III REUNIÃO DA SOCIEDADE BRASILEIRA DE PROTOZOOLOGIA

PZ

PROTOZOOLOGY

PZ-1

Dados morfológicos sobre Licnophora sp. Claparède, 1867 (Ciliophora-Heterotrichida), epibionte de estrela-do-mar.

SILVA NETO, I.D. da; VEIGA, V.F. da; SANTA ROSA, M.R. de & CANHOS, I. Laboratório de Microscopia Eletrônica do Instituto de Microbiologia da UFRJ. Caixa Postal 68040 - Rio de Janeiro,RJ - CEP: 21944.

Licnophora sp. é um ciliado epibionte pela primeira vez assinalado nas estrelas-do-mar Coscinasterias tenuispina, Astropecten armatus braziliensis, Echinaster brasiliensis e Linckia guildinguii comuns no litoral do Rio de Janeiro. Foram encontrados no sulco ambulacrário e superfície das pápulas dos hospedeiros. Têm o corpo alongado, com a região anterior, oral, bem distinta da posterior onde se encontra um disco de fixação. A região anterior, mais entumescida, está ligada à posterior por uma área estrangulada flexível que dá muita mobilidade a primeira.

Licnophora sp. é achatado ventralmente e abaulado no dorso. Suas dimensões variam de 45 a 75 µm de comprimento por 35 a 50 µm de largura. A ciliatura do corpo está limitada a região oral, ao disco adesivo e a uns poucos cílios laterais. O amplo peristoma é contornado por uma A.Z.M. reforçada por raízes cinetosomianas na forma de feixes de fibrilas. O disco basal, de forma arredondada, é ligeiramente côncavo e margeado internamente por duas cinécias quase fechadas. Em nossas preparações, não ficaram muito evidentes as duas cinécias marginais externas, típicas do gênero. O macronúcleo é constituído de cerca de dezoito a vinte e quatro fragmentos. Não evidenciamos micronúcleos.

Agradecemos a colaboração do Instituto de Biofísica Carlos Chagas da UFRJ.

Apoio CNPq - Proc. 30.0653/80.

PZ-2

Aspectos micromorfológicos, ao SEM, de Litonotus sp. Wrzesniowski, 1870 (Ciliophora, Kinetofragminophora, Gymnostomata, Amphileptidae).

SANTA ROSA, M.R. de; SILVA NETO, I.D. da & VEIGA, V.F. da. Instituto de Microbiologia da UFRJ. Caixa Postal 68040, Rio de Janeiro,RJ - CEP: 21944.

Ciliado mesopsâmico proveniente de praia arenosa da Ilha do Fundão, Rio de Janeiro. Tem como habitat areias ricas em matéria orgânica, de granulação fina e com aspecto lamacento. Consideramos, sem maiores dados, que o habitat poderia ser classificado como mesosaprobio ou polisaprobio. O Litonotus, em questão, não coincide com nenhuma espécie até hoje descrita. Seu tamanho é de cerca de 1100 µm de comprimento por 90 µm no seu maior diâmetro. Tem a forma alongada e achatada bilateralmente, principalmente nas extremidades. A parte anterior lembra um escalpelo e a boca localiza-se no lado convexo desta. Apresenta a face direita ciliada com cerca de 25 cinécias longitudinais, sem spica, as quais partem de pequenos sulcos. A face esquerda é acilífera, apresenta uma grande fenda mediana e tem a superfície con cerca de trinta pregas longitudinais intercalados com sulcos pouco profundos.

As cinécias da face direita reduzem-se próximo da parte anterior. Os animais foram fixados em Champy e posteriormente em Da Fano, antes da desidratação e do ponto crítico.

Agradecemos a colaboração do Instituto de Biofísica Carlos Chagas da UFRJ.

Apoio CNPq - Proc. 30.0653/80.

PZ-3 ASPECTOS ULTRAESTRUTURAIS DO MACRONÚCLEO DE PARAMETOPUS CIRCUMLABENS (Biggar & Wenrich, 1932) Grolière et alii, 1980 - CILIOPHORA, ARMOPHORIDA.

Kattar, M.R.
Departamento de Zoologia, ICB, UFMG - Belo Horizonte, Brasil

Parametopus circumlabens é uma espécie de ciliado heterótrico endocomensal de algumas espécies de ouriço-do-mar. Pouco se tem publicado a respeito de sua ultraestrutura. O autor apresenta alguns complementos aos estudos realizados por Grolière et alii (1980) e Neto (1986) em sua tese de mestrado, salientando nesta nota os aspectos ultraestruturais de seu macronúcleo em interfase de reprodução.

De forma ovalada, o macronúcleo mede de 30 a 50 µm de comprimento por 18 a 30 µm de largura. Ao microscópio eletrônico de transmissão observa-se, sob a dupla membrana nuclear provida de numerosos poros, que a cromatina se apresenta sob o aspecto de massas eletrodensas repartidas em massas cromáticas grandes (diâmetro em torno de 0,4 µm) e massas cromáticas pequenas (diâmetro em torno de 0,1 µm). Ambas as massas são desprovidas de envoltórios próprios e se acham imersas num nucleoplasma de baixa densidade eletrônica. Os nucléolos macronucleares, finamente granulosos, aparecem muito mais claros do que as massas de cromatina e são também desprovidos de membranas envoltoras. Apresentam-se sob configurações diferentes. Inclusões intramacronucleares medindo cerca de 5 µm de diâmetro, de aspecto semelhante às observadas por Jenkins & Giese em macronúcleo de Blepharisma japonicum, foram vistas em Parametopus circumlabens. De formato globoso, essas inclusões exibem uma camada cortical eletrodensa envolvendo uma massa medular menos densa. Sua origem e composição não foram determinadas. O autor acredita, todavia, tratar de massas de ribossomos.

PZ-4 PROTOZOÁRIOS ASSOCIADOS AO SISTEMA RADICULAR DO AGUAPÉ, EICHHORNIA sp. (LILIALES, PONTEDERIACEAE), DA LAGOA DA PAMPULHA, BELO HORIZONTE-MG.

Kattar, M.R.; Miranda, P.R.R.; Lemos, M.D.P. & Martins, T.K.

Departamento de Zoologia-ICB-UFMG - Belo Horizonte,MG, Brasil

Coletas de aguapés foram realizadas no período de maio a agosto de 1987 com o objetivo de avaliar a microfauna associada com seu sistema radicular em cabeleira.

Por ocasião das amostragens dos aguapés foram determinadas as variações do pH e temperatura. O material foi examinado apenas a fresco. Associados à fáunula de protozoários foram observadas formas diversas de invertebrados microscópicos e algas unicelulares. Entre os protozoários predominaram os ciliados. A determinação, com algumas exceções, foi feita até o nível de gênero. As formas mais características e passíveis de cultivo vêem sendo mantidas no laboratório. Foram encontrados, até o momento, os seguintes protozoários: Amoeba proteus, Valkampfia, Difflugia sp, Arcella vulgaris, Arcella dentata; Peridinium sp., Phacus sp., Euglena sp., Peranema sp., Chilomonas paramecium, Tetrahymena piriformes, Coleps hirtus, Chilodonella sp., Cyclidium sp., Lionotus sp., Loxodes magnus, Frontonia leucas, Dileptus anser, Stentor coeruleus, Stentor polymorphus, Spirostomum teres, Halteria sp., Stylonychia sp. Holosticha sp., Euplotes sp., Uroleptus sp., Vorticella sp.

Alkaline phosphatase activity in membranes of Hypotrichidae ciliate. Preliminary results. **PZ-5**

Knaippe, F.; De Bonis, M. & Santa Rosa, M.R. de. Setor de Microscopia Eletrônica do Instituto de Microbiologia da UFRJ - CEP: 68040 - Rio de Janeiro, RJ - Brasil.

The presence of specific enzimatic markers of cellular compartiments and membranes, decidedly contributes to the study and increasement of knowledge of several mechanisms of cellular physiology.

Our aim was to detect the alkaline phosphatase, an enzimatic marker of the plasma membrane of special cellular types, in a ciliate belonging to the family Oxitrichidae.

Initial results demonstrated the presence of cytoplasmic vesicles, of varied sizes, with an envolved membrane that is alkaline phosphatase positive . In the same way, an intense positive reaction was observed in some regions of the external face of the plasma membrane. If such results point to the presence of specific compartiments of plasma membrane, characterizing the so called "membrane dominium", as an example, in relation to functional differences of dorsal and central surfaces of the ciliate in general, this can't be afford with such data. We should mention that the negative control used during the experiment, revealed to be positive with respect to the enzimatic reaction, what one could speculate about the existence of endogene substrate evidencing inespecific phosphatase.

CNPq - Process nº 300653/80.

Contribution to the study of commensal ciliates of marine invertebrates of Rio de Janeiro. **PZ-6**

SILVA NETO, I.D. da. Lab. of Electron Microscopy of Microbiology Institute of Federal University of Rio de Janeiro. Caixa Postal 68040 - Rio de Janeiro, RJ- BRAZIL. CEP: 21944.

Several species of ciliophora were found to be associated with marine invertebrates of the phyla Cnidaria, Mollusca and Echinodermata collected from litoral area of the state of Rio de Janeiro, Brazil. The Cnidaria (*Anemonia sargassensis*, *Phylactis flosculifera* and *Bunodosoma caissarum*) lodged in the gastrovascular cavities the Trofont of *Foettingeria actiniarum* (Claparède, 1863)(Apostomatida). The paleal cavity of the bivalve *Anomalocardia brasiliana*, showed great quantities of one atypical ciliate Haplofriidae (?) (Astomatida). The digestive cavity of the *Neoteredo reynei* lodged several *Metanycthoterus rancureli* Laval & Tuffrau, 1973 (Heterotrichida). Numerous individuals of *Euplotes balteatus* (Dujardin, 1841) (Hipotrichida) and *Parametopus circumlabens* (Biggar & Wenrich , 1932) Grolière et al, 1980 (Heterotrichida) were found in the digestive cavity of one Echinoderma *Lytechinus variegatus*. The starfishes *Echinaster brasiliensis* *Coscinasterias tenuispina*, *Astropecten armatus brasiliensis* and *Linckia guildingii* lodged the epibionte *Licnophora sp.* Claparède, 1867 (Heterotrichida). In the *Enoplopatiria stellifera* we found the ciliate *Trichodina sp.* Ehrenberg, 1838 (Peritrichida). For light microscopy we have employed the techniques of Chatton-Lwoff (1930) (Silver impregnation), Bodian (Silver proteinate stain), adapted by Tuffrau (1964 and 1967) and Klein (1958). The Feulfen nuclear stain was also used. The ultrastructure studies (SEM) were limited to the ciliates *F. actiniarum*, *E. balteatus* and *P. circulabens*. The last one was also observed by TEM.

PZ-7 PNEUMOCYSTIS CARINII AND CARLOS CHAGAS.

Bauer,P.G.
Deptº de Protozoologia, Instituto Oswaldo Cruz, C.P. 926, Rio de Janeiro, Brasil.

In his initial description of Trypanosoma cruzi in 1909, Carlos Chagas mentioned schizogenic forms in the lung of guinea pigs which always formed 8 bodies lying inside a pelicula with cyst-like appearance. He believed that they represent the schizogenic cycle of the trypanosomes and created the genus Schizotrypanum with the species Schizotrypanum cruzi. The first autopsy of a human acute case of Chagas' disease revealed also the schizogenic forms in the lung (Chagas 1910, 1911). In the august session of the Sociedade de Medicina e Cirurgia de S. Paulo in 1910 Carini communicated schizogenic forms in the lung of T. lewisi infected rats which were similar with the one described for T. cruzi. Vianna (1911a,b,1912), Buchanan (1911) and Walker (1912), also influenced by the work of Chagas, described schizogenic forms in animals infected with T. gambiense, T. equinuum, T. congolense, T. equiperdum, T. brucei and T. evansi. Carini sent some lung preparations to Mesnil in Paris which gave them to Mr. and Mrs. Delanoe for further investigations. These scientists finding the schizogenic forms not only in the lung of T. lewisi infected rats but also in control animals realized that it must represent a new rat parasite which they named in honour of Carini's observation Pneumocystis carinii (Delanoe & Delanoe, 1912). At the same time Aragão at Manguinhos, which had some doubts about the shizgenic cycle of T. cruzi could often find normal rabbits, rats and guinea pigs with schizogenic forms in the lung. Considering these observations and after reading Delanoes paper he declared that these lung forms should be separated from the trypanosomatid cycles (1913). He thought that these pneumocysts which meanwhile were found in various animals are not pathogenic. Chagas agreed in 1913 with the idea of a new parasite and mentioned, although not convinced, the possibility that its human material could have been exchanged during fixation and staining of the slides. It was only in 1942 when Van der Meer and Brug recognized that Chagas was the first author which described P. carinii in man. In 1962 Brito and Engel studying 3 children which died of P. carinii pneumonia, mentioned in one Chagas' disease myocarditis and thereby confirm that Chagas was also the first scientist which described the human and animal double infection with T. cruzi and P. carinii.

PZ-8 PNEUMOCYSTIS CARINII INFECTION IN AIDS PATIENTS OF THE HOSPITAL GAFFRÉ E GUINLE, RIO DE JANEIRO (1983-1987).

Bianchini,E.X.*, Bauer,P.G.***, Sion,F.S.*, Sá,C.A.M.*, Oliveira,C.A.B.**, Valle,H.A.**, Rachid de la Cerda,M.C.*
*Clinica Médica B and ** Deptº de Patologia, UNI-RIO, and *** Deptº de Protozoologia, Instituto Oswaldo Cruz, Rio de Janeiro.

