PANEL SESSION 2
DIAGNOSIS AND EPIDEMIOLOGY

02-01

EVALUATION OF CLINICAL SPECIMENS FOR DIAGNOSIS OF DIFFERENT CLINICAL FORMS OF PARACOCCIDIOIDOMYCOSIS

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The definitive diagnosis of paracoccidioidomycosis (PCM) is usually made by a combination of microscopic examination and culture. It has been described the preferential use of some clinical specimens in the diagnosis of some mycoses. For instance, the findings of yeast in sputum by direct examination is of no diagnostic value to candidiasis. In PCM, several biological specimens are used in the routine laboratory identification of *Paracoccidioides brasiliensis*. However, a systematic evaluation of the most valuable specimen for mycological examination in different clinical forms of PCM has not been described up to now. In this work, 318 clinical samples obtained from 115 patients at Instituto de Pesquisa Clínica Evandro Chagas–FIOCRUZ, during 5 years, were subjected to mycological examination (KOH mount and culture). Evaluation of different clinical specimens as pus, sputum, biopsy fragments, ulcers scrapings, bronchoalveolar lavage (BAL) showed pus from draining lymph nodes was the preferential clinical specimen to confirm the diagnosis of all clinical forms of paracoccidioidomycosis by the direct examination. For the juvenile form, the diagnostic yields from KOH smears of biopsy and ulcer scrapings were 100% Cultures of pus from lymph nodes obtained of patients with disseminated form, and scrapping of the lesions from juvenile PCM showed 78.57% and 100% sensitivity, respectively, for *P. brasiliensis* isolation. Sputum is the most frequent specimen used for the mycological diagnosis of the chronic pulmonary form. However, both methods, direct examination (54%) and culture (25.95%), show low positivity. Therefore, the testing of serial specimens would increase the accuracy of the diagnosis of chronic pulmonary PCM. Pus and ulcer scrapping must be preferentially used in the definitive diagnosis of PCM.

02-02

DETECTION OF CIRCULATING ANTIGEN (GP43) OF Paracoccidioides brasiliensis IN CEREBROSPINAL FLUID OF PATIENTS WITH NEUROPARACOCCIDIOIDOMYCOSIS AND IN BRONCHOALVEOLAR LAVAGE BY INHIBITION ELISA

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Paracoccidioidomycosis (PCM) is a systemic mycosis caused by the dimorphic fungus *Paracoccidioides brasiliensis* with a spectrum of clinical forms. The most common clinical presentation is the chronic form of the disease and central nervous system involvement occur in about 10% of cases, although such cases are uncommon in the literature. Detection of circulating antigens is very useful mainly when detection of antibodies is not possible such in immunocompromised patients. The test of the inhibition ELISA was standardized to detect and to quantify circulating antigens (gp43) of *P. brasiliensis*, using MAb anti-gp43, in 14 samples in cerebrospinal fluid (CSF) of patients with neuroparacoccidioidomycosis (11 of these patients provided a sample of serum); 13 other patients provided bronchoalveolar lavage samples (BAL). The test was able to detect circulating antigen (gp43) in 100% of BAL and CSF samples, with mean antigen concentration of 16.6 microgram/ml and 17.4 microgram/ml, respectively. In the patients with neuroparacoccidioidomycosis, the antigen concentrations in CSF were higher their in the respective sera. The sensitivity and specificity of the test was 100%. These results indicate that the inh-ELISA is effective to detect circulating antigens (gp43) in BAL and CSF samples and that it may be useful to supplement the diagnosis of the neuroparacoccidioidomycosis and pulmonary paracoccidioidomycosis.

