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Programa de Pós Graduação em Imunologia e Parasitologia Aplicadas

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NOTA 6
AVALIAÇÃO TRIENAL CAPES (2010-2012)

V WORKSHOP PPIPA

ANAIS

20 e 21 de Novembro de 2014

CAROS CONGRESSISTAS,

É com grande satisfação que receberemos vocês para esses dois dias de evento científico organizado por alunos e docentes do Programa de Pós-Graduação em Imunologia e Parasitologia Aplicadas (PPIPA – UFU).
O V Workshop do Programa é um evento que visa proporcionar aos discentes desse Programa a oportunidade de apresentar e discutir os dados referentes aos seus projetos de pesquisa e difundir os conhecimentos atuais das áreas de Imunologia, Parasitologia, Microbiologia e áreas correlatas. Além disso, o evento objetiva discorrer e refletir sobre o estágio atual de desenvolvimento das atividades do curso de pós-graduação em vista do conceito 6 obtido na última avaliação do Programa pela Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).
Agradecemos as instituições que apoiaram e patrocinaram o evento.

Cordialmente,
Comissão Organizadora

O V Workshop do Programa é um evento organizado por alunos e docentes do Programa de Pós-Graduação em Imunologia e Parasitologia Aplicadas (PPIPA – UFU).

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A HIGH THROUGHPUT ANALYSES OF CYTOKINES DETECTION AND ACTIVITIES ALONG *Trypanosoma cruzi* EXPERIMENTAL INFECTION

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Host immune response against *Trypanosoma cruzi* is highly complex and involves many components, both regulators and effectors. This is the firs report of a high throughput kinetics analysis of a broad cytokines and chemokines expression in different organs from mice infected by the virulent *Trypanosoma cruzi* CL strain compared to the non virulent G strain. the results showed that pro-inflammatory cytokines IL-12, IFN-gamma and TNF-alpha were highly expressed in stomach and spleen during the acute and chronic infection with both strains. IL-2, IL-7 and M-CSF were specially expressed during chronic phase, unlikely from IL-3, that was expressed at acute infection. KC and MIP-2 was expressed at acute and chronic phase. MIP-1alpha and beta, IP-10, RANTES, MIG, MCP-1 were expressed at a chronic phase of both strains. IL-4 and IL-9 were less expressed, but where detected in the spleen, at the chronic phase of CL infection, and in the stomach, at the acute phase of infection. In cardiac tissue, the majority of cytokines and chemokines were downregulated; only IL-10 was detected during the acute infection by CL strain. Considering infection by G strain, this cytokine was only observed in samples from chronically infected animals.. Also treatments controlled parasitemia in mice inoculated with Y strain. Thus we have observed that the pattern of cytokines released along *T. cruzi* infection depends on *T. cruzi* strain and host organs.

Key Words: *Trypanosoma cruzi*; cytokines; experimental infection

Financial Suport: FAPEMIG/CAPES/CNPq

COLLECTION TECHNIQUES OF TICK SALIVA AND CYTOTOXICITY AGAINST TUMOUR CELLS

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Ticks are blood-sucking animals, and to support this function, it has several bioactive molecules present in their saliva. Molecule inhibitors of coagulation from hematophagous ectoparasites, such as Ixolaris and Amblyomin-X, have been studied in tumor lines. In this study, we describe the main techniques used for saliva collection and its use in cytotoxicity assays with MTT against Ehrlich cells, a cell line of breast cancer in mice. The main technique of saliva collection is based on implementation of Dopamine in the lateral region of the tick and collecting the secreted substance with automatic pipette, storing content at - 80 ° C until use. The cytotoxicity of the saliva was analyzed by means of observation of staining of the 96-well plate after treatment with saliva and sheer MTT assay, which is a cell viability assay. Our results with respect to the collection of saliva showed that exsistem influences of time of day (morning, afternoon and evening) and stage of engorgement of the female tick (semi or fully) in the amount of saliva obtained in a collection, in addition to species-specific differences. Regarding the MTT assay, the results showed that the pure saliva depth exerts cytotoxic effects on this tumor cell line. Events such as degradation and DNA damage induction and occurrence of necrosis and apoptosis need for studies that can accurately identify as saliva induces the death of cancer cells. The pure saliva of some species of ticks studied stands out as a candidate induction of cell death, with various molecules and substances that can be used in this anticancer therapy.

INFLUENCE OF TROFOBLASTIC CELLS (BEWO LINE) IN MODULATION OF APOPTOSIS ON MONOCYTES (THP-1 LINE) INFECTED BY *Toxoplasma gondii*

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Toxoplasma gondii is an intracellular parasite that causes severe disease when the infection occurs during pregnancy. Trophoblast cells constitute an important maternal-fetal barrier, with monocytes concentrating around them. Thus, interactions between trophoblasts and monocytes are important for maintaining a successful pregnancy, especially in cases of infection. This study aimed to evaluate the role of trophoblast cells (BeWo line) on monocyte (THP-1 line) activity, specifically in monocytes apoptosis, in the presence or absence of *T. gondii* infection. THP-1 cells were stimulated with supernatants of BeWo cells, previously infected or not with *T. gondii,* and then infected with parasites. The supernatant of both cells will be collected and analyzed for cytokine production and FasL secretion, by ELISA kits. Furthermore, the occurrence of apoptosis in THP-1 cells and the expression of death receptor (CD95) at these cells will be evaluated by flow cytometry. Finally, some intracellular proteins, related with apoptotic signaling pathways, will be detected by western blotting. Previous studies supernatant of BeWo cells infected or not, was able to change the cytokine profile secreted by infected THP-1 cells, and this supernatant became THP-1 cells more able to control *T. gondii* proliferation than those that had not been stimulated. So, we expected that trophoblast cells will be able to alter the incidence of apoptosis in THP-1 cells and this event may be related with the maintenance of pregnancy, principally when this gestation occurs concomitant an infection with *T. gondii*.

Key Words: Trophoblast, Monocytes, *Toxoplasma gondii*, Apoptosis

Financial Suport: FAPEMIG, CNPQ, CAPES

THE ROLE OF MIF (MACROPHAGE MIGRATION INHIBITORY FACTOR) IN THE CONGENITAL TOXOPLASMOSIS

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Because macrophage migration inhibitory factor (MIF) is a key cytokine in pregnancy and has a role in inflammatory response and pathogen defense, the objective of the present study was to investigate the role of MIF in congenital toxoplasmosis. For this propose, C57BL/6 $MIF^{-/-}$ and C57BL/6 WT females were orally infected with *T. gondii* ME-49 strain on day 1 of pregnancy and were sacrificed on day 8 post infection. The experimental controls were set up with non-pregnant or/and non-infected mice. The animals were evaluated for mortality and body weight loss. The serum were collected and analyzed for determination of cytokines profile by CBA assay. Also, the uteri were evaluated by western blotting for quantification of MIF receptor and Indoleamine 2,3 Dioxygenase (IDO). Our results demonstrated that C57BL/6 $MIF^{-/-}$ succumbed to infection first than WT. Also, non-pregnant C57BL/6 $MIF^{-/-}$ presented more pronounced body weight loss than WT. Regarding MIF receptor (CD74) expression in uteri it was observed higher expression of the receptor in the C57BL/6 $MIF^{-/-}$ compared with the WT. The analysis of IDO demonstrated that it is expressed in higher amounts in uteri of C57BL/6 $MIF^{-/-}$ in comparison with the WT. Related to the cytokines profile it was observed that higher levels of pro-inflammatory cytokines were detected in the C57BL/6 WT. On the other hand, when the antiinflamatory cytokines were analyzed it was observed that the higher levels of IL4 and IL10 were detected in the serum of the C57BL/6 $MIF^{-/-}$. In conclusion, MIF demonstrated to be important for control of *T. gondii* infection in pregnant and non-pregnant mice. The absence of MIF in C57BL/6 $MIF^{-/-}$ leads to upregulation of MIF receptor demonstrating the importance of MIF during the pregnancy. The mechanism of MIF action in the maternal-fetal interface may be related with IDO suppression and induction of pro-inflammatory immune response.

Financial support: CAPES, FAPEMIG, CNPq

DESCRIPTION OF ANTIGENIC FRACTIONS OF *Neospora caninum* AND ITS POTENTIAL USE FOR DIAGNOSIS

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Neospora caninum, an obligate intracellular protozoan, has epidemiological and economic importance to cause disease in a wide variety of animal species. Our work involved the characterization of antigen recognition from *N. caninum* in murine and bovine models of experimental infection, which were analyzed by IFAT, ELISA and Immunoblot techniques. First, the cross-reactivity profile between *N. caninum* and *Toxoplasma gondii* was evaluated using experimentallly infected mice. Our results indicated the presence of apical recognition of *N. caninum* as indicative of cross-reactivity by IFAT. Animals infected with *N. caninum* showed highcross-reactivity for soluble antigens of *T. gondii* (STAg and STAg-TgESA) by ELISA. On the other hand, soluble antigens of *N. caninum* (NLA and NLA-NcESA) showed less heterologous recognition. The secreted-excreted antigens (NcESA) presented the best potential to distinguish the infection between the parasites. In Immunoblot assay, we observed cross-reactivity to *N. caninum* in NLA with high molecular weight and bands above 64 kDa for NcESA. We then characterized the profile of antigen recognition of *N. caninum* in bovine experimental infection. The animals were determined to be seropositive after 15 days of infection, with increased IgG2/IgG1 ratio to NLA in the beginning of infection and reinfection. Specific IgM and IgA directed towards NLA could not be associated with the acute phase of neosporosis. However, avidity indexes of <50% and >70% were associated with early and late phases of infection, respectively. In immunoblot assay, IgG and IgA recognized proteins with low molecular weight, present in whole antigen lisates, and were associated with reinfection. Antibody recognition of low molecular weight proteins within NcESA by IgG(+) and IgM(-) were associated with the chronic infection. Thus, the use of distinct diagnostic techniques with different antigenic fractions may elucidate the profile of infection with *N. caninum*, favoring more accurate discrimination of the infection stages.

Financial supports: CAPES, CNPq, FAPEMIG, FINEP

Keywords: *Neospora caninum*, Neosporosis, Diagnostic, Cross-reactivity, *Toxoplasma gondii*

PLASMID-MEDIATED QUINOLONE RESISTANCE AND CLASS 1 INTEGRON IN CLINICAL SAMPLES OF *Klebsiella pneumoniae, Escherichia coli* AND *Pseudomonas aeruginosa* FROM UNIVERSITY HOSPITAL

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In recent years, it has been observed the global spread of new genotypes of resistance to antimicrobial drugs among epidemiologically important microorganisms, and as a consequence an increase in morbidity, mortality and costs. The quinolone resistance in nosocomial *Klebsiella pneumoniae, Escherichia coli* and *Pseudomonas aeruginosa* has become an increasing problem and today shows the highest rates of resistance among Latin American countries. Studies in Brazil reported spread of predominant clones in hospitals in different regions nevertheless there are few data in our region. Therefore, due to the rapid worldwide dissemination of resistance genes and the negative impact of prescribed therapy, it is essential to improve understanding of these mechanisms, adequacy of measures for prevention and control, of treatment protocols and study the development of new therapeutic options. The aims of this study are to determine the involvement of integrons and plasmids as vectors and the mechanism of quinolone resistance in strains of *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa*. It will be analyzed the frequency of *qnr*, *aa(6')-Ib-cr*, *qepA* and *IntI* genes; the minimum inhibitory concentration; variation and the profile of susceptibility to quinolones and beta-lactams; and the sequence type and clonal complex of predominant pulsotypes. It will be evaluated strains recovered from patients hospitalized at the Clinical Hospital of Federal University of Uberlândia that showed reduced susceptibility to quinolones. Isolates will be tested through Etest® method for determining the minimum inhibitory concentration. The characterization and detection of genes associated with plasmid-mediated quinolone resistance and class I integrons will be performed by PCR. There will be assessment of genetic similarity by the technique of Pulsed Field Gel Electrophoresis and molecular typing by Multilocus sequence typing. This work expects to determine the mechanisms of plasmid-mediated quinolone resistance in strains recovered from University Hospital, as well as to determine the involvement of integrons.

Keywords: plasmid-mediated; quinolone; resistance.

Financial support: CNPq.

INVESTIGATION OF PROMOTERS PROFILE RELATED TO BREAST CANCER AS POTENTIAL BIOMARKERS FOR INITIAL STAGING

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Breast cancer is the most common malignancy among women and the major cause of cancer death in women younger than 65 years. There are several biomarkers described in the literature that are aberrantly expressed in serum of breast cancer patients and are useful for prognosis and metastasis prediction. Unfortunately, there are few biomarkers for early detection of breast cancer. So, this work aim search specific proteins in the serum and tissue of patients with early stages breast cancer (n=13), pT1pN0 and pT2pN0, compared with two control groups; one of healthy women with no cancer diagnosis (n=20) and the other composed by women with metastatic breast cancer (stage IV) (n=13). Statistical analysis was performedusingGraphPadPrism5. Serum and tissue Epidermal Growth Factor Receptor (EGFR), thrombospondin-1(TSP-1) and Receptor Activator of Nuclear Factor-Kappa B Ligand (RANKL) were investigated by ELISA. Serum levels of EGFR were found to decrease with disease progression, the mean EGFR concentration in healthy woman was 65,7 ng/ml while in initial and metastatic breast cancer the mean was 38 and 31,3, respectively. Regarding TSP-1, women with early breast cancer have higher levels than healthy women. In metastatic breast cancer patients TSP-1 levels were similar than healthy women. Concerning serum RANKL no differences were observed between the groups. Analysis of EGFR, TSP-1 and RANKL expression in tissue showed no differences between the groups, the expression appears to be constitutive. These finds suggest that serum EFGR and TSP-1 levels were altered during early breast cancer development. At this point, such biomarkers must be rigorously studied and validated in the clinical trials to be translated into clinically useful tests for breast cancer diagnosis.

Key words: Breast cancer; Biomarkers; EGFR; TSP-1; RANKL.

Financial Support: Grupo Luta Pela Vida – HC-UFU.

SEROPREVALENCE OF CANINE VISCERAL LEISHMANIASIS IN DIFFERENT SOCIOECONOMIC PROFILES IN UBERLÂNDIA, MINAS GERAIS, BRAZIL

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In Brazil zoonotic visceral leishmaniasis (ZVL) is caused by *Leishmania infantum* which is highly associated to poverty. The dog is the main reservoir of the parasite and euthanasia of seropositive animals has been a strategic key for the ZVL control. The aim of this study was to determine and compare the seroprevalence of canine visceral leishmaniasis (CVL) between dogs attended at private clinics and those assisted in the voluntary castration program of the Veterinary Hospital of the Federal University of Uberlândia (HOVET), using serological diagnosis. From March and December of 2013 owners of dogs attended at private clinics (n=54) and HOVET (n=57) were invited to answer a socioeconomic survey. Following the survey, serum of dogs were submitted to ELISA and indirect immunofluorescence test (IFAT), using crude and whole promastigotes of *Leishmania infantum* (MHOM/BR/1967/BH46) antigens, respectively, and appropriated anti-dog IgG conjugates. Serum dilutions were 1:40 in PBS for IFAT and 1:400 in PBS-CT for ELISA. The criteria adopted to consider CVL serological diagnosis was the combined positive result in both IFAT (≥ 1:40) and ELISA (> cut-off). Socioeconomic survey showed that the owners of dogs attended by private veterinary clinics belonged to A and B economic classes (annual budget upper than U$ 40,000). In contrast, families which looked at the voluntary service of the HOVET belonged to C and D economic classes (less than U$ 7,500/year). Serological tests demonstrated that 07 (13%) dogs from private clinics were positive by IFAT and 02 (3.7%) in ELISA. From those attended at HOVET 22 (38.6%) dogs were positive by IFAT and 23 (40.35%) in ELISA. It was observed that there is a higher prevalence of CVL in dogs among lowincome families (C and D classes) when compared with dogs belonged from families of middle and high economic classes (A, B) in Uberlândia / MG.

