03 IMMUNOLOGICAL DIAGNOSIS

03.001 - PREVALENCE ANALYSIS OF SYSTEMIC AND OPPORTUNISTIC MYCOSES DIAGNOSED IN ADOLFO LUTZ INSTITUTE

Franco, D. L.[1]; Carvalho-Vivi, J. O.[2]; Zamboni, I. M.[3]; Barreto, L. C.[4]; Da Silva, D. F.[5]; Oliveira, L. E.[6]; Assis, C. M.[7]; Vicentini, A. P.[8]

[1,2,3,4,5]Instituto Adolfo Lutz - Laboratório de Imunodiagnóstico das Micoses-Seção de Imunologia; [6]Instituto Adoldo Lutz - Laboratório de Imunodiagnóstico das Micoses-Seção de Imunologia; [7]Instituto Adolfo Lutz - Pós-Graduação; [8]Instituto Adolfo Lutz - Laboratório de Imunodiagnóstico das Micoses-Seção de Imunologia , São Paulo, Brasil. E-mail: adrianavicentini@uol.com.br

Introduction: Systemic and opportunistic mycoses are invasive diseases that can be fatal if not correctly diagnosed and treated. The former is characterized by infections acquired through inhalation, primary lesions and pulmonary manifestations caused by dimorphic fungi as *Paracoccidioides brasiliensis* and *Histoplasma capsulatum*. On the other hand, the opportunistic mycoses are caused by saprophytic fungi and have shown enhanced incidence in parallel with the increased widespread use of antibiotics and immunossupressor drugs, long -lasting parenteral medication and diseases that provoke immunodeficiencies. **Methods and Results:** We evaluated the prevalence of paracoccidioidomycosis (PCM) and histoplasmosis (HP) among the systemic mycoses and of aspergillosis, an opportunistic mycosis. All of them were immunodiagnosed at the Laboratorio de Imunodiagnóstico das Micoses from Adolfo Lutz Institute of São Paulo. Sera samples from 6,041patients with clinical suspicious of PCM, HP or ASP were analyzed by double immunodiffusion technique in a period from April 2001 to May 2004. Among the clinical suspicious, 67.3% corresponded to PCM, 19.4% to HP and 13.3% to ASP. 86.6% of the patients that presented confirmed PCM serology were male between 40 to 60 years old against only 13.4% female patients**.** 78% of the HP patients were male with 30 to 50 years old against only 22% female patients. 82% of the individuals with confirmed AP serology were male against only 18% female patients. **Conclusion:** Among the systemic mycoses**,** PCM presents the highest incidence in Brazil, mainly in some regions of São Paulo state as Campinas, Jundiaí, São José do Rio Preto and Ribeirão Preto. Beside those regions, it is noteworthy the number of positive cases in Mato Grosso and Mato Grosso do Sul states.Opportunistic mycoses**,** as histoplasmosis, are observed frequently associated with patients presenting cellular immunity disturbs, mainly those that developed HIV/AIDS, and in individuals with aspergillosis that present allergic process. **Financial support:** Instituto Adolfo Lutz (Projeto CTC-IAL #107/97)

03.002 - REACTIVITY OF ANTI-*HISTOPLASMA CAPSULATUM SERUM* TO FRACTIONED *H. CAPSULATUM* AND *PARACOCCIDIOIDES BRASILIENSIS* CELL FREE ANTIGENS

Tristao, F. S. M.[1]; Castillo, V. B.[2]; Itano, E. N.[3]

[1,3]State University of Londrina - Department of Pathology Science, Londrina, PR, Brazil; [2]IMT - HC, Lima, Peru

