IV REUNIÃO DA SOCIEDADE BRASILEIRA DE PROTOZOOLOGIA

PZ

PROTOZOOLOGY
PZ-1

E. histolytica ENCYSTMENT ORIGINATED IN TROPHOZOITES KEPT IN AXENIC CULTURE

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A major drawback in the study of amoebiasis today is the lack of an axenic in vitro culture system in which the complete life cycle of E. histolytica can be expressed (Avron, 1986). This problem renders difficult the knowledge of biochemical factors that act in the cellular differentiation.

We were able to obtain a rate of 20% of axenic encystment carrying out alterations in TPS-1 (Diamond, 1968) and TTV-3-33 (Diamond, 1978) media, by the addition of sodium thioglycolate and magnesium sulphate, as well as by variations in the osmotic pressures of those media. This rate is higher than other described in the literature.

The cysts obtained were found to be resistant to detergents, hydrochloric acid and hypotonic media, e.g., distilled water.

Cyst characterization was done by biometric studies and staining by Ferric Hematoxylin. Viability of the cysts was assessed by the Trypan Blue Test, and by their replacement in the original media, after incubation in Triton X-100 (1%), hydrochloric acid (1%), and distilled water, where they transformed again into trophozoites. These trophozoites were able to reproduce in culture media.

In this way, the complete life cycle of E. histolytica (ICB-CSP, ICB-462 and HMI strains) was obtained in vitro, at our laboratories.

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PZ-2

ISoenzyme PATTERNS OF ENTAMOEBA HISTOLYTICA Stocks Grown in AXenic and NON-AXenic Conditions

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Nonpathogenic Entamoeba histolytica stocks (32, 462), isolated from asymptomatic individuals, with negative serology and with no previous clinical presentation of amoebic disease, were cultivated under axenic conditions in our laboratory. Axenization was accomplished by washing faecal cysts in various solutions (KCl, MnO₄, HgCl₂ and acriflavine) and by treating the stocks with various antibiotics after antibiogram sensitivity determinations. Cultures were maintained in TPSI medium (Diamond, 1968). The electrophoretic patterns of four enzymes (GPI, PM, ME and HK) showed that under axenic conditions the stocks could be classified as zymodeme II (Sargeant and Williams, 1982), a 'pathogenic zymodeme'.

Another E. histolytica stock (CSP) isolated from trophozoites of a patient with amoebic dysentery and colon ulcerations, and grown in monoxenic (E. coli) conditions, showed at electrophoresis, a not previously described zymodeme type, with slow HK running bands characteristic of nonpathogenic zymodemes, but with a ⁰⁰⁰⁰⁰ PGM band, thought to be a pathogenic marker. However, a shift to a characteristic pathogenic zymodeme II was observed when the stock was cultured under axenic conditions. Also, axenic CSP cultures directly introduced into hamster liver and re-isolated after 6 days, showed trophozoites actively ingesting red blood cells which at electrophoresis, showed the same group II isoenzyme pattern.

Our results, in agreement with those of Mirelman et al (1986), indicate that culture conditions and bacterial flora can cause changes in E.histolytica isoenzyme profiles.

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DIFFERENTIATION OF AXENIC STRAINS OF Entamoeba histolytica BY USING THEIR GLYCO PROTEIC PATTERN

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In this research five axenic strains of E. histolytica were used: ICB-CSP from patient with symptomatic and severe virulent amebiasis; ICB-32 from patient with non-enteric colitis, starting from cysts, and moderately virulent; ICB-462 from asymptomatic patient, poorly virulent; two other ones were standards: HK9 (USA) and HML (Mexico), the latter being highly virulent. Soluble antigens were obtained after parasite disruption by sonication followed by addition of protease inhibitors (PMSF, TPCK, TLCK) and centrifugation at 20000g at 40C.

After lipid extraction with ether, the soluble antigens corresponding to each strain (60 ug protein) were separated by SDS-PAGE, and the polypeptide bands transferred to a nitrocellulose membrane for detection of glycoproteins, using peroxidase bound to concanavalin A (Con A).

The presence of peroxidase is revealed by a mixture of diaminobenzidine and 4-chloro 1-naphthol in the presence of 30% H2O2.

After reaction with Con A - Peroxidase conjugate, the pattern showed by the strains was complex and apparently homogeneous. The only difference presented was in the highly virulent Brazilian strain, whose band (corresponding to glycoprotein with molecular weight of 58 KDa, approximately) was more evident than in the other strains.

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STUDY OF THE ELECTROPHORETIC PROFILE OF AXENIC STRAINS OF Entamoeba histolytica ISOLATED IN BRAZIL, UNITED STATES AND MEXICO

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The present work was carried out by using five strains of E. histolytica kept in axenic cultures in TPS-1 medium (Diamond, 1968). Three of them were isolated and maintained under axenic conditions in Brazil: ICB-CSP from patient with symptomatic and severe virulent amebiasis; ICB-32 from patient with non-enteric colitis, starting from cysts, and moderately virulent; ICB-462 from asymptomatic patient, poorly virulent. Two other ones were standards: HK9 (USA) and HML (Mexico), the latter being highly virulent.

Soluble antigens from each strain (60 ug protein) were fractionated by SDS-PAGE 5%-12%, and the gel stained by Comassie Blue. Lipid extraction was previously performed by means of ethyllic ether, in order to improve the electrophoretic results.

The electrophoretic profiles of the strains were very similar, showing polypeptides with molecular weight ranging from 200 to less than 29KDa, approximately.

The results obtained demonstrated that the strains isolated and kept under axenic conditions in Brazil presented the same electrophoretic pattern of the standard strains (HK9 and HML).

This work received financial support from CAPES and FINEP.
ENTAMOEBA HISTOLYTICA: PRODUCTION AND ISOLATION OF IgG FOR DETECTION OF CO-PRODUCTS


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Antibodies against 4 different strains of E. histolytica (ICB-CSP and ICB-462 from Brazil and HMI from Mexico) were raised in rabbits. Immunoglobulin G was isolated from immune rabbit sera in two steps: first chromatography in a column containing E. histolytica antigens (strain HMI) bound to Sepharose 4B followed by another chromatography in Sepharose 4B - Protein A.

The specificity of the IgG anti E. histolytica obtained was demonstrated in ELISA assay other enteroparasites such as: Entamoeba coli, Entamoeba hartmanni, Endolimax nana, Giardia lamblia, Ascaris lumbricoides, Ancilostoma duodenalis, Trichuris trichura e Taenia sp. No cross reactivity was observed.

The isolate IgG are able to detect in ELISA assay an amount of antigenic protein equivalent to one trophozoite.

The specific IgG obtained will be used in ELISA assay trying to establish an specific immunodiagnostic test for E. histolytica.

This study was supported by FINEP, FAPESP and CAPES

MOLECULAR CHARACTERIZATION OF Entamoeba histolytica TROPHOZOITES ANTIGENS.

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Cell surface proteins of Entamoeba histolytica strains (HK9 and ICB-UFMG) were radiodinated using chloroglycoluril and their distribution in the detergent-poor (DPP) and in the detergent-enriched phase (DRP) was studied, using a phase separation technique in Triton X-114, and polyacrylamide gel electrophoresis in sodium dodecyl sulphate.

E. histolytica membrane contains at least 5 polypeptides of which 4 could be solubilized in Triton X-114.

Minor apparent qualitative differences between the two strains were detected by autoradiography. One major radiolabelled band with M.W. 95,000 and one minor with M.W. 42,000 was identified exclusively in the DPP of HK9. One polypeptide with 85,000 was detected in the DPP of ICB-UFMG strain. Two bands with M.W. 92,000 and 40,000 were separated into the DRP of the two strains.

No major differences were however detected in the gel with DRP and DPP of the two strains by staining with coomassie or silver. Four major bands with M.W. 42,000, 60,000, 116,000 and 160,000 could be detected in the DPP, whereas in the DRP only one band with M.W. 100,000.

Individual serum with invasive amebiasis recognized two major hydrophilic antigens with M.W. 42 and 45,000. The antigen with M.W. 42,000 was present in the surface cell membrane.
SUZEXIBILITY OF Acanthamoeba spp. TO SOFT CONTACT LENS DISINFECTION SYSTEMS

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Since 1973 corneal ulcers by Acanthamoeba spp have been recognized in increasing number and in association with soft contact lens wearers (more than 80 cases until 1987).

In this study, heat and cold soft contact lens disinfection systems were tested according to the manufacturers' instructions against five species of the genus Acanthamoeba, three isolated from eye infections, one in this country and two in the U.S.A.

The heat disinfection consisted of thermal units that reached 90°C for at least 10 min. The cold disinfection consisted of Hidrocare(R) (Alkyli triethanol amonium chloride 0.330mg, Thiomersal 0.023mg) and Flex-care(R) (Chlorhexidine gluconato 0.005%, Thimerosal 0.001%).

Trophozoites and cysts were placed in 10 ml of the disinfection solution, 8 hr for Hidrocare(R) and 4 hr for Flex-care(R).

All heat disinfection units killed Acanthamoeba spp in one cycle. Hidrocare(R) killed A. castellanii, A. polyphaga, A. astronyx and A. culbertsoni, but A. sp survived. Flex-care(R) killed A. castellanii, A. polyphaga and A. astronyx, but A. sp and A. culbertsoni survived.

Heat disinfection overall appears to be more effective in killing Acanthamoeba as compared to cold disinfection methods.

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ACANTHAMOEBA CORNEAL ULCER (THE FIRST FOUR REPORTED CASES IN LATIN AMERICA)

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The authors reported the first four cases of corneal ulcer due to Acanthamoeba in Latin America.

All patients were contact lens wearers. In spite of several different pharmacological approaches, all four cases required therapeutic corneal transplantation that cured the disease.

The diagnosis was accomplished by pathological studies in two cases and by cytology and cultured in two others.

This entity must be considered in the differential diagnosis of corneal ulcers and, above all, in contact lens wearers.

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SOLUBLE ANTIGENS IN URINE OF MALARIAL PATIENTS

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Although more than 99% of malarial registered cases in Brazil have been acquired in the Amazon region, the migration movements from and to endemic areas are potential factors of dissemination of the disease. Indeed, asymptomatic infected individuals are potential transmitters of malaria through blood transfusion. In order to look for alternative methods for the diagnosis of active infection we have searched for malaria soluble antigens by immunoblotting in urine specimens from: 3 normal individuals, 3 infected individuals without past history of malaria, 3 infected individuals with past history and 13 non-infected with past history of malaria. These individuals originated from an endemic area of malaria in Northwestern Brazil (Ariquemes – RO). The urine was collected and after centrifugation was concentrated by Amicon PM 10 and used 50 times concentrated. At the time of writing this abstract, urine from all the 19 patients which have been screened with sera from 6 hyperimmune patients by immunoblotting, presented at least one antigen that was not observed in normal urines. 13 out of 19 tested urines presented an antigen with approximately 60 KDa, 5 others showed a 50-55 KDa band and 1 individual presented simultaneously antigens of 60, 80 and 116 KDa. 7 presented a 50-55 KDa antigen in addition to that of 60 KDa and 3 patients with past history of malaria presented a 80 KDa antigen besides those of 50-55 and 60 KDa.

Although, up to the moment, the detected antigens seem to be parasite but not species specific and we could not observed a relationship between presence of antigens and active infection, further studies are being carried out in our laboratory and are necessary before a definitive conclusion can be drawn.

ANTIGENURIA IN MALARIOLOGY.

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Antigenuria in malaria has been reported by Kimura et al. (III Meeting Protozoool. PZ 28, 1987).

In this communication we have been able to characterize antigens from urine and compared with total antigenic parasite's extract.