From 109 patients with AIDS (stage IV, CDC classification) studied in our hospital during the period from november 1983 to july 1987, 36 patients showed signs indicative for Pneumocystis carinii infection. The analysis was made by presumptive or definitive diagnosis. The presumptive diagnosis consists, according to the CDC, in a) the history of dyspnea on exertion or nonproductive cough of onset (within the past 3 months), b) chest x-ray evidence of diffuse bilateral interstitial infiltrates, c) no evidence of a bacterial pneumonia and furthermore a good response to treatment with trimethoprim/sulfamethoxazol (Bactrim). The definitive diagnosis is made by cytology or histology using Grocott's silver stain. Up to now, with the exclusion of 7 cases after autopsy, 25 continue with presumptive diagnosis and 4 showed a positive result on histopathological analysis. From these remaining 29 patients 16 (55.2%) are homosexuals, 6(20.7%) are bisexuals, 3 (10.3%) show a blood transfusion history, 2 (6.9%) a transplacentar HIV infection, 1 (3.4%) is drug addict and 1 woman (3.4%) is partner of a bisexual man. To intensify and facilitate the diagnosis of P. carinii and other lung related infections we are actually testing further methods, including sputum analysis, various staining technics and electron microscopy.

ON THE OCCURRENCE OF A GALL'S BLADDER MYXOSPORIDIAN PARASITE OF *Bufo paracnemis*. **PZ-9**

GIOIA, I.; CORDEIRO, N. S. & ARTIGAS, P. T.
Universidade Estadual de Campinas, Deptos. de Zoologia & Parasitologia
C. P. 6109 13081 - Campinas, S.P.

While the investigation was carried out about the parasitic protozoa of Brazilian amphibians from São Paulo State, a myxosporidian parasite has been found to infest the gall bladder of *Bufo paracnemis* Lutz, 1925. The parasite isolated from bile in NaCl 0.9%, treated with Lugol's iodine solution and dry smears stained with Giemsa after fixation in methanol, were carefully studied. The extrusion of polar filament was achivied with KOH 2.5% solution. The myxosporidians (54 yellowish round and thick trophozoites in one host's gall bladder) measured (mean value is given with range within the parenthesis) 1.004(0.687-1.503)mm. The endoplasm is highly vacuolar with fat globules and numerous disporous spores. The spores, elliptical in front view, laterally spindle shaped, with 4-5 transverse ridge striations measured 14.2 x 9.8(13.3 - 15.8 x8.9 - 10.5)µm and contains two normal opposite polar capsules with 4.2(3.6 - 5.0)µm. The polar filament showed 4 coils in each of the polar capsules and when everted measured 39.3(32.8 - 55.7)µm. Sporoplasm granular with two vesicular sporoplasmic nuclei. The iodinophilous vacuole was not found. The only known South American amphibian species of *Myxidium* Bütschli, 1882 is *M. immersum* (Lutz, 1889) Kudo & Sprague, 1940 (Jayarsri & Hoffman, 1982: 74) . The present *Myxidium*, in mensural data, resembles *M. immersum* and *M. serotinum* Kudo & Sprague, 1940, however it differs from this species by trophozoite form and number of the spore's transverse striations (7-9 in *M. immersum* and 10-13 in *M. serotinum*)

CRYPTOSPORIOSIS AT KINDERGARTEN CHILDREN IN SÃO PAULO-SP BRAZIL. **PZ-10**

Mangini ,A.C.S. (*),Cos ta,L.B. ,Dias, R.M.D.S .(*),To rres,D. M.A.G. V.(*),S ucupira ,A.C.S. L.(**) e Chieffi,P.P.(*)

(*) Instituto Adolfo Lutz, Secretária do Estado de Saúde do Estado de São Paulo- Av. Dr.Arnaldo,n 351, 8 andar, CEP 01246 S.Paulo- SP Brasil
(**)Instituto da Criança do Hospital das Clínicas da Universidade de São Paulo

Cryptosporiosis in immunocompetent individuals is characterized by a self-limited watery diarrhoea without mucus or blood.

In Brazil, there are few reports on the frequency of diarrhoea due to Crystosporiosis in immunocompetent children.

It were collected faeces samples from 223 children aged from 4 to 6 years old attending a school located in S.Paulo Municipality, in order to investigate the occurrence of Cryptosporiosis, in addition to other intestinal parasites in immunologically normal individuals.

Each sample was analyses by direct examination, spontaneous sedimentation and concentration method through formaldehyde-ether modified technics, and also by carbol fuchsin with dimethyl sulfoxide staining.

Among 223 samples analysed, three(1.35%) displayed the presence of Cryptosporidium sp oocysts.All of the Three positive samples were diarrhoeal.

PZ-11 Seasonal variation of *Cryptosporidium sp.* in a city with the mild weather of North of Chile.

Araya, J.[1], Hartard, M.E.[2], Sagua, H.[1], González, J.[1] and Arriagada, J.[1]

1. Parasitology Group - Preclinical Science Department - Health Science Faculty - University of Antofagasta, P.O. Box 1240. ANTOFAGASTA, CHILE
2. Pediatrics Section - "Dr. Leonardo Guzmán" Hospital - ANTOFAGASTA, CHILE.

Cryptosporidium sp. a zoonotic aetiological agent of diarrhoea in humans, parasites the gastrointestinal mucosa of a great variety of vertebrated animals, including man. It has been observed with well-defined seasonal variation that infections due to these protozoan increase their frecuencies in hot, rainy and humid seasons, although studies made in this respect are unknown in connection with countries of milder climates.

We report here a systematic study of 2 years term, among milk-fed infants with acute diarrhoea being ingressed in the Pediatrics Section of the Regional Hospital of Antofagasta. In all cases we investigated the presence of *Cryptosporidium sp.* through the modified Zielh-Neelsen acid fast and the Aureamina-Rodamina methodics.

The Antofagasta city presents a mild climate through the whole year long; that of a desertic-coastal kind, with very little variations through the four seasons, recording maximum average temperatures of 24,1 °C and 16,7 °C in summer and winter respectively, meanwhile the minimum average temperatures reach 14,8 °C and 10,2 °C during the same periods. Rainfalls per annum are never superior to 5 mm if not almost nil.

In both summer periods, with the increasing of the pediatric diarrhoea cases higher seasonal frecuencies of the *Cryptosporidium sp.* showed up : 1986 : 18,9 % and 1987 : 22,6 %. Meanwhile from the middle of autum, during winter and early spring, in general, all diarrhoea cases start decreasing and the incidence of *Cryptosporidium sp.* becomes almost null.

The epidemiologic role of the seasonal variation and his relation with the transmissions life cycle of this protozoan are discussed.

SUPPORT : DIEXAT - UNIVERSITY OF ANTOFAGASTA - CHILE.

PZ-12 Isospora sp. (APICOMPLEXA: EIMERIIDAE) FOUND IN THE CANARIES FRINGILLIDAE, MAINTAINED IN THE CAPTIVITY.

Jorge, P. & Kawazoe, U.

Departamento de Parasitologia. IB-UNICAMP, CP. 6109, Campinas, SP, Brazil.

Fourteen examples of wild Brazilian canaries belonging to the Family Fringillidae, with unknown localization, and maintained in the Department of Zoology of UNICAMP (Campinas, SP) were examined for intestinal parasites. The following species were examined: Sporophila albogularis (2), Sporophila leucoptera (1), Sporophila lineola (1), Sporophila caerulescens (1), Sicalis flaveola (6) and Coryophospingus cucullatus (1). Samples of faces were collected in alternated days, during 60 days (may-june/87) for the diagnosis of intestinal parasites using direct examination and Sheater Method. Culture of oocysts positive samples of feces were made using 2% dicromate potassium solution to verify the oocysts sporulation time. Isospora sp. oocysts were found in 4 examples of S. flaveola, 2 of S. albogularis and one of C. cucullatus. The sporulated oocysted measured 24,8 X 22,5 µm in S. flaveola and 24,8 X 23,3 µm in S. albofularis. They presented greenish color, thich wall, presence of Stieda body with no residual body of oocyst or spotocysts. The sporulation time varied from 47 to 50 hours for S. flaveola and from 44 to 46 hours for S. albogularis. The specific identification of the species will be done after more detailed studies of the life cycle and the biology of these parasites.

PZ-13

INTESTINAL COCCIDIA FOUND IN THE WILD BIRDS *Passer domesticus* (PLOCEIDAE) AND *Columba livea* (COLUMBIDAE) IN CAMPINAS REGION, SÃO PAULO.

Jorge, P. & Kawazoe, U.
Departamento de Parasitologia, IB-UNICAMP, CP. 6.109, Campinas-SP., Brazil

Two examples of *Passer domesticus* and two of *Columba livea* were captured in Campinas Region. They were maintained in the captivity during 14 days for feces collection and their examinations to find the intestinal parasites. Direct examination and Sheater method were used for their diagnosis. Dicromate potassium solution (2%) were used for the cultivation of oocysts positive feces to determine the sporulation time of oocysts. *Isospora* sp. oocysts measuring 25,3 X 23,1 µm were found in the both *P. domesticus* and *Eimeria* sp. oocysts with 16,0 X 18,8 µm in size were found in both *C. livea*. The sporulation time in the first specie varied from 74 to 76 hours while in the second, it was 120 hours. Future captures and examinations of these birds will be done for more detailed studies concerning life cycle and biology of these coccidian parasites for the specific identification of them.

PZ-14

Experimental infection of goats with *Toxoplasma gondii*

Vitor, R.W.A.*; Chiari, C.A.*; Lima, J.D.** & Fernandes, M.A.**
*Departamento de Parasitologia/ICB - **Escola de Veterinária - UFMG.

Two crossbred female goats were inoculated subcutaneously with $5x10^6$ to 10^7 tachyzoites of *Toxoplasma gondii* C4 strain. Goat 1 (non pregnant) developed pyrexia and other symptoms from the third day after inoculation (DAI) to 15 DAI. Goat 2 (pregnant) were inoculated 100 days after breeding and remained normal and healthy throught the study. Goat 1 was serologically negative before inoculation and developed titers by the immunofluorescent antibody technique of $\geq$1:16,384 to *T. gondii* within 50 DAI. Titer of goat 2 was 1:64 before inoculation and increased to titer of $\geq$1:4,096 within 64 DAI. A healthy female kid was born 54 DAI of goat 2 and developed titers of 1:16,384. It was killed in apparent godd health one week after birth and necropsy revealed no macroscopic lesions except enlarged mesenteric lymph nodes. Goats were killed 120 DAI and necropsy revealed no macroscopic lesions. *Toxoplasma* was isolated by inoculation in mice from 5 tissues of kid (brain, lungs, spleen, kidneys and heart), from 9 tissues of goat 1 (brain, uterus, kidneys, liver, spleen, lungs, heart, skeletal muscle and mesenteric lymph nodes) and from 8 tissues of goat 2 (brain, uterus, kidneys, spleen, lungs, mammary glands, skeletal muscle and precrural lymph nodes). *Toxoplasma* was also found in milk and urine based on positive serology of mice inoculated with those materials. Additional studies have been carried out to investigate the dissemination of *T. gondii* by contact with excretions and determine the importance of transplacental transfer of this parasite in caprines. Supported by CNPq.

PZ-15 *Toxoplasma gondii* tachyzoits elimination in the urine of experimental inoculated rats and mice.

Rocha, R.J.; Chiari, C.A. & Tafuri, W.L.
Instituto de Ciências Biológicas - UFMG - C.P. 2486 - 31.270 - Belo Horizonte/MG.

Ten female rats were subcutaneously inoculated with 0.3 ml of peritoneal exudate of mice, in the 7th day after infection, containing a low virulent *Toxoplasma* strain (C4). The urine of those rats was collected and inoculated in normal white mice every four days during three consecutive months. These material proved to be positive for tachyzoits of *Toxoplasma*, since the urine of six rats caused the development of cysts in the mice brain, while the inocullum from the other four rats caused the finding of anti-*T. gondii* circulating antibody (IFAT). Frequency of tachyzoits elimination during the period was variable: three rats 3x; two rats 4x; one rat 5x, and four rats 6x. Tachyzoits were not found only in 6 days, out of the 26 in which the urine was collected. In another experiment the authors have already found that four mice among ten experimentally inoculated subcutaneously, were eliminating tachyzoits in their urine during the acute phase of the disease. The demonstration that this infective form had been eliminated either in the beginning of the chronic or in the proliferative phase, suggests that tachyzoits in urine might be a source of infection through direct contact or contamination of food and grains.

PZ-16 **TOXOPLASMA GONDII** IN THE CATS OF ARARAQUARA - SEROLOGICAL AND FECES SURVEY.

Rosa, J.A. da; Buainain, A. & Belda Neto, F.M.
Faculdade de Ciências Farmacêuticas, "Campus" de Araraquara, Universidade Estadual Paulista "Júlio de Mesquita Filho", C.P. 331, Araraquara, SP, Brasil.

Twenty-seven cats of both sexes from Araraquara were examined. The age of them was gressed to be between one and six months old, some of them were settled down and some were not. The propose of this research was verify the infection prevalence of Toxoplasma gondii, through a research of protozoary oocysts in their dejection and a antibodies research int the blood. It was found four cats sheldding structures that could be oocystis of Toxoplasma gondii, but it was not acchieved the segregation of asexual structures in mice. According to indirect immunofluorescence test, seven out of those cats presented titles of antibodies anti-Toxoplasma gondii equal or superior to 1:8. The reaction of indirect haemaglutination test for toxoplasmosis was reagent to the serum of two felids, in the dilution of 1:12,5.

A seroepidemiological study of toxoplasmosis in Trujillo - Venezuela **PZ-17**

Urdaneta, H.; Ramirez, A. & Muñoz, F.
Universidad de Los Andes of Venezuela

It was performed a serological study in order to detect antitoxoplasma antibodies to 650 healthy persons from on urban area of Trujillo city from Venezuela, with age between 0-45 years old. The study gave the foullowing results: 65% of the persons examined were positives with antibody titer as high as or higher than 1:16; 94% of the persons were positive with antibody titer up to 1:2048.