Supported: FAPESP

02-03

DETECTION OF Paracoccidioides brasiliensis-GP43 CIRCULATING ANTIGEN IN SERA FROM PATIENTS WITH PARACOCCIDIOIDOMYCOSIS (PCM) BY INHIBITION ELISA. COMPARISON WITH ANTIBOBY DETECTION IMMUNODIFFUSION TEST

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Techniques for detection of circulating antigens in patients with systemic mycosis are important as complementary diagnostic, mainly when conventional antibody detection tests are inefficients. Several tests to circulating antigen detection have been studied in PCM. However, all of them present low sensitivity and specificity. In this study we standardized the inhibition ELISA test to detect and quantify *P. brasiliensis*-gp43 circulating antigen in 57 samples of sera obtained from patients with comproved paracoccidioidomycosis (by direct and/or serologic test); 33 sera from patients with histoplasmosis, 20 sera from patients with cryptococcosis and 93 sera from healthy individuals (blood donors), were used as control. The test employed monoclonal antibody anti-gp43 (Mab anti-gp43). Our results showed that the test was able to detect gp43-circulating antigen in 94.7% of sera, with antigen mean concentration of the 7.75 microgram/ml. The sensitivity and specificity of the test was of 94.7% and 96.8%, respectively. The sera were also test for antibody detection by immunodiffusion. In 87.7% of the sera was possible to detect antibody. No cross-reaction with heterologous sera was observed. Three human normal sera reacted as positive (false-positive).Theses results indicate that the inhibition ELISA is effective in the detection of circulating antigen (gp43) in sera from patients with paracoccidioidomycosis and that it may be used as complementary diagnosis.

Supported by FAPESP

02-04

EPIDEMIOLOGICAL ASPECTS OF PATIENTS WITH PARACOCCIDIOIDOMYCOSIS DIAGNOSED AT THE INSTITUTO ADOLFO LUTZ, SÃO PAULO

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This study shows the epidemiological profile of PCM patients followed at the Instituto de Infectologia Emílio Ribas with confirmed serological diagnosis by double immunodiffusion assay performed at the Laboratório de Imunodiagnóstico das Micoses do Instituto Adolfo Lutz. We evaluated 86 medical records of patients with positive serology for PCM, the following were analyzed: sex, age, race, habits, visits to rural areas, clinical forms, occupation, associated diseases, and anti-fungal therapy. Of the 86 records, 85% were of males and 15% females. Females showed higher incidence of the disease in the age bracket of 10 to 30 years and males 30 to 60 years. 45.3% percent of these patients were Caucasian, 8.1% Half-caste, 3.5% Black, and 2.3% Oriental. We observed that 54.6% of patients were smokers; 32.5% alcoholics; 12.8% chewed small twigs; 4.65% had ingested armadillo meat; 3.48% used illegal drugs; and 54.6% had visited or lived in rural areas. 50% percent of patients presented chronic multifocal; 34.9% chronic unifocal and 12.7% severe forms. In relation to associated diseases, 19.7% showed parasitosis, 21% Tb, and only one patient was HIV+ (1.2%). The PCM patients occupation were somehow related to agriculture. Analysis of anti-fungal therapy revealed that the most used treatment drugs were sulfonanide derivates and amphotericin B. The above-described epidemiological characteristics are similar and corroborate literature findings. The 6:1 ratio of males and females can be explained by the fact that most studied males occupation was related to agriculture, which favors *P. brasiliensis* infection. It should be emphasized, however, that the presence of b-estradiol receptors in fungal cells protects women from infection, even adult women in contact with rural areas. Other factors that favor infection are closely related to smoking and drinking. Reports of individuals who hunt armadillos, deserves special attention, mainly because these animals are considered as *P. brasiliensis* reservoirs.