In 41 out of 45 patients infected with Plasmodium falciparum and 30 out of 35 patients infected with P. vivax antigenuria was detected through double sandwich dot-blotting in urine samples without concentration. After 100 times concentration by Minicon B 15 the samples were analyzed by Western-blot (two-dimensional or unidimensional); antigens were detected in 41 out of 45 patients infected with P. falciparum with the following molecular weight (MW): 96 kDa, pI 5.1 (30 patients); 180 kDa, pI 6.8 (8 patients); 200 kDa, pI 6.0 (21 patients). In 16 urine samples, 2 or 3 bands with the same MW were detected. Patients infected with P. vivax (30/35) secreted antigens with MW of 200 kDa, pI 6.8; and some of them additional bands with 100 kDa were detected.

The most frequent antigen detected in patients infected with P. falciparum was the 96 kDa. This antigen was already described as a soluble antigen shedded by the parasite. This protein was detected in urine through monospecific immunoserum.

To investigate the origin of antigens eliminated in urine, we have immunoprecipitated cultured medium obtained from erythrocytes infected with P. falciparum, (pre-labelled with [35S]-methionine) with the same immunoserum used in the Western-blot. These materials were analyzed by two-dimensional SDS-PAGE. The same antigens eliminated in urine were detected in the supernatant of culture medium, suggesting that part of the antigens eliminated in urine are shedded (probably S antigens).

Antigenuria in malaria can be used for rapid diagnostic test. However, free antigens as well as immunocomplexes could be also correlated to immunopathological mechanisms.

Acknowledgments: urine samples were obtained by SUCEN.

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A PRELIMINARY STUDY ON THE PREVALENCE AND DISTRIBUTION OF ANTIBODIES TO 72 kDa POLYPEPTIDE OF P. FALCIPARUM IN PATIENTS OF MALARIA SERA.

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Protective immunity against P. falciparum infection in monkeys, can be induced by a 75 kDa SDS-PAGE protein fraction purified from crude extract of schizonts grown "in vitro". This immunity was demonstrated to be mediated by antibodies (Dubois et al., 1985). A 72 kDa polypeptide was identified as a component of this fraction and was produced by genetic recombination by Ozaki et al. (1986). In order to know the prevalence and distribution of antibodies to the 72 kDa polypeptide in human populations of endemic areas of malaria, serum samples of 86 patients who sought medical care at SUCEM (Superintendência de Controle de Endemias da Secretaria de Saúde de São Paulo) were tested by ELISA, using the recombinant protein as antigen. The patients were distributed into 3 groups according to the number of previous episodes of malaria: I (0); II (1-4) and III (>4). Our results showed that there is an increase in frequency and levels of antibodies to 72 kDa polypeptide related to the number of previous infections. Frequency of positive reactions was: 21% (I); 52% (II) and 69% (III). Considering that the acquisition of immunity to malaria is related, among others factors, to the multiplicity of infections, a longitudinal field investigation would be required to evaluate a possible relationship between these antibodies and the development of resistance in humans.

Acknowledgments: sera samples were obtained from SUCEN.

Supported by: CAPES, CNPq, FINEP, FAPESP.
MALARIA: STUDY OF THE PATTERN OF HUMORAL IMMUNE RESPONSE TO *PLASMODIUM FALCIPARUM* POLYPEPTIDES, ACCORDING TO THE IMMUNE STATUS.

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In order to set up a screening test that could discriminate between active and past malaria infections, we studied the ability of sera from individuals with different immune status, reacting with antigen fractions of *P. falciparum*. The following groups of individuals were analysed: patients with active infection with or without past history of malaria, asymptomatic individuals with past attacks of malaria and negative controls from endemic or non endemic areas.

The antigenic extract was prepared from non synchronous "in vitro" culture of *P. falciparum* (schizonts plus trophozoites). After lysis of parasitized red blood cells (RBC) antigen was extracted from the parasites present in the pellet using 2% SDS and fractionated by SDS-PAGE. Non parasitized RBC processed according to the same protocol, was used as control of the antigenic preparation.

Using Coomassie Brilliant blue R 250 it was possible to identify 38 polypeptides with molecular weights (MW) ranging from 7 to 230 Kd. By Immunoblotting analysis a maximum number of 17 polypeptides, with MW ranging from 66 to 230 Kd could be demonstrated. When reacted with control RBC preparation, all tested sera, including those of individuals from non endemic area, revealed one band of 56 Kd that was not present when *P. falciparum* antigen was used. Polypeptides non parasite specific and of high MW (210 and 230 Kd) were recognized by sera from both infected and control individuals. On the other hand, two polypeptides (68 and 103 Kd) reacted with respectively, 85.2 and 74 % of malarial sera independently of the immune status of individuals.

Although no apparent positive relationship was found between the numbers of past attacks and the number of the MW of polypeptides recognized by the IgG present in the sera studied, further studies are necessary and are currently been undertaken in our laboratory to elucidate these points.

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MALARIA TRANSMISSION AND DEVELOPMENT OF IMMUNE RESPONSE IN INDIVIDUALS FROM ARIQUESMES, RONDONIA STATE.

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To evaluate the relationship between malaria transmission and the development of immune status we studied the rate of infected Anopheles (through immunoradiometric assay – IRMA – employing species specific anti-sporozoite monoclonal antibodies), the level of anti-sporozoite antibody (Ab) (by IRMA using (NANP)4 synthetic peptide) and the level of antibodies to blood forms by the Immunofluorescent Antibody Test – IFAT, in four different localities in Rondonia state. In this region uncontrolled migration, mostly from malaria free areas, and remarkable change in the environment has occurred.

Up to the moment 7686 adult female Anopheles have been processed; among 17 species studied only 37 An. darlingi (862%), An. triannulatus (11.6%) and 1 An. braziliensis (24%)were found to carry sporozoites. The frequency of infected Anopheles was 0.2% for Itapó do Oeste, 0.3% for Machadinho, 0.7% for Ariquemes and 1% for Cujubim.

As concerns the serological survey (988 tested sera), the locality of Cujubim presented not only the highest mean titer of anti-sporozoite Ab but also the highest frequency of Ab positive individuals (10%) followed by Ariquemes (4.9%), Machadinho (3.3%), Itapó do Oeste (2.7%) and showing a clear relationship between titers of anti-sporozoite Ab and malaria transmission as reflected by the rate of infected Anopheles. However, IRMA titers were not relate to the age or sex of individuals or the number of past attacks of malaria (PAM) or years of residence (YR), a fact that could indicate that these are not accurate parameters to predict the probable degree of anti-sporozoite immunity. In order to verify the accuracy of the collected data, anti-blood forms Ab were then evaluated and it was found that IFAT titers were not related to the age or sex of individuals but were positively related to the number of PAM and YR.

Taken together these results could suggest that acquisition of anti-sporozoite Ab is related to the rate of infected Anopheles but in the population studied here comprehending mainly non immune migrant individuals, it does not seem to be an identical process to that classically observed in autochthonous inhabitants of hyperendemic areas of malaria.

This work was supported by WHO World Bank, FINEP and CNPq.
PLASMODIUM GALLINACEUM SPECIFIC ANTI-SPOROZITE MONOCLONAL ANTIBODIES INHIBIT THE "IN VITRO" DEVELOPMENT OF THE PARASITE EXOERYTHROCYTIC CYCLE.

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The primary exoerythrocytic cycle (EE) of the avian malaria Plasmodium gallinaceum, originated from the inoculation of sporozoites, takes place "in vivo" in macrophages (Muff & Coulston, 1944). As previously reported by various authors in mammalian malaria specific antibody against the circumsporozoite (CS) protein of the sporozoite surface, partially inhibits parasite penetration in host cells ("in vitro" and "in vivo"). The present work reports the "in vitro" interaction of P. gallinaceum sporozoite with the host cell in the presence of anti-CS stage specific monoclonal antibodies (MoAb). Sporozoites were obtained from the salivary glands of Aedes fluviatilis mosquitoes after 11 days of the infective meal. The cultures of the chicken cells were set up in coverslips 4 to 6 days and then inoculated for 2h with the sporozoites. Parallel experiments were done adding purified anti-CS MoAb or non-relevant MoAb at a concentration of 25 μg/ml to the sporozoite suspension and incubating them with the cells. After cell infections with the parasite, all preparations were allowed to incubate at 37°C/5% CO₂ for different time intervals (up to 30h). The rate of sporozoite penetration and development of the EE parasites were detected in the cultured cells using the immunofluorescence technique and Giemsa staining (see abstract enclosed). The results showed that there were no differences between the number of parasitized cells infected with sporozoites for 2h regardless of the presence of the specific MoAb. The penetration rate ranged from 4-12% in the various experiments. The cultures incubated for 22 hours with medium or with non-relevant MoAb had EE. In the preparations pre-incubated with one specific MoAb (N9BaH₂) there had been observed a complete inhibition of sporozoite development. However, with a second specific MoAb (N9DgA5) the sporozoite cycle was equal to controls. The present experiments parallel and confirmed our previous findings "in vivo" showing that some P. gallinaceum anti-CS MoAb abolish or reduce the infectivity of the sporozoites (Rocha et al, Malaria Meeting, SP, Brazil, 1988). It is surprising that MoAb with the same specificity both recognizing equal epitopes on the sporozoite surface as judged by immuno-precipitation and -blotting techniques (Krettli et al, Parasite Immunology, 1988) have different biological activities "in vivo" and "in vitro". This finding is under investigation.

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PLASMODIUM GALLINACEUM SPOROZOITES: ATTEMPTS TO INFECT PERITONEAL MACROPHAGES OF GERM-FREE MICE "IN VITRO".

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The exoerythrocytic stages (EE) of Plasmodium gallinaceum, an avian malaria, has been reported "in vivo" in mesenquimal tissues (macrophages, fibroblasts and endothelial cells). In artificial "in vitro" conditions this parasite is able to reside in a wide variety of tissues, nevertheless, it does not reproduce as such in natural infections. As we recently reported (Ramirez & Krettli, Abstract, Malaria Meeting, SP, Brazil, 1988) P. gallinaceum sporozoite attach to and enter in peritoneal macrophages of normal mice. No difference in the penetration rate was noted when stage-specific sera or monoclonal antibodies (MoAb) against sporozoites were present during the macrophage invasion. In the various conditions all sporozoites were destroyed after 72 hours incubation. There are evidences that the lack of complex microbial flora in germ-free mice could affect macrophage function. In this study we investigated the "in vitro" interactions between sporozoites and peritoneal macrophages harvested from germ-free mice. The sporozoites obtained from salivary glands of Aedes fluviatilis mosquitoes experimentally infected with P. gallinaceum were incubated with macrophages at 37°C/5% CO₂ for 2, 6, 18, 24, 48 and 72 hours, in a relation of one parasite to 20-30 cells. The cultures were fixed and stained by a sandwich immunofluorescence technique (IF) using anti-sporozoite MoAb as the first antibody, or with Giemsa. The number of infected cells were counted to estimate the parasite penetration rate, the main results being as follows. After 2 hours incubation 54% of the sporozoites were seen attached to and entering the macrophages. The parasitized macrophages contained generally one parasite, but 7% presented 2-3 parasites in their cytoplasms. The cultures fixed at 18 up to 48 hours contained only 0.7% parasitized cells showing therefore 80% reduction in the penetration rate. Preparations fixed at 72 hours contained no parasitized cells. Giemsa or IF techniques showed similar results. These results indicate that although P. gallinaceum sporozoites attach to and enter macrophages from germ-free mice, they were unable to reach further development. This suggests that EE stages may depend on the specific metabolism of the host cells rather than on the presence of specific receptor on its surface as reported previously on mammalian malaria (Mazier et al., Exp. Parasitol. 1987).
"IN VITRO" CULTIVATION OF THE EXOERYTHROCYTIC STAGES OF PLASMODIUM GALLINACEUM IN CHICKEN BONE-MARROW MACROPHAGES SHOW COMMON ANTIGENS BETWEEN SPOROZOITES AND INTRACELLULAR MEROZOITES.