The risk of infection was higher in the children with age lower than five years old.

The positivity of sera was demonstrated to increase with age; sex was not significant different in the immune response to toxoplasma.

A taxa of seropositivity of antibodies against Toxoplasma was higher in persons having cats in their house and a higher percentage of seropositivity was observed in persons with low economic conditions.

Study of the presence of anti-*Toxoplasma* antibodies in butchers in Trujillo - Venezuela. **PZ-18**

Carrillo, T.; Urdaneta, H.; Ramirez, A. & Muñoz, F.
Universidad de Los Andes of Venezuela

It was performed a comparative serological study of antitoxoplasma antibodies using passive haemaglutination. In 115 persons who work killing the animals and manipulations of meat; the same number of persons working an another kind of jobs were used as controls.

The results of the study were as it follows: 37% of the persons working on killing and manipulation of meat were positive, whereas the control group was positive in 6%.

98% of the persons working on killing and manipulation of meat, with anti-*Toxoplasma* antibodies had titers of 1:32768 or less whereas in the other group, the titers were 1:2048 or less.

The seropositivity of the persons working on killing and manipulations of meat, was similar to the persons working on poultry.

PZ-19 ESTUDO SORO-EPIDEMIOLÓGICO DA TOXOPLASMOSE NA POPULAÇÃO FEMININA EM IDADE FÉRTIL, DE SEIS MUNICÍPIOS DO DISTRITO TRUJILLO, VENEZUELA

Diaz de Ramirez, A., Urdaneta, H. & Muñoz, J.F.
Universidade dos Andes, Núcleo Universitário "Rafael Rangel", Trujillo, Venezuela

Determinaram-se anticorpos anti-toxoplasma numa amostra probabilística representativa da população feminina em idade fértil e em mulheres gestantes, da área urbana de seis municípios do Distrito Trujillo, Venezuela, usando a técnica de hemaglutinação indireta. A taxa de positividade em mulheres não gestantes foi de 72.0%, enquanto que em mulheres grávidas atingiu 82,26%. A proporção de indivíduos positivos aumenta com a idade, passando de 68,19% no grupo de 15 a 19 anos de idade a 75.0% nos de 40 a 44 anos. Ao contrário, o risco de adquirir a infecção diminui com a idade. Por outro lado, a grande maioria dos soros da amostra (94,40% em mulheres não gestantes e 83,87% em gestantes) se encontram abaixo do título 1:1024. As pessoas que convivem com gatos, apresentam taxas de positividade mais altas (86,05%) que as que não convivem com esses (59,10%), observando-se além disso, que à medida que aumenta a renda familiar, diminui a taxa de positividade. Os resultados obtidos, demonstram sem dúvida, a necessidade do controle sorológico de toda gestante e a prática de algumas medidas profiláticas derivadas do conhecimento que temos da toxoplasmose.

PZ-20 REAÇÕES DE IMUNOFLUORESCÊNCIA INDIRETA PARA TOXOPLASMOSE EM PACIENTES COM SÍNDROME DA IMUNODEFICIÊNCIA ADQUIRIDA.

TOLEZANO, J.E.[1]; BARLETA, C.[1]; KAWARABAYASHI M.[1]; COELHO J.R.[1]; RAYMUNDO, M.L.[1]. LÍRIO, V.S.[1]; UEDA, M.[1]; SOUZA, A.M.C.[1]; FERRARI, L.[2] & PINTO, W.P.[2]
1- Instituto Adolfo Lutz - Av. Dr Arnaldo - 351 - 8º andar - São Paulo -SP
2- Instituto de Saúde - São Paulo

Em trabalho anterior, apresentado no 10º Congr. Soc. Bras. Parasitologia, os autores da presente comunicação haviam tentado correlacionar informações de pacientes suspeitos de serem portadores da síndrome da imunodeficiência adquirida (AIDS/SIDA), com resultados de sorologia para toxoplasmose.

Neste novo estudo foram coletados dados de 450 pacientes todos com pesquisa positiva para anticorpos anti-HIV. Dentre estes 450 pacientes, 325(72,2%) pertenciam ao Grupo IV da AIDS/SIDA ou seja, estavam na fase ARC da doença; 94(20,9%) eram do Grupo III, correspodente à fase LAS e 31(6,9%) faziam parte do Grupo II ou grupo dos assintomáticos. Do total de pacientes, 105(23,3%) tinham quadro clínico compatível com toxoplasmose.

Foram realizadas 1.050 reações de imunofluorescência indireta (RIFI) no soro e/ou líquido cefalorraquidiano (L.C.R.). Em 689 amostras de soro e 256 amostras de LCR foram dosados anticorpos Ig total e em 71 soros e 34LCR foram dosados anticorpos da classe IgM específico.

Os resultados das dosagens de anticorpos Ig total revelaram reatividade em 69% das amostras de soro e 47% das amostras de LCR. Somente 4,2% das amostras de soro foram reagentes para IgM específico. Dentre os pacientes com quadro clínico compatível com toxoplasmose, 67,6% tiveram RIFI reagente para Ig total no soro e 61,2% para Ig total no LCR. Na fase ARC 73,8% e 66% dos pacientes foram reagentes na RIFI para Ig total no soro e LCR, respectivamente. Cêrca de 64% dos pacientes na fase ARC e com sorologia Ig total positiva mostraram LCR também positivo; se forem considerados dentre estes pacientes aqueles com sorologia $\geqslant$ 1024 a positivação do LCR se eleva para 80%. Nas fases ARC e LAS, 85% dos pacientes com sorologia Ig total negativa, também o foram na pesquisa de anticorpos anti-*T. gondii* no L.C.R.

PZ-21

INFESTAÇÃO POR Cyrilia gomesi (Neiva & Pinto, 1926) (HEMOGREGARINIDAE) E SISTEMA SANGUÍNEO TIPO DUFFY EM SIMBRANQUIDEOS.

Nakamoto, W.; Rubio-Colauto, E.M.; Silva, L.A.G.; Silva, A.J. e Machado. P.E.A.

Faculdade de Medicina de Botucatu, Universidade Estadual Paulista, Botucatu, SP, Brasil.

Nakamoto et alii, 1986; Foresti et alii, 1982 obtiveram dois Fenotipos distintos de *Synbranchus marmoratus* nas eletroforeses em gel de agar-amido de hemolisados totais e na análise dos cariotipos: o Fenotipo I apresentou 3 bandas distintas de hemoglobinas e 44 e 46 cromossomos e o Fenotipo II apresentou até 12 bandas de hemoglobinas e 42 cromossomos.

Lainson, 1981 e Sogayar et alii, 1987 observaram em exemplares de *Synbranchus marmoratus* capturados em diferentes localidades do Brasil, alta infestação por um parasita intraeritrocitário , *Cyrilia gomesi* (Neiva & Pinto, 1926) (Hemogregarinidae).

O sistema sanguíneo Duffy está envolvido no processo de invasão eritrocitária por *Plasmodium vivax*. Indivíduos portadores dos antígenos Fy^a e/ou Fy^b são susceptíveis a invasão por *P. vivax* enquanto que os indivíduos destituídos destes antígenos não são parasitados.

Tendo em vista a alta infestação de *Cyrilia gomesi* em *Synbranchus marmoratus*, o presente trabalho teve o objetivo de estudar a presença dos antígenos eritrocitários Fy^a e Fy^b em hemácias de simbranquídeos da região de Birigui, SP, Brasil.

A análise eletroforética de 49 hemolisados totais, mostrou 27 exemplares do Fenotipo I e 22 exemplares do Fenotipo II.

Os esfregaços sanguíneos com Leishmann, mostraram a presença em média de $4,44 \pm 5,87$ de hemogregarinas em 100 glóbulos brancos contados nos exemplares do Fenotipo I e em média $117,86 \pm 154,30$ de hemogregarinas nos exemplares do Fenotipo II.

A análise dos resultados de aglutinação das hemácias, mostrou que 70,3% dos exemplares do Fenotipo I não reagiram com o antisoro humano anti-Fy^a e 100% dos exemplares do Fenotipo II reagiram com o antisoro humano anti-Fy^a e somente um exemplar do Fenotipo II apresentou aglutinação para o anti-Fy^b.

Os achados mostraram estreita relação entre o grau de infestação dos eritrocitos por *Cyrilia gomesi* e a presença de antígenos eritrocitários humanos Fy^a e Fy^b.

É possível que as hemácias de simbranquídeos tenham antígenos nas membranas eritrocitárias semelhantes aos antígenos Fy^a e Fy^b humanos que possibilitariam a entrada dos parasitas entre o interior das células. Dessa maneira a penetração de C. *gomesi* nos eritrocitos dos simbranquídeos seria de maneira semelhante a que ocorre na infestação por *Plasmodium vivax* em humanos.

PZ-22

"Efeito de plantas medicinais sobre a infecção pelo *Plasmodium berghei* em camundongos".

*ORNELAS, H.M.; **DISTASI, L.C.; ***CURI, P.R.; *SALATA, E.

*Departamento de Parasitologia - IBBMA/UNESP/CAMPUS DE BOTUCATU - 18600 - BOTUCATU/SP.

**Departamento de Farmacologia - IBBMA/UNESP/CAMPUS DE BOTUCATU - 18600 - BOTUCATU/SP.

***Serviço de Estatística e Computação da Faculdade de Medicina de Botucatu/UNESP-18600 - BTU/SP.

Dada a alta frequência da infecção malárica no Brasil e o constante uso de plantas tidas como anti-maláricas, pelo folclore, nos propusemos a reavaliar os efeitos de extratos obtidos de *Cassia occidentalis* (Fedegoso) e de *Mormodica charantia* (Melão de São Caetano) sôbre a infecção de camundongos por *Plasmodium berghei*. Os extratos das referidas plantas foram obtidos em solução hidroalcoólica por Sohxlet. Dose de 1.000mg/Kg de peso, foi ministrada por via oral, 72 horas após a instalação da infecção. A atividade anti-malárica dos extratos foi avaliada em camundongos inoculados por via intraperitoneal com 10^7 eritrócitos parasitados, através da verificação da parasitemia no 7º e 9º dia após a inoculação e da mortalidade nos grupos que receberam os extratos e nos controles. Os resultados demonstraram redução da parasitemia nos lotes tratados com *Cassia occidentalis* e *Mormodica charantia*.

PZ-23 ANTIMALARIAL TESTS OF CHEMICALLY DEFINED MOLECULES OBTAINED BY SYNTHESIS OR FROM PLANT EXTRACTS.

Carvalho, L.H.; Alvim, V.S.; Rocha, E.M.M.; Raslan, D.; Oliveira, A.B. and Krettli, A.U. Dep.Parasitologia ICB and Dep.Chemistry ICEX, University of Minas Gerais and Centro de Pesquisas René Rachou, FIOCRUZ, C.P. 1743, CEP 30.190, Belo Horizonte, MG, Brasil.

In an attempt to find novel antimalarial compounds we have studied the blood schizonticide effect of a total of 12 naphthoquinones chemically defined all related to lapachol. Eight of them were natural products (NP) isolated from plants of the Family Bignoniaceae and 4 were produced by synthesis (S). Different concentrations of drugs were incubated in vitro with Plasmodium falciparum blood cultures. After 24 and 48h the culture medium, with or without drug, was replaced and after 72h blood smears were taken for evaluation of parasitemia. The results of two repeated experiments showed that among the 12 compounds, 3 (2 NP and one S) were very active and completely inhibited parasite growth at the higher concentration; 6 (3 NP and 3 S) were partly active and 3 (NP) were totaly inactive. One of the NP screened was the lapachol which, surprisingly, displayed a very low activity (20% inhibition of schizogony) although it has been considered a potential antimalarial. The antimalarial activity of these naphthoquinones was rather superior than that of chloroquine and quinine used as controls including against two of the chloroquine resistant P. falciparum strains used for the tests. Presently, only one of them was tested as antimalarial in vivo against P. berghei being partly active at a dosage of 200 mg/kg. Since those drugs are easily synthesized once proved active in vivo and of low toxicity they will improve antimalarial chemotherapy. (Results partly presented - FESBE Meeting, Rio de Janeiro, July 1987).

Supported by CNPq.

PZ-24 CIRCUMSPOROZOITE PROTEIN OF *Plasmodium gallinaceum* IDENTIFIED AND CHARACTERIZED WITH MONOCLONAL ANTIBODIES.

Krettli, A.U.*; Rocha, E.M.M.*; Lopes, D.+; Cochrane, A.H.**; Nussenzweig, R.S.** Departamento de Parasitologia, University of Minas Gerais and Centro de Pesquisas René Rachou, FIOCRUZ, CP: 1743 - Belo Horizonte, MG, Brasil*; Instituto Ludwig, São Paulo, Brasil+; Department of Medical and Molecular Parasitology, New York University Medical Center, New York, NY 10016**

Abstract. Monoclonal antibodies (MAbs) were produced against both salivary gland sporozoites (SGS) and oocyst sporozoites (OS) of *Plasmodium gallinaceum*, an avian malaria parasite. By indirect immunofluorescence, all of the MAbs reacted with both SGS and OS of *P. gallinaceum* and two of the MAbs cross reacted with *P. berghei* sporozoites. None of the MAbs reacted with sporozoites of 6 additional species of mammalian plasmodia. MAbs produced against either SGS or OS, in Western blot analysis of extracts of either SGS or OS of *P. gallinaceum*, identified two polypeptides with molecular weights of approximately 76,000 and 64,000 daltons. The results of a MAb inhibition of binding assay and a two-site one antibody immunoradiometric assay indicate that the circumsporozoite protein of *P. gallinaceum*, like those of mammalian malaria parasites, contains a region with one repetitive immunodominant epitope . Two of the anti-*P. gallinaceum* MAbs were tested in a sporozoite neutralization assay and decreased, but did not abolish, infectivity of the sporozoites for chickens, indicating that the two polypeptides of *P. gallinaceum* identified by immunoblot are probably the protective antigens. (Summary presented at the 3rd Int. Conference on Malaria and Babesiosis. Annecy. Sept 87).