Introduction and Objectives: *Histoplasma capsulatum* is the causative agent of histoplasmosis, a systemic infection of significant consequences especially among immunocompromised patients. The *H. capsulatum* antigens are known to cross-react with antibodies to *Paracoccidioides brasiliensis.* The objective of the present investigation was to analyse the reactivity of anti-*H. capsulatum* serum to fractioned cell free antigen (CFA) preparation from *H. capsulatum* and from *P. brasiliensis.* **Methods and Results:** CFA from *H. capsulatum* (IMT/HC) and *P. brasiliensis* (Pb 18) were submitted to gel filtration chromatography in *Sephadex* G75-120 column and the fractions were analyzed by dot-blotting using rabbit anti-*H. capsulatum* serum. Additionaly CFA´s samples were analysed by Western-blotting using anti-*H. capsulatum* serum adsorbed and not adsorbed with *P. brasiliensis*. Chromatography fractions analysis demonstrated positive reaction between .void volume to approximately 58 kDa molecular mass fractions with anti-*H. capsulatum* serum in both CFA´s samples. Also Western blotting analysis showed that most of CFA high molecular mass were recognized with anti-*H. capsulatum* serum and after the adsorbing process didn't present reaction to *P. brasiliensis* antigen. **Conclusion:** With this trial was possible to conclude that high molecular weight fractions from *H. capsulatum* cell free antigens are more immunogenic and less specific and the anti-serum cross-reaction to *P. brasiliensis* can be abolished through the adsorption with this yeast cell.

03.003 - PARACOCCIDIOIDOMYCOSIS - INFECTION IN BOVINE FROM MICROREGIONS OF MATO GROSSO DO SUL - BRAZIL

Silveira, L. H.[1]; Paes, R. C. S.[2]; Medeiros, E. V.[3]; Itano, E. N.[4]; Camargo, Z. P.[5]; Ono, M. A.[6]

[1]Fundação Faculdades Luiz Meneghel - Patologia Geral; [2]Agência Estadual de Defesa Sanitária Animal e Vegetal do Mato Grosso do Sul - Laboratório de Sanidade Animal; [3]Aluno de graduação da Fundação Faculdades Luiz Meneghel - Patologia Geral; [4,6]Universidade Estadual de Londrina - Ciências Patológicas; [5]Universidade Federal de São Paulo - Microbiologia e Imunologia e Parasitologia

Introduction and Objectives: Paracoccidioidomycosis (PCM) is a systemic mycosis endemic in Latin American countries. The etiologic agent *Paracoccidioides brasiliensis* is a thermodimorphic fungus that grows as yeast in the host or at 37 ºC and as mycelia at 25 ºC (Clin Micr Rev 89-117,1993). Despite several attempts to find the *P. brasiliensis* habitat, until now, it is unknown although that the fungus lives in soil. Also, the role of other animal species in the fungus ecology remains unclear. *Paracoccidioides brasiliensis* was isolated from frugivorous bats (*Sabouraudia* 4:124-125, 1965), penguin [Inst Venez de Invest Cient, Abstract B-2, 1989) and armadillos (Med Mycol; 38(3): 193-199,2000). Epidemiological studies suggest that other species as cows (Antioq Med; 24: 339-358, 1974), sheeps (*Sabouraudia*; 16:93-101, 1978), monkeys (Vet. Pathol. 1977; 14:368-371) and dogs (Med. Mycol. 2001; 39: 277-282, 2001) may be infected by *P. brasiliensis*. The aim of this work was to evaluate the humoral immune response in bovine immunized with *Paracoccidioides brasiliensis* and realize a seroepidemologic study of paracoccidioidomycosis in bovine from four microregions of Mato Grosso do Sul, Brazil. **Methodes and Results:** Two bovine were inoculated with suspension of *P. brasiliensis in Freund incomplete adjuvant.* Samples of blood were collected periodically to evaluate humoral immune response by immunodiffusion and ELISA, using exoantigen and gp43 as antigens, respectively. The production of antibody was detected by immunodiffusion and ELISA, in both animals 14 days after immunization. The soroepidemiologic study was carried out in 400 bovines of Mato Grosso do Sul from 4 regions: Dourados, São Gabriel d´ Oeste, Corumbá and Nova Andradina. The reactivity to gp43 was 17,5% and municipalities of Corumbá (30%) and Nova Andradina (28%) showed higher positivity than São Gabriel d´ Oeste(4%) and Dourados (8%). **Conclusion:** In this study we concluded that bovines immunized with *P. brasiliensis* can elicit humoral immune response against gp43, remaining with high titles of antibodies and that this animal species could be an epidemiologic indicator of paracoccidioidomycosis. **Financial support:** CAPES, FUNDAÇÃO ARAUCÁRIA, CNPq E FUNDAÇÃO FACULDADES LUIZ MENEGHEL