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The exoerythrocytic (EE) schizogony of the avian malaria parasite *Plasmodium gallinaceum* was previously obtained "in vitro" infecting embryonic chicken cells with sporozoites (Dubin et al, 1949). In natural infections primary EE stages have been found in skin macrophages at the inoculation site or less often in a distant organ. The present work reports the successful "in vitro" development of *P. gallinaceum* sporozoites in chicken bone marrow phagocytic mononuclear cells. Sporozoite were obtained from salivary glands of *Aedes fluviatilis* mosquitos experimentally infected. The phagocytic cells were harvested from normal chicken bone marrow, placed in coverslips and grown for 4-6 days at 37°C/5% CO₂. Cultured cells at near confluency were infected with sporozoites (50x10⁵ sporozoites per coverslip) and cultured. At various time intervals the cultures were fixed and stained either by a sandwich immunofluorescence (SIF) technique using specific anti-sporozoite monoclonal antibody as the first antibody or with Giemsa. Fifteen minutes after incubation 3% of the cells showed sporozoites attached or interiorized. After 2 hours of infection the penetration rate ranged from 4-12% within the various experiments. The sporozoites had their characteristic morphology at this time as well as before, however, after 15h of incubation they were completely round. Each parasitized cell contained in general only one form but in the next hours (22,30h) 3.6 to 5.0% contained 4 to hundreds round shaped parasites in the cytoplasm. Such parasites shared common antigens with the sporozoites since they were revealed by SIF using an anti-sporozoite specific monoclonal antibody. The distribution of the infected cells in the cultures was irregular tending to occur in groups in one area, but infected cells were also found scattered throughout the culture. This "in vitro" method to culture EE stages of *P. gallinaceum* would be useful to elucidate whether anti-sporozoite antibodies play a protective role in the anti-sporozoite induced immunity.

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A NEW TRYPSANOSOMATID ISOLATED FROM CRYSSOMYA MEGACEPHALA (DIPTERA: CALLIPHORIDAE)

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A small number of flagellated trypanosomatids was found parasitizing the Malpighian tubules from *Crysomya megacephala* (DIPTERA: CALLIPHORIDAE) caught in the vicinity of Alfenas-MG, Brazil. The Roitman's complex medium, supplemented with tween 80 (50mg%) gentamycin (0.028mg%) and "oxiconazol" (20mg%), was used for the isolation. The morphological studies were based on Giemsa-stained smears and showed opisthomastigote forms in natural infections as well as in the culture forms. Length 6 to 10 um (7.8); maximum width 4.5 um: flagellum 4 to 10 um (6.9). We also observed a singular characteristic: the presence of a small round region, localized posteriorly to the nucleus, which did not stain with Giemsa. Light microscopy of the fresh material showed this region as a refringent point. The culture is in a clone stage and ultrastructural studies by transmission electron microscopy are to be realized. The data above led us to suggest that it is a new trypanosomatid.

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TRYPANOSOMES OF BRAZILIAN FISHES. VIII. TRYPANOSOMA ITOI SP. N. FROM ARMORED CATFISH HYPOSTOMUS SP. I (PISCES, LORICARIIDAE), FROM MOGI-GUAÇU RIVER, MUNICIPALITY OF PITANGUEIRAS, SP, BRAZIL.


Faculty of Pharmaceutical Sciences of Ribeirão Preto - USP

Faculty of Dentistry of Ribeirão Preto - USP

Museum of Zoology - USP

The authors described two types of trypanosomes in the armored catfish Hyphostomus sp. I, captured in Mogi-Guaçu river, municipality of Pitangueiras, SP, in 1984. The smears were stained with Leishman and the parameters values were expressed in micrometers. The type I was similar to the Trypanosoma strigaticeps Fonseca & Vaz 1928 type II: body narrow, attenuated at both ends; cytoplasm granular; nucleus ovoid; kinetoplast round and terminal; undulating membrane less visible continuing as visible free flagellum. The type II is of different shape: Trypanosoma itoi n.sp.: total length values varied from 51.5 to 59.3 and body length from 42.0 to 45.3; the free flagellum is from 9.5 to 11.0; its nucleus is at variable distance from this anterior extremity (20.0-22.0) and, from the posterior extremity (21.3-22.0); its body breadth is from 2.6 to 4.7; the kinetoplast breadth varied from 0.6 to 1.1 and the nuclear breadth varied from 1.5 to 2.3; the nucleus length is from 3.0 to 4.2 and the nuclear index was found to vary from 0.9 to 1.1; the kinetoplast-posterior extremity length is zero. The mean nuclear volume was 8.89µm³, and the mean kinetoplast volume was 0.38µm³.

TRYPANOSOMES OF BRAZILIAN FISHES. IX. TRYPANOSOMA PINHOI SP. N. FROM ARMORED CATFISH HYPOSTOMUS SP. II (PISCES LORICARIIDAE), FROM MOGI-GUAÇU RIVER, MUNICIPALITY OF PITANGUEIRAS, SP, BRAZIL.

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The authors described a new trypanosome in the armored catfish Hyphostomus sp. II, captured in Mogi-Guaçu river, municipality of Pitangueiras, SP, in 1984. The smears were stained with Leishman and the parameters values were expressed in micrometers. Trypanosoma pinhoi n.sp.: total length values varied from 53.5 to 65.7 and body length from 42.5 to 48.1; the free flagellum is from 11.0 to 20.0; its nucleus is at variable distance from this anterior extremity (17.0 - 23.5) and from the posterior extremity (22.2 - 29.4); its body breadth is from 2.7 to 3.7; the kinetoplast breadth varied from 0.5 to 1.0, and the nuclear breadth varied from 1.5 to 2.5; the nucleus length is from 3.6 to 4.7 and the nuclear index was found to vary from 0.9 to 1.6; the kinetoplast-posterior extremity length is zero. The mean nuclear volume was 14.40µm³ (7.70 - 21.09), and mean kinetoplast volume was 0.46µm³ (0.22 - 0.69).
PZ-21  

TRYPANOSOMES OF THE SUBGENUS MEGATRYPAHUM FROM ARMADILLOS (XENARTHRA: DASYPUDIDAE)

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Although it has been known for some time that armadillos are hosts of undescribed trypanosomes belonging to Hareel's subgenus Megatrypanum (Barrett, PhD Thesis, Univ. London, 1979), these have only very rarely been observed, probably because of extremely low parasitaemia in natural infections. With the recent discovery of reproductive forms of Trypanosoma (Megatrypanum) sp. in the lymphatic system of Dasypus novemcinctus, studies on this group of parasites are likely to advance in the near future. The purpose of the present communication is to illustrate the trypanosomes of this subgenus known from armadillos and to summarize the information available on their biology. A taxonomic paper is in preparation.

A single large trypanosome was observed in a preparation of fresh blood from a juvenile Euphractus sexcinctus from Bahia State, but no parasites were seen in 25 stained bloodsmears made at the same time. One of four cultures, made by inoculating a drop of tail blood onto a fortified NNN blood-agar slant with T 29 medium as overlay, became positive within 10 days. Overlay from the positive culture failed to infect laboratory mice but infected a second E. sexcinctus. Xenodiagnosis with Rhodnius prolixus and Triatoma infestans was negative on both armadillos. Growth in blood-agar culture medium was rapid and luxuriant.

Twenty-nine Dasypus novemcinctus and one Dasypus kappleri from southern Pará State were subjected to haemoculture with NNN Difco blood-agar medium, xenodiagnosis with Rhodnius prolixus, examination of fresh blood, and examination of impression smears of subcutaneous lymph nodes. Large trypanosomes including epimastigote forms were seen in fresh and stained preparations of lymph nodes from 10 D. novemcinctus. The only trypanosome isolated by haemoculture and xenodiagnosis was T. cruzi, from 9 D. novemcinctus. Cultures of material from infected lymph nodes were also all negative for Megatrypanum.

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PZ-22  

CONTINUOUS GROWTH AND TRANSFORMATION OF TRYPANOSOMA (MEGATRYPANUM) FREITASI "IN VITRO" 

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The study of T. (N.) freitasi isolated from naturally infected opossums (Deane & Jansen, 1986) confirmed the difficulty to maintain this trypanosome in the usual culture media (Rego, Magalhães & Siqueira, 1957).

Several tentatives with different axenic, liquid or biphasic media and various cell systems were made; some promoted rapid initial growth that, however, could not be maintained. We finally devised conditions that support continuous exuberant growth and differentiation of T. freitasi. These conditions are, briefly: 1. Cultures of the cell line L929 (mouse fibroblasts) with the medium of Baltz et al (1985) plus 10% fetal bovine serum as nutrient; 2. Leighton tubes with flying coverslips; 3. Incubation temperatures of 27, 25° and 37°.

When incubated at the lower temperature the flagellate grows in very large agglomerates of small epimastigotes, practically motionless and firmly attached to the glass surfaces below the coverslip, while the feeder layer of cells proliferates on its upper side; the substitution of the coverslip, i.e., of old for new feeder layers, permits continuous growth in the same tube for a long time. Transformation into the small metatrypanomastigotes already described (Deane & Jansen, 1986) follow detachment of the epimastigotes from the glass surface.

The transference of the cultures to 37° is followed by the appearance of the large blood stream type trypanomastigote. There are, however, some intermediate phases hitherto undescribed for any trypanosome in culture, which might correspond to the initial reproductive phases that have been found in vertebrate hosts of species of sub-genera Megatrypanum and Beripetosoma. Detailed descriptions and illustrations will be presented in a Poster.

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ULTASTRUCTURE AND STEREOLGY ANALYSIS OF SOME Endotrypanum spp.

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Parasites of the genus Endotrypanum are flagellate protozoa of the family Trypanosomatidae. The members of this genus are intracytoplasmic parasites of two and three toed sloths, and only few papers are available dealing with their ultrastructure. In the present study, culture forms of four strains of both species of Endotrypanum (E. schaudinni and E. monteregi) were processed for transmission electron microscopy and analyzed at the ultrastructural level. Quantitative data about some cytoplasmic organelles were obtained by stereology, using a MDP-Videooplan microcomputer. All culture forms were promastigotes. In their cytoplasma three different organelles could be found: a) lipid inclusions (mean diameter = 0.2-0.4 μm); b) membrane bounded vacuoles (mean diameter = 0.2-0.8 μm); c) glycosomes (mean diameter = 0.2-0.3 μm). The kinesion appears as a thin rod, except for the strain IM201, which possesses a broader structure. Clusters of virus-like particles were seen in the cytoplasm of the strain LV88; the particles were rounded, with a mean diameter of 25nm. The relative volume of the tubular mitochondrion ranged from 6.2 to 8.5% and that of the glycosomes from 0.8 to 1.4%. However, in the strain IM201 these values were 11.7 and 6.0% respectively. Lipid inclusions occupied a relative volume of about 0.4% (2.5% in strain LV88), while the relative volume of membrane bounded vacuoles was about 0.7-0.8%. The data obtained show that all strains analyzed have the typical morphological features of the trypanosomatida. Only strain IM201 (Endotrypanum sp.) could be differentiated from the others, due to its larger kinesion, DNA network and larger mitochondrial and glycosomal relative volume. The morphometrical data did not allow the differentiation between E. schaudinni (strains IM217 and M5226) and E. monteregi (strain LV88).