Keywords: Canine visceral leishmaniasis, *Leishmania infantum*, Dog, Economic survey, Seroloprevalence

MODULATION OF FORMYL PEPTIDE RECEPTORS EXPRESSION IN HIV INFECTION

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Human Immunodeficiency Virus (HIV) infection leads to a severe depletion of CD4+ T-cells and systemic activation, providing opportunities for secondary infections and chronic inflammation. The infection process is established with the virus entry through the interaction of the viral envelope proteins (gp120, gp41) with surface cell receptors (CD4 and CCR5) of the host. However, the Formyl Peptide Receptor 2 (FPR2/ALX) has also been described as an alternative co-receptor for HIV, a promiscuous G-protein coupled receptor that belongs to the FPR receptor family, which also includes FPR1 and FPR3 that are abundantly found in several cells of the human immune system. The FPRs control several processes that support or prevent the inflammatory response according to the available ligand. Thus, we have focused on their expression pattern in HIV-1 positive subjects, in order to assess the involvement of all three FPRs in HIV pathogenesis. Samples of peripheral blood of 47 HIV-1 positive patients in chronic phase and 18 healthy control subjects were collected at the *Clinical Hospital* of Uberlandia. Total RNA samples were extracted and reverse transcription-qPCR (RT-qPCR) assay was performed using Taqman probes to quantify FPR1, FPR2/ALX and FPR3 mRNAs. The GAPDH gene was used as endogenous control. No *significant difference between patients and controls* was observed for FPR1 ($p=0.41$). However, FPR2 had significantly lower levels in HIV-positive subjects ($p<0.001$), while the FPR3 was 5.3-fold higher ($p<0.0001$) than controls. Interestingly, the increased FPR3 expression in patients was correlated with opportunistic diseases ($R = 0.31$, $p <0.001$). We hypothesized that the FPR2/FPR3 expression disequilibrium caused by the HIV infection may have contributed to the disruption of the FPR signaling pathway, which has led to molecular mechanisms that are still unknown, but with consequences that resulted in opportunistic infections. The fine-tuning of FPRs signaling during HIV infection still needs to be thoroughly evaluated, and it may represent a key event in the HIV pathogenesis.

Keywords: Human Immunodeficiency Virus (HIV), Formyl Peptide Receptors (FPRs), Immune activation

Financial Support: FAPEMIG, CNPq, CAPES

EFFECT OF SILICON TREATMENT ON THE *Artemisia annua* GLANDULAR TRICHOME AND ITS ARTEMISININ CONTENT, AND THE ROLE OF THE PLANT TEA INFUSION IN THE CONTROL OF *Toxoplasma gondii* INTRACELLULAR REPLICATION

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Toxoplasmosis is an important zoonotic disease due to *Toxoplasma gondii* ability to infect large number of vertebrates. As the traditional treatment has shown adverse effects, low-toxicity compounds as artemisinin have been researched in *Artemisina annua* tea infusion. This study aimed to investigate the effects of silicon on *A. annua* plant physiology and the role of its tea infusion in the control of *T. gondii* infection in cell culture. The experimental design was a completely randomized design (CRD), in which *A. annua* was planted in the soil with five different doses of calcium/magnesium silicate (0, 200, 400, 800 and 1600 kg ha^{-1}) and five replications. Analysis of foliar macronutrients showed a significant increase only for nitrogen, in the presence of the highest dose of silicate in the soil. The foliar micronutrient, Si concentrations and plant height were not significantly changed with any silicate doses in the soil. The use of 400 kg ha^{-1} of silicate induced the highest total glandular trichome area and intact glandular trichomes, as observed by scanning electron microscopy, and the highest artemisinin content in plant leaves and tea infusion determined by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC), respectively. HeLa cell treatments along with or after *T. gondii* infection and infusion of *A. annua* grown in the soil without or with silicate (400 kg ha^{-1}) and pure artemisinin induced a decrease of parasite proliferation in a dose-dependent manner. In conclusion, the use of silicon had positive effect on the glandular trichome areas and artemisinin contents, but this outcome was not associated with a better efficacy of *A. annua* tea infusion on *T. gondii* replication. These findings suggest that other components, such as flavonoids present in its leaves may act in synergism with the artemisinin improving its efficacy.

Keywords: *Toxoplasma gondii*, *Artemisia annua*, artemisinin, silicon, herbal alternatives.

LATE ONSET SEPSIS IN NEWBORN BABIES: EPIDEMIOLOGY AND EFFECT OF A BUNDLE TO PREVENT CENTRAL LINE ASSOCIATED BLOODSTREAM INFECTIONS IN THE NEONATAL INTENSIVE CARE UNIT

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Late-onset neonatal sepsis (LOS) remains an important cause of death, morbidity and long-term complications among premature infants, they are associated with prolonged hospital stay and increased health-care costs and the incidence and effort for prevention of LOS is of great interest of neonatal intensive care units (NICUs). We assessed late onset sepsis (LOS) rates of neonates in a neonatal intensive care unit(NICU) before and after implementing an evidence-based bundle to prevent these infections in a country with poor resources. We evaluate trends of LOS between October 2010 and August 2012 in a large tertiary hospital in Brazil. We designed a protocol based of CDC guidelines for insertion of maintenance of central venous catheter targeted to reduction of bloodstream infections. During this period two major events occurred: a great increase of LOS rates in January months and relocation of the unit to a provisory place. Additionally we evaluated the risk factors and etiology of these infections. A total of 112 (20.3%) cases defined as LOS were found. The overall incidence rate of LOS in the study was 16.1/1000 patient/days and 23.0/1000 CVC-days. Our monthly rates data of LOS/1000 patient-day reveal fluctuations over the studied period, with incidence rates of these infections in staff vacation period (January 2011 and 2012) significantly higher (59.6/1000 patients-days) than compared with the other months rates (16.6/1000 patients-days) (IRR=3.59; P<0.001). As opposite, the incidence rates of LOS during relocation period was lower (10.3/1000 patients-days) when compared with baseline period 26.7/1000 patients-days (IRR=2.59; P=0.007). After the intervention period, these rates decreased in the post intervention period, when compared with preintervention 14.7/1000 patients-days and 23.4/1000 patients-days respectively (IRR=1.59; P=0.04). Through simple infection control measures, LOS can be successfully controlled especially in NICUs of limited resources countries such as ours.

Key-words: neonates, bloodstream infection, bundle, surveillance

SINGLE STEP OF GEL FILTRACTION CHROMATOGRAPHY TO EXCLUDE HIGH-MOLECULAR-WEIGHT POLYPEPTIDES RELATED TO CROSS-REACTIVITY IN NEUROCYSTICERCOSIS IMMUNODIAGNOSIS

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Neurocysticercosis (NC), caused by *Taenia solium* metacestode, affects the central nervous system and is a devastating parasitic infection. Diagnosis is based on symptoms, imaging, serology and epidemiology data. Due to the importance of diagnosis and the need of alternative antigens in sensitive and specific tests, fractionation of these antigens is an important tool that allows diagnosis even in the absence of homologous antigen. Current preparations present variable sensitivity and specificity, frequent cross-reactions and are not able to discriminate NC clinical forms. The aim of this study was to evaluate the cross-reactivity of high-molecular-weight fraction obtained from the total saline extract (SE) of *Taenia saginata* metacestodes by gel filtration chromatography for human NC immunodiagnosis. The SE was fractionated by gel filtration chromatography using Sephacril®S-100 resin. For IgG detection by enzyme-linked immunosorbent assay (ELISA), 210 serum samples were analyzed: 50 from patients with NC (G1), 100 from patients with other parasitic infections (G2), and 60 (G3) from healthy individuals. Data were analyzed using the McNemar test for paired samples. Statistically significant differences were considered when $P < 0.05$. Sensitivity and specificity were calculated. The high-molecular-weight fraction showed one clearly defined component with molecular size of 95kDa. These polypeptides showed high cross-reactivity with patients from G2, with positivity rate of 46% (46/100). The ELISA sensitivity and specificity were 86% and 72.5%, when using SE, respectively and 82% and 60.6% for the high-molecular-weight fraction, respectively. Gel filtration chromatography can be useful tool to exclude high molecular weight polypeptides generating antigenic fractions potentially applicable in NC immunodiagnosis.

Keywords: Cross-reactivity, immunodiagnosis, Neurocysticercosis, *Taenia saginata metacestodes.*

Financial Support: CNPq, CAPES, FAPEMIG.

IDENTIFICATION OF PROTEINS ASSOCIATED WITH THE DEVELOPMENTOF LOCOREGIONAL METASTASIS OF SQUAMOUS CELL CARCINOMA OF THE ORAL CAVITY USING PROTEOMIC TECHNOLOGY.

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Squamous cell carcinoma (SCC) of the oral cavity accounts for 90% of all malignant lesions. As it is normally diagnosis lately, this neoplasm presents high morbidity and mortality rates. The aim of this project will be to detect tumor biomarkers that predict metastases, the mainly cause of death of SCC-affected patients. Samples from metastatic (M) and non-metastatic (NM) patients diagnosed and treated at Federal University of Uberlândia hospital complex will be used in this study. To investigate the presence of possible protein biomarkers, a highly sensitive tool called Proteomic will be employed. Fresh SCC samples will be collected during surgical resection of primary tumor and immediately frozen at -70 C. Also, other samples from each patient will be routinely processed to confirm SCC diagnosis. From frozen samples, protein extractions will be performed according to the protocol previously established in the literature and then submitted to two-dimensional gel electrophoresis protocol aiming to identify proteins differentially expressed between M and NM groups. Next, gel spots differentially expressed will be cut and subjected to mass spectrometric analysis for protein identification. To confirm these results, Western blotting and immunohistochemical tools will be employed. The impact that these results on public health will be enormous by reducing the cost of SCC treatment as well as its mortality and morbidity.

Keywords: Squamous cell carcinoma, tumor biomarkers, proteomic, mass spectrometric.

Financial support: Fundação de amparo, pesquisa e ensino de Minas Gerais. FAPEMIG.

GENETIC RISK MARKERS FOR STRONG BIOFILM-FORMATION IN CLINICAL METHICILLIN-RESISTANT *Staphylococcus aureus* AND ITS THE ASSOCIATION WITH THE CLONAL PROFILE.

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is the leading cause of hospital infections worldwide, and the biofilm production has a crucial role in many of these infections. This study aimed to determine the biofilm production of the most common MRSA SCC*mec* types, and its relationship with antimicrobial resistance, virulence genes and the genetic background of MRSA isolates. Fifteen strains carrying different chromosomal cassettes, recovered from patients hospitalized in the Teaching Hospital of the Federal University of Uberlandia were selected: five SCC*mec*II, five SCC*mec*III and five SCC*mec*IV. The SCC*mec* type, *agr* group and the presence of the virulence genes (*bbp, clfA, icaA, icaD, fnbB, bap, sasC* and *IS256*) were assessed by PCR. The genetic relationship between the isolates was investigated by pulsed field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). The initial adhesion and biofilm formation were examined by quantitative assays. The surface tension and hydrophobicity of the strains were measured by contact angle technique to evaluate the association between these parameters and adhesion ability. There was association between the values of the electron acceptor parameter, the degree of hydrophobicity and adhesion ability. SCC*mec*III and IV strains were less hydrophilic, showed higher values of the electron acceptor parameter and adhered better than SCC*mec*II strains. In the analysis of biofilm production, only SCC*mec*III strains were characterized as strong biofilm producers. The PFGE showed five major pulsotypes (A-E) however, biofilm production was related to the dissemination of one specific PFGE clone (C) belonging to MLST ST239 (BECC, Brazilian epidemic clonal complex). The genes *agr*I, *fnb*B and *IS*256 in clinical MRSA SCC*mec*III strains, were considered as genetic risk markers for strong biofilm-formation by an *ica*-independent biofilm pathway. This study contributes to the understanding of biofilm production as a virulence factor potentially involved in the persistence and severity of infections caused by MRSA belonging to this genotype.

Keywords: MRSA, SCC*mec*, biofilm, hydrophobicity, BECC

Financial support: FAPEMIG e CAPES

IMMUNOBLOTTING USING *Strongyloides venezuelensis* LARVAE, PARTHENOGENETIC FEMALES OR EGGS EXTRACTS FOR THE DIAGNOSIS OF EXPERIMENTALLY INFECTED IMMMUNOSUPPRESSED RATS.

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Strongyloides stercoralis is widespread throughout the tropics and subtropical regions. This geo-helminthiasis can occur asymptomatic or as a potentially fatal hyperinfection or disseminated infection due to immunosuppression. Diagnostic of the infection occurs for detection of larvae in feces, but low elimination of larvae hampers the detection of disease particularly in cases of immunosuppression. Immunodiagnostictests have been developed but there is difficulty in obtaining larvae of *S.stercoralis* for the production of homologous antigen extract, thus the use of *S.venezuelensis* and their different developmental forms for the production of antigen extracts become an alternative.The aim of this study was to evaluate immunoblotting front L3 larvae, parthenogenetic females or eggs alkaline extract of *S. venezuelensis* in experimental strongyloidiasis associated with immunosuppression. Non-immunosuppressed and immunosuppressed male rats were experimentally infected and serum samples from all animals were obtained at day 0 and 5th, 8th, 13th and 21th days post infection (d.p.i.). Immunoblotting was evaluated for detection of IgG anti *S. venezuelensis* in bothgroups. Larvae extract showed in the immunoblotting profile with immunoreactive fractions in immunosuppressed group from the 5th d.p.i.while the non-immunosuppressed group reactivity begins in 8thd.p.i. Parthenogenetic females and eggs extract showed an early reactivity in the immunosuppressed group compared with non-immunosuppressed group. Immunoreactiveprotein fractions of 17 kDa present in larvae alkaline extract presented as possible markers of infection in immunosuppressed animals. It is concluded that all extracts using immunoblotting have diagnostic potential in experimental strongyloidiasis especially the larval extractin cases of immunosuppression.

Financial support: CNPq, CAPES and FAPEMIG.

Keywords: rats, *S. venezuelensis*, immunoblotting, immunossupression.

DIAGNOSTIC POTENTIAL OF ANTI-rNcROP4 ANTIBODIES FOR THE DETERMINATION OF PHASE INFECTION DURING NEOSPOROSIS

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Neospora caninum is an obligate intracellular parasite which is able to infect a wide range of hosts. This parasite has emerged as an important disease of cattle and dogs. The neosporosis diagnosis is classically performed by serological tests such as IFAT, the gold standard, and ELISA. However, there are no commercial tests designed to assess the stage of infection, once the main clinical signs are reported during the acute phase or during infection reactivation. Thus, we aimed to study the potential of NcROP4 protein as a stage marker of *N. caninum* infection. We evaluated the expression and localization of NcROP4 during infection in HeLa cells to investigate the possible exposure of this protein to antibodies. Subsequently, bioinformatic analysis and phage display were developed to predict and determine B-cell epitopes of the NcROP4. Mice and cattle were experimentally infected to investigate the producing of IgG through indirect immunoenzyme assay against recombinant NcROP4 (rNcROP4) during infection. It was observed that the NcROP4 protein is secreted into the cytoplasm of HeLa cells during the invasion process, which allows its extravasation to the extracellular environment after cell lysis. Bioinformatics analysis showed that NcROP4 displays 23 regions that are potential B-cell epitopes and analysis of phage display have showed that mAb 20D2 binds to the region 360-400 amino acids of this protein. Futhermore, in mice was observed high avidity antibodies against rNcROP4 after 30 days of infection (chronic phase) as well as no difference between the IgG subclass (IgG1 and IgG2) in this recognition process. Experimentally infected cattle recognize rNcROP4 after 44 days of infection, featuring the recognizing of chronic phase and produce high avidity antibodies. Thus, we concluded that NcROP4 can be used as chronic phase marker during *N. caninum* infection, being an additional strategy in the immunological diagnosis of neosporosis.