03.004 - PARACOCCIDIOIDOMYCOSIS - INFECTION IN DOGS SEROPOSITIVE AND SERONEGATIVE TO LEISHMANIOSIS

Silveira, L. H.[1]; Camargo, Z. P.[2]; Ono, M. A.[3]; Domingos, I. H.[4]; Kouchi, K.[5]; Silva, E. A.[6]; Landgraf, V. O.[7]; Werneck, S. M.[8]

[1]Fundação Faculdades Luiz Meneghel - Patologia Geral; [2]Universidade Federal de São Paulo - Microbiologia e Imunologia e Parasitologia; [3]Universidade Estadual de Londrina - Ciências Patológicas; [4,5,6]Prefeitura Municipal de Campo Grande - Centro de Controle de Zoonoses; [7,8]Fundação da Saúde do Mato Grosso do Sul - LACEN - Laboratório Central

Introduction and Objectives: Paracoccidioidomycosis (PCM) is a systemic mycosis endemic in Latin American countries. The etiologic agent *Paracoccidioides brasiliensis* is a thermodimorphic fungus that grows as yeast in the host or at 37 ºC and as mycelia at 25 ºC. The individuals that develop PCM are mainly male agricultural workers. The granulomatous lesions are frequently observed in lungs, lymphonodes, spleen, liver, skin and mucosa. Probably infection occurs by fungus propagule inhalation (J. Med. Vet. Mycology, 23:323-334,1985). The ecoepidemiological aspects of PCM remains poorly understood. *P. brasiliensis* was isolated from frugivorous bats [*Sabouraudia* 4:124-125, 1965], penguin [Inst Venez de Invest Cient (IVIC), Abstract B-2, 1989] and armadillos (Med Mycol; 38(3): 193-199,2000). Recently the first case of natural PCM in dogs was reported [Medical Mycology 42: 379-383, 2004]. Taking into account that endemic areas for PCM can be endemic for other diseases that affect dogs as leishmaniosis. The aim of this study was to evaluate the infection by *P. brasiliensis* in dogs seropositive and seronegative to leishmaniosis. **Methods and Results:** Sera from 836 dogs (449 positive and 387 negative to leishmaniosis) were analysed by ELISA and immunodifusion test using gp43 and crude *P. brasiliensis* exoantigen respectivelly. The analysis of the 836 serum samples by ELISA and immunodiffusion test showed a positivity of 67.8% and 7.3%, respectively for *P. brasiliensis* infection. The dogs positive to leishmaniosis (n= 449) showed a higher reactivity to gp43 (79.95%) and to exoantigen (93.5%) by ELISA. Four out 61 dogs seropositive by ID test were examined for clinical signs of paracoccidioidomycosis. One dog showed cough and dyspnea and the other ones showed haematological alterations. Pulmonary discrete radiological alterations were observed in two dogs. **Conclusion:** The higher reactivity to *P. brasiliensis* antigens may be due to a cross-reactivity or a co infection of dogs by *Leishmania sp* and *P. brasiliensis*. The lower correlation (0.040) observed between reactivity to gp43 and *Leishmania* antigen reinforce the latter hypothesis. Probably the dogs seropositives by ID were in an initial phase of PCM with very discrete signs of disease. Probably dogs at higher risk of infection by *Leishmania* also are more exposed to *P. brasiliensis* infection. Unfortunately the following of a greater number of animals was not possible taking into account that the dogs positive in leishmaniosis test were killed. The results of this study suggest that co infection of dogs by *P. brasiliensis* and *Leishmania* are occurring. The association between these diseases in dogs is very interesting taking into account that both pathogens are controlled by a cellular immune response. Additional studies are necessary to elucidate the possible association between PCM and leishmaniosis in dogs. **Financial support:** CAPES, FUNDAÇÃO ARAUCÁRIA, CNPq E FUNDAÇÃO FACULDADES LUIZ MENEGHEL