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CULTURE DEVELOPMENT AND ISOENZYME PATTERN OF Trypanosoma rangeli STRAINS FROM HONDURAS

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Trypanosoma rangeli is a non-pathogenic mammalian parasite. Because T. rangeli and T. cruzi occur in the same hosts and have common vectors, these two species can be confused unless they are correctly identified and differentiated. At this study differential characterization of the two species was made based on development in culture and isoenzyme pattern. For culture development the following parameters were considered: the growth curve profile, the duplication time, differentiation and morphology. The morphometry was obtained measuring 50 epimastigotes in the exponential growth phase and 25 trypanomastigotes in the stationary phase in CIEMSA stained smears of each strain. The isoenzyme pattern was studied for the enzymes ALAT, ASAT, GPI and POM. We studied the strains H-8, H-9 and H-14 of T. rangeli isolated by hemoculture of infected patients from Honduras. The patients from which the strains H-8 and H-9 were isolated had negative serology for Chagas' disease. The patient from which H-14 strain was isolated showed positive serology for Chagas' disease, turning this strain suspect of being a mixture T. rangeli-T. cruzi. The Y T. cruzi strain kept routinely in culture in our laboratory was used for comparison. The strains duplication time were: H-8 = 7.1 days, H-9 = 6.9 days, H-14 = 4.5 days, and Y = 3.1 days. The trypanomastigotes percentage in the 14th day culture were: H-8 = 11.7%, H-9 = 11.7%, H-14 = 12.2% and Y = 1.9%. The results of the morphometry of 5 internal cellular parameters showed: a) epimastigotes of T. rangeli and T. cruzi showed identical values; b) trypanomastigotes of T. rangeli had the same values, though they could be differentiated from T. cruzi trypanomastigotes in the posterior end-kinetoplast, free flagellum and total length measurements. The isoenzyme pattern of the T. rangeli strains were identical but distinct from T. cruzi. Our results showed that T. rangeli strains were identical among them and could be differentiated from T. cruzi Y strain through development in culture and isoenzyme pattern, being the latter easier for differentiation. Although suspected of mixture, the data did not indicate any presence of T. cruzi in the H-14 T. rangeli strain. Further biological, immunological and molecular parameters will be tested in order to have a complete characterization of these T. rangeli strains.

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BLOCKING FACTORS TO TRYPANOSOMA CRUZI GROWTH IN SOME PROTEOSE-PEPTONE PREPARATION
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Many complex media developed for routine cultivation of T. cruzi can use, without loss of efficacy different commercial protein hydrolysates, as proteose, proteose or proteose-peptone that on the other hand can be produced by different producers of biological goods. Proteose-peptone is indeed produced for bacterial growth and frequently has been used for T. cruzi cultivation, instead of tryptose in the common liver-infusion tryptose media (LIT). We observed excellent growth (2x10⁵) in culture of T. cruzi Y strain in media containing proteose-peptone Difco or Oxoid (4x10⁵) but the growth was inhibited (1x10⁵) when Merck proteose-peptone was used. A high rate (70%) of spontaneous transformation epimastigote-to-trypanosomal tigote was observed in a Difco proteose-peptone (or typtose) medium in which the usual Na/K relation was inverted.

In a similar assay using Herpetonema samuelpessoa and Merck proteose-peptone, the growth curve was also inferior but less dramatically than that observed with T. cruzi.

To test an eventual and potentially toxic excess of heavy metals, we investigated the levels of contamination with lead and iron. Proteose-peptone Merck showed the greatest level of iron, about 6 times the concentration presented by Difco proteose-peptone. No great difference was observed when we compared the contamination by lead in the three products assayed. Contamination by lead and iron was measured by Atomic Absorption Spectrophotometric assay. Further studies are being carried out to test this hypothesis.

EFFECT OF CRYSTAL VIOLET ON TRITRICHOMONAS FOETUS
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Low concentrations of crystal violet (50-100 μM) inhibited completely T. foetus growth in culture. In addition, crystal violet was reduced by intact T. foetus cells to a carbon-centered free radical as demonstrated by electron spin resonance spectroscopy. Anaerobic T. foetus hydrogenosomes supplemented with pyruvate and CoA effectively reduced crystal violet to its carbon-centered free radical derivative. The linewidth (4.2 G) of this radical was less than the linewidth (10 G) of the free radical obtained in T. foetus intact cells incubations. No crystal violet radical could be detected under aerobic conditions. However, crystal violet was found to convert oxygen to superoxide anion under aerobic conditions in the presence of hydrogenosomal preparations, although at a slower rate than nitrofurane. This was probably due to the conversion of the dye to fully reduced leuco crystal violet. Accordingly, crystal violet only slightly stimulated oxygen consumption by the hydrogenosomal preparations. Very high concentrations of crystal violet (>500 μM) were necessary to demonstrate this effect. In contrast lower concentrations of crystal violet (<50 μM) inhibited oxygen uptake by intact cells, probably as a consequence of cell death. These results suggest that redox cycling of crystal violet is not involved in its trichomonicidal action.

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IN VITRO INFLUENCE OF HORMONES ON THE ADHESION OF *Trichomonas vaginalis* TO EPITHELIAL CELLS.

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*Trichomonas vaginalis*, the causative agent of human trichomoniasis, interact with its hosts through the squamous epithelia lining urogenital cavities. The adhesion of the parasite to the epithelial cells seems to be a prerequisite for the expression of its cytotoxic effect (Infect. Immun. 50: 701, 1985). The hormonal milieu which may alter the host susceptibility to a variety of infections, may also be related to the trichomonad-host cell interaction. Microorganisms from cultures of *T. vaginalis*, which were previously frozen or not at -80°C, were labelled with 3H-thymidine and allowed to interact with epithelial monolayers formed by Madin-Darby canine kidney (MDCK) cells (Cell Struct. Funct. 13: 301, 1988), which in turn, were incubated previously to the interaction for 18h with each one of the hormones (nanomolar concentrations): estrone, 17β-estradiol, α-estradiol, progesterone and testosterone. A high increase (about 92%) in parasite cyoadherence was observed when epithelial cultures were treated with 17β-estradiol, being that treatment of 17β estradiol-treated cells with the antiestrogen tamoxifen (TMX) lead to a partial reversion of the effect (46%). Incubation of the epithelial monolayers with cycloheximide, 2,4-dinitrophenol, puromycin or 2-deoxy-D-glucose for 4h was succed by 18h additional incubation with 17β estradiol. All these metabolic inhibitors induced decreases in parasite adhesion. The more pronounced effect was found in monolayers which have been treated with protein synthesis inhibitors. These results suggest that trichomonas infection may be characterized by an intense estrogenic activity of the host.

FREEZE-FRACTURE OF *Trichomonas vaginalis*

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*T. vaginalis* is a pathogenic protozoan of the urogenital tract of human. Although it has been studied previously in thin sections they do not reveal details of the molecular structure of the cell membrane. In replicas of freeze-fractured membranes, the inner part of the cell membrane is exposed, allowing the examination of either the inner or the outer membrane halves. For the experiments, cells were grown in TIN medium for 24 to 48h at 37°C. They were washed once in phosphate-buffered saline solution, fixed for 2h at room temperature in 2.5% glutaraldehyde in 0.1M cacodylate buffer, pH 7.2. After fixation, the cells were rinsed in buffer, impregnated during 30 min with glycerol in cacodylate buffer up to a concentration of 30%. Specimens were mounted on Balzer’s support disks and rapidly frozen in the liquid phase of partially solidified Freon 22 cooled by liquid nitrogen. Freeze-fracture was carried out at -115°C in a Balzer’s apparatus equipped with a turbomolecular pump. The specimens were shadowed, cleaned, mounted on grids and examined with a Jeol 100 CX electron microscope. Examination of freeze-fracture replicas showed large surfaces of the inner or outer membrane halves or the cytoplasm, exposing membranes of cellular components and other non-membranous structures. Observations of fracture faces of *T. vaginalis* plasma membrane revealed a marked heterogeneity in the size and distribution of membrane particles. The P face - with represents the outer aspect of the inner membrane half presented a higher density of membrane particles than the E face. A large number of areas of pinocytosis were seen through the plasma membrane. The flagellar membranes of the anterior flagella differ in structure from the cell membrane in that they have fewer intramembranous particles and clusters of 7-13 intramembranous particles, forming rosettes. No rosettes were ever observed on the membrane of the recurrent flagellum. On the P face of the membrane of the recurrent flagellum, organized arrays of particles, arranged as 4 ribbons were localized only in the portion of the flagellum facing the body and following the along axis of the flagellum. In many cases, the fracture plane deviated from the plasma membrane and entered the cell cytoplasm, exposing faces of the membrane of intracellular organelles. The Golgi complex was observed as well developed structure, presenting many lamellae and small vesicles associated with them. When the nuclear membrane was fractured, typical nuclear pores were found in both faces. In some areas it was possible to see many membrane bounded-organelles, probably lysosomes, hydromegosomes and other vesicles. In some cases, double membrane bounded-organelles were seen - they probably correspond to hydromegosomes.

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ANIONIC SITES AT THE ELECTROKINETIC SURFACE OF TRICHOMONADS.

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Trichomonas vaginalis and Trichomonas foetus are both mucosal parasites of the urogenital-vaginal tract of humans and cattle, respectively. Colloidal iron oxide and ferritin particles carrying a net positive charge bind by coulombic attraction to the negatively charged sites on trichomonad surfaces (J.Prototool.29:551,1982), which in turn, seems to be partially due to the presence of sialic acid residues (Cell Biophys.8:161,1986). Both phospholipase C and neuraminidase treated parasites were incubated in the presence of cationized ferritin. Their net surface negativity was reduced by incubation with the enzymes, as indicated by measurements of their electrophoretic mobilities (EPM). From the measurements of EPM, an estimate has been made of the charge density (6) at the cellular electrokinetic surface, expressed in electron charges (/e^-)/10^-6A^2, by use of the Helmholtz-Smoluchowski equation (J.Theor.Biol.34:21,1972). A correlation between the data obtained from ultrastructural cytochemistry with cationic particles and the measurement of the EPM of parasites was done. Assuming that the charge of isolated phosphate and carboxyl ions are -0.25 e and -0.4 e (Pauling,1960), being 1 e = 4.8 x 10^-10 e.s.u., it is possible to infer that for each 10^6A^2 of the trichomonad electrokinetic surface, there are 803 e^- in the case of T.vaginalis and 788 e^- in the case of T.foetus. The carboxyls of neuraminidase susceptible groups account for 28.3% (T.vaginalis) and 31.2% (T.foetus), while the phosphates related to phospholipase susceptible groups account for 17.4% (T.vaginalis) and 23.0% (T.foetus).

ISOLAMENTO E CARACTERIZAÇÃO DE GANGLIOSÍDIOS DE TRITRICHOMONAS FOETUS

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Glicolipídios de T.foetus, protozoário patogênico do trato urogenital bovino, foram extraídos sucessivamente com clorofórmio-metanol (2:1, 1:1 v/v) e clorofórmio-metanol-água (4:8:3 v/v/v) e fracionados em coluna cromatográfica de silica gel 60. As frações identificadas como gangliosídio, reveladas por resorcinol, por cromatografia em camada fina (TLC), foram purificadas em coluna trocadora de íons DEAE Sephadex A-25 e analisadas em TLC e cromatografia líquida gasosa (GLC). Foram obtidas duas frações (A e B) que submetidas a hidrólise ácida revelaram, em comum, por GLC, a presença de galactose, como único componente osídico. Da hidroesfingosina liberada após hidrólise foi a base de cadeia longa caracterizada em TLC e também compartilhada por ambas as frações. Contudo, os gangliosídios diferiram quantitativamente na composição de ácidos graxos identificados em GLC: enquanto na fração A a razão dos ácidos mirístico, palmitico e esteárico foi de 1:3:3, na fração B uma proporção de 1:4:2 foi constatada. Tal diferença no conteúdo de ácidos graxos entre os gangliosídios justificaria, em parte, a diferente migração em TLC.