Supported by CNPq (Process 407093/86), UNDP-TDR/WHO and AID-USA.

PZ-25

Plasmodium falciparum CYTOADHERENCE INHIBITION BY MONOCLONAL ANTIBODY OKM 5.

Kloetzel, J.K., Malafronte, R., Silva, S.R. da & Lourenço, A.M.
Instituto de Ciências Biomédicas - Dept. de Parasitologia/USP and Instituto de Medicina Tropical de São Paulo, Av. Dr. Enéas de Carvalho Aguiar 470, 05403 São Paulo, S.P., Brasil.

Monoclonal antibody (Moab) OKM 5, which reacts with human monocytes, venular endothelial cells and C 32 melanoma cells, the latter used in the P. falciparum in vitro cytoadherence test, was shown to inhibit cytoadherence of FMG and Malayan Camp strains (Barnwell et al., 1985). We have tested cytoadherence inhibition by OKM 5 Moab with 3 Brazilian strains of P. falciparum, using the FMG strain as a control. Strains 369 and RO 48 were isolated from patients at Ariquemes - State of Rondonia, and strain ADA was isolated by Dr. C.E. Tosta, from a patient from the State of Amazonas, several years ago. These strains, as well as FMG, were kept in continuous culture in RPMI 1640 medium and human erythrocytes. C 32 target cells (TC) were seeded on 13 mm diameter round coverslips (1.5×10^4 cells per coverslip).After 48 hs they were washed, overlayed with 0.20 ml Moab in PBS at 5 ug protein/ml, incubated for 1 h at 25ºC, washed and overlayed with 0.25 ml infected erythrocytes (IRBC), 3% hematocrit. The IRBC contained 3 - 7% mature trophozoites. Ninety min. incubation at 37ºC, with light swirling every 15 min. was followed by gentle washing in Hanks' balanced salt solution, fixation in glutaraldehyde, staining with Giemsa. TC pre-incubated with PBS were included in all reactions. Average number of adherent IRBC/TC was calculated, counting at least 200 TC per coverslip. All tests were carried out in duplicate and repeated at least twice. Moab inhibition % was obtained by $\frac{A - B}{A} \times 100$, where A = Average nº adherent IRBC/TC of control; B = Average nº adherent IRBC/TC with Moab. In all experiments a test with the FMG strain was included. Inhibition was 82.50% ± 7.99 for FMG; 95.84% for RO 48; no inhibition for 369; 44.40 ± 11.59 for ADA. These results suggest that binding sites of C 32 for IRBC, as defined by blocking with Moab OKM 5, may vary for individual strains and this model may be useful in the study of receptors for cytoadherence.

This work was supported by UNDP/World Bank/WHO Special Program for Research and Trainig in Tropical Diseases - Project 860115 and CNPq PIDE VI Grant 400906/85.

PZ-26

ISOTYPIC PATTERN OF THE POLYCLONAL B cell RESPONSE DURING PRIMARY INFECTION BY Plasmodium chabaudi AND IN IMMUNE-PROTECTED MICE*

D'Império Lima, M.R.[1], Falanga, P.B.[2], Coutinho, A.[2] and Pereira da Silva, L.[2]
1. Departamento de Imunologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo
2. Département d'Immunologie, Institut Pasteur, Paris

The primary infection by Plasmodium chabaudi induces an increase of the numbers of splenic immunoglobulin (Ig)-secreting B cells in both athymic and euthymic BALB/c mice. The isotypic pattern of the polyclonal response is restricted only in euthymic mice where IgG2a, IgG2b and IgM plaque-forming cells (PFC) predominate. In immunized animals, protected against a parasite challenge, the isotypic pattern of splenic PFC is completely different, the IgG1 and IgM isotypes constituting the main part of the response. Reinoculation of immune-protected animals induces a PFC response which is dose dependent and accentuates the characteristic isotypic profile of the immune-protected mice.

* The research was supported by grants for Ministère de l'Industrie et de la Recherche (grant nº 0.06.84.L.1276)

PZ-27 HYPOGLYCEMIA AS A TERMINAL EVENT IN **PLASMODIUM BERGHEI** INFECTION ON BALB/C MICE.

ANDRADE Jr.,H.F., LIMA, S.T.C., LAURENTI, M.D. & CORBETT, C.E.P.

Tropical Medicine Institute of São Paulo and Dept. Pathology - Fac. Medicine - Univ. São Paulo
São Paulo - SP - BRASIL - CEP: 05403

Hypoglycemia has been detected in patients with severe falciparum malaria associated with hyperparasitemia and explained by starvation, depletion of glycogen stores and by competition from the malaria parasites (White et al.,1983, N.Engl. J.Med 309:61-66). Hypoglycemia can be induced by antimalarial drugs. Neurological clinical signs of hypoglycemia and cerebral malaria can masked each other. In order to analyse the influence of glucose serum levels in the evolution of malarial infection we used Balb/C mice infected with 10^7 erythrocytic forms of P.berghei (ANKA strain,provided by Dr.Walliker, Edinburg University) submitted to two diets schems, one feeded ad libitum (non-restricted group) and other with same diet with 23 hours fast period (restricted group). Weight, parasitemia and glucose serum levels were detected at time intervals as the disease progressed. Determination of glucose serum levels were made using a reactive strip (Destrostix), tail blood and a glucometter to quantify the glucose levels. The non-restricted groups developed progressive parasitemias with initial letality in the 8th day of infection with mean parasitemias of 10,41% ± 3,35 (SEM), but 5 of 7 animals survived ten days. The weight loss in this group were not significant when compared with controls. In the restricted group, the infection behaviour was quite different with early mortality (all 7 animals dead at 8th day) low parasitemias with mean of 6,1% ± 1,3(SEM) and a great wight loss. None of the dying animals presented neurological signs, but a hypoactive pattern were detected, with no search of food and water, but reactive to external stimula. Animals of both groups have glucose levels very low in this hypoactive status, dying generally few hours later. The glucose levels in the restricted group were lower (80,72 ± 12,13 mg/100ml)when compared with non-restricted animals (126,97 ± 11,42) (MEAN ± SEM)

We think that hypoglycemia could be an important terminal event in this experimental model, and can contribute to death early in the disease. The feeding habits of animals can interfere in the course of this infection and more studies are necessary to detect the exact nature of this involvement.

We gratefully thanks the technical assistance of M.L.G.B.Barits.
Financial support provided by CNPq-PIDE IV and INST.LAB.INVEST.MED. HC/FMUSP.

PZ-28 **ANTIGENURIA IN MALARIA**

KIMURA, E.A.; ALEXANDRE, C.O.P.; PERES, V.J.; KATZIN, A.M.
Deptº de Parasitologia. ICB. USP. São Paulo.

Antigenuria has been described recently associated with some parasite infections. During the course of malaria infection different antigens can be detected in the plasma. In this work we describe the presence of **Plasmodium vivax** and **Plasmodium falciparum** antigens in the urine of pacients infected with these species.

Urine samples (200 ml) from infected individuals (parasitemia positive) were concentrated 100 times with Lyphogel(R). Each sample was analyzed by immunoblotting utilizing a pool of positive human sera (IFA 1/300. An antigen of 170 kDa could be detected in 20 from 30 of the analyzed urines of **P. vivax** infected individuals. In 17 of the 20 analyzed urines from individuals infected with **P. falciparum** an antigen of 180 kDa could be detected. Some of the urine samples were radioiodinated with ^{131}INa and immunoprecipitated (Kessler, S.W., 1975 J. Immunol., 115:1617) with homologous and heterologous sera. The immunoprecipitate was then fractionated with SDS-PAGE. Out of 12 urines from **P. vivax** infected individuals 7 showed a band with the same molecular weight detected by immunoblotting. Similar results were obtained with 10 of the 13 urines from **P. falciparum** infected individuals. This fact suggest the presence of an antigen (probably S antigen) that is eliminated in the urine. **P. falciparum** antigen and **P. vivax** antigen probably have some common epitopes due to the fact that they can be precipitated by heterologous sera.

Plasmodium antigens were also detected in urine samples, without concentration, utilizing doube-sandwich dot blotting. The capture antibody was a rabbit hiperimune serum. Out of 11 **P. falciparum** samples 7 were positives, and out of 10 **P. vivax** samples 5 were positive. Ten samples of normal urines were analyzed by the above described methods and none showed a positive reaction for all the utilized probes. Antigenuria associated with malaria could be due to renal lesions and may be could also be used as test for evaluating the treatment efficiency.

STUDIES IN PROGRESS ON MALARIA TRANSMISSION IN RONDONIA STATE, BRAZIL. **PZ-29**

Lourenço-de-Oliveira,R.*; Oliveira-Ferreira,J.**; Guimarães,A.E.*; Daniel Ribeiro,C.** & Deane,L.M.*. Departments of Entomology* and Immunology**, Instituto Oswaldo Cruz.

In order to study the malaria transmission in Ariquemes,State of Rondônia, the most endemic municipality in Brazil, a survey of anophelines was carried out to determine the local species, their prevalence, habits and natural infection rates with the different species of *Plasmodium* through an immuno-radiometric assay(IRMA). The field work was performed, trimestrally, from November 1985 to May 1987,and includes captures of adult mosquitoes on human bait indoors and outdoors(close and far from the houses and in the forest)and on animal bait(cow) outdoors, generally from 6 to 9 p.m.A total of 4726 anophelines of 17 species was collected. *An.darlingi* was by far the most abundant species(73.9% of the total),followed by *An.triannulatus*(7.4%),*An.evansae*(5.7%),*An.oswaldoi*(5.3%),*An.strodei* (2.2%),*An.braziliensis* (1.9%) and *An.albitarsis*(1%), all remaining species being scarse. Inside houses, *An.darlingi* was found in small numbers(19.7 specimens/10 hours),but represented 98.6% of the total anophelines.In the close vicinity of houses, biting humans *An. darlingi* was abundant(90.3 specimens/10 hs.),accounting for 83.3% of the total,followed by *An.triannulatus*(6.1 specimens/10 hs.) and 5.6% of the total collected. On the other hand, outdoors, on human(far from dwellings "a" and in the forest "b" and on animal baits "c" other species were much more numerous.*An.darlingi* was the third in frequency in "a" and in "b" and the seventh in "c",corresponding,respectively,to 22.9, 9.6 and 2.6% of the total in each modality.*An.oswaldoi* and *An.triannulatus* were the most frequent species in "a" and in "b" and *An.evansae* in "c". In a total of 2245 anophelines examined by IRMA only 10 were found infected with sporozoites of *Plasmodium*, 8 with *falciparum* and 2 with *vivax*, all of them being *An.darlingi* (1327 specimens tested). At the time of writting this abstract another lot of mosquitoes is being analysed by the IRMA but up to the present the main conclusion of this survey is that *An.darlingi* seems to be the principal or the only important vector of malaria in the area, for being the most antropophilic and abundant species indoors and in the close vicinity of the houses and the only found naturally infected.

Supported by CNPq and WHO.

VIRULENCE OF Entamoeba histolytica. PERFORMANCE OF VARIOUS SAMPLES IN AXENIC CULTURE INOCULATED INTO HAMSTERS AT DIFFERENT AGES. **PZ-30**

SILVA, E.F. - Departamento de Parasitologia, ICB/UFMG, Cx.P. 2486, Belo Horizonte,MG, Brasil

Six samples of Entamoeba histolytica in axenic cultures were isolated from patients with different clinical forms of the disease. These samples were used in this research aiming at verifying their infectivity and virulence levels in hamsters. Three of them were isolated and kept under axenic conditions in Brazil (ICB-CSP, ICB-32 and ICB-462: isolated, respectively, from patients with amebic dysentery or non-dysenteric colitis, isolated and maintained under germ-free conditions from cysts obtained from asymptomatic patients without passages through bacterial cultures), the other two were isolated in the United States of America (HK9 and NIH 200), and the last onde in Mexico (HM1).

Hamsters aged 1, 3, 6, 10, and 20 days were used in the experiments, and the inoculations were performed with and without laparotomy, intrahepatic route, and at the dorsal region, subcutaneously. Inocula used were, respectively: $10_4 x 10^3$, $50_5 x 10^3$, 25×10^4, and 50×10^4 for intrahepatic inoculations, and 10×10^3, 50×10^4, $10x\ 10^5$ and 10×10^6 for subcutaneous inoculations. The animals were sacrificed 6, 8 and 14 days after inoculation for macroscopic (all the organs and subcutaneous sites where the inoculations were performed) and microscopic examinations (by means of liver macerate and histological cuts). Cultures in TPS_1 medium were carried out for all animals.

The forthcoming of lesions in trahepatic inoculations varied according to the size of inoculum and virulence of the strain. The number of infected animals ranged from 38.5 to 92.2% for the ICB-CSP strain, the infection grade varying from II to IV, according to JARUMILINTA & MAEGRANTH, 1961. The number of infected animals was lower for HM1 and ICB-32 strains (0.7 and 4.3%, respectively), and the maximal infection grade (G.I.) was II. No infection could be detected for the other strains, the same occurring with the inoculations performed subcutaneously.

This work was supported by CPq-UFMG and FAPEMIG.

PZ-31 CARACTERIZAÇÃO DE QUITINA EM TROFOZOÍTAS DE ENTAMOEBA INVADENS.

Ribeiro,S.*, da Silva,E.F.***, De Souza,W**, Alviano,C.S.* & Angluster,J.*
*Instituto de Microbiologia e **Instituto de Biofísica Carlos Chagas Filho, C.C.S. Cidade Universitária, Ilha do Fundão, 21941 - Rio de Janeiro - RJ. *** UFMG.