03.005 - NEGATIVE IMMUNODIFFUSION IN PARACOCCIDIOIDOMYCOSIS PATIENTS´ SERUM ANALYZED BY IMMUNOBLOTTING ASSAY

Da Silva, D. F.[1]; Assis, C. M.[2]; Zamboni, I. M.[3]; Oliveira, L. E.[4]; Vicentini, A. P.[5]
[1,3]Instituto Adolfo Lutz - Laboratório de Imunodiagnóstico das Micoses-Seção de Imunologia ; [2]Instituto Adolfo Lutz - Pós-Graduação; [4]Instituto Adoldo Lutz - Laboratório de Imunodiagnóstico das Micoses-Seção de Imunologia; [5]Instituto Adolfo Lutz - Laboratório de Imunodiagnóstico das Micoses-Seção de Imunologia , São Paulo, Brasil. E-mail: dfragata@yahoo.com.br

Introduction and Objectives: Paracoccidioidomycosis (PCM), the main systemic mycosis in Brazil, requires long-term and high-cost treatment. A definitive diagnosis of PCM includes direct observation of the characteristic multiple budding cells in biological fluids and tissues sections or isolation of the fungus from clinical materials. For cases in witch *P. brasiliensis* remains undetected in direct examination, serological assays have been used. Double immunodiffusion test (DI) is used routinely by clinical laboratories, due to its easy procedures, its high specificity and its sensitivity (65-100%). The purpose of this work was to evaluate two serological tests: double immunodiffusion and immunoblotting in immunodiagnosis of PCM. **Methods and Results:** We evaluated by immunoblotting assay (IB) 23 serum samples from patients with clinical confirmation of PCM, all of them with negative DI results against metabolic antigen from 113 isolate of *P. brasiliensis*. For IB assay we employed soluble component cell wall outer surface of *P. brasiliensis* (SCCWOS of Pb) from 113 isolate of *P. brasiliensis* cultivated at 36°C in Fava-Neto´s agar medium for 5 and 10 days as well as for comparative DI assay. Among the 23 serum samples analyzed by DI, 11 (47.8%) were negative and 12 (52.1%) were positive against SCCWOS of Pb obtained from 5 and 10 days, however, by IB assay, all serum (100%) were positive and reacted strongly to gp43 and gp70. **Conclusion:** Our results had demonstrated that the use of an immunoenzimatic assay, significantly improve the sensitivity of PCM immunodiagnosis and also suggest that at least two serological tests for antibody detection should be adopted in cases of questionable diagnosis. **Financial support:** Instituto Adolfo Lutz (Projetos # 107/97, # 13/02, # 05/04) and CAPES.

03.006 - STABILITY ANTIGENS OF *PARACOCCIDIOIDES BRASILIENSIS*: COMPARISON OF THE REACTION PROFILE PARACOCCIDIOIDOMYCOSIS PATIENTS SERUM AMONG RECENTLY PRODUCED ANTIGENIC PREPARATIONS AND ANTIGENIC PREPARATIONS PRODUCED 15 YEARS AGO

Da Silva, D. F.[1]; Assis, C. M.[2]; Zamboni, I. M.[3]; Benard, G.[4]; Vicentini, A. P.[5]
[1,3]Instituto Adolfo Lutz - Laboratório de Imunodiagnóstico das Micoses-Seção de Imunologia; [2]Instituto Adolfo Lutz - Pós-Graduação; [4]USP - Laboratório de Alergia e Imunologia Clínica e Experimental, Faculdade de Medicina; [5]Instituto Adolfo Lutz - Laboratório de Imunodiagnóstico das Micoses-Seção de Imunologia. São Paulo, Brasil. E-mail: dfragata@yahoo.com.br