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INTERACTION OF Trichomonas foetus AND Trichomonas vaginalis WITH PHAGOCYTIC CELLS.

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The fine structure of the in vivo and in vitro interaction process between T. foetus and T. vaginalis with mouse peritoneal macrophages and rat peritoneal neutrophils was studied. For in vivo experiments, the parasites were suspended in PBS pH 7.2 in order of obtain a density of 10⁷ parasites/ml. Then 3ml of the suspension were inoculated into the peritoneal cavity of the mouse and rat so that a parasite to cell ratio of about 5:1 was achieved. Interaction times varied from 5 to 30 min. After interaction the animals were sacrificed, the peritoneal cavities were washed with PBS, the cells collected and immediately fixed in Karnovsky solution for macrophages and in 2.5% glutaraldehyde solution in 0.1M phosphate buffer, pH 7.2, for 1h at room temperature for neutrophils. They were then washed in the same buffer and post-fixed for 1h at room temperature with 1% OsO₄. After fixation, the cells were dehydrated in acetone and embedded in Epon. For in vitro interaction, the macrophages were collected from peritoneal cavities of normal Swiss mice and plated in culture flasks. They were allowed to adhere to the glass surface for 30 min at 37°C and then washed with Hank's solution to remove those cells which did not adhere. Culture medium (199 medium plus 10% fetal calf serum) was added, the cells were incubated for 24h at 37°C and then, after washing used for the experiments. Then the cells were incubated at 37°C in the presence of the parasite (parasite-cell ratio of 5:1). Interaction times varied from 5 to 30 min. After, the cells were fixed Karnovsky solution and processed for electron microscopy as described above. The neutrophils were collected from the peritoneal cavity of rats, washed with PBS at 4°C and the incubated at 37°C in the presence of the parasite (parasite-cell ratio of 5:1). Interaction times varied from 5 to 30 min. After interaction, the cells were fixed in 2.5% glutaraldehyde solution in 0.1M phosphate buffer and processed for electron microscopy as already described.

Our results obtained in the experiments in vivo and in vitro were similar and show that macrophages and neutrophils attach to the surface of T. foetus and T. vaginalis. At the attachment sites condensation of microfilaments was seen beneath the plasma membrane of the phagocytic cells. This process is followed by destruction of some parasites with sub-sequent ingestion so that damaged parasites are seen within cytoplasmic vacuoles of the phagocytic cells.

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DIFFERENÇAS EM CARBOIDRATOS DE SUPERFÍCIE ENTRE AMOSTRAS DE Trichomonas vaginalis
SENSÍVEL E RESISTENTE AO METRONIDAZOL.

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Os açúcares de superfície em amostras de T. vaginalis, sensível e resistente ao metronidazol, foram analisados com o uso de lectinas de várias especificidades osídicas, através de testes de aglutinação celular. D-Glic NAc, D-Gal NAc, D-Gal e D-Man e similares, foram os resíduos osídicos expostos na superfície de ambas as amostras. No entanto marcadas diferenças foram detectadas. A amostra sensível a droga foi seletivamente aglutinada pela lectina SBA (específica para D-Glic NAc) enquanto que a T. vaginalis resistente ao metronidazol interagiu preferencialmente, com as lectinas PNA (específica para D-Gal) e LCL (específica para D-Man e similares). Também, em contraste com a amostra sensível, os protozoários resistentes à droga evidenciaram acentuada atividade aglutinante com as lectinas RCA I e Con A, específicas, respectivamente, para D-Gal e D-Man e similares. Tais pronunciadas diferenças nas estruturas de carboidratos de superfície, podem estar relacionadas, em parte, com a resistência de T. vaginalis ao metronidazol, e possível, pois que em T. vaginalis resistente ao metronidazol ocorra um decréscimo na permeação ou uma alteração nos receptores específicos da droga.

Auxílio Financeiro: CNPq, CAPES, CEPR-UFRJ e FINEP.
ACTION OF DIFFERENT PLANT EXTRACTS ON Phytoponas serpens CULTURE FORMS.


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Phytoponas serpens is a parasite of tomato fruits, not demonstrable in other parts of the plant. In order to understand why it does not occur in other parts, we tested the action of extracts of different parts of the tomato plant on culture forms of Phytoponas serpens. The parasites were put together with the crude extracts and microscopically observed. Except for mature tomato pericarp, all other extracts immediately paralyse the promastigote forms of P. serpens, indicating harmful action on the parasites. On the other hand, the pericarp extract alone did not maintain the cultures. P. serpens is transmitted by the bite of phytophagic hemipterans as Pthia pica and Nezara viridula. These insects are not exclusive to tomatoes and are frequently found on many other crops, some of them, as leguminosae, are also infected with trypanosomatid protozoans. We, then, tested the extracts from Soya, beans and peas on P. serpens culture forms. The results are essentially the same as tomatoes, with the parasites not being affected by the fruit and seeds, except that the action of the extracts are slower, paralyzing the culture forms within 2 hours. This action of plant extracts can be used as a criterion to exclude strains of Phytoponas as parasites of a specific plant or part of it.

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COMPOSIÇÃO DE ÁCIDOS GRAXOS EM Phytoponas sp.

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Os lipídeos foram extraídos de Phytoponas sp., protozoário patogênico de tomate, cultivada em meio complexo, a 28°C, por 48 horas, com clorofórmio/metanol/água (4:8:3) e saponificados com NaOH 5N a 45°C. Os ácidos graxos, da fração saponeificável, foram convertidos em ésteres metílicos e analisados em cromatografia líquida gasosa, com o uso de coluna capilar. Foram identificados ácidos graxos pares, ímpares, saturados e insaturados. Os ácidos C18:2, C18:1 e um de cadeia longa, provavelmente maior do que C18:4, foram os principais ácidos graxos identificados. Esse perfil de ácidos graxos diferiu, acentuadamente, daquele encontrado em estudo prévio em Phytoponas davidi, onde ocorreu preferencial síntese dos ácidos graxos na faixa de C16. Tal fato pode ser atribuído à diferenças de espécies e/ou do meio de cultivo. Também, a predominância do aludido ácido de cadeia longa, não usual, ainda não completamente identificado, pode estar associado com a fitopatogenicidade do flageloado. É aceito, no presente, que componentes de superfície estão envolvidos em fenômenos de interação célula-hospedeiro e de patogenicidade, entre outros.

Auxílio Financeiro: CNPq, CAPES, CEPG-UFRJ e FINEP.
NUTRITION OF HERPETOMONAS ANGLUSTERI N.Sp. ISOLATED FROM Liopygia ruficornis (DIPTERA: SARCOPHAGIDAE).

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Previous studies showed that Herpetomonas angulisteri is a new trypanosomatid species. The nutritional study of this flagellate organism showed that it was able to grow in a defined medium without carbohydrate at 28°C. At 37°C, the growing was observed only in a complex medium. Raffinose, fructose, sucrose, glucose and maltose showed a stimulatory effect on the growth. Ten amino acids (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, tryptophan, tyrosine and valine), six vitamins (nicotinamide, pantothenate, riboflavin, pyridoxamine, thiamine and folic acid), hemin and a source of pyruvate were also required. It was observed that the flagellate could grow in a range of pH between 3.0 and 8.0 (optimum 6.5) and of osmolarity between 250 and 1110 mOsM (optimum 490).

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ISOLATION OF A FLAGELLATE PHYTOZOA FROM COCONUT (Cocos nucifera) FROM FRENCH GUIANA "HARTROT" SYMPTOMS

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The isolation was made from macerating the base of the leaf of a coconut tree showing "hartrot" symptoms in complex medium (2% sucrose, 1% KCl, 0.5% yeast extract, 0.5% Phyton, 0.5% Trypticase, 0.001% folic acid and 0.001% hemin, pH 7.0) at 28°C. In order to obtain an axenic culture of the protozoan, the elimination of contaminant bacteria (susceptible to chloramphenicol, salifidix acid and rifampicin) lead to a drastic decrease in the number of protozoa, up to their elimination. In tests performed with dead bacteria, bacteria culture filtrate (Pseudomonas sp) as well as the use of separated chambers with millipore filter (avoiding the contact between bacteria and the protozoans) no stimulus to the flagellate growth was observed, even in different defined and complex culture media. Several bacterial species (Sphingomonas, Pseudomonas, Lactobacillus, Klebsiella, etc) temporarily introduced in the axenic culture (24-48 hours) of the flagellate are seen to stimulate its growth. These observations show the need of living bacteria for the growth of this protozoan.

The observation of these flagellates by scanning electron microscopy showed that they have two flagella emerging from a single flagellar pocket. One flagellum projects anteriorly and the other runs along the cell body. The cell body measures 6-7 µm. Thin sections observed with the transmission electron microscope showed the presence of structures such as subpellicular microtubules, a large and spherical kinetoplast DNA network, and the presence of a peraxial body along the flagella. In many cells vacuoles containing bacteria were seen. These flagellates show, next to its anterior side, an arrangement of microtubules that must correspond to the cytopharynx.

So far, based on these observations we would suggest that these flagellates belong to the suborder Bodonina.

Supported by FINEP, CNPq, CEPG.
PZ-37 RESPIRATION OF PHYTOMONAS

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The consumption of O₂ was measured in several strains of Phytomonas (Trypanosomatidae): P. francai (isolated from Manihot esculenta), P. sps. (from Euphorbia pinea; P. characias; K. hyssopiphila), P. sp. (from tomato - Lycopersicon esculentum), P. sp. (from Phthia picta - vector of the infection in the tomato). Cells were obtained in a biphasic medium (4-5 days incubation at 28°C) and washed in phosphate buffer 0.1M, pH 6.5. The respiration was estimated by polarography. Substrate tested: glucose, fructose, manose, lactose, sucrose, sorbitol, threose, xylene, glycerine, aspartate, glutamate, proline, serine, citrate, succinate. Only 3 substrates - glucose, fructose and manose - stimulate the endogenous respiration in all strains tested. Proline showed a respiratory stimulation in all strains excepted those from the tomato and vector. KCN (10⁻³ M) inhibit O₂ consumption in the absence and presence of glucose; however the inhibition was partial with variations among the strains tested.

PZ-38 PRELIMINARY STUDIES ON POLYSACCHARIDE OF PHYTOMONAS ISOLATED IN LONDRINA.

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Strains of Phytomonas (9T, 10T, 15T, 30T) have been isolated from tomatoes of different areas, digestive tract (1TD) and salivary glands (1G) of the bug Phthia picta and salivary glands (6G) of Nezara viridula. In order to isolated polysaccharide fractions, cells of these trypanosomatids were cultivated in a biphasic blood agar medium during 5 days at 28°C and extracted with 3% aqueous KOH for 3 hours at 100°C, followed by precipitation with ethanol. Monosaccharides were freed by hydrolysis of the polysaccharides with 1N H₂SO₄ for 18 hours at 100°C and quantitative estimations were carried out by gas-liquid chromatography of the corresponding alditol acetates.

Our preliminary results showed the presence of rhamnose and mannose in a ratio of 1:7 in strain 1TD. The main sugar components of strains 1G and 6G were rhamnose, fucose and glucose in a proportion of 2.7:1.1:2 and 1:0:1.0, respectively.