Financial supports: CAPES, CNPq and FAPEMIG

Keywords: *Neospora*; Roptry; Immunological diagnosis

PARTICIPATION OF LEUKOTRIEN B_4 DURING EXPERIMENTAL *Toxoplasma gondii* INFECTION

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Toxoplasma gondii induces type 1 immune response in infected hosts and the disease occurs mainly in immunocompromised individuals. Leukotrien B_4 (LTB_4) is a lipid mediator synthesized by 5-lipoxygenase with proinflammatory proprieties. Once LTB_4 and 5-lipoxygenase participate in others parasite infections, such as leishmaniosis and Chagas's disease, they may have important participation during *T. gondii* infection. Additionally, elucidation of the participation of LTB_4 during toxoplasmosis could be useful for development of pharmacological treatments and vaccines. The aim of the study is to investigate the participation of 5-lipoxygenase and LTB_4 during experimental toxoplasmosis. Methods: C57BL/6 mice will be daily treated by oral route with MK886 (inhibitor of 5-lipoxygenase) or CP105696 (antagonist of LTB_4 receptor – BLT1). After one hour of the first treatment, mice will be infected with 20 cysts of ME49 strain of *T. gondii*. Clinical signs and mortality will be observed daily. At day 8 post-infection, serum sample, peripheral organs and brain will be collected for histological analysis and parasite detection. Cytokine, FoxP3 and BLT1 expression, as well as LTB_4 levels, will be analyzed in tissue samples of ileum and lung. Cytokine levels will be verified in serum and tissue samples of mice. Furthermore, Reactive Oxygen Species (ROS) production and *T. gondii* proliferation will be analyzed in bone marrow derived macrophages of treated mice. Expected results: We expected that 5-lipoxygenase inhibition or antagonism of BLT1 will reduce tissue inflammation and ROS production and will alter cytokine production. Additionally, parasite burden could be altered by treatments, indicating the important participation of 5-lipoxygenase and LTB_4 during experimental toxoplasmosis.

Key words: Toxoplasmosis, ROS, lipid mediator, LTB_4, 5-lipoxygenase

Financial support: CAPES and FAPEMIG.

CANCER AND EXOSOMES: PHENOTYPIC CHANGES INDUZED AND TUMORAL PROGRESSION INDUZED BY CANCER-DERIVED EXOSOMES

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Metastasis is a serious complication in several cancers. In advanced breast cancer, is the most common debilitating and comorbidity factor, and the leading factor of death. Is thought that metastasis is drived by epithelial-to-mesenchymal transition (EMT), a phenomenon marked by loss of expression of epithelial factors like E-cadherin, and expression of mesenqchymal factors like N-cadherin, vimentin and expression of several factors of invasiveness and migration. The exossomes are small vesicles physiologically secreted by the cells and contains several proteins, lipids and nucleci acids derived from their source cell. Cancer-derived exosomes are capable to induce several changes in normal/pre-cancerous cells, and is thought that these changes are sufficient to trigger EMT in others cells in the cancer niche and drive metastasis. We cultured MCF-7 lineage cells, a breast cancer, hromone-depedent, cell lineage, in standard conditions with calf serum lacking exosomes (ultracentrifugation pre-treatment) and isolated the MCF-7-derived exosomes. These exosomes were added in MCF-10A lineage cells, a non-trasformed breast epithelial cell lineage, culture in standard conditions, with bovine serum lacking exosomes, for 48 hours. After this, we captured photos for morphological study and extracted the RNA. We performed qRT-PCR (SYBR and TaqMan systems) and analyzed the expression patterns of genes involved in EMT, invasiveness and angiongenesis, migration and chemotaxis, growth factor receptors and stemness markers. We observed an overexpression of invasiveness markers (metalloproteinaeses 2 and 9, CD44, Tsp-1), overexpression of HER-2, overexpression of MT2A, and decrease in the expression of EMT transcription factors. These data altogether suggest us that the MCF-7-derived exosomes were capable to induce an invasive phenotype that favour the cancer niche to progress, possibly contributing to metastasis in an indirect way.

EVALUATION OF FUNCTIONAL ACTIVITY OF DIFFERENT POPULATIONS THE TROPHOBLASTIC CELLS INFECTED BY *Toxoplasma gondii.*

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Toxoplasmosis is occasioned by *Toxoplasma gondii.* Infection by *Toxoplasma gondii* during pregnancy can lead to congenital toxoplasmisis, affecting the normal fetus development. The effectiveness of maternal-fetal interactions that begins during embryonic implantation is critical for a successful pregnancy. During the early placentation, cytotrophoblast cells present in floating villi proliferate and differentiate by fusion to form multinucleated syncytiotrophoblast layer. The extravillous trophoblast cells invade the uterine wall in the deciduous region. Therefore it is of great importance to evaluate the differences of these cell lines to *T. gondii* infection. This knowledge will enable the understanding of the complex interactions operative in placental microenvironment in the presence of *T. gondii*. This study aims to evaluate the susceptibility of the three cell lines to *T. gondii* infection. Thus we will evaluate the profile of infection of three cell lines (BeWo, syncythialized BeWo and HTR-8) to infection with *T. gondii*. Afterwards the profile of cytokines and the expression of intracellular proteins MAPKs will be compared in these three cell lines. BeWo and HTR-8 cells, which are maintained under normal culture conditions will be used. BeWo cells will be used for the process of cell syncythyalization. The treatment will be done with the forskolin and PMA reagent at a concentration of 20µm and 10nM respectively. ELISA assay will be performed for detection of the cytokines profile (MIF, TGF-β1, IL-6, IL-10, IL-12, IFN-γ and TNF-α) Also, Western-Blotting will be performed for evaluation of the intracellular signaling (ERK 1/2), c-JUN, p-38). It is expected that the results will contribute to the understanding of the differences on susceptibility of these cell lines to *T. gondii* infection.

Keyword: Trophoblast, syncytiotrophoblast, cytotrophoblast, extravillous trophoblast, *Toxoplasma gondii.*

Financial Support: CAPES (Coordination of Improvement of Higher Education Personnel*).*

USE OF SYNTHETIC PEPTIDES DERIVED FROM IMMUNODOMINANT *Toxoplasma gondii* ANTIGENS FOR SERODIAGNOSIS OF HUMAN TOXOPLASMOSIS

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Serological methods usually used in the diagnosis of human toxoplasmosis are based on the reactivity of specific IgG, IgM and IgA antibodies against parasite antigens present in different antigenic preparations, mainly total antigens, which can generate many false-positive and false-negative results, and allow weak differentiation between acute and chronic phases of the infection. So, this study aimed to identify B cell epitopes within different immunodominant antigens from *Toxoplasma gondii* using software-based prediction, and evaluate the diagnostic performance of synthetic peptides representative from these predicted epitopes in the diagnosis of human toxoplasmosis. A total of 22 B cell epitopes of antigens from surface (SRS), rhoptries (ROP), micronemes (MIC) and dense granules (GRA) were identified, and 15 residues from their amino acid sequences were used to synthesize peptides chemically linked to bovine serum albumin backbone. The diagnostic performance of these synthetic peptides was evaluated in immunoassays to detect specific IgG antibodies in sera of two groups of patients; G1 (n=42) with suspected acute phase, and G2 (n=42) with chronic phase of *T. gondii* infection. All synthetic peptides were recognized by IgG antibodies from these sera, showing mean absorbances higher than cut off values, high percentages of positivity, and good differentiation from seronegative samples. The peptides derived from SRS (Pep1-Pep4) and ROP (Pep5 and Pep7) antigens showed high mean reactivity and positivity rates. Pep13 (GRA4) and Pep21 (M2AP) were significantly more recognized by sera from G1, making these epitopes potential markers of acute phase of infection. Pep12 (MIC14) was significantly more recognized by sera from G2, characterizing it as potential chronic marker of infection. In conclusion, synthetic peptides designed from B cell linear epitope prediction constitute promising antigens in serological assays to diagnose toxoplasmosis and differentiate acute from chronic phases of infection, representing an alternative to the use of native or recombinant antigens.

Keywords: Toxoplasmosis; synthetic peptides; B cell epitopes; immunodominant antigens.

Financial support: CAPES, CNPq, and FAPEMIG.

INVOLVEMENT OF TUMOR NECROSIS FACTOR ALPHA IN MODULATING HUMORAL IMMUNE RESPONSE DURING *Neospora caninum* INFECTION

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The protozoan parasite *Neospora caninum* has been associated to abortions in cattle since the early 1990's, and the infection leads to major economic impact to the segment. TNF-α is rapidly elicited during the acute phase of infections. Produced mainly by activated macrophages, its actions are required for the induction of systemic inflammation. Given the importance of this cytokine during acute infectious processes, we here aimed to verify its role in antibody production during *N. caninum* infection. C57BL/6 wild type (WT) and genetically deficient mice in TNF-α receptor I (TNF-αRI$^{-/-}$), were intraperitoneally infected with a sublethal dose ($1x10^6$) of *N. caninum* tachyzoites of the Nc-1 strain. The animals were monitored during four weeks and bled every week for serological analysis by ELISA and Western blotting. We found a role for TNF-α in the production of immunoglobulins during infection by this protozoa. We observed that, regardless of the presence of TNF-α signaling, mice showed normal production of specific IgM to soluble antigens of the parasite. However, the recognition of antigens by specific IgG, as well as its subclasses, were compromised in TNF receptor deficient mice. WT, Interferon gamma-deficient (IFN-$\gamma^{-/-}$) and TNF-αRI$^{-/-}$ mice were immunized with total antigens of *N. caninum*, receiving three doses. Specific antibody production was measured by ELISA. Serological tests showed in IFN-$\gamma^{-/-}$ mice an impairment in the production of IgG and its subclasses, while TNF-αRI$^{-/-}$ mice produced less IgG to parasitic antigens, with severely compromised IgG1 production. These results demonstrate that TNF-α signaling is required for regular B cell class switch during infection by *N. caninum*.

Keywords: *Neospora caninum*;TNF;Antibody

Financial Support: CAPES; CNPq; FAPEMIG

ASSESSMENT OF *Taenia crassiceps* ANTIGENIC FRACTIONS OBTAINED BY HYDROPHOBICITY IN THE IMMUNODIAGNOSIS OF NEUROCYSTICERCOSIS IN HUMAN CEREBROSPINAL FLUID SAMPLES

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Neurocysticercosis (NCC) is an infection caused by *Taenia solium* metacestodes that affects the central nervous system. Justification: Despite advances in neuroimaging and immunological tests, NCC diagnosis still is a challenge due to the nonspecific clinical manifestations. The aim of this study will be to obtain antigens from *Taenia crassiceps* metacestodes and to develop an alternative immunological method for NCC which may be technically and financially feasible. *T. crassiceps* metacestodes will be maintained in female BALB/c mice by intraperitoneal inoculation. It will be performed the production of the total saline extract from *T. crassiceps* metacestodes of this parasite by a standardized method. *T. crassiceps* metacestodes total saline extract will be fractionated by Triton X-114 to obtain its detergent and aqueous fractions. After characterization of total saline extract and its fractions by SDS-PAGE and immunoblotting, the fractions will be used in the IgG detection from human cerebrospinal fluid samples by ELISA, to determine the best fraction that could be applied to the NCC diagnosis. For this purpose the sensitivity, specificity and diagnostic efficiency will be calculated. Expected results: It is expected that *T. crassiceps* metacestodes antigens fractionating could offer good immunogenic fractions which potential application in the immunological diagnosis of NCC which could help the neuroimaging.

Keywords: Neurocysticercosis, heterologous antigens, fractionation, immunodiagnosis.

Support: CNPq, CAPES, FAPEMIG.

KINETIC ANALYSIS OF ANTIBODY PRODUCTION OF ANIMALS EXPERIMENTALLY INFECTED AGAINST SYNTHETIC PEPTIDES DERIVED FROM IMMUNODOMINANT MOLECULES OF *Toxoplasma gondii*

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Toxoplasma gondii is a member of the phylum Apicomplexa, which comprises a diverse group of intracellular parasites. It can infect a wide range of hosts and cause different symptoms depending on the host immune system, being especially important for the pregnant and immunocompromised humans because it can cause abortion and neural complications. In contrast, only the members of the *Felidae* family are the definitive hosts. Investigations of the biology of this parasite have provided us with new information that can be used to improve diagnostic, treatment and prevention, being the study of the parasite-host of extreme importance, even though more research is necessary to achieve better understanding of this interaction. In the present work, we used 22 syntetic peptides derived from immunodominant proteins (SAG, ROP, MIC and GRA), which were selected by in silico analysis taking into consideration the higher scores for B epitope prediction. It was determined the kinetic of antibody production in C57Bl/6 experimentally infected animals by ME-49 strain of *T. gondii*. It was analysed serum samples from five animals by an ELISA protocol to detect total IgG against *T. gondii*. The results showed that the animals produced IgG against 17 of the 22 peptides tested, being that 16 of them demonstrated higher levels of antibody production at day 49 post-infection, characterizing as good markers to chronic infection. In contrast, only 1 peptide was recognized at earlier days of infection, being considered a molecular marker for acute infection. Overall, it can be concluded that these peptides constitute useful tools for antibody kinetic determination and further studies will be necessary to develop new vaccines strategies and diagnostic tools.

Keywords: *Toxoplasma gondii*, sorology, antibody kinetic

Financial support: CAPES, CNPq and FAPEMIG

IMMUNOCHEMICAL CHARACTERIZATION AND APPLICATION IN STRONGYLOIDIASIS DIAGNOSIS OF DEGLYCOSYLATED *Strongyloides venezuelensis* ANTIGENS

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Strongyloides venezuelensis filariform larvae were treated with sodium metaperiodate (MP) to characterize immunochemically and evaluate MP-treated antigen in the diagnosis of human strongyloidiasis. Larvae were treated with MP and stained to detect carbohydrates. Saline extract (SE) treated or not with MP was evaluated by one-dimensional gel electrophoresis and used for IgG and IgG subclasses detection in serum from patients with: definitive diagnosis of strongyloidiasis (n = 50), other parasitic diseases (n = 60) and endemic normal individuals (n = 50). ROC curves were done and two-way ANOVA was applied. Sensitivity, specificity and likelihood ratio (LR) were calculated for IgG. Larvae showed differential localization of carbohydrates: in the untreated ones internal structures (esophagus and intestine) were marked, while those treated with MP cuticle was evident. After MP-treatment no carbohydrates were measured in SE. Electrophoretic profiles of polypeptides were similar between SE before and after MP treatment. ELISA sensitivity reached 90% for SE and 92.5% for MP while the specificity values were respectively 88.2% and 94.6%. Resulting LR + of 8.25 for SE and 16.9 for MP. Significant interaction between IgG subclasses and extracts was observed for patients with strongyloidiasis (P = 0.02), i.e., the reduction in detected levels, particularly IgG1 and IgG3, was due to MP treatment. Chemical deglycosylation of larval antigen decreased mean IgG in patients with strongyloidiasis and also overcome cross-reactivity in the control groups, demonstrating the role of carbohydrate residues in the recognition of anti-*Strongyloides* IgG and its subclasses.

Keywords: deglycosylation, strongyloidiasis, *S. venezuelensis*, sodium metaperiodate, diagnosis.

Financial Support: CAPES; CNPq; FAPEMIG.

