with PMSF looking for the amount of PMSF that did not reduce cell survival at high extent. 2 x 10^5 ml^{-1} trophozoites were inoculated in encystment medium supplemented or not with PMSF. The resulting rates of cysts formation

appeared to be not affected by the presence of the protease inhibitor. Similarly, the growth of trophozoites in PYG medium supplemented or not with PMSF did not result in growth inhibition. Altogether, the results here reported indicate that amoebic proteases inhibited by PMSF do not are involved in both *in vitro* proliferation and encystment.

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BC79 - CULTIVATION OF *Entamoeba histolytica* IN TRIDIMENSIONAL MATRICES MADE OF TYPE I COLLAGEN

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Trophozoites of the human parasite *Entamoeba histolytica* recognize and bind to extracellular matrix (ECM) components such as collagens, fibronectin and laminin. Such interaction process that lead parasite to perform adhesion and spreading *in vivo* may induces ECM remodelling. Data available on the interaction between parasitic protozoa and ECM underlie the existence of a bidirectional signal exchanging from ECM to parasites and from parasites to ECM. Both chemistry and mechanics concerning networks formed by ECM triggers the activation of many cell functions, including invasiveness. The objective of the present study is to explore the mechanochemical interaction between trophozoytes of *E. histolytica* and tridimensional (3D) matrices made of collagen I (COL). *E. histolytica* trophozoytes (strain HM1:IMSS) were cultured in each one of 1,5 (low density) and 3 (high density) $mg.ml^{-1}$ COL which was leaded to polymerize for 1 h at $37^{o}C$. Just after, the resulting COL matrices were rinsed, and allowed to interact with parasites for 24h and 48h. Alternatively, parasites were associated to COL during its polymerization. The parasite-3D matrix interactions were followed by scanning electron microscopy. Zimography assays were carried out in order to investigate the expression of secreted proteases by the parasites during their cultivation in such 3D environment. Throughout cultivation in 3D, parasites presented quantitative differences in terms of protease secretions. The resulted zymograms clearly showed a direct correlation between COL density and amount of secreted proteases by the parasites. At 48h cultivation in a 3D matrix made of $3mg.ml^{-1}$ COL *E. histolytica* exhibited a strong protease activity. The results here presented demonstrated that the physical architecture of the environment where *E. histolytica* is found influence the protease activity of the parasite. Furthermore, such protease activity may be related to parasite invasiveness and/or its remodeling of the surrounding ECM.

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BC80 - POTENTICAL OF ASYMPTOMATIC DOGS ON THE PARASITE TRANSMISSIBILITY TO THE VECTOR

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Since there are a large number of asymptomatic dogs with tissue parasitism in endemic area of visceral leishmaniasis which could be a source of parasites to the vector, the ability of the symptomatic and asymptomatic dogs naturally infected by *L.(L.)i.chagasi* on the transmissibility of the parasite to the vector was investigated using the xenodiagnosis.

Animals referred to the Center of Zoonosis Control of Araçatuba city (SP), Brazil, endemic region for visceral leishmanisis were submitted to aspirative biopsy of lymphnodes for parasitological diagnosis. Forty dogs with positive parasite search in lymphnodes were divided according to clinical signs in symptomatic (n=24) and asymptomatic (n=16) and selected for the study. The animals were sedated and anesthesized. For each animal, a round plastic box with an open side covered by a fine-mesh nylon screen countaining around 50 *Lu. longipalpis* from F1 laboratory rared were placed over the skin of the internal ear. After 60 minutes, the sand flies were transferred to holding cages that were kept at room temperature and humid air. On the fourth day after the blood meal, female flies were dissected and examinated for visible promastigotes in the gut in 50% of samples. In another 50% of samples, DNA was extracted for

Leishmania PCR. After euthanasia the blood samples were collected and the sera were processed by anti-*Leishamania* immunoglobulin detection, as well as viscera fragments for amastigotes detection.
High levels of immunoglobulins as well as amastigotes forms of *Leishmania* in skin or víscera were confirmed in all dogs submitted to the xenodiagnosis. Promastigotes forms were observed in 27% of female which fed in symptomatic dogs and in 42% of female which fed in asymptomatic dogs. *Leishmania* DNA was detected in 24% of female which fed in symptomatic dogs and in 34% of female which fed in asymptomatic dogs.
The high infectivity rates of asymptomatic dogs, bigger than symptomatic dogs, showed that asymptomatic dogs could be a expressive source of parasites to the vector.