Phytomonas isolated from tomatoes showed quantitative and qualitative differences in terms of sugar components. Xylose and galactose in a ratio of 1:1 were detected in strains 10T, 15T. Strain 9T showed fucose, galactose and glucose (1.5:3.5:1) and rhamnose galactose and glucose in a proportion of 2:2:1 were found in strain 30T.

Structural studies of these purified polysaccharides and comparison with glycoconjugates isolated from other trypanosomatids, will be carried out to a better understanding of their biological functions and also for phylogenetic studies.

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TOPOISOMERASE II FROM LEPTOMONAS SAMUELI: AN EVIDENCE FOR AN ATP-INDEPENDENT DECATENATION ACTIVITY.

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Decatenation of the mitochondrial DNA (dDNA) of trypanosomatids is a suitable method to assay for the presence of eukariotic type II topoisomerases, since type I enzymes are unable to catalyze this reaction.

DNA topoisomerases are ubiquitous enzymes that change the DNA topology and play an important role in many cellular processes such as replication, transcription, recombination and transposition of DNA molecules. Topoisomerases are classified into two types (I and II) based upon their action mechanism and energy requirements.

We have purified a topo II activity from cellular extracts of *L. samueli* by a sequence of ion exchange and affinity chromatography. Such activity is able to decatenate dDNA even in the absence of ATP (in general, eukariotic type II topoisomerases require ATP as a cofactor). In addition, the known inhibitors of *E.coli* DNA gyrase such as novobiocin, nalidix acid, oxolinic acid and ofloxacin have poor inhibitory effects over the decatenation activity.

An antiserum against this purified fraction was obtained by immunizing mice and was used for the screening a Lambda gt11 expression library in an attempt to isolate the genes coding for this enzyme.

Supported by CNPq

ANALYSIS AND COMPARISON OF THE KARYOTYPES OF SEVERAL TRYPANOSOMATIDS.

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Chromosome-sized DNA fragments from several trypanosomatids were compared by Ortagonal Field Alternating Gel Electrophoresis (OPAGE). The results showed that the chromosomes of monogenetic trypanosomatids are smaller than those of *T. cruzi* which range from 550Kb to more than 2,000Kb. Some of the chromosomes might correspond to mini-chromosomes in view of their small size ranging from 250Kb to 350Kb. In addition, with the exception of the *Crithidia* species analysed, monogenetic trypanosomatids displayed less large sized chromosomes than *T. cruzi*.

The comparison of *Crithidia deanei, Crithidia luciliae* and *Crithidia guilliermei* showed that they can be distinguished in terms of their karyotypes.

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SCHIZOTRYPANUM FROM THE BAT PHYLLOSTOMUS HASTATUS: ISOENZYMATIC CHARACTERIZATION

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Three stocks (M5, M29, WB) of Schizotrypanum isolated from the bat Phyllostomus hastatus were unable to produce detectable parasitaemia in normal and irradiated C3H young mice (Pinto et al., 1987). They showed lectin interactions patterns with carbohydrate surface determinants very similar to the ones observed with T. (S.) cruzi (Andrade et al., 1987). Isoenzymatic patterns of these stocks were determined by thin-layer starch gel electrophoresis.

The cells were grown in LIT medium (Camargo, 1964) and the soluble enzyme extracts, prepared according to Kilgour & Godfrey (1973), were stored as beads in liquid nitrogen (Godfrey & Kilgur, 1976). The electrophoresis conditions and the detection of enzyme activity were adapted from Miles et al., (1980). Five enzymes were studied: GPI (EC 5.3.1.9); ALAT (EC 2.6.7.2); MDH (EC 1.1.1.37); PMG (EC 2.7.5.1) and ME (EC 1.1.1.40).

The M5 and M29 stocks showed similar isoenzymatic profiles, except for GPI, while the WB stock presented patterns different from the other two stocks. The isoenzyme profiles of the enzymes studied of M5, M29 and WB stocks were different from the ones presented by the T. (S.) cruzi A, B, C, 21, 22 and 23zymodemes.

Supported by grant from FINEP.

MONOCLONAL ANTIBODIES FOR THE IDENTIFICATION OF TRYPANOSOMATIDS OF THE GENUS PHYTOMONAS

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Trypanosomatids of the genus Phytomonas are plant parasites thought to be transmitted through the bite of phytophagous insects. However, the same insects also bear species of other genera of trypanosomatids like Leptomonas and Herpetomonas which are morphologically indistinguishable. In order to distinguish species of Phytomonas from other trypanosomatids, monoclonal antibodies (MoAbs) have been produced against cultured forms of Phytomonas serpens isolated from tomatoes and Phytomonas franci from cassava roots.

Seven MoAbs have been tested by IFA against 7 other Phytomonas spp. isolated from plants and 14 species of trypanosomatids of various genera. None of the monoclonals antibodies were capable to react with all species of Phytomonas, however, the joint utilization of 3 MoAbs permitted the identification of all trypanosomatids investigated. No other genera of trypanosomatids was ever recognized by these MoAbs. Tests with new isolates from insects and with flagellates recovered from artificially infected tomatoes or from salivary glands of insects are resulting in additional confirmation of these results.

Since MoAbs recognized exclusively Phytomonas spp. among various species of trypanosomatids tested, we may consider as a valuable tool for the identification of the genus Phytomonas.

Supported by: FINEP, FAPESP, CNPq.
CHARACTERIZATION OF MONOCLONAL ANTIBODIES DIRECTED AGAINST ANTIGENS OF TETRAHYMENA PYRIFORMIS

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The vaccination of the commercially valuable channel catfish (Ictalurus punctatus) against the parasitic protozoan Ichthyophthirius multifiliis using cross-reacting antigens from Tetrahymena pyriformis is under investigation in our laboratory. To determine the nature of the Tetrahymena antigens inducing protection against Ichthyophthirius in catfish, eleven hybridomas were previously produced, each secreting antibodies reactive to antigens present in whole Tetrahymena pyriformis. Three of these hybridomas were selected for characterization in this study, using various techniques. Monoclonal antibody (Mab) 4 produces IgG, class antibodies and Mabs 2 and 8 produce IgM class antibodies as determined by Ouchterlony and ELISA (Enzyme-Linked Immunosorbent Assay) techniques. The biochemical properties of the determinants recognized by the three Mabs were tested by pronase digestion and periodate oxidation. Results indicate that a glycoprotein is recognized by Mab 2 while a protein is recognized by Mabs 4 and 8. Affinity chromatography using cyanogen-bromide (CNBr) Sepharose beads was performed. It was not possible to elute any antigen from the columns, probably due to the high affinity of the Mabs for the antigens. Indirect immunofluorescent tests demonstrate a pattern of surface staining for Mab 2, and cytoplasmic staining for Mabs 4 and 8. Antigens specified by Mabs 2, 4 and 8 were characterized by immunoblotting experiments. A glycoprotein of apparent molecular weight 52kd is recognized by Mab 2. Monoclonal antibody 4 recognizes a component of approximately 28kd. Monoclonal antibody 8 recognizes one main band of approximate molecular weight 41kd, four less prominent bands of 43kd, 42kd, 31.5kd and 30kd, and two components appearing as very faint bands of molecular weight approximately 45kd and 37kd.

INTERIORIZATION OF SURFACE ANIONIC SITES AND PHAGOSOME-LYSOSOME FUSION DURING INTERACTION OF Toxoplasma gondii WITH MACROPHAGES

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With the objective to analyze the process of phagosome-lysosome fusion, we have undertaken a cytochemical study, at ultrastuctural level, using albumin-colloidal gold as a marker for secondary lysosomes and colloidified ferritin as a marker of anionic sites on the surface of cell membranes. Resident macrophages obtained from peritoneal cavities of normal Swiss mice were incubated for 24 hours at 37°C, were incubated with albumin labeled with colloidal gold for 2 hours at 37°C, rinsed and incubated with colloidified ferritin for 20 minutes at 37°C. Thereafter, the cells were washed and incubated with untreated or specific antibody-coated parasites for periods varying from 10-60 min at 37°C. The cells were then rinsed, fixed and processed for electron microscopy.

After incubation with antibody-coated parasites, the colloidal gold particles were observed only in the parasitophorous vacuoles while the colloidified ferritin particles were observed only in the cytoplasmic vesicles. However, when the interaction was carried out with untreated parasites, the parasitophorous vacuoles exhibited colloidified ferritin particles while the colloidal gold particles were observed in cytoplasmic vesicles.

This work has been supported by CNPq, FINEP and CEPG-UFRJ.
ACUTE TOXOPLASMOsis: THIN-LAYER IMMUNOASSAY FOR DETECTION OF IgM ANTIBODIES TO Toxoplasma gondii.

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A solid phase method, thin-layer immunoassay (IgM-TIA) was standardized and evaluated for the serodiagnosis of acute toxoplasmosis, through the detection of IgM antibodies to *T. gondii*. A total of 160 serum samples, showing or not serological pattern I of acute toxoplasmosis, was investigated by IgM-TIA and analysed qualitative and quantitatively in comparison with conventional tests of immunofluorescence for the detection of IgM antibodies (IgM-IFT) and passive hemagglutination test which uses 2 mercaptoethanol serum treatment (2ME-HAT). IgM-TIA proved to be as efficient as these two conventional tests, with the advantage that this assay is very practical and economic. Also, the correlation of coefficient obtained were 0.91 and 0.86, respectively found for the comparisons done between the diameters of reactive areas of IgM-TIA and the titers obtained in IgM-IFT and in 2ME-HAT. The influence of antigenic concentration on the relative index of sensitivity and specificity showed that ideal concentration was 72 µg/ml to 100 µg/ml.

SERODIAGNOSIS OF ACUTE TOXOPLASMOsis BY PASSIVE HEMAGGLUTINATION TEST (HAT) WITH THE USE OF A NEW IgM REACTIVE IMMUNOREAGENT (HA-A Toxo)

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A new IgM reactive immunoreagent (HA-A Toxo) was standardized for the serodiagnosis of acute and congenital toxoplasmosis by HAT. The use of HA-A Toxo obviated the conventional serum processings, required in most of serological tests, for evidencing IgM antibodies found among highly competitive IgG antibody excess and rheumatoid factor. This immunoreagent consisted of formalin-stabilized human group O, Rh-negative erythrocytes, sensitized with heat-stable *T. gondii* alkaline extract which predominantly reacted with IgM antibodies, seldom with IgG from acute cases and none from chronic cases. The evaluation of HA-A Toxo performed, in a total of 585 serum samples, including from patients with toxoplasmosis showing serological patterns I, II and III, from non-related diseases and from healthy individuals with no previous *T. gondii* infection, provided relative indices for sensitivity, specificity and efficiency of 1,000, 0.977 and 0.981, respectively. The IgM antibodies eluted from the HA-A Toxo immunoreagent reacted with a 28KD component of *T. gondii* alkaline-extract antigen, in immunoblotting technique.

Supported by FAPESP and CNPq.
THE SURFACE CHARGE AND CARBOHYDRATES OF SOME SPECIES OF Crithidia WHICH ADHERE TO SUBSTRATES

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Crithidia fasciculata, C. guihemel and C. lucilise are insect trypanosomatids which strongly
adhere to glass and plastic surfaces, suggesting that they possess a highly negative surface charge.
To visualize the attachment of the cells to the substrate, they were allowed to adhere to glass
coverslips laid in the culture tubes and then processed for scanning electron microscopy. The
parasites of all three species appeared spread on the glass, attached by the plasma membrane, either
of the cell body or the flagellum.