MOLECULAR EPIDEMIOLOGICAL SURVEY IN QUINOLONE AND CARBAPENEM-RESISTANT GENOTYPE AND ITS ASSOCIATION WITH TYPE III SECRETION SYSTEM IN *Pseudomonas aeruginosa*

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This study evaluated the predictors of mortality and the impact of inappropriate therapy on patients outcomes with bacteremia and ventilator-associated pneumonia (VAP). Additionally, itwas evaluated the correlation among the Type Three Secretion System (TTSS) effector genotype with the resistance to carbapenems and fluoroquinolones, mutations in the Quinolone Resistance Determining Regions (QRDRs), Metallo-Beta-Lactamase (MBL) and virulence factors. A retrospective cohort was conducted at a tertiary hospital in patients with multidrug-resistant (MDR) *P. aeruginosa* bacteremia (157 patients) and VAP (60 patients). Genesbla_{IMP}, bla_{VIM}, bla_{SIM}, bla_{GIM} and bla_{SPM} and virulence genes (*exoT*, *exoS*, *exoY*, *exoU*, *lasB*, *algD*, *toxA)* were detected; the sequencing was conducted for QRDR genes on fluoroquinolone-resistant strains. The multivariate analyses showed that predictors independently associated with death in patients with bacteremia were cancer and inappropriate therapy. Carbapenem resistance was more frequent among strains of VAP (53.3) and we observed in blood 66.6% bla_{SPM} genotype and 33.3% bla_{VIM} genotype. *exoS* gene was found in all isolates, while for the *exoU*, the frequency was low (9.4%). Substitution of threonine to isoleucine at position 83 in *gyrA* was the most frequent mutation among fluoroquinolone-resistant strains. Our study showed a mutation at position 91 in *parC* gene (Glu91Lys) associated with mutation in *gyrA* (Thre83Ile) in a strain of extensively drug-resistant *P. aeruginosa*, $exoT^{+}exoS^{+}exoU^{+}$ genotype, not yet described in Brazil. This comprehensive analyze of resistance mechanisms to carbapenem and fluoroquinolones and their association with TTSS virulence genes, covering MDR *P. aeruginosa* in Brazil is the largest reported, up to now.

Keywords: *Pseudomonas aeruginosa*, Carbapenem Resistance, Fluoroquinolone Resistance, Type Three Secretion System, Metallo-beta-Lactamase

ALLELIC LOSS IN AMELOBLASTOMAS AND AMELOBLASTIC CARCINOMAS

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The aim of the present study is to comparatively evaluate frequency of allelic loss (FAL) in 3p, 9p and 17p chromosomal regions in ameloblastic carcinomas (CA) and ameloblastomas (AM). FAL was evaluated in 14 CA and 13 AM by using capillary electrophoresis. The FAL for AM and CA were 26.0% and 50.4%, respectively. Mean FAL in chromosome 3, 9 and 17 were 21.4%, 29.2 and 20.0% for AM, and 46.4%, 59.0% and 66.8% for CA. Two AM (23.0%) showed allelic loss in > 50% of the loci; similar loss rate was observed in 57.1% of the loci for CA. Loci with higher FAL in AM were D3S1234 (40.0%); D9S1751 (55/6%) and TP53 (40%); for CA, higher FAL (>50,0%) were observed in D3S1029, D3S1293, D9S157, D9S162, D9S1751, D9S171, TP53 and AFM238F2. Allelic loss was absent in AFM238F2 locus for AM. Higher FAL in CA than AM point allelic loss as an important phenomenon in CA pathogenesis and biological behavior.

Keywords: Ameloblastic carcinoma; ameloblastoma; allelic loss; loss of heterozigosity; prognosis.

Financial support: FAPEMIG and CNPq

EVALUATION OF COINFECTION BY *Strongyloides venezuelensis* AND *Syphacia muris* IN WISTAR RATS (*Rattus norvegicus*)

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Syphacia muris is a nematode with a simple and direct life cycle that infects albino rats (*Rattus norvegicus*). *Strongyloides venezuelensis* is the species used to elucidate the parasite/host relationship in rodents infected with helminths, and also as an alternative for obtaining heterologous antigen for diagnosis of human strongyloidiasis. The present study aimed to evaluate coinfection parameters and cross-reactivity by ELISA and Immunoblotting in coinfected Wistar rats (*R. norvegicus*) naturally infected with *S. muris* and experimentally infected with *S. venezuelensis*. Forty rats were used in the study. The Feces were collected daily for quantification of eggs per gram of feces and after 21 days the rats were sacrificed by anesthetic overdose, and the intestines were collected from each animal for quantification of helminths. In ELISA and Immunoblotting, the serum samples obtained 21 days after helminth infection howed high cross–reactivity with 100% of samples obtained from rats infected with *S. muris* reacted to the total saline extract of *S. venezuelensis* and 100% of samples obtained from rats infected with *S. venezuelensis* reacted to the saline extract of *S. muris*. For *S. venezuelensis* saline extract, the predominant bands of cross-reactivity were 36, 68, 76, 83, and 102 kDa, whereas in the saline extract of *S. muris* the predominant bands of cross-reactivity were 36, 91, and 149 kDa. *Syphacia muris* coinfection significantly decreased egg kinetics, as well as the quantity of parthenogenetic female *S. venezuelensis* recovered in the small intestine. We conclude that antigenic cross-reactivity occurs in serum samples from rats naturally infected with *S. muris* and experimentally infected with *S. venezuelensis*, as determined by ELISA and Immunoblotting. Periodic monitoring of these animals is thus fundamental for evaluation, since *S. muris* infection may interfere with and compromise experimental results of studies conducted with *S. venezuelensis.*

Keywords: *Strongyloides venezuelensis*; *Syphacia muris*; Cross–reactivity; ELISA; Immunoblotting.

Financial Support: CAPES and FAPEMIG.

MIXED FORMULATIONS CONTAINING LIPOSOMAL MILTEFOSINE AND AMPHOTERICIN B FOR TREATMENT OF LEISHMANIASIS

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Leishmaniasis is a group of neglected diseases caused by the protozoa *Leishmania*, which are endemic worldwide. Main clinical manifestations are cutaneous leishmaniasis, mucosal leishmaniasis and the visceral leishmaniasis, which is potentially lethal if untreated. The treatment of infected patients is remains the first line control measure of leishmaniasis, and the drugs used to treatment are pentavalent antimonials, amphotericin B, miltefosine or pentamidine. Although currently used these drugs have limitations, such as collateral effects and reduced efficacy in some cases. Recently, therapeutic alternatives have been proposed to overcome these limitations: encapsulation of drugs in liposomes, targeting the sites of infection, using lower doses and better efficacy and less side effects than the "free" drug; combination of drugs to obtain synergistic effect and improving its leishmanicidal effects. The aim of this study is to evaluate the efficacy *in vivo* and *in vitro* of a mixed liposomal formulation containing amphotericin B and miltefosine for the treatment of tegumentar and visceral leishmaniasis. Stealth(PEGlytaded) liposomes containing miltefosine and conventional liposomes containing amphotericin B will prepared and characterized. Initially, mixed formulations will tested against murine and macrophages (J774A.1), *Leishmania amazonensis* (IFLA/BR/1967/199) and *L. infantum* (MHOM/BR/1967/BH46) promastigotes in order to obtain the therapeutic index (TI), using the resazurin-based colorimetric assays. Following the TI determination, therapeutic efficacy *in vitro* will be determined against intracellular amastigotes of the two speciesin macrophages. Based on these results a therapeutical protocol will be designed and used in murine models of tegumentar and visceral leishmaniasis. Main expected results are the optimization and characterization of the formulations and that the mixed formulation presents better efficacy both *in vitro* and *in vivo* than the free drugs. Also we expect that results can provide subsidies for proposing efficacy preclinical tests in other expected models, such as dogs, non-human primates, and finally human beings.

Keyword: Leishmaniasis, treatment, amphotericin B, miltefosine e liposomes.

Financial Support: CNPq, CAPES e FAPEMIG.

CONTAMINATION BY Toxocara sp IN SOIL FROM DIFFERENT LOCATIONS IN UBERLÂNDIA, MINAS GERAIS

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Toxocariasis is a common parasitic disease in animals and neglected helmintozoonose. Street dogs and cats infected are the main transmitters and soil contaminants by this parasite. This study aimed to determine the prevalence of Toxocara sp. by conventionalmethods, in soil from different areas located in Uberlândia - MG. The sample onllection was performed in squares, parks, clubs, gardens and municipal preschools (EMEIs). Sand, soil and grass of different areas were collected from January to March 2014. At each site, samples were collected on five diferents sides forming homogenized "pool". The samples were placed in labeled plastic bags and transported to the Parasitology Laboratory of the Federal University of Uberlândia (UFU) to be processed. Microscopic examination for observation of Toxocara sp. was perfomed using theformalin-ether and sedimentation techniques. P values <0.05 was considered statistically significant. The 93 samples collected belonged to five parks, eight clubs, 38 squares, 12 gardens and 30 EMEIs. Of the total, 19 (25.8%) were positive for Toxocara: (19) 59.26% were detected from the formalin-ether technique and (11) 40.74% by sedimentation. The lower positivity was demonstrated in samples collected in the northern region of the city. There was moderate agreement between the methods, from the application of the kappa statistic (p=0,002). It is concluded that the soils of Uberlândia are considerably contaminated with Toxocara sp., contributing to the spread of disease among animals and humans.

Keywords: Toxocara; toxocariasis; epidemiology; helmintozoonose.

Financial Support: CAPES

EFFECT OF *Trichoderma stromaticum* EXTRACT IN THE TACHYZOITES OF *Toxoplasma gondii* PROLIFERATION *IN VITRO* AND *IN VIVO*

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Trichoderma is well known by inhibit the growth and development of a variety of plant pathogens. Previous studies demonstrated that the fungal spore from *T. stromaticum* downmodulated the response of murine phagocytes by decreasing the production of nitric oxide (NO) and reactive oxygen species (ROS). *Toxoplasma gondii* is an obligate intracellular parasite that induces a strong Th1 response which activates the production of IFN-γ which in turn activates several innate immune mechanisms in *T. gondii* infection, such as NO production. In order to investigate the effect of *T. stromaticum* extract (ExtTs) in the control of RH strain of *T. gondii* in treated cells (J774 mcarophages) or directly in the parasite viability, we treated cells before or after infection; or treated parasites with ExtTs and infected cells or mice and observed parasitism. It was observed that the ExtTs do not interfered in cytokine productin in infected cells, despite decreasing the NO production and autophagic vesicles detection. However, the treatment of parasites with ExtTs decreased the parasitism in the cells and mice. Thus, these results showed that the ExtTs is a good candidate to control *T. gondii* proliferation.

Key Words: ExtTs, J774 macrophage, *Toxoplasma gondii*, *Trichoderma stromaticum*

IMMUNOMODULATORY EFFECT OF *Synadenium carinatum* (ScLL) AND *Artocarpus heterophylus* (ArtinM) LECTINS IN TOXOPLASMOSIS TREATMENT

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Advances in glycobiology and immunology enabled greater understanding between lectins and the immune system interaction. Lectins are carbohydrate-binding proteins that interact with immune system cells. Therefore lectins play an important role in the activation of immune response. ScLL and ArtinM are lectins present in plants, the first present in Synadenium Carinatum latex and the second in Artocorpus heterophylus seed. Much is know about potential of these lectins in pathological processes such as parasitic infections and cancer. However there are no reports of these lectins in toxoplasmosis treatment. Toxoplasmosis is a zoonosis caused by the protozoan Toxoplasma gondii. High infection rates by this parasite put the disease as serious public health problem. So, this study aims to determine the immunomodulatory role of ScLL and ArtinM lectins in toxoplasmosis treatment. ScLL was obtained from crude extract by affinity chromatography using immobilezed D-galactose column on agarose. ArtinM was gently provided by Maria Cristina Roque Barreira USP / Ribeirão Preto. Cytotoxicity assays were performed using murine primary macrophages. Proinflammatory and anti-inflammatory cytokines and NO production were assessed in macophages stimulated by lectins. Based on cytotoxicity experiments optimal lectins dose was established for in vitro stimulation, 1,8 µg/ml for ScLL and 1 µg/ml for ArtinM. Analysis of immunomodulatory ability of these lectins show that ScLL induces pro-inflammatory cytokines, such IL-12, while ArtinM has greater potential to induce anti-inflammatory profile, by IL-10 induction. Further, ScLL was able to increase macrophages NO levels. Next steps include in vivo experiments C57BL/6 mice infected with T. gondii, strain ME-49. The animals will be allocated in control group and treated with sulfadiazine and ScLL and ArtinM lectins, separately and/or together. Hopefully we will assess the lectins potential in toxoplasmosis treatment.

Key words: *Toxoplasma gondii*. Lectins.ScLL. ArtinM.

Financial Support: CAPES, FAPEMIG and CNPq.

COMPARISON OF PARASITOLOGICAL, IMMUNOLOGICAL AND MOLECULAR METHODS IN FECAL SAMPLES OF IMMUNOSSUPPRESSED RATS EXPERIMENTALLY INFECTED WITH *Strongyloides venezuelensis*

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Definitive diagnosis of strongyloidiasis is typically achieved by detection of parasitic larvae in fecal samples. However the number of parasites can be small and as such the elimination of larvae is often irregular. Improvement of diagnostic methods is thus a necessary goal for achieving greater sensitivity and success in diagnosis. The objective of this study was to compare the diagnostic value of several methods including egg detection in feces by the Egg Counts per Gram of Feces (EPG) technique the detection of coproantigens by enzyme linked immunosorbent assay (ELISA) and DNA detection by conventional Polymerase Chain Reaction (PCR) in feces of both non-immunosuppressed and immunosuppressed rats infected with *S. venezuelensis* at 5, 8, 13, 21 and 39 days post-infection (d.p.i.). Results from statistical analysis of egg counts are expressed as mean ±SEM and statistical variations were analyzed using ANOVA followed by the Bonferroni test. The criterion for statistical significance was set at $p<0.05$. For non-immunosuppressed rats the EPG test detected eggs in the feces from days 5 to 13 d.p.i. ELISA detection of coproantigens occurred on days 5, 8, 13, 21 and 39 d.p.i.; PCR amplification was verified by the presence of bands which were visible on the same days. For immunosuppressed rats eggs were detected by EPG on days 5, 8, 13 and 21 d.p.i. Coproantigen positivity was observed on days 5:39 d.p.i. while PCR bands were observed daily for the duration of infection. We conclude that ELISA detection of coproantigens and PCR are more sensitive alternatives to the EPG method for precise and definitive diagnosis of strongyloidiasis.

Keywords: *Strongyloides venezuelensis*, fecal antigen, PCR, Immunosuppression, Diagnosis

Financial support: CAPES, FAPEMIG and CNPq.

STUDY OF THE MESENQUYMAL CELLS PARACRINE EFFECTS ON THE MCF-7 CELLS

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Breast câncer has varied etiology influenced by genetic and environmental factors. The conversion of primary tumors in invasive malignantis associated with activation of epithelial-mesenchymal transition (EMT). The acquisition properties of mesenchymal by EMT can promote the separation of cancer cells from the primary tumor and to facilitate their subsequent migration, thus allowing the emergence of metastases. This study aims to analyze the potential for malignant transformation of mesenchymal cells in vitro, by performing co-culture Transwell system with human mammary carcinoma cells (MCF-7). The stimulation of mesenchymal cells (MSCs) by tumor cells (MCF-7) can establish a tumor phenotype, but this research also in tends to verify that the MCF-7 can acquire mesenchymal phenotype. For this purpose, MCF-7 will be co-cultured with MSC in a Transwell system. The DNA damage and proliferative index will be assayed by flow cytometry. The mRNA expression of E-Cadherin, N-Cadherin, CD44 and Vimentin will be performed by real time PCR in MCF-7 and MSC. This experiments aim demontrate the paracrine effects of MCF-7 in MSC and vice-versa trying to understand the complexicity of the tumor microenviroment.