Intoduction *P. brasiliensis* (Pb) is a dimorphic human pathogenic fungus that causes paracoccidioidomycosis (PCM), the most prevalent deep-seated mycosis in Latin America, occurring predominantly in Brazil, Venezuela and Colombia. PCM presents a broad spectrum of clinical and pathological manifestations reanging from asymptomatic pulmonary infection to severely disseminated disease. Serum antibodies against antigens of *P. brasiliensis* constitute one of the major diagnostic indivators of PCM, and their detection is especially important in cases of patients with crypitc lesion in internal organs. In addition, serologic procedures have proven valuable in monitoring the PCM patient´s response to treatment. **Methods and Results:** We analyzed the reaction profile, employing double immunodiffusion assay (DI), of 30 serum samples of patients' with PCM against nine differents antigenic preparations of Pb: somatic antigen (AgSo) and cell free antigen (AgCFA), produced from 113 and B-339 isolates, cultivated in Fava-Netto's agar medium for 7 days at 36°C and metabolic antigen (AgM), produced from 113 and B-339 isolates, but cultivated in NGTA liquid medium for 20 days at 36°C; soluble component cell wall outer surface of *P. brasiliensis* (SCCWOS of Pb) from 113 isolate cultivated at 36°C in Fava-Neto´s agar medium for 5,10, 15 and 20 days, antigen Pb113 Negroni and antigen Pb113 NGTA, cultivated for 20 days, at 36°C. The AgSo, AgCFA and AgM were made two years ago and the others were produced 15 years ago. By ID assay, we observed that the reaction profile of the patients' serum with PCM was 90% to AgSo and SCCWOS of Pb with 5, 10, 15 and 20 cultivation days; 86.6% to CFA; 83.3% to AgM; 80% to Ag 113 NGTA and 76.6% to Ag 113 Negroni. **Conclusion:** The results' analysis confirms that specificity and sensitivity of the DI are closely linked with the kind of antigenic preparations used. The results also support, the previous datas showed, related the antigenic stability of SCCWOS of Pb, which demonstrate the same reaction profile of the AgSo recently produced. **Financial support:** Instituto Adolfo Lutz (Projetos # 107/97, # 13/02, # 05/04) and CAPES.

03.007 - EVALUATION OF *HISTOPLASMA CAPSULATUM* SOLUBLE ANTIGEN FOR SEROLOGICALS REACTIONS

Freitas, R. S.[1]; Assis, C. M.[2]; Martins, J. E. C.[3]; Zamboni, I. M.[4]; Vicentini, A. P.[5]
[1]Instituto de Medicina Tropical - Laboratório de Micologia Médica-LIM53; [2]Instituto Adolfo Lutz - Pós-Graduação; [3]Instituto de Medicina Tropical - Laboratório de Micologia Médica-LIM 53; [4]Instituto Adolfo Lutz - Laboratório de Imunodiagnóstico das Micoses-Seção de Imunologia; [5]Instituto Adolfo Lutz - Laboratório de Imunodiagnóstico das Micoses, São Paulo, Brasil. E-mail: roselifreitas403@hotmail.com

Introduction and Objectives: *H. capsulatum* (Hc) is the etiologic agent of histoplasmosis (HP), a mycosis that principally affects the lungs. Proven diagnostic modalities include cultures, fungal stains of tissues or body fluid, and tests for antibodies and antigens. The aim of this study was to obtained a mycelial-phase soluble antigen of Hc as well as evaluate the usefulness of this antigenic preparation in standards serologicals reactions. **Methods and Results:** Soluble antigens were obtained from the RP, 49, 200, 212, 268, 299, 340, 361, 406, 584 802 and 2030 Hc isolates cultivated at 27ºC on Sabouraud-dextrose agar for 15 and 33 days. After incubation mycelial cells were suspended in aqueous solution of thimerosal (1;5000) at room temperature for 24 h. Antigens were concentrated by lyophilization procedure and stored at -20^0 C. The specificity and sensitivity of the different Hc soluble antigens were evaluated, using the immunodifusion (ID) assay against a panel of sera from: HP patients (illness or infection); individuals with clinical suspicion of HP but non-reactive with Hc reference antigen by ID; paracoccidioidomycosis, aspergillosis and leishmaniasis patients; sera anti-exoantigens of Hc, *P. brasiliensis and A. fumigatus* and reference positive control serum anti-Hc (H and M fractions). Moreover, the electrophoretic profile of those antigens were analyzed by SDS-PAGE and the immunoreactivity, by immunoblotting (IB). Through ID, it was verified that the 20-fold concentrated soluble antigens presented reactivity only against serum anti-Hc and anti- H and M Hc fractions, being observed the presence of H and M bands, and also against sera from patients with HP infection or illness. The best pattern of reactivity was observed for antigens obtained with 33 days of culture from the isolates 200 and 406 and for the antigen 200 with 15 days of culture. The analysis of the electrophoretic profile by SDS-PAGE disclosed great proteic complexity, presenting antigenic components of apparent molecular mass from 17 to 119 kDa. Through the IB, it was observed intense reactivity of the sera from patients with HP (infection or illness) against epitopes with molecular mass from 74 to 119 kDa. It is noteworth that the antigen from sample 200, with 15 or 33 days of culture, presented the best pattern of recognition against the homologous sera. **Conclusion:** The results suggest the employment of the soluble antigen from sample 200 in the ID assay due to its good capacity to discriminate both sera from patients with HP illness and HP infection, besides its high specificity (100%) against heterologous sera. **Financial support:** Adolfo Lutz Institute (Projetos # 107/97e # 06/04)