Foi feito um estudo da presença de quitina em formas trofozoíticas de E. invadens. As amebas foram cultivadas em meio TPS-1 (Diamond,1968) por 10 dias à temperatura ambiente (25ºC). Após crescidas, foram deixadas em banho de gelo por 15 minutos, separadas por centrifugação e lavadas 3 vezes em tampão "PBS" 0,01M, pH 7,2.Os polissacarídeos foram extraídos com KOH a 6%, por 3 horas, a 100ºC. Os carboidratos insolúveis em álcali foram hidrolisados quimicamente com HCl 6N/24 horas/30ºC, e os produtos resultantes caracterizados em cromatografia de papel. A hidrólise ácida formou glicosamina como único componente e a digestão enzimática, com quitinase, resultou somente na produção de diacetilquitobiose. Esses dados evidenciaram que o polissacarídeo álcali insolúvel corresponde a quitina, um homopolímero linear formado de unidades de N-acetilglicosamina associadas por ligações β1,4. É conhecido que a quitina é o principal componente do envoltório cístico de E. invadens. Pode-se,pois, concluir que a quitina detectada nas formas de trofozoítas é a precursora daquela que compõe a fase cística. É possível, também, que esse polissacarídeo aminado seja importante no processo de diferenciação de E. invadens

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PZ-32 **THE RELATIONSHIP BETWEEN ABO BLOOD AND GIARDIA IN A COMMUNITY SITUATED IN THE CITY OF RIO DE JANEIRO.**

Silva J.P., Carvalho S.A., & Marzochi M.C.A.
Departamento de Ciências Biológicas - Escola Nacional de Saúde Pública - FIOCRUZ, CEP 21041, Rio de Janeiro.

A parasitológical study of 130 families with 510 individuals was carried out in the "favela" of Manguinhos with the objective of determining the prevalence of **Giardia lamblia** and its correlation with the ABO Blood Group System. Of the 510 individuals studied, 173 (33,92%) were found to be infected with **Giardia lamblia**, 197 (38,62%) were negative and 140 (27,45%) had other parasites.

The age groups with the highest prevalence of **Giardia lamblia** were the 0-5 (19,6%) and the 6-10 (10,6%).

In the population studied the distribution of the ABO Blood Group was as follows:

A= 193 (37,84%), B= 90 (17,64%), O= 217 (42,57%) and AB= 10 (1,96%)

The following relationship between individuals infected with **Giardia lamblia** and the ABO Blood Group System was observed:

Group A = 68 (39,30%)
Group O = 71 (41,04%)
Group B = 30 (17,34%)
Group AB= 4 (2,31%)

No significant difference was found in the prevalence of **Giardia lamblia** infection to the ABO Blood Group, among the 510 individuals studied.

"Study of prevalence of *Giardia* from domestic and wild animals of rural zone of Itaporanga, SP." **PZ-33**

*SOGAYAR, M.I.L.; *RAMOS, M.A.M. & *YOSHIDA, E.L.A.

*Department of Parasitology - IBBMA - UNESP - CAMPUS DE BOTUCATU - 18600 - BOTUCATU/SP.

The lack of information in the literature about the prevalence of *Giardia* from domestic rodents and the scarcity of information about the prevalence of *Giardia* from wild animals in our country, stimulated us to do this work. 183 animals were examined: 101 rodents (93 *Rattus rattus*, 7 *Bolomys lassiurus* and 1 of the Cricetidae family); 72 opossums (45 *Didelphis albiventris*, 27 *Didelphis marsupialis*); 6 lizards (*Tupinambis teguixin*); 2 coatis (*Nasua nasua*); 2 carnivores (1 *Procyon cancrovorus*, 1 *Lutreolina crassicaudata*). The animals were sacrificed and the intestinal material examined. Mucosa smears were made and stained by the Heidenhain iron haematoxylin method. Only the rodents were infected by *Giardia*. The domestic rodents (*Rattus rattus*) were infected by *G. muris* (29,0%) and *G. duodenalis* (18,3%). In *Bolomys lassiurus* was encoutered only *G. muris* (14,3%). There is related for the first time in this work the presence of *Giardia duodenalis* type in domestic rodents (*Rattus rattus*) in our country. It is necessary to have more studies made to verify if *Giardia duodenalis* type from *Rattus rattus* is infective to man and its eventual importance to contaminate the water fountains and food in view of its proximity to man.

SUPPORTED BY FAPESP AND CNPq

INFECÇÃO DE CAMUNDONGOS POR **GIARDIA sp**: ESTUDO HISTOLÓGICO. **PZ-34**

Martini, A.S.; Utrilla, L.S.; Possato, T.A.C. & Fernandes, M.Z.T.
Faculdade de Ciências Farmacêuticas de Araraquara, UNESP. C.P. 331, Araraquara, São Paulo, Brasil.

A infecção do Homem por Giardia sp, em relação à sua ação patogênica e ao seu aspecto biológico, tem sido pouco estudada. SOGAYAR, M.I.T.L., 1983, realizou interessante estudo quanto à identidade de espécies de Giardia em animais de laboratório, peridomiciliares e silvestres. Verificou também seus aspectos morfológicos e sua prevalência em área limitada. Entretanto os fatores responsáveis pela variada patogenicidade, relacionados ao parasito e ao hospedeiro, ainda não estão bem esclarecidos. Considerando-se que a giardíase é frequente em nossa região, que o Homem possa se infectar pela ingestão de cistos de Giardia de outros hospedeiros e que a ocorrência de sintomas em portadores humanos nem sempre é frequente, resolvemos estudar a ação desse flagelado em animais de laboratório. Através de cortes do duodeno e jejuno dos camundongos infectados por Giardia sp, pudemos constatar pontos de destruição do epitélio de revestimento da mucosa entérica, determinando área de efração epitelial e expondo ocasionalmente o meio interno ao meio externo; moderado infiltrado linfoplasmocitário com presença frequente de linfócitos na camada basal, entre as células epiteliais e mesmo na luz intestinal e presença de trofozoitos de Giardia sp próximos a esses pontos de efração.

PZ-35 THE SURFACE CHARGE OF Giardia lamblia TROPHOZOYTES.

Silva Filho, F.C.[1,2], Ward, H.[3], Pereira, M.E.A.[3] and de Souza, W.[2]

1. Instituto Oswaldo Cruz - Deptº Protozoologia; 2. Instituto de Biofísica Carlos Chagas Filho, UFRJ, Rio de Janeiro, Brasil; 3. Tufts University, MA, USA.

The surface charge of Giardia lamblia trophozoytes was analyzed by direct measurements of their electrophoretic mobility (EPM), agglutination in the presence of different concentrations of cationized ferritin (CF) particles and ultrastructural cytochemistry by using CF particles. Microorganisms from Pl and WB strains of G. lamblia, grown in the TYI-S-33 medium, were washed and allowed to react with CF particles. Agglutination in the presence of the particles was performed at pH 6.8 and the results indicate that WB strain microorganisms agglutinate when incubated in the presence of 0.01 $mg.ml^{-1}$ CF particles whereas parasites from Pl strain agglutinated at concentrations higher than 0.01 $mg.ml^{-1}$. The binding of CF particles to the surface of trophozoytes from both strains was homogeneous; the cationic particles were distributed over the entire cell body. EPM measurements were made with trophozoytes which were previously treated with trypsin, neuraminidase or phospholipase C. Incubation of the parasites in the presence of enzymes, except phospholipase C, did not induce morphological alterations. The mean EPM values of -0.875 $\mu m.s^{-1}.v^{-1}.cm$ and -0.932 $\mu m.s^{-1}.v^{-1}.cm$ were obtained for non-treated trophozoytes from Pl and WB, respectively. Treatments with trypsin and phospholipase C significantly reduced the mean EPM of the trophozoytes (about 40-50%) whereas neuraminidase treatment reduced the mean EPM in a smaller extent (about 10-20%).

Supported by CNPq, FINEP and CEPG-UFRJ.

PZ-36 INQUÉRITO SUMÁRIO SOBRE A PREVALÊNCIA DO TRICHOMONAS TENAX E DA ENTAMOEBA GINGIVALIS.

Giazzi, J.F.*; Buainain, A.* & Ribeiro, R.D.**

* Faculdade de Ciências Farmacêuticas de Araraquara - UNESP.

** Faculdade de Ciências Farmacêuticas de Ribeirão Preto - USP.

R E S U M O: A prevalência do Trichomonas tenax e da Entamoeba gingivalis tem sido objeto de estudo de numerósos autores tentando correlacionar sua presença maior ou menor conforme a higiene bucal dos pacientes e conforme as várias patologias da cavidade oral apresentadas.

Procurando verificar a prevalência destes protozoários em clientes que demandam às Clínicas Odontológicas, colheu-se material de 100 indivíduos, escolhidos ao acaso, dos quais 39 eram do sexo masculino e 61 do sexo feminino. A coleta consistiu em passar uma mecha de algodão esteril no colo dentário, em cáries e cavidades e imediatamente mergulha-las em solução de Ringer. Procedeu-se o exame através da observação direta entre lâmina e laminula e da semeadura em meio de cultura de Kupferberg e cols para o Trichomonas tenax e o meio de Pavlova modificado para a Entamoeba gingivalis.

Observou-se que nenhum paciente do sexo feminino estava parasitado, entretanto o índice de positividade dentre os indivíduos do sexo masculino foi de 2,5% para o Trichomonas tenax e de 7,6% para Entamoeba gingivalis.

IMMUNOCYTOCHEMICAL LOCALIZATION OF ACETYLATED ALPHA-TUBULIN IN Tritrichomonas foetus and Trichomonas vaginalis. **PZ-37**

Batista, C.M.C.*, Benchimol, M.*, Cunha e Silva, N.L.*, Piperno, G.** & de Souza, W.*

* Instituto de Biofísica Carlos Chagas Filho, UFRJ, Rio de Janeiro.

** The Rockefeller University, New York, 10021, USA.

We have used monoclonal antibodies specific for acetylated and non-acetylated alphatubulin to detect and to localize microtubules containing acetylated alpha-tubulin (stable microtubules) in the pathogenic protozoa Tritrichomonas foetus and Trichomonas vaginalis. SDS-PAGE analysis showed that tubulin is a major protein of both parasites, being enriched in cytoskeletal preparations of whole cells extracted with Triton X-100. Monoclonal antibodies specific for acetylated tubulin (6-11B-1) or which recognizes all isoforms of alpha-tubulin (B-5-1-2) bind to the tubulin of T. foetus and T. vaginalis as seen by immunoblotting. Tubulin-containing structures were localized using immunofluorescence microscopy and transmission electron microscopy of the whole cytoskeleton previously incubated in the presence of the anti-tubulin antibodies and á second antibody-gold complex and then processed using the negative staining or replica techniques. The results obtained indicate that in addition to the flagellar microtubules those which from the peltar-axostylar system represent stable microtubules containing acetylated alpha-tubulin (or the alpha-3 tubulin isotype).

LOCALIZATION OF LAMININ AND FIBRONECTIN BINDING SITES ON THE CELL SURFACE OF TRICHOMONADS. **PZ-38**

Silva Filho, F.C.[1,2], Mota, G.[3], Lopes, J.D.[3] and de Souza, W.[2]

1. Instituto Oswaldo Cruz - Deptº Protozoologia; 2. Instituto de Biofísica Carlos Chagas Filho, UFRJ, Rio de Janeiro; 3. Instituto Ludwig de Pesquisa sobre o Câncer, São Paulo, Brasil.

Laminin (LMN) and fibronectin (FN) are glycoproteins involved in several phenomena of biological significance, such as those related to cellular recognition. In a previous study the presence of LMN receptores on cell surface of the human parasite Trichomonas vaginalis was shown (Silva Filho et al., manuscript in preparation). In the present study we extended our previous work analyzing the presence of LMN and FN receptors in the cattle's parasite Tritrichomonas foetus. Both trichomonads were incubated during 30 min in the presence of each one of the following substances: (a) whole LMN or (b) its P1 fragment, (c) whole FN or (d) its 120 kD fragment. After incubation the parasites were washed, incubated in the presence of polyclonal antibodies anti LMN or anti FN, and then incubated in the presence of colloidal gold particles (mean diameter of 20 nm) which were complexed with goat anti rabbit IgG. After this incubation the cells were dehydrated , critical point dried and replicated at 45º using platinum-carbon. The replicas were then analyzed in a transmission electron microscope. The results obtained indicated that either T. vaginalis as T. foetus possess numerous LMN and FN binding sites on their surfaces, whose distribution did not revealed any particularty. The immunocomplexes were observed over the whole cell bodies and flagella.

Supported by CNPq, FINEP, CEPG-UFRJ and Ludwig Foundation for Cancer Research.

PZ-39 PARTIAL LOST OF FIBRONECTIN BINDING SITES ON THE CELL SURFACE OF Tritrichomonas foetus AFTER TREATMENT OF THE PARASITE WITH ∝ -MANNOSIDASE.

Silva Filho, F.C.[1,2] and De Souza, W.[2]

1. Instituto Oswaldo Cruz, Deptº Protozoologia; 2. Instituto de Biofísica Carlos Chagas Filho, UFRJ, Rio de Janeiro, Brasil.