Keywords: breast cancer, epithelial-mesenchymal tansition, Transwell system, cancerization model, mesenchymal cells

Apoio Financeiro: CNPQ and FAPEMIG

THE GENETIC BACKGROUND AND MHC HAPLOTYPE OF BALB/c MICE AFFECTS POSITIVELY THE OUTCOME OF PREGNANCY IN CONGENITAL TOXOPLASMOSIS

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Successful pregnancy is related to Th2 immune response profile; however, *Toxoplasma gondii* infection induces Th1 immune response that is associated with adverse pregnancy outcome. To investigate the influence of MHC haplotype in pregnancy outcome in *T. gondii*-infected animals, congenic mice, C57BL/KsJ and CB10-H2 females, were orally infected with ME-49 strain on the first day of pregnancy (dop) and sacrificed on 8 and 18 days post-infection (dpi). The uterus/placenta were evaluated for foetal resorption rate, parasite load, immunological changes and mast cells migration. C57BL/KsJ showed higher number of parasites in the lungs on 8dop/8dpi compared with CB10-H2; and CB10-H2 showed higher number of parasites in the brain on 18dop/18dpi compared with C57BL/KsJ. Parasites were not found in the uterus/placenta in both lineages of mice irrespective of the day of infection and there was no difference in abortion rate of infected mice, although C57BL/KsJ females had higheraverage of normalimplantationsitescomparedwithCB10-H2 on8dop/8dpi. Infection of non-pregnant C57BL/KsJ increased mast cell infiltration in the uterus, and gestation decreased this cell numbers. Furthermore, C57BL/KsJ presented higher IFN-γ levels systemically on 8 dpi; and TNF and IL-6 on 8 and 18 dpi compared with CB10-H2, despite gestation or not. Additionally, in the uterus/placenta of CB10-H2, pregnancy increased foxp3 and under infection IL-10, IL-13 and IL-17 expression levels. Previous results showed that BALB/c presented better pregnancy outcome in *T. gondii* infection compared to C57BL/6, our data suggest that both genetic background and MHC haplotypes are essential to protect against reabsorption rate and abortion in congenital toxoplasmosis. CB10-H2, which present the same genetic background of BALB/c, however the same MHC haplotype of C57BL/6 presented poor pregnancy outcome under *T. gondii* infection; and C57BL/KsJ, that present the same genetic background of C57BL/6 but the same MHC haplotype of BALB/c, also presented poor pregnancy outcome.

Key words: Congenital toxoplasmosis, Abortion, Congenic mice, Mast cells, MHC haplotype.

Financial support: CAPES AND FAPEMIG.

INSECTS OF MEDICO-LEGAL FORENSIC INTEREST IN TWO ENVIRONMENTS OF A RURAL AREA OF UBERLÂNDIA-MG.

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Studies of Forensic Entomology have increased in Brazilan contributed to the knowledge of the diversity and biology of insects colonizing carcasses and human corpses in decaying process. These information associated with the environmental factors of each region, can help in determining the post-mortem interval (PMI) and may be important for forensic medicine. However,Brazil presents continental dimensions and its carrion fauna are poorly known in the majority of regions and biomes, including the Cerrado, a biodiversity hotspot. The present research examined the carrion fauna of two environment sand seasons in a rural area of Uberlândia-MG, situated in a region of Cerrado in southeastern Brazil. Samples were collected in a pasture and in a fragment of semideciduous forest, during the dry and humid seasons of 2010. The study emphasizes the diversity, relative abundance and entomological succession of species of adult and immature insects along the decomposition of eight pig carcasses (*Susscrofa*) (10±2 kg) exposed in each environment and season. A total of 92,489 adult insects were collected in traps, belonging at least 41 families and 189 species. The larval forms resulted in emergence of 32,577 insects, belonging to 8 families and 19 species. Among the insects attracted, Diptera was the most abundant order (92.22%), followed by Coleoptera (4.40%), Hemiptera (1.92%), Lepidoptera (0.91%) and Hymenoptera (0.55%). Diptera and Coleoptera were the main groups considered of forensic importance by the large number of species that used the carcasses as breeding substrate. Several of these species are considered potential indicators of PMI, such as: *Chrysomyaalbiceps*, *Chrysomyaputoria*, *Hemilucilia segmentaria*, *Ophyraaenescens*, *Peckia* (*Pattonella*) *intermutans*, *Hermetiailluscens*, *Dermestesmaculates* and *Necrobiarufipes*. The pig carcasses attracted a wide variety of insects and several species were considered potential indicators of environment and seasonality.

Keywords: Forensic Entomology, Calliphoridae, Sarcophagidae, Muscidae, Cerrado.

DETERMINATION OF KAPLAN-MEIER SURVIVAL CURVES IN BALB/C MICE INFECTED BY TWO NEW *Toxoplasma gondii* ISOLATES FROM FREE RANGE CHICKENS IN UBERLÂNDIA, MG, BRAZIL.

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Two new isolates of *Toxoplasma gondii,* named UDI-3 and UDI-4, were obtained from free range chickens (*Gallus domesticus)* in Uberlândia-MG using a mouse bioassay. The virulence of both isolates was tested against mice in order to observe the mortality of these animals during the period of 30 days. Groups of BALB/c (n = 5) mice were inoculated with 10, 10^2, 10^3, 10^4 and 10^5 tachyzoites (intraperitoneally in a volume of 200 uL per animal) of *T. gondii* isolates from chicken number 162 (UDI-3) and 193 (UDI-4). Another group of mice BALB/c (n = 5) was also inoculated with tachyzoites of a highly virulent strain, RH type I strain, in the same conditions described for the groups inoculated with both new isolates. The susceptibility of these mice to infection for tachyzoites from UDI-3 isolate varies depending on the amount of parasite inoculation, as at 12 post-infection all mice that received 10^5 parasites died, while those animals receiving doses of 10^4 and 10^3, died at 23^{th} and 26^{th}, respectively. In contrast, for all groups of mice inoculated with tachyzoites of isolated UDI-4 no deaths were observed during the 30 days, in comparison with the groups of mice that were inoculated with the RH strain, as 100% of mortality rates occurred between 6 to 8 days post-infection in the all groups mortality, except for those animals receiving 10 parasites. In conclusion, both *T. gondii* isolates showed significant differences in terms of the virulence degree for mouse model, suggesting a phenotypic variation among isolates and RH strain. Also, additional pieces of information will be necessary to associate genomic characterization of *T. gondii* isolates with its virulence degree from free-range chickens, as useful tools to determine their population structure, evolution, and transmission.

Key Words: *Toxoplasma gondii*, survival curve, isolates, Free range chickens.

Financial support: CAPES, FAPEMIG and CNPq.

ASSESSMENT OF IMMUNODOMINANT PROTEIN AND IMMUNE RESPONSE OF ISOLATED FROM DIFFERENT *Neospora caninum* IN MURINE MODEL

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Neospora caninum is a protozoan parasite that infects many domestic and wild animals. Notably, this parasite causes clinical diseases in dogs and cattle, due to neuromuscular disorders and abortions, respectively. These abortions cause relevant economic losses and may be related to genomic differences amongst different isolates. The present study aimed to comparatively evaluate the immune response and pathological alterations induced by two different isolates of *N. caninum*, in a murine model. The isolates used in this study were Nc-1 and Nc-Liv. C57BL/6 mice were infected intraperitoneally and checked for morbidity scores, based on weight loss and temperature variations. Additionally, comparative production of nitric oxide (NO) and specific IgG antibodies were also determined, along with the parasite load, during distinct phases of the infection. We observed a clear distinction in weight gain in the Nc-Liv infected group during the first week of infection, which was concomitant with increased parasitism. However, NO levels were similar between both groups. We also observed differential recognition of heterologous antigens in the serum of chronically infected mice with the different strains, by Indirect ELISA and Western Blotting. In that sense, we conclude that there are significant differences in antigenicity between both of *N. caninum* isolates and, consequently, the induced immune response elicited.

Financial supports: CAPES, CNPq and FAPEMIG

Key words: *Neospora caninum*, virulence factors, isolates, immune response

INTERACTION BETWEEN BmooMP-alpha-I, A METALLOPROTEASE ISOLATED FROM *Bothrops moojeni*, AND TNF-ALPHA CYTOKINE INTERFERES IN THE INFLAMMATORY RESPONSE.

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BmooMP-alpha-I is a metalloprotease fibrin(ogen)olytic with 24.5 kDa isolated from *Bothrops moojeni* snake venom. Considering that this metaloprotease does not present phospholipase A_2, haemorrhagic and thrombin-like activities, several studies are focusing on its fibrin(ogen)olytic activity. In the present study, it was investigated the interaction of BmooMP-alpha-I metalloprotease and Tumor Necrosis Factor (TNF-alpha), because this cytokine is able to convert to its active form by a metalloprotease denominate as TNF-alpha converting enzyme (TACE). To determine the similarity between metalloprotease and cytokine, we first proceed to an alignment approach using ClustalW, followed by a dock approach using the program Cluspro to verify protein interaction. Additionally, it was chose the top ten structures resulted from clustering, which was aligned using a multiple (protein) structural alignment algorithm (MUSTANG). The best structural alignment was further analysed by Swiss Pdb Viewer. Also, the interaction was verified *in vitro*. BmooMP-alpha-I and recombinant murine TNF-alpha were incubated to for 45' at 37ºC and after this ELISA, SDS-PAGE and Western Blot were performed. It was obtained a score of 68 in ClustalW alignment. Moreover, the model 2 was the best structure according to Mustang alignment and Fit alignment in Swiss Pdb Viewer. Moreover, it was observed an interaction between A: GLN31 - A: VAL171, A: ARG43 - C: GLU127 and A: ARG32: HE - A: GLU33. Also, in ELISA test the levels of TNF-α were significantly reduced in the presence of the enzyme BmooMPα-I suggesting that it exerts a proteolytic action on TNF-α, which was confirmed by the disappearance of this protein cytokine profile bands in SDS-PAGE and Western Blot. Overall, it is possible to conclude that BmooMP-alpha-I effectively interacts with TNF-alpha, as it has similar structure with TACE, indicating that this metalloprotease is a useful tool to be evaluated in treatment protocols against inflammatory disorders.

Keywords: BmooMP-alpha-I, Metalloprotease, TNF-alpha, TACE.

Financial support: CAPES, CNPq, FAPEMIG and INCT.

IN VITRO EFFICACY OF AN INNOVATIVE STEALTH LIPOSOMAL FORMULATION CONTAINING MILTEFOSINE FOR THE TREATMENT OF VISCERAL LEISHMANIASIS

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Visceral leishmaniasis (VL) is a disease which represents serious public health problem. The current treatment of VL presents many side effects and is poorly effective. Studies have suggested new approach based on the leishmanicidal drugs in prolonged blood circulation liposomal formulations (stealth liposomes) in order to increase its therapeutic efficacy and diminish side effects. The aim of this study was evaluate the efficacy *in vitro* of an innovative stealth liposomal formulation containing miltefosine for the treatment of VL. In order to obtain stealth liposomes containing miltefosine (HePC-PEG), DSPE-PEG 2000 polymer was added in the composition of the vesicle membrane and liposomes were prepared using "Dehydration Rehydration Vesicle" method. Then the mean of hydrodynamic diameter, zeta potential (Z) and polydispersity index (PI) of vesicles were determined by photon correlation spectroscopy. The cytotoxicity assay (CC_{50}) was performed on murine macrophage using the MTT colorimetric method. Then, the efficacy of HePC-PEG against intracellular *Leishmania infantum* amastigotes was performed in a 72h kinetic assay. The mean of diameter of the HePC-PEG vesicles was 196.8nm, and formulation was monodispersed (PI = 0.125) and presented excellent stability (z = -63mV). The CC_{50} of HEPC-PEG was 226μM, being 2.20 times greater than the commercial miltefosine (103μM). The intracellular amastigotes test showed that HEPC-PEG formulation (10 mM) presented better efficacy than commercial miltefosine, but not significant, on reducing infection rates of macrophages, as follows: 24h - 20% for HePC-PEG and 22.7% for miltefosine; 48h - 3.8% for HePC-PEG and 17.7% for miltefosine; and 72h - 3.8% for HePC-PEG and 5.7% for miltefosine. However, both drugs presented statistical significant reduction on infection rates ($p<0.05$) when compared to non treated controls. In conclusion, the stealth liposomal formulation of miltefosine presented lower toxicity than the commercial drug and a significant efficacy on reducing infection rates of *Leishmania infantumin vitro*.

Keywords: miltefosine, stealth liposomes, treatment, visceral leishmaniasis
Financial Support: CNPq, CAPES, FAPEMIG

STRUCTURAL CHARACTERIZATION OF THE BINDING BETWEEN HEAT SHOCK PROTEIN (HSP60) OF *Strongyloiodes* sp. AND SINGLE-CHAIN VARIABLE FRAGMENT (scFv) SELECTED AGAINST *Strongyloides venezuelensis* TOTAL PROTEINS BY PHAGE DISPLAY

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Human strongyloidiasis is an intestinal helminthiasis of neglected condition mainly due to the low sensitivity of the parasitological diagnosis. The aim of this study was to select single-chain variable fragment (scFv) against *Strongyloides venezuelensis* total proteins by Phage Display and to perform the structural characterization of the binding between the selected scFv and its ligand by bioinformatic. Two cicles of selection were performed and after confirmation of clones reactivity to *S. venezuelensis* total proteins by ELISA, it was carried out the sequencing reaction to obtain the scFv sequence and the mass spectrometry, after pull-down assay and 15% SDS-PAGE, to obtain the amino acids sequence of ligand. Amino acids sequences were submitted to the bioinformatics tools raptor-x and PyMOL to obtain and analyse the 3D structure from both molecules. Later it was performed a docking for the structural characterization of the binding between the scFv and its ligand. After two cicles of selection4 out of 96 clones were expressed and reactive to *S. venezuelensis* total proteins and sequencing analyses showed that these clones were identical which it could be confirmed by the characteristic structure of an scFv molecule. Mass spectrometry showed that the scFv bound to the heat shock protein 60 (HSP60) of *Strongyloides* sp. The 3D structure analyze of scFv-HSP60 demonstrated that only the amino acids from CDR2 variable region did not bind to the HSP60 and an analyze of binding site showed that 25% from all interactions were made by the amino acid serin, which it could indicates high affinity to the target. In this study it was selected and obtained an specific antibody fragment by Phage Display with efficiency. This monoclonal fragment may be applied in the development of novel serodiagnosis method that enable a sensitive and specific detection of the infection by the parasite.

Keywords: *Strongyloides*, *Phage Display*, scFv, bioinformatic.

Financial support: CNPq and FAPEMIG

MOLECULAR IDENTIFICATION OF *Cryptosporidium* spp. AND G*iardia* spp. IN BRAZILIAN WILD BIRDS

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Parasitic infections are among the commonest health problems that affect birds. The identification of *Cryptosporidium* spp. and *Giardia* spp. in wild birds is relevant, since these animals can act as disseminators of these parasites to humans through environmental contamination. The aim of this study was to determine the molecular occurrence of *Cryptosporidium* spp.and *Giardia* spp. in wild birds in southeastern Brazil and genetically characterize the isolates obtained. A total of 256 fecal samples were collected from 172 captive and 84 free-living wild birds. The DNA extracted was subjected to nested-PCR and semi-nested PCR analysis for amplification of fragments of the 18S rDNA and *gdh* genes of *Cryptosporidium* spp. and *Giardia* spp., respectively. Results showed that out of 256 samples collected, 10 (3.9%) were positive for *Cryptosporidium* spp., and 8 (3.1%) for *Giardia*spp.. RFLP and sequencing analysis have identified *C. meleagridis* in two Muscovy duck (*Cairina moschata*), in two *Aratinga leucophthalma* (White-eyed parakeet), and in two *Athene cunicularia* (Burrowing Owl). They have also found *C. baileyi* in three *Amazona aestiva* (Blue-fronted Parrot) and in one *Amazona amazonica* (Orange-winged Parrot). Only three positive samples for *Giardia* spp. – classified as *Giardia duodenalis* assemblage B – were sequenced, two of them found in *Rupornis magnirostris* (Roadside Hawk), and one in *Theristicus caudatus* (Buff-necked Ibis). The presence of *C. meleagridis* and *G. duodenalis* assemblage B suggests that epidemiological studies involving wild birds and humans are needed to better understand the impact of avian cryptosporidiosis and giardiasis on avian health and their possible implications for public health.