03.008 - MORPHOLOGICAL AND IMMUNOCHEMICAL CHARACTERIZATION OF *PARACOCCIDIOIDES BRASILIENSIS* SAMPLES ISOLATED FROM *DASYPUS NOVENCINCTUS* AND HUMAN CLINICAL SAMPLES

Oliveira, L. E.[1]; Assis, C. M.[2]; Freitas, R. S.[3]; Zamboni, I. M.[4]; Vicentini, A. P.[5]
[1]Instituto Adoldo Lutz - Laboratório de Imunodiagnóstico das Micoses-Seção de Imunologia; [2]Instituto Adolfo Lutz - Pós-Graduação; [3]Instituto de Medicina Tropical - Laboratório de Micologia Médica-LIM53; [4]Instituto Adolfo Lutz - Laboratório de Imunodiagnóstico das Micoses-Seção de Imunologia; [5]Instituto Adolfo Lutz - Laboratório de Imunodiagnóstico das Micoses-Seção de Imunologia, São Paulo,Brasil. E-mail: larryend@yahoo.com.br

Introduction: Paracoccidioidomycosis (PCM) is a deep mycosis prevalent in Latin America. In Brazil, PCM has been the 8th reason of morbidity among the chronic infectious diseases and it mainly affects male agricultural workers. Epidemiological records demonstrate that the habitat of *P. brasiliensis* (Pb), has remained undefined, though *D. novencinctus* armadillos have been considered as PCM sentinels animals because they have lived in PCM endemic areas. These animals have been recognized as a natural host to Pb on account of the finding of fungi samples isolated from armadillo internal organs. **Objective:** To perform morphological and immunochemical characterization of samples of Pb isolated from *D. novencinctus* and from human clinical samples in order to get the antigens potentially applicable for PCM immunodiagnosis. **Materials and Methods:** Pb was isolated from 3 human clinical samples (Bot1, Bot2, and Bot3) and from 4 armadillos (Imr1, Imr2, Bot3/1 and Imr3/1). Yeasts were cultivated into Fava Netto agar medium at 36ºC for 5, 10, 15 and 20 days, according the procedure described by Kaufman & Standard (1978) and Assis (1990). Antigens reactivity profiles were determined by means of immunodiffusion assay (ID) applying each antigen sample at 3mg/mL protein concentration, and assaying specific and heterologous polyclonal antibodies, and Pb antibody positive patient serum samples (titre of 16). Fungal morphologic features were following the methodology reported by Riddel (1950). **Results:** Antigens yield from samples Imr1, Imr2, Bot1 and Bot2 concentrated by vaccum-dried technique revealed best reactivity pattern when assayed with anti- Pb exoantigen polyclonal serum sample (titres of 16, 32, 128, and 256), and anti- Pb gp43 antibody sample. Morphological analysis revealed that the majority of sample presented cottony texture except the Bot3 sample which showed a coriaceous aspect. **Conclusions:** The discovery of P.b infected armadillos *D. novencinctus* has been of great importance for studies on ecoepidemiology, in order to delineate areas presenting the occurrence of infected people and risk factor for PCM. For decades ID and Western blot techniques have successfully been employed for detecting anti-Pb antibodies for routine diagnosis. Although some difficulties have been found in producing this fungal antigens. Therefore, it has been demanded to conduct a study on Pb immunochemical characterization, and also to evaluate the rates of sensitivity and specificity of serological assays employing the produced Pb antigens and analyzing PCM positive patients from both endemic and non-endemic areas. **Financial support:** Adolfo Lutz Institute (Projeto CTC # 107/97 e #13/02)