To evaluate the surface charge of the parasites, the mean electrophoretic mobility (EM) of
the cells was measured in a Zeta cytrophotometer. The EM of C. fasciculata, C. lucilise and C.
guilihmel was respectively -1.383, -0.915 and -0.873 µm. s⁻¹. V⁻¹.cm. Treatment of the cells with
neuraminidase (from Vibrio cholera, 0.1U/ml for 5 min.) decreased the EM in all three species,
suggesting that the negative charge is partially due to sialic acid.

A further analysis of the surface carbohydrates was undertaken by lectin agglutination, using
several lectins specific for N-acetyl-D-glucosamine, N-acetyl-D-galactosamine, D-galactose,
D-mannose and D-fucose. The data obtained showed that D-fucose and GlcNAc residues seem to be absent
in all three species, C. guihemel has the highest titers for D-mannose, while C. lucilise has the
lowest titers for BPL, DSL, and SRL (DolcNe).

The data obtained show that all Crithidia spp here analyzed have a net negative surface charge
which varied according to the species. Further studies are necessary to determine whether surface
anionic sites are involved in the adhesion process.

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PHORBOL ESTER EFFECTS ON FLAGELLAR STRUCTURE OF Herpetomonas megaloleae AND Crithidia deanei

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In a previous study it was shown that 20ng/ml TPA (12-O-Tetradecanoyl-phorbol-13 acetate) an
exogenous protein kinase C activator, can induce several structural alterations on L. mexicana
amazonesis (Vannier-Santos et al., J. Submicrosc. Cytol Pathol. 20:583, 1988). TPA caused
remarkable plasma membrane detachment from subpellicular microtubules. TPA also caused flagellar
membrane displacement from axoneme microtubules, specially when filipin was added to the fixative
solution. During this alteration the intraflagellar structure of some cells may be twisted inside
the flagellar membrane and the paraxial structure seemed to be more strongly linked to the plasma.
We started to study the TPA effects on H. megaloleae mainly because of its typical paraxial
enlargement in the flagellar area near the flagellar pocket opening. Flagellar pocket desmosomes
appeared to participate in the process and the membrane was detached from axoneme microtubules but
remained tightly bound to the paraxial structure. In order to evaluate the role of the paraxial
structure in this phenomenon we repeated the TPA-treatment using C. deanei, a trypanosomatid which
lacks this structure. We observed wider flagella specially at the basal regions corroborating the
participation of flagellar pocket desmosomes in the alteration. Many flagella of TPA-treated C.
deani had a "winged" appearance presenting lateral expansions at both sides. Although not so
frequent and conspicuous, this alteration was also seen in control preparations. Intensification of
flagellar alterations by TPA was also observed in T. cruzi (Carvalho and de Souza, Parasitol. Res.,
74:11, 1987). Taken together these data suggest that flagellar pocket desmosomes and the
paraxial structure modulate the TPA-induced alterations in trypanosomatid flagella which may be analogous to
the flagellar enlargement observed when parasites attach to invertebrate hosts.

Supported by CNPq, FINEP, CEP-G-UFRJ.
A trypanosomatid was isolated from the bug *Ornithodoros omarum* and cloned in blood agar (Mem. Inst. Oswaldo Cruz, Suppl., 81, 1986, pág.58). After the cloning, a nutritional study was realized and showed that the flagellate has a very fast exponential growth rate (24 h. at 28°C) in Roitman’s defined medium. The organism was able to grow without carbohydrate at 28°C and several of them showed a stimulatory effect, mainly maltose and fructose. Hemin, adenine, and the amino acids valine, isoleucine, lysine, threonine, tryptophan and phenylalanine, and the vitamins pyridoxamine and folic acid, are not required for growth. Lactose also showed a stimulatory effect for growth at 28°C. At 37°C no growth was observed. Transmission electron microscopy showed that this trypanosomatid harboured a bacterium-like and a virus-like particle in its citoplasm.

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**THE INCIDENCE OF INTESTINAL PROTOZOA PARASITES AMONGST ZERO TO SEVEN YEARS OLD CHILDREN.**

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In one of the slums of the city, Campina Grande, Paraíba (North-east-Brazil), 203 children of the age between 0 and 7 years were registered.

All the registered children received sterile containers to collect fecal material to be submitted for parasitological examination.

Total number of 192 containers with fecal samples were returned, the same being examined in the laboratory, it showed 138 (71.8%) positive cases for intestinal protozoan parasites with the occurrence of 121 (63.0%) *Entamoeba histolytica* and 41 (21.3%) *Giardia lamblia*.

Amongst 54 (28.2%) negative cases for the intestinal protozoan parasites are also included newborn babies and that of few weeks of age, those were fed only with milk and/or mussels and have had no contact with the soil outside the domiciles.

The infection index is high considering, that the slum is benefited with treated tap-water, electricity, domiciles of briks and latrines of fossa type.
CRYPTOSPORIDIUM sp INFECTION IN CENTERS FOR MALNOURISHED CHILDREN IN SANTIAGO, CHILE.

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Cryptosporidium sp., a coccidion whose medical importance is already acknowledged, is a cause of acute diarrhea in immunocompetent persons and of chronic diarrhea in the immunocompromised.

Since 1985 our group has been working in the diagnosis of this infection by means of the Ziehl-Neelsen (ZN), Auramine and Saffronin techniques, finding a 6.4% rate of infection in hospitalized children with digestive symptoms, and absence of the parasite in asymptomatic children.

An epidemiologic study performed in 4 of these centers revealed 2 outbreaks of cryptosporidiosis in 2 centers for the recovery of malnourished children in Santiago. In one center 17 out of 70 children (24.3%) were infected, and in the other 12 out of 51 (23.5%) harbored the parasite. A control group had 3 infected children among 54 (5.5%), p<0.01.

These findings stimulated us to study the prevalence of Cryptosporidium sp in malnourished children interned in centers for their recovery in Santiago. Three stool samples were taken from each of 201 children from 3 centers, fixed in Phenol-Alcohol-formaldehyde fixative, and processed by Burrows' and ZN techniques. A group of 126 eutrophic children of similar ages, from 5 day care centers situated in the same geographic zones was the control group.

The positive ZN smears for Cryptosporidium sp. were distributed as follows: Center A 4/46 (8.7%); Center B 8/36 (13.9%); Center C 0/29; Center D 2/48 (4.2%); and Center E 1/42 (2.4%). The overall rate of positivity was 6% (12/201). The positivity rate in the control group was 0.8% (1/126); p<0.05.

The Burrows' technique showed infection by other enteric parasites in 16.4% of the malnourished children, and in 48.6% of the control group. Probably this fact is due to a better control and treatment of enteric parasites in the malnourished. Our results show that Cryptosporidium sp. is a frequent parasite in malnourished children, and that it is able to cause epidemic outbreaks in these patients, probably contributing to maintain and/or worsen their condition.

We feel that new studies should be done in this respect, in order to establish the real importance of this parasite in these patients, specially relating it with their immunologic status.

ACUTE DIARRHEA ASSOCIATED WITH Cryptosporidium oocysts IN CHILDREN ATTENDING A HOSPITAL AMBULATORY.

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Stool samples from 116 children with acute diarrhea were analysed for Cryptosporidium oocysts from August 1987 through July 1988. In view of the increased reporting of this parasite over the past few years, we decided investigated the diarrheal illness in a group of infants and toddlers attending a hospital ambulatory to provide epidemiological information. Of the 116 children examined, 21 (18.10%) had oocysts of Cryptosporidium sp. in their stools; the highest prevalence (ten of twenty six children) was in children between 7 and 12 months. (Table)

<table>
<thead>
<tr>
<th>Age group (months)</th>
<th>n of patients</th>
<th>Cryptosporidium positive</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-6</td>
<td>57</td>
<td>6</td>
<td>11.7</td>
</tr>
<tr>
<td>7-12</td>
<td>28</td>
<td>10</td>
<td>8.62</td>
</tr>
<tr>
<td>13-18</td>
<td>21</td>
<td>2</td>
<td>9.52</td>
</tr>
<tr>
<td>19-24</td>
<td>10</td>
<td>3</td>
<td>30</td>
</tr>
<tr>
<td>Total</td>
<td>116</td>
<td>21</td>
<td>18.10</td>
</tr>
</tbody>
</table>

In 18 children, Cryptosporidium was the only parasite found. Other three children with Cryptosporidium, also presented cysts of Giardia lamblia. Our results show a high occurrence of Cryptosporidium oocysts in stool samples from children with acute diarrhea who live in Sao Paulo city; and the highest prevalence rate in children from 7 to 12 months agree with other reports.
IMMUNOCYTOCHEMICAL LOCALIZATION OF ACETYLATED ALPHA-TUBULIN IN TROPHozoITES OF Giardia lamblia

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The identification and localization of acetylated alpha-tubulin became possible with the production by Pippemo and Fuller (1985) of a monoclonal antibody, designated as 6-11B-1, that is specific for this kind of tubulin.

Giardia lamblia trophozoites (Giardia intestinalis, Portland-1 strain) cultivated axenically at 37°C in modified TYI-S medium containing 10% heat-inactivated bile, were fixed in methanol at -20°C for 30 min on poly-L-lysine (0.1%) coated glass slides. Tubulin-containing structures, as the median body, flagella and the fusions were localized using immunofluorescence microscopy by previous incubation in the presence of anti-tubulin antibodies and in a second antibody labeled with fluorescein. We have used antibodies specific for acetylated (6-11B-1) and non-acetylated (8-5-1-2) alpha tubulin.

Tubulins were also detected in immunoblotting, confirming the existence of both post-translational forms in all lysates of this parasite.

Our observations show the presence of stable microtubules containing acetylated alpha-tubulin (or the alpha-3-tubulin isotype) in median body, flagella and fusions.

This work has been supported by CNPq, CEPG-UFRJ and FINEP.

Prevalence of Giardia in small laboratory rodents.

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In Brasil there are few cases or literature work in reference to Giardia infection in small laboratory rodents, having reference in relation to rabbits and chinchillas. 30 Wistar white rats, 30 Swiss white mice, 27 Norfolk rabbits, 17 guinea-pigs and 37 golden hamsters were selected at random for examination. The intestinal contents of each animal were smeared on a slide, fixed in Schaudinn's solution and stained by the Heidenhain iron hematoxylin method. Fresh smears of intestinal contents were also examined by direct observation with the microscope. Giardia has not been found in rabbits and guinea-pigs. The prevalent rate of infection in rats was 16,6% and the trophozoites were of the Giardia duodenalis type; 26,6% of mice were infected of Giardia muris type. 37 hamsters were 100% infected of which some had Giardia duodenalis type trophozoites and others had Giardia muris type trophozoites. There were some hamsters which had both Giardia types. It has been discussed that the possibility of these small rodents with Giardia duodenalis type were possible reservoirs of human giardiasis.
Preliminary Study of the Ciliatofauna of Briofits found in Rio de Janeiro State.

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It was promoted the observation of the briofits ciliatofauna representatives, by the deposition of little portions of the mass in groups of Petri dishes with mineral water.

Later, it was stated the appearing in the water mass of a moss community, where the existence of more representative specimens and the gradual substitution of this representativity were observed. It is necessary to distinguish, that among all the agents of the microscopic invertebrates community associated to the mass which was studied, the most plentiful and frequent are the ciliates and rotifers.

For this initial study, it was used some specimen of ciliates numerically more expressive. The use of culture mediums, has being trying to establish the maintenace of these organisms in laboratory. The morphological data needed to the diagnosis are being concluded with the guidance of the microscopy known techniques, being already established the presence of a specimen from the Colpodidae family, one from the Spathidium genus and another one from the Hypotrichida order.