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BC81 - EXPRESSION OF T LYMPHOCYTES IN THE SKIN OF SYMPTOMATIC AND ASYMPTOMATIC DOGS NATURALLY INFECTED WITH *L. (L.) chagasi*

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Introduction and Aim: We have been studying aspects of the immunity in dogs naturally infected with *L.(L.)chagasi* and their correlation with clinical forms and tissue parasitism. Previous studies showed more intense inflammatory reaction, parasitism and T CD3 cells in viscera of symptomatic dogs. In order to study the cellular immunity in the skin, the expression of CD3, CD4, CD8 and CD45RA T cells was evaluated in the dermis of parasite-positive symptomatic and asymptomatic dogs naturally infected with *L. (L.) chagasi*.
Material and Methods: Frozen sections of skin from symptomatic (n=6) and asymptomatic (n=6) dogs and control (n=5) animals from non-endemic area for visceral leishmaniasis were submitted to the immunohistochemistry reaction (Avidin-Biotin method) using mouse anti-human CD3 (DAKO, USA), rat anti-canine CD4 and CD8 (Serotec, USA), and mouse anti-canine CD45RA (Serotec, USA) as primary antibodies. Semi-quantitative analysis was done, as follows: discreet = 1-10 cells; moderate = 11-20 cells and intense = more than 20 cells by microscopic field, using 40X objective. Parasitism was determined using the same criteria.
Results: T lymphocytes in the dermis infiltrate of dogs naturally infected with *L. (L.) chagasi* were enhanced in comparison with the controls. The intensity of CD3, CD4 and CD8 cells was higher in the dermis of symptomatic than asymptomatic dogs. Discreet CD45RA cells were observed in both clinical groups. Direct correlation was observed between the T cellular immune response in the skin and parasitism.
Conclusion: The data suggest that T lymphocytes found in the dermis infiltrate of dogs infected with *L. (L.) chagasi* could be associated with the spreading of the infection.

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BC82 - THE BIOLOGICAL BEHAVIOR OF *Leishmania (Viannia) shawi IN VITRO*

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Introduction: *Leishmania (V.) shawi* were recently described by R. Laison and J. J. Shawi in 1989 and a few studies has been developed since then. The aim of this work was to characterize the biological behavior of *L. (V.) shawi in vitro*.
Material and Methods: Promastigote forms (10^6 promastigote/mL) were plated in a flask of 25cm^2 and the number of parasites measured daily for 15 days using a Neubauer chamber to standardizing the growth rate curve. Macrophage from BALB/c and C57BL/6 mice were isolated and infected with promastigote forms in stationary phase using the ratio of 5 promastigotes to 1 macrophage. After 24 hours, the infection index was available and nitric oxide (NO) production in the macrophage culture supernatant was measured by the Griess method.
Results: The promastigote forms started the stationary phase at the 6th day and its length was approximately 72 hours. After this time, the growth decreased. Comparing the infectivity of peritoneal macrophages, BALB/c mice showed an index of infection (155.2) higher than the mice C57BL/6 (113.4). Infected macrophages from BALB/c mice produced 92.7μM of NO while infected macrophages from C57BL/6 produced 111.7μM of NO.

Conclusion: These results indicate that *L. (V.) shawi* has a similar *in vitro* behavior with others cutaneous *Leishmania* species, thus BALB/c mice showed signals of susceptibility with higher infection index and lower NO levels compared to C57BL/6 mice. Other studies has been done to verify if *in vivo* this biological behavior is maintained, moreover, this work open perspective to the study of *L.(V.)shawi* in the field of immunology, biochemistry, new drugs technology and in the host cell/parasite interactions.

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