03.009 - PRELIMINARY STUDY IN THE IDENTIFICATION AND CHARACTERIZATION OF NEW SEROLOGICAL MARKERS TO HISTOPLASMOSIS

Pizzini, C. V.[1]; Almeida-Paes, R.[2]; Albuquerque, P. C.[3]; Bailao, A. M.[4]; Soares, C. M. A.[5]; Medeiros, A. I.[6]; Sá-Nunes, A.[7]; Faccioli, L. H.[8]; Zancope-Oliveira, R. M.[9]

[1,2,3,9]Instituto de Pesquisa Clínica Evandro Chagas - FIOCRUZ - Serviço de Micologia-DEMIP ; [4,5]Universidade Federal de Goiás - Bioquímica e Biologia Molecular ; [6,7,8]Faculdade de Ciências Farmacêuticas de Ribeirão Preto - USP - Análises Clínicas, Toxicológicas e Bromatológicas

Introduction and Objective: Histoplasmosis, a systemic fungal disease caused by *Histoplasma capsulatum*, is one of the most common systemic mycoses in Brazil where epidemiological surveys carried out using the histoplasmin skin test indicate that this mycosis is endemic in all surveyed areas. Definitive diagnosis of histoplasmosis is still reliant on the visualization of the organism and/or isolation of the fungus in culture; these methods are time-consuming and lacking in sensitivity. Serological tests, which consist in useful tools for the detection of either antibodies and/or antigen in clinical fluids specimens (such as serum, urine and liquor), have been developed and they offer a rapid alternative in order to diagnosis of histoplasmosis. The H and M glycoproteins constituents of the culture filtrate of *H. capsulatum* are considered pluripotent antigens that elicit both humoral and T-cell mediated immune responses, and suitable molecules to diagnosis of histoplasmosis. However, the characterization of new antigenic proteins and their heterologous production will allow a broader spectrum of molecules to be used in the diagnosis of histoplasmosis. In an attempt to contribute to the discovery of useful biomarkers for its diagnosis and therapeutic monitoring new potential antigens are studied for our group. An immunoproteomic approach was taken to separate and identify proteins from a crude extract of *H. capsulatum*. **Methods and Results:** In this study, the SDS-PAGE, Western blot test and the 2D electrophoresis was used to analyze this antigen. A protein profile composed by 7 bands was identified in silver stained SDS-PAGE gels with molecular masses ranging from 117 to 14,7 kDa. When these gels were analyzed by W*estern blot* probed with human serum from mycollogically confirmed histoplasmosis, positive reactions occurred with protein profiles ranging in size (120, 110, 60, 57, 44, 32 kDa). There were not reactive bands pattern in the negative control. After immunoproteomic approach (2D electrophoresis) 16 spots were observed in *western blot* test, 80 kDa (pI 6.2), 70 kDa (isoforms of 6.3, 6.5), 68 kDa (pI 4.8), 66 kDa (pI 7.0), 63 kDa (pI 7.1), 57 kDa (isoforms of 6.1, 6.8), 50 kDa (pI 6.5), 45 kDa (pI 5.0), 43 kDa (isoforms of 5.7, 6.8), 30 kDa (pI 6.9), 25 kDa (isoforms of 6.0, 6.2, 6.4). **Conclusion:** Further studies using proteomics tools must be done in order to achieve a possible relationship of such proteins as a new serological marker and its application in immunological diagnostic assay. **Financial support:** CNPq and FAPERJ

03.010 - IMMUNOPROTEOMIC IDENTIFICATION OF SPECIFIC ANTIGENS OF THE DIMORPHIC FUNGUS *SPOROTHRIX SCHENCKII*

Almeida-Paes, R.[1]; Pizzini, C. V.[2]; Pimenta, M. A.[3]; Bailao, A. M.[4]; Soares, C. M. A.[5]; Peralta, J. M.[6]; Zancope-Oliveira, R. M.[7]

[1,2,3,7]Fiocruz-Instituto de Pesquisa Clínica Evandro Chagas - Serviço de Micologia-DEMIP; [4,5]Universidade Federal de Goiás - Bioquímica e Biologia Molecular; [6]Universidade Federal do Rio de Janeiro - Instituto de Microbiologia Prof. Paulo de Góes