Occurrence of Metacyctothus rancureli, Laval and Tuffrau, 1973, on Neoteredo from Rio de Janeiro State littoral.

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Metacyctothus rancureli is a bactivorous ciliate endocommensal of Neoteredo reiney (Mollusk-Bivalvular). The hosts were collected in the Coroa Grande, Mangrove, Mangaratiba, Rio de Janeiro. On its gills, we observed numerous Trichodina sp. which sometimes got loose to swim free. Examining the content of the digestive apparatus in sterile sea water, we found M. rancureli and Boveria sp.

The Heterotrichida upon which is based this paper, when seen "in vivo", performs slow and spiral movements, without particularities. These ciliates are easily recognized though the AZM. When seen in the Protargol, present an egg-shaped and laterally flat body. The left face is slightly convex and the right one, almost plane. It has near 100 (one hundred) longitudinal kinetics and two drying anterior systems guided longitudinally being the one from the left face, longer than the right face one. The peristomial area, with a three-cornered opening, has close by 80 membranelles which are ranked transverse to the long paraoral that extend themselves up to the cytostome.

The macronucleous is triangular-roundish placed in the anterior half of the cell body. A well-marked cytoproct, duct-shaped, opens itself obliquely in the posterior pole of the ciliate.
PZ-57 DEVELOPMENTAL CYCLE AND HISTOPATHOLOGICAL STUDIES OF *Thelohania* sp. (MICROSPORIDA: THELOHANIDAE) IN LARVAL BLACKFLIES (DIPTERA: SIMULIIDAE)

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The order Microsporida Balfouri, 1882 of the phylum Microspora Sprague, 1977 is one of the most important from the standpoint of the parasitism of insects. In fact, most of protozoa known to be pathogenic for insects belongs to the Microsporida, and this is sufficient to warrant study. The development and histopathological studies of a microsporidium identified as *Thelohania* Henneberg, 1902 are presented with a brief discussion. The material used in this study come from Brazilian blackflies *Simulium* (Parsomosomus) sp. larvae collected from a stream in the vicinity of the city of Itupé, São Paulo State, Brazil. Infected larvae showed anomalous behaviour such as decreasing of the normal movements. Fresh smears were prepared and studied using phase contrast microscopy and dark field illumination. Various life-cycles stages were also examined in preparations stained with 10% (v/v) solution of Giemsa (pH 7.2). For histological observations, infected individual were fixed in Bouin-Dubosq - Brazil solution overnight, washed and dehydrated in an ascending series of ethanols, and embedded in wax. Sections were cut sagittally at 5μm, and stained with Heidenhain’s hematoxylin and eosin. The fat body is the site of the infection and the development conforms with the characteristics of Thelohanidae: eight sporoblasts, formation of eight spores, typically produced, always within a panesporoblastic membrane. Measurements were made with an eye-piece micrometer at 1,000. Unfixed spores were puriform, and a vacuole in the posterior part, was the only internal structure visible. All the spores, fresh, fixed and stained, were of the same shape, 4.8 X 3.2 μm. Mechanical pressure as other commonly used techniques (Vavra & Maddose, 1976) to force protrusion of polar filaments were unsuccessful. On sagittal sections of infected larvae it was seen that other tissues, except the fat body, were free from the parasite. Larvae of *Simulium* (Parsomosomus) sp. parasitized with *Thelohania* sp. have a characteristic white spots in abdomen. Nuclei of infected cells were hypertrophied.

PZ-58 USE OF FLUORESCIN LABELLED ANTI-TUBULIN MONOCLONAL ANTIBODIES TO DETECT CILIARY STRUCTURES IN THE VEGETATIVE AND CYSTIC FORMS OF *GASTROSTYLA STEINII*

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Several studies concerning aspects of the ciliature and infraciliature of *Gastrostyla steinii* (Ciliate, Hypotrichida) have been made since the last century. All of them have contributed, like pieces of a jigsaw puzzle, to form the whole picture of our present understanding of the structure of this ciliate. In this study, we obtained more information about the oral and somatic ciliature in the vegetative cell of *G. steinii*. These microtubular systems were visualized by immunofluorescence microscopy, by using anti-tubulin monoclonal antibodies coupled to fluorescein. Additionally, attempts were made to detect primordia of ciliary structures and microtubules in the resistant form (cyst). The strain of *G. steinii* used was isolated, in the cystic form, from the digestive apparatus of *Rhinocricus padbergii* (Diplopoda). The gut contents were placed in petri dishes containing mineral water to trigger the excystment process. The excysted ciliates were maintained in culture using mineral water plus lettuce powder or infusion as the nutritive medium. The cysts were collected from advanced aged cultures where most of the free swimming cells had already metamorphosed into resistant forms. As expected, none of the primordia of ciliary structures were visualized in the resting cyst by using this methodology. In contrast, intensive positivity was found in the vegetative cell. In the ventral surface, the oral ciliature (adoral zone membranelles and oral membranes) could be distinguished from the somatic structures (fronto-ventral-transverse and marginal cirri). Dorsally, the 6 rows of longitudinal cilia that comprise the dorsal kineties were also seen. We believe that these preliminary findings can be used to shed more light on the role played by the cytoskeleton elements during the cell differentiation process in protozoa.
HEMOPARÁSITOS ENCONTRADOS EM ALGUNS MAMÍFEROS DE BALDEINA, ESTADO DO AMAZONAS.

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Com o intuito de verificar a ocorrência de hemoparasitos em mamíferos da área da Usina Hídrelétrica de Baldeira, Estado do Amazonas, examinamos os seguintes animais capturados durante a operação de salvamento efetuada na época do enchimento do seu reservatório no rio Uatumã: 41 macacos, sendo 31 guirias vermelhas (Alouatta seniculus straminea), nove macacos-de-cheiro (Saimiri sciureus) e um paraucú (Pithecus pithecus); duas preguiças reais (Chloropithecus didactylus); uma capuchinha-de-quatro-olhos (Philander oposum) e um gato-maracajá-assu (Felis pardalis). A pesquisa dos parasitos foi através do exame de gotas espessas, esfregações finas, hemocultivo e xenodiagnóstico com ninhas de Rhodnius prolixus.

Plasmodium brasiliense foi achado em 15 dos macacos examinados (36,6%), sendo 10 guirias (30,3%), quatro macacos-de-cheiro (44,4%) e em nenhum. Quatro espécies de Trypanosoma foram encontradas: T. cruzi em apenas um macaco-de-cheiro (2,3% do total), T. evectae em 15 guirias (51,6%) e em nenhum, T. saimiri em seis macacos-de-cheiro (66,7%) e T. lebichi em duas guirias (6,5%). Endotrypanum schublinii foi achado em uma das preguiças reais examinadas. Microfílias foram vistas apenas no sangue de macacos das três espécies, sendo: 17 guirias, cinco macacos-de-cheiro e em nenhum. Todas as microfílias encontradas nos macacos-de-cheiro eram compatíveis com as de Dipetalonema gracile. Nenhum hemoparasito foi encontrado na depenta, nem no gato-maracajá-assu.


TRANSFUSIONAL MALARIA.
RISK OF CONTAMINATION IN NON-ENDEMIC AREAS.

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& Chamone, D.F.A.**
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Extensive migration from areas where malaria is endemic may increase the risk of acquiring this disease through blood transfusions. In 1985, 500,000 new cases of malaria were diagnosed in Brazil, mainly (90%) in the Amazon basin. Many of these individuals will migrate to the more industrialized southern states. In order to assess the risk of transfusional malaria we conducted a pilot study at Fundação Hemocentro de São Paulo. In more developed countries, such as England, screening for malaria in blood banks has become routine. We tested 1889 blood donors for the presence of anti-P. falciparum antibodies using a serum dilution of 1/20 and indirect commercial* immunofluorescence assay. In this first screening 36 sera were reactive (+), and 74 were weakly reactive (±), giving a total of 110 (5,6%) reactive sera. From this total, 7 sera had antibodies against T. cruzi; 5 against T. pallidum; and 2 against both.

Confirmatory tests were performed at Instituto de Medicina Tropical de São Paulo, Brazil. [Immunofluorescence with commercial and non commercial slides containing P. falciparum with serum dilutions of 1/20 and 1/40, and ELISA test using antigenic extracts of P. falciparum]. Of the 36 reactive sera 9 (25%) were non reactive and 27 (75%) were confirmed as reactive. Of the 74 weakly reactive sera only 40 (54%) were confirmed, showing an estimated 3,5% of blood donors as having antibodies reacting with P. falciparum antigen. This high observed frequency is may be in part due to the cut off used, to the fact that immunofluorescence involves subjective reading, to the fact that when screening for blood transfusions is done one tends to play safe, and perhaps due to cross reactivity with other antigens.

The clinical and epidemiologic study of these blood donors with positive serology, is under way in order to understand our results and establish better screening methods.

*BIOL-MÉRIEUX - Slides, distributed by BIOLAB - S.A.
Toxoplasma gondii: EPIDEMIOLOGY IN MARINE ECOSYSTEMS OF THE II REGION, CHILE.

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Toxoplasmosis is one of the most widely distributed zoonosis in the animal world where various homeothermal species of land and water environments are exposed to infection.

With the purpose of contributing to a better knowledge of the probable role of Toxoplasma gondii in marine ecosystems, an epidemiological study of the coastal littoral of Antofagasta, II Region, Chile, is presented.

261 persons coming from coastal communities were studied, from among these, blood was collected for serological studies by means of Indirect Fluorescence Antibody Test for Toxoplasmosis, according to Camargo, 1964 (IFAT-Toxo). Furthermore, 47 wild marine fowl of different species were captured and subjected to a serological study by means of the Dye test of Sabin-Feldman, 1968 (SFT) and a parasitological study of the encephalic mass through experimental inoculation and histopathology. All of them, human beings and fowl, presented as the principal and/or only source of food, marine species.

Of the 261 investigated persons, 83 (31.8%) were seropositive, while among the fowl an incidence of 12.8% (6/47) was observed. The experimental inoculation was negative in all fowl, the histological analysis was positive in 3 cases, but in only one of them correlation with SFT was noted.

The epidemiological importance of these results, is here discussed.

PRIMEIRA COMPROVAÇÃO PARASITOLÓGICA NO BRASIL DA INFEÇÃO EXPERIMENTAL PELO T. cruzi EM CAPRINOS.

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Na procura de novos reservatórios do T. cruzi, tem-se conseguido demonstrar experimentalmente a infecção pelo parasito em diversas espécies, entre elas, mamíferos sinantrópicos de grande porte, tais como suínos, bovinos, ovinos e caprinos.

Enquadra-se nesta linha de pesquisa, se efectua estudo experimental inicial que objetivou a comprovação parasitológica da infecção pelo T. cruzi na espécie caprina. Assim sendo, foram utilizadas diferentes cepas, doses e vias de inoculação dos parasitos.

Havendo-se inoculados 5 caprinos machos castrados, de 6 meses de idade, o seguimento foi feito semanalmente mediante o exame sanguíneo direto, esfregaço corado, hemocultura e xenodiagnóstico. Simultaneamente foi feito seguimento sorológico por meio das reações de hemaglutinação e imunofluorescência indireta.

Aos quatro meses de seguimento obteve-se a comprovação parasitológica da infecção, isolando-se o parasito num dos animais estudados. Este exemplar foi inoculado pelas vias endovenosa e intraperitoneal, com 1,05 x 10⁷ parasitos da cepa FL (Rio Grandeense). O mesmo caprino apresentou, a partir da segunda semana de infecção, título sorológico superior a 1 : 640 (RIFI), mantendo-se até a 12ª semana.

Trabalho realizado com o apoio do TDR/WHO e ENSP/FIOCRUZ.