Tritrichomonas foetus, a parasite which inhabits the urogenital tract of the cattle, was grown in TYM medium supplemented with bovine serum depleted of fibronectin (FN). Parasites obtained from the late log phase of growth were washed and powdered into 50 $\mu g.ml^{-1}$ FN-coated or uncoated plastic dishes. Adhesion of the parasites to the substrate was estimated by microscopical counting. The adhesion to FN-coated dishes was almost tottaly abolished when the interaction medium (a rich salt solution) was supplemented with the tetrapeptide RGDS or with the FN fragment 120 kD which retains the cell binding domain of the whole FN molecule. It was observed that the presence of 120 kD fragment of FN molecule induced agglutination of the parasites. Taking into consideration that receptors for Concanavalin A (ConA) are involved in some adhesive properties of the human parasite Trichomonas vaginalis and that T. foetus possess receptors for such lectin on its surface, we search for a possible correlation between receptor to FN and to ConA in T. foetus. Into multiwell plates 20 μl of a parasites's suspension, which were previously treated or not with FN, were allowed to react with each one of the reagents: ConA, RGDS, 120 kD. In a second step parasites were previously treated with ∝-mannosidase from Canavalia ensiformes and allowed to react with each one of the reagents: ConA, RGDS, 120 kD fragment , FN and Laminin. The results indicate that treatment of T. foetus with 16 $U.ml^{-1}$ ∝ -mannosidase inhibited the agglutination of the parasites induced by FN or the FN fragment of 120 kD.

Supported by CNPq, FINEP and CEPG-UFRJ.

PZ-40 OXIDATIVE DECARBOXYLATION OF 2-OXOACIDS BY PYRUVATE:FERREDOXIN OXIDOREDUCTASE OF TRITRICHOMONAS FOETUS

Moreno,S.N.J. & Docampo,R.

Instituto de Microbiologia, UFRJ, Rio de Janeiro 21941, R.J.

We have demonstrated the formation of two substrate free radicals derived from pyruvate and CoA as intermediates in the generation of acetyl-CoA by T. foetus hydrogenosomes (Docampo, Moreno and Mason, J. Biol. Chem. 262, 1987). In the present communication we report that other 2-oxoacids such as 2-oxoglutarate and 2-oxobutyrate also give rise to free radical intermediates during their oxidative decarboxylation by T. foetus hydrogenosomes. Aerobic incubations of the T.foetus hydrogenosomal fraction containing 2-oxoglutarate (or 2-oxobutyrate), CoA, and the spin trap 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) gave spectra containing contributionsfrom two adducts. One was a carbon-centered radical adduct of DMPO derived from 2-oxoglutarate (or 2-oxobutyrate). The other radical was identified as the CoA thiyl radical adduct of DMPO. Deletion of CoA led to an increased stability of the carbon-centered radical adducts, dissapearance of the thiyl radical adduct and appearance of a hydroxyl radical adduct of DMPO. Superoxide dismutase suppressed the appearance of the DMPO-hydroxyl radical adduct but did not have any inhibitory effect on the appearance of the other adducts. Catalase had no significant effect on any of the adducts. Addition of 2-oxoglutarate or 2-oxobutyrate to these hydrogenosomal preparations stimulated O_2 consumption. Addition of CoA led to a further increase in the rate of O_2 uptake but had no effect in the absence of 2-oxoacids. The oxidative decarboxylation of 2-oxoglutarate by T. foetus hydrogenosomes may be an important pathway for the formation of succinate which is excreted by this protozoa.

Supported by CNPq and FINEP.

Roystonea regia (O.F. Cook) A NEW HOST PALM FOR Phytomonas staheli. **PZ-41**

Attias, M.*, Vilela Resende, M.L.**, Bezerra, J.L.** and de Souza, W.*.
* Instituto de Biofísica Carlos Chagas Filho, UFRJ.
** CEPEC - CEPLAC Itabuna, BA.

Phytomonas staheli is a trypanosomatid that has been associated to palm diseases since the early 1930's. Two diseases are attributed to the presence of P. staheli in the sieve tubes of coconut (Cocos nucifera) and oil palm (Elaeis guineensis), namely hartrot and marchitez sorpresiva. Now it seems that this protozoan can also parasite the phloem of other palm species as Roystonea regia (palmeira real). The observed specimens were collected at Una, in the south of the state of Bahia, Brazil, where hartrot and marchitez are quickly spreading. Fragments of the stem of diseased Roystonea trees, were processed for transmission (TEM) and scanning (SEM) electron microscopy. The presence of sieve elements clogged with parasites was evident in SEM, and younger tubes were preferentially infected. As previously observed in coconut and oil palm, parasites are thin and convoluted, measuring 15 um average. Thin sections showed Phytomonas with an oblique kinetoplast as was previously observed in oil palm. Many parasites were observed close to the sieve plate and although it was not observed, they must be able to cross it through the plate perforations. It is to be determined if the three hosts are harboring a single species of Phytomonas and if there is a single common vector. The occurrence of different hosts in the same geographic area leads to the suggestion that the three diseases should have some common ecological features.

Supported by CNPq, FINEP, CEPG and CEPLAC.

PHYTOMONAS SP FOUND IN LEGUMINOUS CROPS **PZ-42**

Itow Jankevicius, S.*, Jankevicius, J.V.*, Dutra Menezes, M.C.N.*, Torrezan, H.C.* Menezes, J.R.** & Rezende, M.I.*
* - Depto de Patologia Geral, Centro de Ciências Biológicas, Un. de Londrina
Caixa Postal 6001 - CEP 86055 - Campus - Londrina, PR
** - Instituto Agronômico de Londrina - IAPAR

While searching for Phytomonas infected plant species in Paranã state - Brasil, we examined Phaseolus vulgaris cultivar carioca after 80 days of germination, collected at the F.T. Experimental Station, Ponta Grossa-PR. These plants have numerous Nezara viridula bugs and pods with evident symptoms of bug's bite were selected. The seeds from these pods were crushed in a pestle mortar with phosphate buffered saline and the direct microscopic examination revealed small trypanosomatids wich when stained with Giemsa showed typical nucleous and kinetoplast, measuring 8.1 ± 1.5um lenght and 1.6 ± 0.3um widht. Only a few forms had flagella, measuring up 3.8um lenght. Pods of Glycine max cultivars with the same symptoms collected near Londrina-PR, also revealed similar trypanosomatids using the same procedure. These parasites morphologically resembled those found in Solanaceae but were very different from those encountered in Euphorbiaceae.

Supported by grants from CNPq (40.6843/84) and CPG (UEL)

PZ-43 TRYPANOSOMATIDS ISOLATED FROM NEZARA VIRIDULA COLLECTED ON PHYTOMONAS INFECTED LEGUMINOUS CROPS

Jankevicius, J.V.*, Itow Jankevicius, S.*, Dutra Menezes, M.C.N.*, Menezes, J.R** Jurca, M.* , Maeda,L.A.* & Oliveira,E.L.*

* - Depto de Patologia Geral, Centro de Ciências Biológicas - Un. de Londrina
Caixa Postal 6001-CEP 86055-Campus-Londrina,PR.

** - Instituto Agronômico do Paraná - IAPAR

Nezara viridula bugs collected on Phaseolus vulgaris and Glycine max crops infected by Phytomonas sp.at Ponta Grossa, PR and Londrina, PR were dissected and the digestive tract and salivary glands examined revealed flagellates with typical kinetoplast, extremelly pleomorphyc.Bugs from G. max cultivars had, in the digestive tract trypanosomatids measuring 18.6±1,0um lenght , 2.1±0.6um width and flagella with 9.9±7.0um lenght while in the salivary gland , measures 9.1±6.7um x 1.4±0.5um widht, without free flagella. When cultivated in biphasic medium at 28ºC, the culture forms measures, in um

	Source	lenght	widht	free flagella
G. max	Digestive tract	14.6+2.0um	1.5+0.3um	few, up to 2,0um
	Salivary gland	7.0±0.7um	1.5±0.2um	absent
P.vulgaris	Digestive tract	13.0+4.7um	1.6+0.4um	few, up to 11.1um
	Salivary gland	13.4±1.7um	2.2±0.5um	absent

Current experiments are leading to the biochemical identification of these organism and to demonstrate the KOCH's postulate and the possible pathogenesis involved in the plant host.

Supported by grants from CNPq (40.7933/85) and CPG (UEL)

PZ-44 DETECTION OF THE MONKEY CALLITHRIX GEOFFROYI (HUMBOLDT, 1812) NATURALLY INFECTED BY TRYPANOSOMA (MEGATRYPANUM) MINASENSI CHAGAS, 1909, IN THE STATE OF MINAS GERAIS.

RIBEIRO, R.D.; PINTO, F.H.; GARCIA, T.A.R. & RIBEIRO, G.M.C.A.

Faculty of Pharmaceutical Sciences of Ribeirão Preto, USP.

The detection of Trypanosoma (Megatrypanum) minasensi in Callithrix geoffroyi monkeys in Brazil was first reported by Ribeiro and Barretto (1979) for a specimen captured in the State of Espírito Santo. During a field study conducted in the State of Minas Gerais, we had the opportunity to capture eight C. geoffroyi specimens and to perform blood tests on them without killing them. In one specimen we detected trypomastigote forms in the bloodstream. In an attempt to isolate the sample, we submitted the animal to xenodiagnosis and blood culture and inoculated blood into laboratory animals. The results, however, were negative. The morphology of the bloodstream forms was studied in blood smears stained with Giemsa. The mean measurements, obtained by micrometry of drawings of projected images using a curvimeter, were as follows: total length, 34.8 um; posterior end/kinetoplast distance, 9.5 um; kinetoplast/nucleus distance, 7.8 um; body width, 2.8 um, and free flagellum length, 5.5 um. These data permitted us to the identify the trypanosome as Trypanosoma (Megatrypanum) minasensi Chagas, 1909 according to the criteria adopted by Hoare, and to actually consider this species to be a natural host of the parasite in Brazil, in the States of Espírito Santo and Minas Gerais.

OCCURRENCE OF TRYPANOSOMA REGANI TYPE IV FONSECA & VAZ,1928 IN THE ARMORED CATFISH OF PARDO RIVER, MUNICIPALITY OF RIBEIRÃO PRETO, SP, BRAZIL. **PZ-45**

RIBEIRO,R.D.; LOPES,R.A.; SATAKE,T.; NUTI-SOBRINHO,A. & GARCIA,T.A.R.

Faculties of Pharmaceutical Sciences and Dentistry of Ribeirão Preto, University of São Paulo,Ribeirão Preto, SP, Brazil.

The authors describes the occurrence of Trypanosoma regani Type IV in the armored catfish Hypostomus regani, capture in Pardo River, municipality of Ribeirão Preto, SP, in 1984. The smears were stained with Leishman and parameters values of 6 samples were expressed in micrometers: total length values varied from 36.2 to 77.8 and body length from 26.0 to 57.8; the free flagellum is from 10.2 to 21.0; its nucleus is at variable distance from this anterior extremity (8.5-30.6),from kinetoplast (16.9-38.2) and from the posterior extremity (17.4-38.9); its body breadth is from 2.0 to 4.0; the kinetoplast breadth varied from 0.5 to 1.0 and the nuclear breadth varied from 1.1 to 2.5; the nucleus length is from 3.0 to 4.5 and the nuclear index was found to vary from 0.83 to 2.04; the kinetoplast - posterior extremity length is from 0.3 to 0.7. The mean nuclear volume was 17.56μm^3 (10.97-33.51), and mean kinetoplast volume was 0.33 μm^3 (0.10-1.10). The parasite here described is large than that described by Fonseca & Vaz 1928.

NEW TRYPANOSOME SPECIE FROM ACARI RHINELEPIS ASPERA AGASSIZ,1829 (PISCES, LORICARIIDAE). **PZ-46**

RIBEIRO,R.D.; SATAKE,T.; NUTI-SOBRINHO,A.; BRETENGANI,L.G. & LOPES,R.A.

Faculties of Pharmaceutical Sciences and of Dentristy of Ribeirão Preto, University of São Paulo Ribeirão Preto, SP, Brazil.

The authors describes a new trypanosome specie from acaris (Rhinelepis aspera) captured in Paracatu River, municipality of Brasilândia, MG, in 1987. The smears were stained with Leishman. The parameters values of 5 sample are expressed in micrometers. Trypanosoma lopesi n. sp.: total length values varied from 34.2 to 42.1 and body length from 23.6 to 40.3; the free flagellum 0.4--13.0 long, in some samples varies from a were stub to a short whip; its nucleus is at a variable distance from this anterior extremity (7.0-21.0), from kinetoplast (14.0-25.0) and from the posterior extremity (14.5-24.5); its body breadth is from 2.0 to 3.0, the kinetoplast breadth varied from 0.6 to 0.8 and the nuclear breadth varied from 1.0 and 1.6; the nucleus length is from 2.9 to 4.0; the nuclear index was found to vary from 1.5 to 3.2; the kinetoplast - posterior extremity length was zero. The mean nuclear volume, obtained with the cariometry, was 7.33μm^3 (4.70-10.50) and the mean kinetoplast volume was 0.25μm^3 (0.17-0.49).

PZ-47 NEW TRYPANOSOME SPECIE IN THE ARMORED CATFISH HYPOSTOMUS ALATUS CASTELNAU,1855 (PISCES, LORICARIIDAE), FROM PARACATU RIVER, MINAS GERAIS, BRAZIL.

LOPES,R.A.; SATAKE,T.; BRENTEGANI,L.G.; NUTI-SOBRINHO,A. & RIBEIRO,R.D.

Faculties of Dentistry and Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil.

The authors describes a new trypanosomose in the arwored catfish Hypostomus alatus, captured in Paracatu River, municipality of Brasilândia, MG, in 1987. The smears were stained with Leishman and the paremeters values of 15 samples are expressed in micrometers. Trypanosoma dominguesi n. sp.: total length values varied from 20.6 to 41.0 and body length from 20.6 to 34.5; the free flegellun was absent in 4 samples, short in 3 (0.6-1.0) and 4.0-14.3 in 8 samples;its nucleus is at a variable distance from this anterior extremity (6.1-17.9), from kinetoplast(7.1-18.6) and from the posterior extremity (7.9-19.0); its body breadth is from 1.6 to 2.7; the kinetoplast breadth varied from 0.5 to 0.8 and the nuclear breadth varied from 1.0 to 1.7; the nucleus length is from 2.3 to 6.0; the nuclear index was found to vary from 0.76 to 2.11; the kinetoplast posterior extremity length is from zero to 1.0. After the cariometry, the mean nuclear volume was 6.94μm^3 (2.88-14.56), and the mean kinetoplast volume was 0.17μm^3 (0.04-0.32).