Key words: *Cryptosporidium* spp.; *Giardia* spp.; wild birds; 18S rDNA; *gdh*

Financial support: FAPEMIG

THE ME-49 AND RH *Toxoplasma gondii* STRAINS INDUCE LIPID BODIES FORMATION IN THE MURINE MACROPHAGE LINEAGE

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Lipid droplets (LDs) are active organelles linked to biological functions, in addition to being important sites for lipid mediators storage. Adipocyte differentiation-related protein (ADRP) is known to be a lipid droplet-associating protein, and was originally found in the early stages of adipocyte differentiation and is expressed in a variety of tissues and cells. This work aimed to investigate the relationship of LDs formation and *Toxoplasma gondii*, type I and type II infection of hematopoietic (J774.1) cells. For this purpose, J774.1 macrophages were infected with RH or ME-49 *T. gondii* strains and LDs formation was analyzed by oil red staining and ADRP expression by immunocitochemistry. It was observed that macrophages presented LDs formation constitutively, and IFN-γ stimulus increased LDs formation in this cell line. Infection with *T. gondii* increased the LDs formation in cell line compared with uninfected cell and ME-49 strain was able to induce higher LDs formation compared with RH strain. Lipid droplets inhibition by aspirin (ASA) increased parasitism in macrophage (J774.1) compared with untreated macrophages. Additionally, cells infected with ME-49 strain presented higher levels of ADRP expression compared with cells infected with RH strain of *T. gondii* or treated with IFN- γ. The results obtained here suggest that the *T. gondii* infection induces LDs formation that seems to be involved in the control of parasite proliferation.

Key words: Hematopoietic cells; Lipid droplets; *Toxoplasma gondii*.

Financial Support: CAPES and FAPEMIG

MHCII KNOCKOUT IMPAIRS *Leishmania amazonensis* EXPERIMENTAL PATENT INFECTION

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L. amazonensis are obligate intracellular protozoan, engulfed by host immune cells and multiply in parasitophorous vacuole (PV). The PV biogenesis involves acquisition of markers as lamps, RabGTPases, cathepsin, proton ATPases, and MHC class II. In these PVs MHC II molecules are located in the same binding site of *L. amazonensis* amastigotes. In this context, we aimed to verify whether the MHC II molecule is important in the VP biogenesis and its absence would affect the course of infection. We performed *in vivo* assays in which we infect Paws of MHC II (*mhcii*$^{-/-}$) knockouts and wild type (WT) C57BL / 6, and after 6 weeks, we collected spleen, popliteal lymph node and paw for histopathological analysis, cytokine profile and parasitic load. We made a multiplication assay with peritoneal macrophages of mice *mhcii*$^{-/-}$ and WT infecting them via intraperitoneal, with amastigotes of *L. amazonensis*. The results of the histological analysis showed that infected animals *mhcii*$^{-/-}$ showed no significant difference compared to WT. Regarding cytokine production, the results showed increased production of IL -4 and IL -12 in *mhcii*$^{-/-}$ and more significant increase in IFN-γ in WT, even with a reduction of TNF- α in two groups analyzed. In the proliferation assay with peritoneal macrophages, it was observed decreased in the parasitophorous vacuole area and lower parasite load in *mhcii*$^{-/-}$ animals compared to WT mice. Therefore , the process of colocalization of MHC II molecules on the membrane of the VP seems to be not only a mechanism of subversion of the parasite , whereas in the absence of this molecule was highly significant decrease in the VP area and lower parasite load , featuring less progression of disease compared to WT mice. Thus, we believe that the MHC II molecule may also play a role in the biogenesis of the VP.

Key Words: *Leishmania amazonensis*; MHCII; parasitophorous vacuole; megassomes; leishmaniasis

Financial Suport: FAPEMIG; CAPES; CNPq.

ROLE OF DECTIN-1 IN HOST DEFENSE AGAINST *Neospora caninum* AND RELATED APICOMPLEXA

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Apicomplexan parasites as *Neospora caninum, Plasmodium berghei* and *Toxoplasma gondii* have been associated with disease in humans and animals. Dectin-1 is a pattern recognition receptor (PRR) which recognize 1, 3 and 1,6 beta-glucans and it is related with immunity against fungal species. This work aimed to evaluate the role of the C-type lectin receptor Dectin-1 during the infection by *N. caninum* and correlated Apicomplexa. Groups of C57BL/6 mice (WT), pretreated or not with Laminarin (LAM; competitive inhibitor of Dectin-1), and mice genetically deficient in Dectin-1 (Dectin-1$^{-/-}$) were infected with *N. caninum*, *P. berghei* and *T. gondii*, for determination of survival rates, acute and chronic parasitism, tissue inflammation, cytokine and reactive oxygen species (ROS) production. Our results showed that Dectin-1$^{-/-}$ mice are more resistant to *N. caninum* infection, since these mice present increased survival, associated with reduced acute and chronic parasitism. Additionally, Dectin-1$^{-/-}$ mice present high production of IL-12p40 by peritoneal and Spleen cells. Similar results were found when WT mice were treated with Laminarin and challenged with *N. caninum*. Treated animals exhibited reduced acute and chronic parasitism, along with attenuated brain inflammation, and high IL-12p40 and ROS production by peritoneal cells, associated with increased survival. In comparison, we observed that Dectin-1$^{-/-}$ mice are also resistant to infection by *P. berghei*, presenting lower parasitaemia and prolonged survival. However, in *T. gondii* infection, Dectin-1 receptor does not appear to play an important role, since Dectin-1$^{-/-}$ and WT mice displayed similar chronic phase parasitism and survival rate. The gathered data suggest that Dectin-1 can be a possible target for the development of therapeutic and prophylactic measures against *N. caninum* and *P. berguei*, but not for *T. gondii* infection.

Key Words: immunomodulation, CLEC7A, toxoplasmosis, neosporosis, malaria

Financial Suport: CAPES, CNPq, FAPEMIG

ROLE OF EPSTEIN-BARR VIRUS INDUCED 3 (EBI3) ON THE RESISTANCE OF MICE INFECTION WITH *Neospora caninum.*

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Neospora caninum is an obligate intracellular parasite and has the dog as it's definitive host and causes abortion in infected cattle, which causes great economic loss to farmers. *Epstein-Barr* virus-induced gene 3 (EBI3) is a component of the IL-12 family and, combined with other genes as p28 and p35, results in the production of IL-27 and IL-35, respectively. In *Listeria monocytogenes* infection, EBI3 is described as an inhibitor of the Th17 cytokines. The aim of this work is to describe the role of EBI3 during the infection with *N.caninum*in a murine model. First, we will perform a survival analysis to characterize the differential resistance profile of infected wild type (WT) and EBI3 deficient mice ($EBI3^{-/-}$), using a tachyzoite dose lethal for 50% (DL50) of the mice. Additionally, we will analyze the kinetics of the infection: hyperacute, acute and chronic phases, using a sub-lethal dose of tachyzoites of *N. caninum*. In those experiments, we will observe the tissue parasite loads by qPCR and quantify nitric oxide (NO) production and cytokine profile, in bodily fluids and tissues. Also, inflammatory alterations in affected organs will be determined in histological sections, as well as specific IgG with its subsets, in serum samples. We expect that EBI3 might play a relevant role during *N. caninum* infection, which may lead to therapeutical and/or prophylactic strategies against the disease.

Key Words: *Neospora caninum*, EBI3, IL-27, IL-35, immune response.

Financial Suport: CNPq, CAPES, FAPEMIG.

SELECTION AND APPLICATION OF RECOMBINANT AND SYNTHETIC PEPTIDES OBTAINED BY PHAGE DISPLAY IN THE IMMUNODIAGNOSIS OF HUMAN STRONGYLOIDIASIS

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Human strongyloidiasis is an important intestinal parasitic infection worldwide, 50% of infected individuals are asymptomatic, however it can cause hyperinfection and dissemination in immunocompromised hosts leading to death. Early detection of the disease prevents the development of hyperinfection and dissemination syndromes, so the use of an efficient diagnostic tool has great importance to identify and control this parasitic disease. Due to the lack of efficiency in parasitological and serological tests currently available to detect human strongyloidiasis it is necessary to improve immunodiagnostic tests using recombinant and synthetic antigens once there are limitations to obtain and use homologous antigens produced from the parasite. The aim of this study was to select using Phage Display technology *Strongyloidesstercoralis* mimetic peptides ligands to immunoglobulin G from patients with strongyloidiasis. The PhD^{TM}-C7C library was used in the selection process and the DNA of the selected clones was extracted, sequenced and analyzed using bioinformatics tools. ELISA tests were done by using five distinct phage clones, which presented significant similarity with proteins from *S. stercoralis*, and the two synthetic peptides corresponding to the sequences displayed on two phage clones. Sensitivity, specificity, diagnostic efficiency, area under curve and likelihood ratio were calculated for each antigen. All phage clones presented high diagnostic potential achieving area under curves higher than 0.8, the C9 clone presented reasonable sensitivity (87.5%), specificity (80%) and diagnostic efficiency (82.5%).Synthetic peptides C10 and D3 showed superior diagnostic performance, with areas under the curve greater than 0.9 and excellent sensitivity (95%, 95%), specificity (86.3%, 92.5%) and diagnostic efficiency (89.2%, 93.3%) respectively. It was concluded that the selected peptides by Phage display can mimic *S. stercoralis* epitopes and represent promising alternative to the currently available antigens for human strongyloidiasis diagnosis.

Key Words: strongyloidiasis, immunodiagnosis, peptides, phage display, mimotopes.

Financial Suport: FAPEMIG, CAPES, CNPq

EFFECT OF PLANT EXTRACTS:*Zingiberofficinale Roscoe* (GINGER), *Camellia sinensis* (GREEN TEA), *Annonamuricata L.* (GRAVIOLA), *Chamomilla Recutita* (Chamomile), *Momordicacharantia L.* (BITTER MELON) IN THE CONTROL OF *Toxoplasma gondii* INFECTION *IN VITRO* AND *IN VIVO*

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Toxoplasmosis is an infection caused by obligate intracellular parasite *Toxoplasma gondii*, which is common in all warm-blooded animals, including humans. The immune system has the ability to adapt to the recognition of molecules that is unique to this parasite. Sulfadiazine and pyrimethamine or atovaquone, are considered the drugs of first choice for treatment, however, they are associated with many side effects including bone marrow suppression. Previous studies have shown that the plant Extracts have anti-parasitic action to other species of parasites, for example, *Camellia sinensis* and *Momordicacharantia L.*, with trypanocidal action. Studies of less toxic products that are not teratogenic and can control the parasite is of utmost importance. Therefore, this study intend to using plant extracts *in vitro* experiments using fibroblasts and macrophages to verify the effect of the extracts against *T. gondiiin vitro*. We also intend to evaluate the effect of these plant extracts in the control of experimental *T. gondii* infection *in vivo*, by treating C57BL/6 mice with extracts and infect them with the parasite. The morbidity, mortality, parasitism and immunological parameters will be analyzed.

Key Words: Toxoplasmosis, extracts, anti-parasitic, cell culture, immune response.

Financial Suport: CAPES, FAPEMIG

GENOTYPIC VARIABILITY OF *Giardia duodenalis* ISOLATES FROM DIFFERENT SPECIES OF ANIMALS

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Giardia duodenalis is actually a species complex and comprises eight assemblages (A-H). Research into the molecular epidemiology of this parasite is based on the analysis of genes such as β-giardin (*bg*), glutamate dehydrogenase (*gdh*), and triose phosphate isomerase (*tpi*), to name several examples. However, gene polymorphism can lead to divergent results when different loci are sequenced, making multilocus genotyping the main ally in trustworthy attribution of assemblages and subassemblages to isolates. This study aimed to perform molecular characterization of cysts of *Giardia duodenalis* from dogs, cattle, lambs and pigs using three genes (*gdh*, *tp*i and *bg*). Fecal samples of the four groups of animals were analyzed using the zinc sulphate centrifugal flotation technique. Cyst pellets were stored and submitted to PCR and nested-PCR reactions with *gdh*,*tpi* and *bg* primers. Fragment amplifications of thesegenes were observed in 38 (33.3%), 42 (36.8%) and 31 (27.2%) samples, respectively. Regarding the sequencing, 34 (48.6%) sequences were obtained with *gdh*, 25 (35.7%) with *tpi* and 11 (15.7) with *bg*. For all the genes, there was a prevalence of specific species assemblage, although some isolates were identified as A and B by the *tpi* sequencing. They also revealed a large number of heterogeneous sequences, which are attributed to mixed infections between assemblages B and E. In the present study, of the four samples that were sequenced simultaneously in all three genes, only three showed complete concordance of assemblages. These findings raise concerns about the interpretation of genotyping data based on single markers.

Key Words: *Giardia duodenalis*, multilocus, assemblage, genotypic variability

Financial Suport: FAPEMIG, CAPES, CNPq

POLYCLONAL DISSEMINATION OF ESBL AND KPC-PRODUCING *Klebsiella pneumoniae* VIRULENT AND MULTIRESISTANT STRAINS IN A BRAZILIAN HOSPITAL

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Klebsiella pneumoniae is a common cause of urinary tract infections, pneumonia and bacteremia, and the problems related to resistance to β-lactams in these organisms appear to be most important in Brazil and in Latin America than in other regions of the world. The aims of this study were to determine the occurrence of *fim*H, *fimA* and *wabG* virulence genes in 6 KPC-producing *K. pneumoniae* isolates as well as 24 not KPC-producing isolates from patients, and also to analyse the clonal relationship of the isolates by PFGE. Additionallyit was evaluated the presenceofresistance genesbla_{KPC}, bla_{SHV}, bla_{CTX-M}, $bla_{TEM}$by PCR. The presence of TEM, SHV or CTX-M ESBL types was detected in 27/30 (90%) of the isolates. The dominant ESBL types werebla_{SHV} (n=22), bla_{TEM} (n=22) and bla_{CTX-M} (n=21). Four of the 6 bla_{KPC}+ isolates carried bla_{SHV}, bla_{CTX-M} and bla_{TEM} concomitantly. Eighteen strains were positive for the three virulence genes evaluated, most isolated in the blood (8/30), and the presence of at least two virulence genes was observed in 23 ESBL-positive isolates (76.7%), particularly among the bla_{KPC}+ (5/6). PFGE genotyping revealed two main genetic patterns in *K. pneumoniae* isolates, types A (mainly in bla_{TEM} isolates) and B (isolates containing the genes bla_{SHV}, bla_{CTX-M}, bla_{TEM}). Even with two clonal featured profiles, it was observed a polyclonal spread among the strains (24 profiles), including in KPC isolates. Among the 24 ESBL-producing strains, 95.8% were resistant to the fourth-generation cephalosporin cefepime, and 83.3% were multiresistant. Our results confirm that the SHV, TEM and CTX-M ESBL types are prevalent in the hospital and the spread of KPC is worrying. The accumulation of virulence genes of *K. pneumoniae* isolates, observed in this study, requires careful monitoring along with the multi-resistance that impose significant therapeutic limitations on the treatment of infections caused by *K. pneumoniae*.

Key Words: *Klebsiella pneumoniae*; ESBL and KPC genes; virulence; PFGE

Financial Suport: FAPEMIG

RECRUITMENT OF DYSFERLIN DURING THE INVASION OF *Trypanosoma cruzi* IN C1C12 CELLS.

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Trypanosoma cruzi, the intracellular protozoan, which causes the seriously debilitating Chagas' disease in Latin America, able to invade cells to replicate within its vertebrate host, with tropism for cardiomyocytes and smooth muscle cells. The process of cell internalisation activates the host cell structures that interact during invasion. The intense movement of the parasite injures the outer membrane of the cell and cause cell damage. The repair process of a muscle's cell is associated with the recruitment of dysferlin, molecules that acts as a mediator in vesicle fusion by interacting with membrane phospholipids. The initial aim of this paper was to observe the recruitment of dysferlin molecules and the mobilization of lysosomes during invasion of C2C12 cells (myoblasts) by metacyclic trypomastigotes (TCT) of *Trypanosoma cruzi* G strain. Was found, by indirect immunofluorescence reaction that, during the process of internalization of TCT in myoblasts forms, a significant increase in the recruitment of dysferlin molecules and formation of lysosomes (LAMP-1). The recruitment was visualized in the initial invasion of time (between 15 and 30 minutes), indicating that the process is quick and important for both *Trypanosoma cruzi* and for the viability of the host cell.