Introduction and Objective: Sporotrichosis is the most prevalent subcutaneous mycosis in Brazil, especially in Rio de Janeiro State, where an outbreak, related to zoonotic transmission from infected cats to human patients has been described. Although the definitive diagnosis of this infection requires the isolation of the etiologic agent in culture, serologic evidence of these fungal infection is important since the isolation of *Sporothirx schenckii* is time-consuming and lacking in sensitivity, mainly in unusual clinical manifestations of the disease. In an attempt to contribute to the discovery of useful biomarkers for its diagnosis and therapeutic monitoring, we embarked on a mapping of *S. schenckii* immunogenic proteins specifically recognized by antibodies produced during the natural course of this infection. **Methods and Results:** An immunoproteomic approach was taken to separate and identify proteins from an aqueous extract from yeast form. About 154 protein spots were identified in silver stained gel with molecular mass ranging from 110kDa to 11 kDa. The most prominent protein species were those of 70 kDa (isoforms of 5.0 and 5.5), 60 kDa (pI 4.8), 50 kDa (pI 4.7), 39 kDa (isoforms of 5.3 and 5.4), 35 kDa (pI 4.6), 30 kDa (pI 4.8), 25 kDa (pI 5.0) and 13 kDa (pI 6.8). The protein species of 13 kDa (pI 6.8 and 7.1), 42 kDa (pI 6.4), 50 kDa (pI 4.7), 53kDa (pI 5.1), 70 kDa (pI 5.0 and 5.5) were reactive to sera of infected patients, but not with sera from patients with paracoccidioidomycosis, histoplasmosis or American tegumentary leishmaniasis. **Conclusions:** The use of proteomics can provide useful information on the antigenic make up of this fungus and the purification and characterization of these specific proteins can improve the differential diagnosis between sporotrichosis and other related diseases.

03.011 - ANTIBODY DETECTION IN SPOROTRICHOSIS USING MYCELIAL PHASE *SPOROTHRIX SCHENCKII* EXOANTIGENS IN AN ELISA TEST

Almeida-Paes, R.[1]; Pimenta, M. A.[2]; Reis, R. S.[3]; Monteiro, P. C. F.[4]; Pizzini, C. V.[5]; Zancope-Oliveira, R. M.[6]

[1,2,3,4,5,6]Fiocruz-Instituto de Pesquisa Clínica Evandro Chagas - Serviço de Micologia-DEMIP

Introduction and objective: Infections caused by *Sporothrix schenckii* have increased in recent years, especially in immunocompromised patients. *S. schenckii* can cause either limited cutaneous lesions or invasive, disseminated infections. Systemic sporotrichosis may be due to bloodstream dissemination from a cutaneous lesion or conidia inhalation. Risk factors, such as alcoholism, diabetes, use of immunosuppressive drugs and chronic granulomatous disease may predispose to severe infections, including pulmonary and osteoarticular sporotrichosis. The diagnosis of these clinical manifestations is often difficult and the therapeutic follow-up of patients is usually made by clinical findings, without any knowledge about their immunologic responses against the fungus. In order to solve these questions, we have developed an immunoassay for the serodiagnosis of sporotrichosis. **Methods and results:** An enzyme-linked immunosorbent assay was developed as a method for specific antibody detection in serum specimens of sporotrichosis patients. The assay was made with mycelial phase *Sporothrix schenckii* exoantigens and tested against 90 sera from patients with several clinical forms of sporotrichosis. Cross reactivity was analyzed with 72 heterologous sera from patients with paracoccidioidomycosis, cryptococcosis, aspergillosis, histoplasmosis, tuberculosis and American tegumentary leishmaniasis, as well as 76 sera from healthy controls. Sensitivity of 97% and specificity of 89% were observed in this assay. These parameters were higher than previously published data relating the use of ELISA in serodiagnosis of sporotrichosis. Also, we have observed that all cutaneous forms of this disease responded well in the described ELISA, indicating the useful of this assay in the serodiagnosis of sporotrichosis, especially in cutaneous forms that are not promptly diagnosed with immunoprecipitation or agglutination techniques described up to now. **Conclusion:** These results suggest that the ELISA using mycelial phase *S. schenckii* exoantigens is a very sentitive diagnostic tool that could be applied to the serodiganosis of sporotrichosis.