PZ-48 NEW TRIPANOSOME SPECIE IN THE ARMORED CATFISH HYPOSTOMUS PAULINUS IHERING,1905 (PISCES,LORICARIIDAE) FROM PARDO RIVER, MUNICIPALITY OF RIBEIRÃO PRETO, SP, BRAZIL.

LOPES,R.A.; RIBEIRO,R.D.; SATAKE,T.; NUTI-SOBRINHO,A. & GARCIA,T.A.R.

Faculties of Dentistry and Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil.

The authors describes a new trypanosome in the arwored catfish Hypostomus paulinus, captured in Pardo River, municipality of Ribeirão Preto, SP, in 1984. The smears were stained with Leishman and the parameters values of 6 samples were expressed in micrometers. Trypanosoma barrettoi n. sp.: total length values varied from 32.2 to 72.4 and body length from 23.2 to 48.4; the free flagellum is from 7.0 to 24.0; its nucleus is at variable distance from this anterior extremity (9.1-22.7), from kinetoplast (12.1-24.0) and from the posterior extremity (13.9-26.4); its body breadth is from 1.1 to 6.7; the kinetoplast breadth varied from 0.4 to 1.2 and the nuclear breadth varied from 1.0 to 5.0; the nucleus length is from 2.9 to 7.0 and the nuclear index was found to vary from 0.8 to 1.4; the kinetoplast-posterior extremity length is from zero to 2.4. The mean nuclear volume was 28.06μm^3 (3.76-111.92), and mean kinetoplast volume was 0.29μm^3 (0.03-1.02).

PZ-49

ULTRASTRUCTURAL STUDIES OF *BLASTOCRITHIDIA TRIATOMAE*

Schaub, G.A.
Inst. Biologie I (Zoologie), Univ. Freiburg, Albertstr.21 a, D-7800 Freiburg
Federal Republic of Germany

The homoxenous trypanosomatid *B. triatomae* is pathogenic for several vectors of Chagas'disease (Schaub, J. Invertebr. Pathol., in press). Cannibalistic or coprophagic infection of bugs is facilitated by the development of resistant cysts, which can survive for at least 3 years. In contrast to other insect trypanosomatids, *B. triatomae* develops enormous numbers of cysts, facilitating ultrastructural studies of excystment (Schaub & Pretsch, Trans. R. Soc. Trop. Med Hyg. 75, 168-171, 1981) and encystment (Peng & Wallace, J. Protozool. 29, 464-467, 1982). Comparison of epimastigotes and cysts by transmission-electron-microscopy of ultrathin sections indicated no substantial differences of mastigotes to other trypanosomatids. Rarely, alterations of nuclear ultrastructure were seen in mastigotes. Final cyst stages showed no ultrastructural details and resembled spores of microsporidia. In intermediate stages ultrastructural alterations of kinetoplast, nucleus, cytoplasm, and subpellicular microtubules were observed, but fixation artefacts could not be excluded. Freeze-cleavage studies of cysts frozen immediately and of those fixed and cryoprotected confirmed the alterations of the internal organelles (Reduth & Schaub, unpublished). In the subpellicular region multiple rows of particles were seen (which appear in electron microscopy of ultrathin sections as a homogeneous region).In addition, the intramembrane particle distribution of the surface membrane was highly asymmetric: the ectoplasmic face had very few, the protoplasmic face numerous, closely packed particles. Since no external cyst wall exists, this peculiarity and the specialized subpellicular region must be responsible for the desiccation resistance of cysts.

Supported by the Deutsche Forschungsgemeinschaft.

PZ-50

Crithidia fasciculata: the influence of U.V. irradiation and culture medium on the growth and survival of a trypanosomatid.

A.M.S. Barros(*) & P.P. Andrade(**)
* Depto. Biologia Geral & (**) Depto. Biofisica e Radiobiologia, Universidade Federal de Pernambuco, RECIFE, PE, BRASIL.

As previously reported (1), U.V. irradiation of C. fasciculata induces a lag-phase in liquid culture medium which is dose-dependent. Parasites which were ressuspended in fresh culture medium 1 hr before irradiation restarted division with the same growth ratio as unirradiated controls. These results were in apparent contradiction with a sharp decrease in parasite survival as measured by colony formation in agar plates. We report here the effect of U.V. light when the parasites are not ressuspended in fresh culture medium before irradiation. The influence of the medium on the survival of the parasites after irradiation is also described. Cell concentrations in culture medium were measured with a Coulter Counter model ZBI. Parasites were maintained in a complex medium containing sucrose, bacto-peptone, yeast extract, hemin and folic acid. A G.E. germicidal lamp (253.7 nm) was used in all experiments. U.V. flux was adjusted at 0.5 $J/m^2.s$. Plates and tubes were incubated at 26°C. Colony formation was visually accessed 5 days after plating.

Again, as previously reported (1), the lag-phase was dose-dependent. The growth ratios after cell division reinitiation, however, changed after U.V. irradiation, being progressively smaller with increasing U.V. doses. Also, cell survival rates after U.V. irradiation were significantly smaller than the observed when parasites were ressuspended in fresh culture medium 1 hr before irradiation. These results suggest that U.V. induced lesions are not repaired or are wrongly excised by one or more error prone mechanisms, acting possibly at the parasite kDNA or nDNA.

This work was partially supported by a CNPq grant. P.P.A. is a CNPq research fellow.

ref.1. Barros,A.M.S. & Andrade, P.P.- Crithidia fasciculata:U.V. induced growth inhibition in a trypanosomatid. Braz. J. Med. Biol. Res. (accepted).

PZ-51

ULTRASTRUCTURAL CHANGES OF Crithidia fasciculata AFTER SHORT U.V. IRRADIATION.

Allana E. Nascimento (*), M.A.M.Santos(**), A.M.S. Barros (***), P.P. Andrade (**)& I.Padovan(*)
(*) Lab. Imunopatologia Keizo Asami, (**) Depto.Biofisica e Radiobiologia & (***) Depto. Biologia Geral, Universidade Federal de Pernambuco, Recife, PE, Brasil.

Ultrastructural changes after U.V. irradiation were described previously for Herpetomonas samuelpessoai (1). We report here changes in kinetoplast ultrastructure induced by exposure of Crithidia fasciculata to short U.V. light. The flagellates were grown in a complex medium containing sucrose, bactopeptone, yeast extract, hemin and folic acid. They were harvested by centrifugation, ressuspended in fresh culture medium and incubated for an additional 1 hour before exposition to U.V. light (200 J/m). Parasites were collected for transmission electron microscopy at 24 and 48 hours after irradiation. They were washed 3X in PBS, fixed for 1 hour in 2.5% glutaraldehyde in PBS pH 7.4, washed again 3X and post-fixed in 1% OsO4. After dehydration, the cells were embeded in EPON; the blocks were cut and the material stained with lead citrate and uranyl acetate. The grids were observed in a JEOL 100 transmission electron microscope.

No changes could be observed immediately after irradiation. Therefore, after 24 and 48 hours ultrastructural abnormalities of the kinetoplast (low electron density, drastic changes in shape, fuzzy fibrilar network) would be detected in almost all parasites. Large vesicles similar to peroxisomes or glycosomes but with low electron density were some times observed.

Mitochondrial cristae were also reduced in size and relative number. The other cellular structures were apparently unchanged. A correlation between these findings and the parasites survival after U.V. irradiation is presently under
investigation.

This work was partially supported by CNPq grant and by the Japanese International Cooperation Agency.

Ref.(1)- Penido et al.(1980)- Herpetomonas samuelpessoai effects of U.V. radiation and survival curves. Abst. BI31 Annals VII. An. Meet. Basic Res. Chagas' Disease. Caxambu, MG, Brasil.

PZ-52

PHOTOREPAIR OF U.V.-INDUCED DNA LESIONS: A POSSIBLE EXPLANATION FOR INCREASED SURVIVAL OF U.V.-IRRADIATED Crithidia fasciculata WHEN EXPOSED TO VISIBLE LIGHT.

Aline E.Nascimento(*), A.M.S.Barros(**) & P.P.Andrade(*).
(*)Depto. Biofisica e Radiobiologia & (**) Depto. Biologia Geral, Universidade Federal de Pernambuco,Recife, PE, Brasil

Photorepair enzymes(PRE) have been described in many eukaryotic cells.PREs cut the cyclobutane ring of thymidine dimer when the DNA is exposed to photoreactivating light(400-500nm). The photorepair of dimers usually leads to improved survival, as measured by colony formation or growth rate in liquid medium. We described here the effect of post-exposition of U.V.-irradiated C. fasciculata to photoreactivating light on the survival rates, as evaluated by colony formation in solid medium. Effective U.V. doses were corrected by a factor of 10 due culture medium absorption. Parasites were grown in a complex medium containing sucrose, bactopeptone, yeast extract, hemin and folic acid. They were ressuspended in fresh medium 1hr before irradiation. After being exposed to differents U.V.doses up to 20J/m^2, the parasites were post-exposed for 1hr to the photoreactivy light of a white 100W lamp or kept in the dark. They were then diluted and plated as described elsewhere(1). Survival rates were significantly higher in post-exposed parasites then in those kept in the dark, for all doses studied.These results closely correspond to the photorestauration in bacteria post-exposed to photoreactivating light, which is known to be due to the action of PRE; they suggest that C.fasciculata has a DNA photorepair mechanism possibly similar to those described for other eukaryotes.

This work was partially supported by CNPq grant. P.P.Andrade is a CNPq research fellow.

Ref.(1)-Tanuri et al(1981)- J.Protozool.28:360-363

PZ-53

EFFECT OF LITHIUM CHLORIDE ON LECTINS RECEPTORS IN *Herpetomonas samuelpessoai*.

Nakamura, C.V.[1]; Pinto, A.S.[2]; Hamanaka, S.I.[2]; Esteves, M.J.G.[3] & Andrade, A.F.B.[3]

1. Departamento de Farmácia-Bioquímica, Universidade Estaduál de Maringá, Caixa Postal 331, Maringá, PR, Brasil.
2. Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Caixa Postal 2486, Belo Horizonte, MG, Brasil.
3. Instituto de Microbiologia, UFRJ, C.P. 21941, Rio de Janeiro, RJ, Brasil.

Previous studies showed that LiCl interfere with the growth and inhibit the cell differentiation in *H. samuelpessoai*. We report here the effect of this salt on lectins-mediated agglutination of this flagellate, when incubated in a defined medium at 28 and 37 C for 72 h. Lectins with the following specificities were used: DGlcNAc, DGalNAc, DGal, DMan and L-fucose. Agglutination assays were performed with a Takasky microtitrator using 0.025 loops and suspensions containing 2×10^8 cells/ml. Equal volumes of the cells suspension and lectin dilution were mixed , placed at room temperature (24 C) for 1 h, and read. Of those lectins only Con A (DMan-like residues) agglutinated the cells. The minimal concentration (µg/ml) of the lectin required for agglutination cells incubated at 28 C in presence of: No add=62.5, LiCl 15mM=62.5, LiCl 150mM=15.6 and cells incubated at 37 C in presence of: No add=62.5, LiCl 15mM=62.5, LiCl 150mM=7.8 . The distribuition of Con A-binding sites in the LiCl treated cells was similar to that found in the controls.

Supported by CNPq.

PZ-54

EFEITO DO DIMETILSULFÓXIDO (DMSO) EM HERPETOMONAS SAMUELPESSOAI.

Soares,R.M.A., Angluster,J. & Alviano,C.S.
Instituto de Microbiologia da UFRJ, Bloco I,Cidade Universitária,Ilha do Fundão, 21941 - Rio de Janeiro, RJ.

Glicoproteinas de H. samuelpessoai cultivada em meio quimicamente definido, a 28ºC, por 48 horas, na presença e ausência de DMSO (2%) foram extraídas com fenol a 90% em PBS, pH 7,2, 0,01M, a 67ºC por 10 minutos e caracterizadas por mobilidade eletroforética em gel de agarose, análise química e pelo uso da cromatografia líquido-gasosa e do analisador de amino ácidos. As glicoproteínas de H. samuelpessoai em gel de agarose evidenciaram uma banda ampla principal e uma menor, ambas com caracter negativo. No extrato fenólico foram detectados através,dosagem química,ácido siálico, fosfato e ácido urônico. Fucose, ribose, xilose, manose, galactose, glicose e inositol foram os componentes osídicos identificados. Xilose, ribose e glicose foram os açúcares predominantes. Glicina, serina e os ácidos glutâmico e aspártico foram os proeminentes amino ácidos caracterizados. Esses resultados indicam a presença de ligações do tipo N e O-glicosídicas. Tais características das glicoproteínas de H. samuelpessoai foram sensivelmente modificados pelo DMSO. A droga induziu o aparecimento de outra glicoproteína, aumentou o teor de galactose, diminuiu de fosfato, da glicose e inositol. Essas significantes altenações no perfil de glicoproteínas provocadas pelo DMSO podem ser importantes para o processo de diferenciação celular induzido pela droga em H. samuelpessoai.

Financiamento: CNPq, FINEP, CEPG-UFRJ.

PZ-55 LECTIN BINDING TO SURFACE SACCHARIDES ON ENDOTRYPANUM STRAINS.

Lopes,A.H.C.S.,* Esteves,M.J.G.*, Angluster,J.* & Andrade,A.F.B.**
*Instituto de Microbiologia-UFRJ, Deptº Microbiologia Geral, ** Departamento de Imunologia, Bloco I, C.C.S. Cidade Universitária, Ilha do Fundão, 21941 Rio de Janeiro RJ.