Key Words: *Trypanosoma cruzi*, Dysferlin, Cell repair

INDUCIBLE NITRIC OXIDE SYNTHASE MODULATE CYTOKINE PRODUCTION DURING *NEOSPORA CANINUM* INFECTION

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Neospora caninum is an obligate intracellular parasite that is capable of infecting a wide range of hosts. Since its first description, *N. caninum* infection has emerged as an important cause of neuromuscular disease in dogs and abortion in cattle worldwide, leading to significant economic losses in beef and dairy cattle industries. Inducible Nitric Oxide Synthase (iNOS) is the most important enzyme responsible for the generation of Nitric Oxide (NO), which is classically described as an important effector mechanism in the killing of intracellular pathogens. Here, we aimed to evaluate the role of iNOS during *N. caninum*infection. For that purpose, we have followed the production kinetics of the major elicited cytokines, mortality rate, inflammation and parasitism after parenteral infections with live tachyzoites, using mouse models with a targeted genetic disruption of enzyme gene ($iNOS^{-/-}$), along with its wild type C57BL/6 counterparts. Our results obtained from experimentally infected mice indicate that WT mice had a lower mortality rate, parasitism and inflammation if compared to $iNOS^{-/-}$ mice, along with lower early Th1 cytokine profile levels and Th2/Th17 cytokines in intermediate stages of infection. These results indicate that iNOS is an important resistance mechanism in *N. caninum* infection, by inducing the control of acute and chronic parasitism, cytokine production and consequent exacerbation of the inflammatory responses.

Key Words: *Neospora caninum*; iNOS; Cytokine; Inflammation; Parasite load

Financial Suport: CAPES, CNPq and FAPEMIG.

THE IMPORTANCE OF DYSFERLIN PROTEIN, ACID SPHINGOMYELINASE ENZYME AND TRANSCRIPTION FACTOR EB AT EXOCYTOSIS OF LYSOSOMES AND CELLULAR REPAIR DURING THE INVASION OF MYOBLASTS AND CARDIOMYOCYTES *in vitro* BY THE *Trypanosoma cruzi* (Y STRAIN).

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Trypanosoma cruzi, etiologic agent of Chagas disease is an obligate intracellular parasite, and present three evolutionary stages, being able to invade and replicate within a wide variety of nucleated cells and exhibit tropism for cardiomyocytes and smooth muscle cells of the vertebrate host. The process of cellular internalization by *T. cruzi* is dependent on their adherence and internalization into the host cell. The repair process of a muscle cell during parasite internalization may be associated with the recruitment of dysferlin molecules that acts as a mediator in vesicle fusion by interacting with membrane phospholipids. The expression of transcription factor EB (TFEB) is a master regulatory gene lysosomal biogenesis and the extracellular release of acidic sphingomyelinase enzyme (ASM), appear to play a key role in the repair mechanism of the membrane. The objective of this study is to investigate the importance of dysferlin protein, acid sphingomyelinase enzyme and transcription factor EB exocytosis of lysosomes and cellular repair during the invasion in myoblasts and cardiomyocytes by Y strain of *T. cruzi*. For this we use immunofluorescence techniques, RNAi and quantification of gene expression, thus trying to understand what tricks the parasite uses the invasion process and which are used for intracellular maintenance of this host-parasite relationship. With this work we intend to evaluate the interaction of trypomastigote forms of Y strain of *T. cruzi* with host cells.

Key Words: *Trypanosoma cruzi*, Dysferlin.

MESSENGER RNA PROFILE FOR CYTOKINES EXPRESSED BY B CELLS PRODUCING IgE, IgG4 AND IgGA FROM PATIENTS SENSITIZED TO *Blomia tropicalis* ALLERGENS

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Introduction, Justification and Objectives: The knowledge about the cell biology involved on cytokines messenger RNA expression promotes the understanding of amplification or modulation of allergic responses B cells. Among these, are the regulatory B cells-IgG4 producing, which are an important source of IL-10 e play a key role in immunoregulation, since they suppress the production of other cytokines and chemokines, as well as antigen-presentation [1]. With the significant increase of prevalence and gravity of allergic diseases, especially the respiratory allergy, mainly caused by mites, studies intending to modulate these responses are important to create immunotherapeutic strategies [2,3]. Therefore, this study aims evaluate the messenger RNA profile expressed by IgE-, IgG4- and IgA-producing B cells from patients sensitized with *Blomia tropicalis* allergen Blo t 5. Methodology: The total mite extract will be obtained according to the methodology described by PEREIRA et al. (2005) with concomitant production of recombinant protein of the allergen. Will be selected adults patients with a history of allergic respiratory symptoms caused by house dust mites confirmed through a positive skin prick test. Blood samples will be collected for subsequent isolation of PBMC to be used for cell sorter. Then, the cells will be stimulated with the total antigen and recombinant protein of *B. tropicalis*, collected and the messenger RNA extracted using Trizol solution. The cDNA library will be made for furthers analysis by qPCR. Statistical analysis will be employed by GraphPad Prism 4.0 (GraphPad Software, Inc., USA). Expected results: It is expected that different levels and profiles of cytokines are obtained by stimulation of both total antigen as recombinant antigen. Furthermore, should be some differences on the expression of cytokines from various types of B cells to atopic and non-atopic patients, particularly IL-10 and TGF-β.

Key Words: Allergy; *Blomia tropicalis*; Regulatory B cells; Recombinant antigen; messenger RNA.

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POLARIZATION OF HUMAN MACROPHAGES INFECTED WITH *Toxoplasma gondii* BRAZILIAN STRAIN: AN IN VITRO ANALYZE OF THE IMMUNE RESPONSE TRIGGERED BY THESE CELLS

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Several immunological mechanisms are responsible for the control of the *T. gondii*infection. The immune response occurs initially by the participation of cells from innate immunity, with macrophages have an important role in this early response. Macrophages can be activated and polarized according to the type of stimulation and substances produced at the site of inflammation. In this sense, if the microenvironment predominates Th1-type cytokines, the macrophages will be polarized to macrophage M1 (classical activation), and to macrophage M2 if they are alternatively activated in a microenvironment with the presence of Th2 cytokines. In addition, the great advance in molecular tools has allowed the characterization of different strains of *T. gondii*, known as atypical strains. In Brazil, there was a greater genetic variability in isolated lineages of strains. In Uberlândia (Minas Gerais, Brazil), two isolates of *T. gondii* were found in samples of heart from chickens (*Gallus gallusdomesticus*), which were named TgChBrUD1 and TgChBrUD2. The high virulence and the severe symptoms caused by the atypical strains of *T. gondii* show the importance of elucidating the immune response generated by macrophages infected with the atypical strains compared with clonal strains of *T. gondii*. The aim of this study is to analyze the polarization of macrophages (THP-1 line) infected with TgChBrUD1. For this purpose, THP-1 monocytes will be treated with PMA for processing the differentiation to macrophages. The cells will be infected with TgChBrUD1 or the clonal strains (RH or ME-49). The supernatant will be collected for posterior cytokine, arginase and nitritte measurement. Phenotypic markers of the polarization were assessed by flow cytometry. The expected result from the research is to verify if the atypical strain of *T. gondii*will be able to change the macrophage polarization when compared to clonal strains.

Key Words: macrophages, polarization, *Toxoplasma gondii*, atypical strains

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IMMUNIZATION WITH *Toxoplasma gondii* HEAT SHOCK PROTEIN 70 KDA (*TG*HSP70) INDUCE B CELL-MEDIATED PROTECTIVE IMMUNITY

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Toxoplasma gondii is an obligate protozoan parasite widely distributed that may represent a risk factor in immunocopromised hosts, among them HIV-positive patients and pregnant women. Under stress conditions, *T. gondii* tachyzoites converts to bradyzoites, and vice versa, and express *Tg*HSP70, which is known to represent a danger signal and a virulence factor in *T. gondii* infection. The aim of this work was to determine the effects of the immunization with recombinant *Tg*HSP70 in the development of toxoplasmosis. C57BL/6 mice were immunized with 10 µg *Tg*HSP70 alone or combined with aluminum hydroxide (alum), together with control groups. After 45 days of infection, a group of animals were used for spleen *ex vivo* analyses, and the remaining were infected with 10 cyst of ME49 *T. gondii* strain and sacrificed 30 days later for parasitism analyses. It was observed that the immunization with *Tg*HSP70 reduced 76.9% the number of cysts in the brain of infected mice, regardless alum addition. Moreover, we observed that *Tg*HSP70 was capable to induce B cells proliferation, but not T cells, regardless the groups of immunization. Interestingly, the stimulus of spleen cells with *Tg*HSP70 was able to reduce CD80 and increase CD86 cell co-stimulatory molecules expression, respectively, in B cells, which are directly correlated to the production of neutralizing antibodies. Thus, the immunization with *Tg*HSP70 promotes B lymphocyte-mediated protective responses against *T. gondii* infection.

Key Words: *Tg*HSP70, toxoplasmosis, immunization, vaccine

Financial Suport: CNPq, CAPES and FAPEMIG

THE REINFECTION BY BRAZILIAN STRAINS OF *Calomys callosus* CHRONICALLY INFECTED BY CLONAL TYPE II *Toxoplasma gondii* PROMOTE REACTIVATION AND VERTICAL TRANSMISSION OF THE PARASITE

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Congenital toxoplasmosis results mainly from primary maternal infection during pregnancy, but it can be occasionally caused by reinfection. *Toxoplasma gondii* has shown high genetic diversity in Brazil. *Calomyscallosus* is resistant to *T. gondii* type II clonal ME-49 strain, but susceptible to TgChBrUD1 and TgChBrUD2 Brazilian strains. The aim of this study was to determine whether *C. callosus* chronically infected by ME-49 strain might be susceptible to reinfection by these Brazilian strains, including to vertical transmission of the parasite.The survival curves were analyzed in non-pregnant females chronically infected with ME-49 and reinfected with TgChBrUD1 or TgChBrUD2 strain. The vertical transmission was analyzed after reinfection with TgChBrUD1 or TgChBrUD2 strain, which was carried out in the first day of pregnancy. At 19th day of pregnancy, placentas, uteri, fetuses, liver, spleen and lung were processed for detection of the parasite. The uteri and placentas were evaluated for the fetal resorption rates. Blood samples were collected for humoral and cellular immune response analyses. All non-pregnant females chronically infected by ME-49 strain survived after reinfection. In pregnant females, parasites were detected in placenta and fetuses after reinfection by TgChBrUD1 and TgChBrUD2 strains. TgChBrUD2 reinfected females showed more impaired pregnancy outcomes presenting higher number of animals with embryonic lossand resorption rate, in parallel with higher levels of pro-inflammatory cytokines and IgG2a subclass. In conclusion, our results clear demonstrate that, during pregnancy, protection against *T. gondii* can be breached after reinfection with parasites belonging to different genotypes, particularly when non-clonal strains are involved in this process. Also, the reinfection promoted the ME-49 strain reactivation and *T. gondii* vertical transmission of type II and Brazilian strains.

Acknowledgements: FAPEMIG, CNPq, CAPES.

Keywords: *Toxoplasma gondii*, congenital toxoplasmosis, parasite genotypes, Brazilian strains.

ENROFLOXACIN CONTROLS *Toxoplasma gondii* INFECTION AND INDUCES IL-6 PRODUCTION IN HUMAN TROPHOBLAST CELLS

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Toxoplasma gondii is an obligate intracellularprotozoan parasiteresponsible fortoxoplasmosis. The classical treatmentof toxoplasmosisis based on theassociation ofpyrimethamineandsulfadiazine, but pyrimethamine has teratogeniceffectswhen administeredduringthe firsttrimester of pregnancyand mayalter the normalbone marrow activity. In this sense, the establishment ofalternative therapeuticstrategies isvery necessary in order to minimizethe undesirable sideeffectsandbetter controlthe infection. Enrofloxacinis an antibioticcommonly usedin veterinary medicineandhas shownexcellent resultsin controlling infectionby*Neosporacaninum*and *T. gondii*in human fibroblast cells. The objectiveof the present study was to evaluatethe efficacyofenrofloxacinin the control of *T. gondii* infectionin humantrophoblast cells(BeWolineage), a model of treatment of the congenital toxoplasmosis. Initially,BeWo cellswere grownin cultureand treated or not withdifferent concentrations ofenrofloxacin, sulfadiazine,pyrimethamine or pyrimethamine plussulfadiazinetoverify thecellular cytotoxicity by MTT. Next, BeWo cells were infected with *T. gondii* (2F1 clone), treated or not with the same antibiotics and analyzed to *T. gondii*intracellular proliferation and cytokine production by beta-galactosidase assay and ELISA, respectively. The results showed that all antibiotics, including enrofloxacin, did not reduce the cell viability in BeWo cells. Furthermore, enrofloxacin significantly decreased the parasite intracellular proliferation when compared to untreated cells and, interestingly, this reduction was more effective in controlling infection when compared to the other antibiotics used. In addition, enrofloxacin induced a high production of IL-6 when compared to untreated cells. Therefore, it is possible to conclude that enrofloxacinis a potential alternative for the treatment of congenital toxoplasmosis, contributing for the control of the vertical transmission of this parasite.

Key Words: *Toxoplasma gondii*; enrofloxacin; treatment

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Pseudomonas aeruginosa BACTERAEMIA: INDEPENDENT RISK FACTORS FOR MORTALITY AND IMPACT OF RESISTANCE ON OUTCOME

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The rates of multidrug-resistant, extensively drug-resistant and pandrug-resistant isolates among non-fermenting Gram-negative bacilli, particularly *Pseudomonas aeruginosa*, have risen worldwide. The clinical consequence of resistance and the impact of adverse treatment on the outcome of patients with *P. aeruginosa*bacteraemia remain unclear. To better understand the predictors of mortality, the clinical consequence of resistance and the impact of inappropriate therapy on patient outcomes, we analyzed the first episode of *P. aeruginosa* bacteraemia in patients from a Brazilian tertiary-care hospital during the period from May 2009 to August 2011. Antimicrobial susceptibility testing was conducted; phenotypic detection of Metallo-β-Lactamase (MBL) and PCR of MBL genes were performed on carbapenem-resistant strains. Amongst the 120 *P. aeruginosa* isolates, 45.8 % were resistant to carbapenem and 36 strains were tested for MBL detection. A total of 30 % were phenotypically positive and, of these, 77.8 % expressed an MBL gene, $bla_{\text{SPM-1}}$ (57 %) and $bla_{\text{VIM-type}}$ (43 %). The resistance rates to ceftazidime, cefepime, piperacillin/tazobactam, carbapenem, fluoroquinolone and aminoglycoside were 55, 42.5, 35, 45.8, 44 and 44 %, respectively. Previous antibiotic use, length of a hospital stay $\geq$ 30 days prior to *P. aeruginosa*, haemodialysis, tracheostomy, pulmonary source of bacteraemia and Intensive Care Unit admission were common independent risk factors for antimicrobial resistance. Cefepime resistance, multidrug resistance and extensive drug resistance were independently associated with inappropriate therapy, which was an important predictor of mortality, being synergistic with the severity of the underlying disease.