The cell surface of the Endotrypanum spp. strains were characterized comparatively by using 18 lectins with different sugar specificities. The IM-201, IM217, LV-88 and M-6226 strains were obtained in a complex medium and used for lectin-binding activity. All the strains were agglutinated by Canavalia ensiformes (Con-A) and Lens culinaris (LCL) D-Man-binding lectins. However, important quantitative differences were observed in the Con-A agglutination pattern between the IM-201 (31.25 ug/ml), IM-217(500 ug/ml), LV-88 (125 ug/ml) and M-6226 (15.62 ug/ml) strains. Qualitative differences were also detected. The Bandeiraea simplicifolia I (BS-I, 62.5 ug/ml) and Glycine max (SBA, 73.7 ug/ml) D-GalNAc-binding lectins interacted selectively with the IM-217 strain. On the other hand, Arachys hypogaea (PNA, 62.5 ug/ml) D-Gal-binding lectin and Bandeiraea simplicifolia II (BS-II, 31.7 ug/ml) D-GlcNAc-binding lectin reacted only with the M-6226 strain. The Artocarpus integrifolia (jacalin) D-GalNAc-binding lectin did not agglutinate the IM-207 strain. The ^{125}I-labeled lectin binding to strains was consistent with the agglutinated data. The association constant (K_o) and the average number of lectin binding sites per cell (n) will be reported. Our results suggest that the lectins can be used as a tool in the identification of strains within the genus Endotrypanum.

PZ-56 IDENTIFICATION AND PARTIAL CHARACTERIZATION OF PLASMA MEMBRANE POLYPEPTIDES OF Crithidia guilhermei, Crithidia deanei and Crithidia oncopelti.

Giovanni De Simone, S. (1), Soares, M.J. (2), De Souza, W. (3) and Goldenberg, S. (4).

1. Deptº Imunologia, FIOCRUZ, Rio de Janeiro, RJ and Deptº Biol. Celular e Molecular, UFF, Niteroi, RJ; 2. Deptº Ultraestrut. e Biol. Celular, FIOCRUZ, Rio de Janeiro, RJ and Inst. Biofísica, UFRJ, Rio de Janeiro, RJ; 3. Inst. de Biofísica, UFRJ, Rio de Janeiro, RJ; 4. Deptº Bioquímica e Biol. Celular, FIOCRUZ, Rio de Janeiro, RJ, Brazil.

Components of the cell surface of C. guilhermei, C. deanei and C. oncopelti were radioiodinated by the iodogen technique. The distribution of proteins in the detergent-poor (DPP) and detergent-enriched phase (DRP) were studied using a phase separation technique in Triton X-114 and one-and-two-dimensional polyacrylamide gel electrophoresis in sodium dodecyl sulphate (1D and 2D SDS-PAGE). Significant differences were noted in the proteins present in the DRP when the three species were compared. Two major bands with mol. wt. 28,000 and 56,000 and motility in the pH gradient of 7.4 and 6.3, respectively, were observed in C. guilhermei, but not discernible in C. deanei and C. oncopelti. One polypeptyde with mol. wt. 50,000 and pI 4.9 was identified in the DRP of C. deanei. A broad of C. deanei and one or two polypeptides only present in the DPP were observed in the three Crithidia species analyzed. Our observations show that C. guilhermei has characteristic surface polypeptides not found in C. deanei and C. oncopelti. Our results, in association with those reported by others, show that the phase separaction using Triton X-114 offers a simple approach to the separation and further analysis of a selected group of proteins from the bulk of the cellular proteins.

This work has been supported by CNPq, FINEP and WHO-World Bank.

ISOLATION OF INTRACELLULAR SYMBIONTS BY PERCOLL GRADIENT OF CRITHIDIA DEANEI AND CHARACTERIZATION OF THEIR DNA. **PZ-57**

E.Novak, E.F.Haapalainen, S.da Silva & J.Franco da Silveira
Disciplina de Parasitologia - Escola Paulista de Medicina S.P. Brasil.C.P.20342

The method described here allows the isolation of pure and intact symbionts in sufficient amounts for biochemical studies. The flagellates were disrupted by sonication and the symbionts were separated from unbroken cells and cellular debris by centrifugation through a discontinous Percoll gradient. The isolated symbionts retained their characteristic morphological features and they were reasonably free of subcellular debris.

The ornithine carbomoyltransferase enzyme (OCT), a symbiont enzymatic marker, present in the symbiont fraction has a specific activity 3 fold higher than in crude homogenates and ~6% of total OCT activity of crude homogenate was recovered in the symbiont fraction. We estimate that 20% of symbionts present in the homogenate are recovered in this fraction.

The incorporation (^{3}H)-thymidine into acid-insoluble symbiont fraction was linear up to 75 min showing that isolated symbionts can synthesize DNA. This result suggests the presence in the symbionts of a dependent thymidine kinase salvage pathway for DNA synthesis.

The purity and integrity of DNA extracted from isolated symbiont were analysed by agarose gel electrophoresis. It comigrates with intact E. coli DNA as a single heavy band. Southern blot analysis showed that the symbiont DNA hybridizes to ^{32}P-E. coli RNA.

These results reinforce the notion of the prokaryotic nature of endosymbionts.

Supported by CNPq.

PROTEIN SYNTHESIS IN THE ISOLATED SYMBIONTS FROM THE FLAGELLATE PROTOZOAN CRITHIDIA DEANEI. **PZ-58**

E. Novak, E.F. Haapalainen & J.Franco da Silveira.
Disciplina de Parasitologia, Escola Paulista de Medicina, CP: 20342, SP, Brasil.

The symbionts in the flagellate C. deanei were isolated by Percoll density gradient. The isolated symbionts incorporated ^{3}H-leucine and ^{35}S-methionine actively into acid-insoluble fraction for the first 90 and 120 min, respectively. In order to characterize protein-synthesis machinery operating in the symbionts, we studied the effects of chloramphenicol and rifampicin on the incorporation of leucine into proteins. Chloramphenicol and rifampicin at 20µg/ml decrease the incorporation by about 70% and 50%, respectively. These antibiotics at 50µg/ml inhibited the incorporation almost completely. The inhibition of protein synthesis by chloramphenicol and rifampicin provides direct evidence for the existence of a prokaryotic protein-synthesizing system in this unusual subcellular structure.

The labeled synthesized polypeptides are in the molecular weight range 200,000 - 18,000, as determined by SDS-polyacrylamide gel electrophoresis. The most heavily -labeled polypeptides were those with apparent molecular weight of 90,88,78,60 and $58x10^{3}$. The profile of symbiont labeled polypeptides differs from that obtained with the symbiont strain of C. deanei. Several symbiont polypeptides are absent or poorly represented in the flagellate, for example, the 88,78,60 and 58 KDa polypeptides. This result suggests that the synthesis of these polypeptides is inhibited in the intact flagellate.

Supported by CNPq.

PZ-59 CHARACTERIZATION AND PARTIAL SEQUENCING OF TUBULIN GENES IN TRYPANOSOMA RANGELI.

Esquenazi,D., Morel, C.M., Traub-Csekö, Y.M.
Departamento de Bioquímica e Biologia Molecular - Fundação Oswaldo Cruz
Av. Brasil 4365 - Manguinhos, Rio de Janeiro, RJ, Brasil

T.cruzi and T.rangeli are the only trypanosomes known to infect humans in America. T.cruzi is the causative agent of Chagas' disease and although T.rangeli does not seem to be responsible for a specific pathology, there is some evidence for serological cross-reaction between these two organisms which may hinder epidemiological studies. We have done some experiments to investigate the resemblance of T.rangeli and T.cruzi at the genomic level by studying genes already well characterized in other trypanosomatids, the tubulin genes. It has been shown that in Leishmania the organization of the genes is in separate clusters of alfa or beta genes and in african trypanosomes in clusters of alternating alfa and beta genes. In T.cruzi the structure is more complex, but at least part of the genes seems to be in an alternating alfa and beta cluster. In T.rangeli genomic Southern blot experiments using DNAs of various isolates cut with different restriction enzymes and hybridized to alfa and beta tubulin genes of L.enrietti show that there is a band of 3800bp that lights up with both probes, indicating that these genes are linked and repeated in tandem.
We have cloned Bam HI DNA fragments of 3800bp in pBR322 and isolated 3 clones containing tubulin genes, pRM1, pRM2 and pRM4. Clones pRM1 and pMR2 have identical inserts. We have mapped clones pRM2 and pRM4 and found only one difference, the presence of a Hind III site in pRM4. This is interesting in view of our observations that two isolates of T.rangeli, Venezuela and José, differ at the genomic Southern blot analysis level only at the HindIII site, which suggests a differential amplification of polymorphic tubulin genes. Pst I digestion of the Bam HI insert of pRM2 gives rise ot 3 bands that were cloned in M13mp8 and M13mp9 in both directions and sequenced through the Sanger technique. These sequencing data permitted the exact positioning of the genes on the repeat. We have compared our sequences to published T.brucei rhodesiense sequences through computer homology matrix constructions, and found high levels of homology. In conclusion, we have shown in T.rangeli a structure of tubulin genes amazingly similar to the one found in the african trypanosomes. If in T.cruzi, as predicted, we find a structure of tubulin genes at least in part consisting of alternating alfa and beta genes, this structure will be so far a general feature of the genus Trypanosome.

CNPq, FINEP, WHO

PZ-60 IMMUNOCYTOCHEMICAL ANALYSIS OF LANGERHANS CELLS IN MURINE CUTANEOUS LEISHMANIASIS.

Tapia, F.J., Rojas, E., Mosca, W.
Instituto de Biomedicina, Apartado 4043, Caracas 1010A, Venezuela.

Langerhans cells (LC) are immunocompetent cells of the skin that function as potent antigen-presenting and accesory cells. The importance of these cells in cutaneous leishmaniasis remains uncleared. The present study involves the characterization and quantification of LC at different stages of the experimental Leishmania infection. C57BL/6 mice were infected with Leishmania mexicana amazonensis (L.m.a.) strain M1132. Some of the infect mice were treated with monobenzyl ether of hydroquinone (MBEH), an agent that causes a marked increase in the population density of LC. The C57BL/c mice (n = 60) were inoculated s.c. in the footpad with 10^3 amastigotes of L.m.a.. To the group of treated animals (n = 30), 20% MBEH was topically applied 7 days before infection. The analysis was performed on frozen section and EDTA-separated epidermis. The immunocytochemical characterization was carried out using anti-murine LC monoclonal antibody NLDC-145 (kindly donated by G. Kraal, The Netherlands) and an avidin-biotin immunoperoxidase technique. Infected non-treated mice showed a significant increase in the number of epidermal LC/mm^2. These values were the double of those obtained for the MBEH treated mice and four times those of healthy animals. A similar analysis was carried out on infected BALB/c mice. These animals showed a marked increase in LC density during the course of infection. In each animal the non-inoculated footpad always showed an increase in LC similar but in a higher scale to that observed in the infected foot pad. The latter emphasizes the concept of the skin as an integral organ. These results demonstrate an association between LC density and Leishmania infection.

Financed by Consejo de Desarrollo Científico y Humanístico, Universidad Central de Venezuela, Project M-10.16/87.

PZ-61

CUTANEOUS LEISHMANIASIS IN GERMFREE AND CONVENTIONAL MICE

Guimarães, I.T., Silva, M.E., Nicoli, J.R., Afonso, L.C.C., da Costa, C.A., Mayrink, W., Bambirra, E.A. & Vieira, E.C.

Departamentos de Bioquímica-Imunologia e de Parasitologia, Instituto de Ciências Biológicas; Departamento de Anatomia Patológica, Faculdade de Medicina, Universidade Federal de Minas Gerais, C. P. 2486, Belo Horizonte, MG, Brasil.

Fifteen germfree (GF) and 16 conventional (CV) 21 days old CFW mice were infected, 1cm above the root of the tail with 10^6 promastigotes of Leishmania mexicana amazonensis. Both GF and CV groups were maintained in isolators and fed the same heat-sterilized diet. After 75 days, the experiment was interrupted due to a monocontamination of the isolator by a fungus. There was no difference in weight gain between the two groups of mice. Macroscopically, the lesion was much more prevalent and intense in the CV group. All of the 16 CV mice had ulderated lesions. In the GF group, only one animal exhibited ulcerated lesions and two had non-ulcerated nodules. Histopathologically, the CV animals showed a severe infection reaching the skeletal muscles. There was an intense inflammatory reaction by mononuclear leukocytes. Areas of intense necrosis were observed. Numerous amastigotes were found in volumous intracellular nests. GF mice, on the other hand showed discrete cutaneous lesions restricted to epidermis and subcutaneous tissue. An inflammatory reaction consisting fo focal mononuclear leukocytes was observed. Small nests of intracellular amastigotes were observed.

(Supported by FINEP, CNPq, CAPES, and FAPEMIG)

PZ-62

COMPARISON OF COMPLEMENT FIXATION AND DIRECT AGGLUTINATION TEST WITH 2 MERCAPTOETHANOL (AD2ME) IN THE SEROLOGICAL DIAGNOSIS OF CHAGAS' DISEASE.

Diaz de Ramirez, A., Urdaneta, H. & Mancilla, I.
Universidad de los Andes, Nucleo Universitario "Rafael Rangel", Trujillo, Venezuela.

The Direct Agglutination Test using 2-mercaptoethanol (AD2ME) was compared with the Complement Fixation Test (CF) on sera of 319 blood donors from the city of Valera Blood Bank, in the State of Trujillo, and on sera of 25 persons who had been affected with tegumentaria americana leishmaniasis and had been clinically cured. The agreement observed between the two tests was 92,16%. The disagreements between the tests involved 25 sera of which 22 presented positive titers only with AD2ME, and 3 only with CF. The statistical analysis using Chi squared revealed that the differences observed between the results of the methods were not significant. Consequently, the conclusion was reached that, considering the advantages of AD2ME, this test is more practical in the Blood Bank Laboratories for screening out blood with anti *Trypanosoma cruzi* antibodies.