Keywords: *Pseudomonas aeruginosa*, bacteremia, resistance, risk factors, mortality

Financial support: CAPES and FAPEMIG

PROBIOTICS PREVENT THE BACTERIA TRANSLOCATION AND DECREASE THE ILEITIS THAT DEVELOPS IN ORAL *Toxoplasma gondii* INFECTION

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Toxoplasma infection serves as a trigger for inflammatory pathology caused by intestinal bacteria, which interferes in the interaction between microbiota and intestinal mucosal immune system and results in mucosal inflammation. Probiotic treatment may recover the commensal bacteria and normalize the host-microbiota interaction. Oral infection with *T. gondii* in certain mouse strains induces ileitis which lesions resemble to those of human Crohn's disease. In the present study, C57BL/6 mice were treated with *Lactobacillus casei* or *Lactobacillus acidophilus* before and during *Toxoplasma gondii*infection in order to evaluate immunological parameters and bacteria systemic translocation. Animals treated with *L. casei* or *L. acidophilus* one day before and 8 days after oral infection with 30 ME-49 *T. gondii*cysts survived longer and presented reduction in parasite burden. *L. casei*-treatment decreased macrophage activity ($p > 0.05$, n= 4). In addition, none of the treatments were able to prevent Paneth cell loss and do not interfere in IgA^+ cell numbers in the small intestine in *T. gondii*-infection, however, these probiotics avoid the goblet cell loss in the ileum of non-treated mice ($p > 0.05$, n=3). Microbiological culture of organs, blood and feces indicated that treatment with *L. casei* or *L. acidophilus* prevents bacterial translocation of intestinal lumen to liver, lung and blood. The qPCR analysis revealed that treatment with *L. acidophilus* decreased IFN-γ and TNF-α mRNA expression in the ileum induced by *T. gondii* infection, and *L.casei* increased Foxp3 and IL-10 expression. The results demonstrated the ability of probiotics to control the inflammatory immune response and reduce mortality caused by ileitis.

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EFFECT OF TREATMENT WITH IL3, IL7 OR IL9 IN MICE EXPERIMENTALLY INFECTED WITH *Trypanosoma cruzi*

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Chagas disease, caused by *Trypanosoma cruzi* infection, is ranked as the most serious parasitic disease in Latin America and has huge potential to become a worldwide problem. A bulk of studies substantiates that Th1-associated cytokines are essential elements in early resistance against the parasite and are associated with the development of the chronic cardiac form, while the balance between Th1 and Th2 responses is suitable for overcome the infection. Although cytokines have a key role in the immune response against *T. cruzi*, little is known about IL3, IL7 and IL9. Then the aim of this study was to analyze the role of IL3, IL7 and IL9 in the acute phase of *T. cruzi* experimental infection. For this purpose, parameters indicative of improvement in clinical status of the animals were studied, such as: parasitaemia, morbidity, mortality, histopathology and biomarkers of organs injury. Our data revealed that the treatment with IL3, IL7 or IL9 control the parasitaemia with consequent improvement of clinical parameters in mice infected with a virulent strain of *T. cruzi* (Y strain). However this profile did not promote the reduction of *T. cruzi*-induced inflammation in peripheral organs neither modified kidney and liver functions of these animals. Taken together, our results underline the importance of these cytokines in modulation of *T cruzi* infection.

Keywords:*Trypanosomacruzi*; interleukin-3 (IL3); interleukin-7 (IL7); interleukin-9 (IL9).

Financial support:CNPq, CAPES and FAPEMIG.

HIGH PREVALENCE OF IS*Aba1*-ASSOCIATED $bla_{\text{OXA-23}}$GENE IN *Acinetobacter baumannii* CLONES IN A TERTIARY CARE HOSPITAL

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Acinetobacter baumannii has been considered one of the major nosocomial pathogens worldwide, most often isolated in intensive care units (ICUs), where extensive antibiotic use has enabled selection of resistance against almost all known antimicrobials. The aims of this study were to investigate potential mechanisms that contribute to antimicrobial resistance and its association with high-level resistance to carbapenems. This study verified the presence of the insertion sequence IS*Aba1* and its association with $bla_{\text{OXA-51-like}}$ and $bla_{\text{OXA-23-like}}$ genes in 23 isolates of *A. baumannii* recovered from clinical and environmental samples in an adult ICU. In addition, it was evaluated the presence of outer membrane proteins (OMP) and oxacillinases genes by PCR and the Minimum Inhibitory Concentration (MIC) by Etest®. All carbapenems-resistant isolates were OXA-23 producers and were typed by Pulsed-field Gel Electrophoresis. Molecular typing revealed a polyclonal profile, however, clone A (clinical) and H (environment) were the most frequents. Of the 23 isolates analyzed, 43.5% (10/23) harbored all the resistance genes associated or not with the element IS*Aba1* as well as the genes evaluated for OMPs (porins). Of these 10 isolates, 60% were recovered from the environment. Lower MICs were observed in the strains with the absence of $bla_{\text{OXA-23}}$ gene (n = 3), although two of them presented the association *ISAba1/OXA-51*. All carbapenems-resistant isolates had lower MICs for imipenem (47.4%; 9/19) when compared to meropenem and 12 of them carried *ISAba1/OXA-23*. We concluded that, in these isolates, the major carbapenem resistance mechanism was due to OXA-23 carbapenemase activity associated or not with IS*Aba1*. There was the coexistence of multiple clones with the prevalence of clone A, between clinical strains, and H, between environmental ones. Our results demonstrate the complexity in addressing the role of different mechanisms in carbapenem resistance and highlight the possible influence of porins in this phenotype.

Keywords: *Acinetobacter baumannii*, carbapenem resistance, oxacillinases, IS*Aba1*

Financial support: FAPEMIG

SPOROZOITE/OOCYST SPECIFIC RECOMBINANT PROTEINS TO DIFFERENTIATE SOURCES OF *Toxoplasma gondii* INFECTION

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Toxoplasma gondii is transmitted horizontally mostly through ingestion of tissue cysts in undercooked/ uncooked meat, or by ingesting food or water contaminated with sporulatedoocysts from infected felidae feces. The diagnosis of toxoplasmosis can be established by serological tests that detect exposure to *T. gondii*, although the current immunoassays are not able to distinguish the sources of infection. This lack of information hampers the implementation of measures to reduce the transmission of the parasite. The diagnosis of toxoplasmosis may be improved by using stage specific antigen in serological assays and epidemiological surveys. We used 16 sporozoite/oocyst specific recombinant proteins against serum samples from chickens (naturally infected by oocyst or experimentally infected with tissue cysts), pigs (experimentally infected with oocysts or tachyzoites), mice (experimentally infected with cysts or oocysts) and humans (individuals infected by oocyst from an outbreak of toxoplasmosis or serum samples of pregnant women positive for IgG anti - *T. gondii* from unknown source of infection).The antibody response was evaluated by ELISA and Western blotting. Among the 16 proteins, the CCp5A protein was able to differentiate the parasite stage that infected chickens, pigs and mice, since the stage-specific antibodies was detected in serum samples only in those animals infected with oocysts. In humans serum samples that acquired infection by oocyst (outbreak of toxoplasmosis) the IgG positivity for CCp5A protein was 82% (n = 78), whereas samples from patients infected by unknown sources showed 52% of positivity (n = 78). The IgM positivity to CCp5A protein in serum samples from an outbreak was 80% (n = 42), while in serum samples from pregnant women were 16% (n = 42). These data suggest that CCp5A protein may be useful to differentiate the stage of the parasite causing infection, as the antibody detection was mainly directed to this component of the parasite.

Key words:*Toxoplasma gondii*, oocyst/sporozoite, outbreak, diagnosis.

Financial support: CAPES, CNPq and FAPEMIG

ANALYSIS OF IMMUNE BIOMARKERS AND THEIR ASSOCIATION WITH CLINICAL ASPECTS OF THE CONGENITAL TOXOPLASMOSE IN CHILDREN FROM MINAS GERAIS, BRAZIL.

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Toxoplasmosis is one of the most common zoonosis caused by protozoan parasites in the world, and the etiologic agent is *Toxoplasma gondii*. The congenital toxoplasmosis is an important public health, since it can lead severe symptoms to the embryo or newborn, including miscarriage. The clinical manifestations of the congenital toxoplasmosis depend on the stage of gestation at which the transmission of the parasite to the fetus occurs. Chemokines and cytokines are extremely important to the immune response against *T. gondii i,* since the presence of these inflammatory mediators triggers the control of the infection and protects against possible overreaction that could induce tissue damage. Thus, this study aims to characterize immunologic biomarkers that best determine important clinical manifestations of congenital disease caused by T. gondii. Eighty newly born children that are experiencing positive for anti-*T.gondii* IgM will be selected in a period of 30 to 60 days after birth in Belo Horizonte, Minas Gerais, Brazil. It will be collected for this study 4.5 ml of peripheral blood and serum cytokine analysis (IL-2, IL-4, IL-6, IFNg, TNFa, IL-17A, IL-10) and chemokines (IP-10, MIG, MCP1, RANTES and IL-8) will be held through cytometric Bead Array-Becton Dickinson (CBA) kit. Data analysis will be done using the specific software. Statistical analysis will be performed according to the categorization of results in parametric and non-parametric. Differences between the production of circulating cytokines and chemokines in newborns with congenital toxoplasmosis are expected and maybe these differences can be associated with the distinct clinical manifestations observed.

Keywords: congenital toxoplasmosis, biomarkers, newborn, *Toxoplasma gondii*.

Financial Suport: CAPES, CNPq, FAPEMIG.

Leishmania amazonensis PHAGOLYSOSOME FUSION: A MAP-KINASE SIGNALING-DEPENDENT EVENT

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Introduction: *Leishmania* spp are causative protozoa of leishmaniasis, which affects millions of people worldwide. The host-pathogen interaction involves the parasite surface molecules and cellular receptors that culminate in phagocytosis. One mechanism that promastigotes use to evade the microbicidal effect of phagocytosis is to inhibit phagolysosome biogenesis by delaying lysosome fusion using LPG. However, the molecular mechanisms underlying the *Leishmania*-mediated inhibition of phagosome-lysosome fusion are still poorly understood. This study aimed to understand which signaling pathways are involved in the formation of the phagolysosome process, the invasion and multiplication of the parasite in the host cell.Methods:Kinetics of recruitment to the phagosome vesicles, invasion and multiplication assays were performed using specific inhibitors of cell signaling PI3K, Akt, MEK1/2, ERK2, mTOR and NRAS. In addition, western blot of invasion kinetics at different times were performed to understand what pathways are activated in each step of the invasion. Results: In the invasion assay there was a reduction in the rate of invasion and parasite load murine peritoneal macrophages when were treated with inhibitors of PI3K, MEK1/2, ERK2 and AKT signaling pathways. In addition, inhibition of signaling involving AKT, ERK, MEK1/2, mTOR and RAS delayed phagolysosome maturation. Furthermore, western blot showed that the AKT pathway was activated by *L. amazonensis* entry into the host cell 15 and 30 hours post *L. amazonensis* invasion. The ERK pathway was activated at 15 minutes, 30 minutes, and 1 hour after invasion. However, the intracellular multiplication was not affected by inhibition of the pathways. Conclusion:*L. amazonensis* cell invasion and phagolysosome fusion relies on different host cell signaling pathways. It also appears likely that different pathways temporally regulate fusion of lysosomes, and that ERK-2 signaling is a major contributor during both processes.

Keywords: Phagolysosome; *Leishmania amazonensis*; Signaling pathways.

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SCFV ANTIBODIES AS A POWERFULL TOOL TO SELECT HIGHLY SENSITIVE AND SPECIFIC ANTIGENS APPLICABLE IN NEUROCYSTICERCOSIS DIAGNOSIS

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Human neurocysticercosis (NC) is an important but neglected cause of epilepsy in developing countries where the parasite occurs. Selection of antibodies from combinatorial libraries displayed on the surface of bacteriophages is important to generate molecules applicable in diagnosis. A phage scFv antibody library was selected against *Taeniasolium* mimotopes displayed on phages ($NC_2$2 and $NC_4$1) coupled in beads and total saline extract of *T. solium*m etacestodes (S) immobilized on microtiter plate wells. After two rounds of selection, 96 phage clones were evolved and validated against each target by enzyme linked immunosorbent assay (ELISA), dot-blot, sequencing, indirect fluorescent antibody test (IFAT) assays and tested for antigenic capture. Captured antigens were elucidated through mass spectrometry and tested by enzyme linked immunosorbent assay (ELISA) for IgG detection in serum samples. Diagnostic parameters (sensitivity, specificity, likelihood ratio and area under curve)were calculated by using TG-ROC curves. Three specific antibodies (B6, G10 and A4) were selected. IFI revealed tegument staining for the B6, while the others showed a non-uniform staining in the whole parasite. The selected scFvs achieved sensitivities greater than 97% and specificities above 95% in ELISA. Test performance, indicated by AUC for A4 target (0.998) almost reached the maximum value (1.00) of efficiency; likelihood ratio conferred to this antigen a very high diagnostic value (60.0). Diagnostic parameters obtained were much higher than those from saline extract (S) or other antigens described in the literature. Antibody fragments specific to *T. solium*antigens were successfully selected and used for the first time to capture antigens applicable in NC diagnosis being potentially promising diagnostic tools.

Keywords:scFv antibodies, antigenic capture, serodiagnosis, neurocysticercosis and immunofluorescence.

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THE ROLE OF ADAPTER MOLECULE INDUCING INTERFERON-B WITH TIR DOMAIN (TRIF) ON RESISTANCE OF *Neospora caninum* INFECTION IN MICE

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Neospora caninum is an obligate intracellular parasite that has the dog as definitive host and other mammals, especially cattle, as intermediate hosts. Economically, neosporosis is considered a disease of great importance in veterinary medicine due to the fact that the parasite is able to cause potentially serious complications, like abortion in cattle and neuromuscular paralysis in dogs. The mechanisms of the innate immune response related to neosporosis are still largely unknown, thus further studies are needed to describe the participation of various signaling pathways that culminate in production of inflammatory mediators, enabling the development of preventive and therapeutical effective methods against this infection. Thus, the objective of this study is to evaluate the role of the adapter molecule inducing interferon-β with TIR domain (TRIF) in resistance to *N. caninum* infection *in vivo* and *in vitro*. For this, initial assays of mortality and morbidity will be performed in C57BL/6 wild type (WT) mice and genetically deficient in TRIF (TRIF-/-). In addition, determination of parasite loads will be performed in the different experimental groups using quantitative real-time PCR. The acute and chronic phases of infection will be evaluated by serological tests for quantification of antibodies, besides of cytokines and inflammatory alterations analyzed by ELISA, CBA and histological sections of different tissues, respectively. Thus, we expect that the adapter molecule TRIF might be responsible for the development of protective immune responses against *N. caninum*, deduced by probable susceptibility of knockout mice. That hypothesis is based on the supposition that this molecule plays an important role in the induction of the host's innate recognition by Toll like receptors.

Key words: *Neospora caninum*, innate immune responses, TRIF.

THE ROLE OF MACROPHAGE MIGRATIONINHIBITORY FACTOR IN THE INFECTION WITH *Toxoplasma gondii* AND *Neospora caninum*

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Macrophage Migration Inhibitory Factor (MIF) is a pleiotropiccytokine produced by various cell types including monocytes, macrophages and dendritic cells. It is preformed intracellularly, which makes itan important factorin acute inflammatory responses and as a central mediator in innate immunity. Since its discovery, MIF has been shown to be important in the immune response against intracellular protozoa. Thus,the present study aimed to evaluate comparatively the role of this cytokine during infection with *Toxoplasma gondii* and *Neospora caninum*. C57BL/6 wild type (WT) and genetically deficient mice in MIF ($MIF^{-/-}$) were intraperitoneally infected with *T. gondii* tachyzoites (PRU strain) and *N. caninum* tachyzoites (Nc-Liv strain). Different groups of infected mice were monitored up to 30 days, and samples were collected for the determination of survival rates, cytokine production, cell collection and acute and chronic phase parasite loads. As a result, it was observed that animals MIF-/- infected with *T. gondii* presented increased amounts of parasites in the brain associated with lower serum concentration and spleen cell production of IFN-γ, compared to WT littermates. In comparison, $MIF^{-/-}$ mice inoculated with tachyzoites of *N.caninum* presented surprisingly lower parasite loads during acute and chronic phases, increased survival and recovery of body weight, compared to wild type litte mates. There were no significant alterations in cytokine production between wild type and genetically deficient mice under *N. caninum* infection. These results demonstrate that, while MIF presents a protective role for hosts during infection by *T.gondii*, it may take part of *N. caninum* evasion mechanisms of the host's effector immune responses.

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