02-05

WHOLE ANTIGEN VS PARTIAL PROTEIN ANTIBODY RESPONSE IN ACUTE AND CHRONIC PARACOCCIDIOIDOMYCOSIS

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Paracoccidioidomycosis (PCM) is a sistemic granulomatosis disease caused by *Paracoccidioides brasiliensis* (Pb). This disease is endemic in the majority of Latin American countries. The majority of infected and asymptomatic individuals show a subcutaneous reaction to Pb antigens. The individuals with symptoms show one of the two clinical principal forms of the disease. The acute or sub-acute (juvenile) form involving the reticuloendothelial system, and the chronic form (adult type) which is predominantly pulmonary, with or without muco-cutaneous involvement. The profile of the imunoglobulins isotypes produced against the soluble Pb antigens (whole Pb antigen), and also those produced after the treatment with sodium meta-periodate to destroy the carbohydrate epitopes (protein Pb antigens) were evaluated in this work. Sera from 15 patients presenting the acute form of PCM, 14 with the chronic form of PCM, 5 patients showing Leishmaniosis or Chagas disease, and 5 normal individuals was tested by ELISA against the whole Pb antigen and against the protein Pb antigen. The results show, in the sera from both acute and chronic PCM patients, a higher reactivity of the immunoglobulins (total IgG, IgM, IgA, IgG1, IgG2) when whole Pb antigen total was used, in comparison to the Pb protein antigenic preparation ($p<0.05$). However, the IgG total and IgM production was higher in the sera from acute form patients when partial protein was used ($p<0.05$). The production of IgA was greater in the sera of chronic PCM patients when the whole Pb antigen was used ($p<0.05$). The production of IgG1 and IgG2 by acute and chronic PCM patients showed no significant difference, but the production of these isotypes by patients with PCM was much higher when compared with control individuals ($p<0.05$). There was no significant difference between acute and chronic patients with relation to the production of IgG3 and IgG4. The analysis by western blotting of the specific isotype IgG1 against isolated antigen protein of Pb, indicate that the patients presenting the acute form have sera immunoglobulins capacity to bind antigens that show molecular weights of approximately 19, 27 and 31 KDa.

02-06

ATTEMPTED ISOLATION OF *Blastomyces dermatitidis* FROM NORTHERN WISCONSIN, USA SHREWS

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Paracoccidioides brasiliensis has been isolated from armadillos. We attempted isolation of *Blastomyces* from shrews, North American ground dwelling insectivores. Thirty-nine masked (*Sorex cinereus*) and 13 northern short-tailed (*Blarina brevicauda*) shrews were collected in Northern Wisconsin using pitfall traps, 1998-2001, kept frozen for various intervals, then necropsied. Cultures of nasopharynx, lungs, liver, spleen and intestines were placed on yeast extract phosphate agar with NH_4OH. Cultures for Blastomyces were negative from all 52 shrews; and two mice (Peromysus maniculatus) and three voles (*Clethrionomys gapperi*). Minimal contaminating fungi were present on the culture plates when two drops of NH4OH was used. This methodology appears feasible for use in larger studies attempting to isolate Blastomyces from small mammals.

02-07

LEVELS OF ANTI-gp43 IgG, IgG-gp43 IMMUNE COMPLEXES AND SOLUBLE gp43 OF Paracoccidiodes brasiliensis IN EXPERIMENTALLY INFECTED MICE.

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The levels of circulating anti-gp43 antibodies, soluble antigen and immune complexes (IC) have been used for diagnosis and treatment monitoring of paracoccidioidomycosis in human. The present study analyses plasma levels of anti-gp43 IgG, soluble gp43 and gp43-IgG IC and fungemia in 78 experimentally infected ddY mice. The blood samples colected soon after the inoculation and 6, 12, 24, 48 and 72 hours and 5, 7, 10, 14, 17, 21, 24, 28 and 56 days after the infection were analysed by ELISA and by blood culture. The results showed increased levels of IgG anti-gp43 after 14 days with the peak on the 28th and 56th days, increased levels of soluble gp43 and IC on the 28th day after infection and fungemia up to 7 days. This study reports the first detection of soluble gp43 and IgG-gp43 immune complexes in experimental model and also anti-gp43 IgG in ddY mice.The lack of anti-gp43 IgG and fungemia 10 days after infection show the necessity to use them in association with another method for paracoccidioidomycosis diagnosis and treatment monitoring.

02-08

PRELIMINARY PARACOCCIDIOIDOMYCOSIS EPIDEMIOLOGICAL STUDY BY USING SALIVA SAMPLES

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Skin test with paracoccidioidin is the most used test for epidemiological studies of paracoccidioidomycosis (PCM). In this study we used saliva samples to detect anti- *P. brasiliensis* IgG, that could be an alternative to intradermal reaction or serological tests in children. A total of 152 saliva samples were obtained from individuals from Cambé and Rolândia Rural Villages (Paraná State, Brazil), which were grouped as follows: I (0 to 10 years-old), II (11 to 20 years-old), III (21 to 40 years-old), IV (31 to 40 years-old) and V (> 40 years-old. Seventy five serum samples from groups IV and V were also analyzed. Positive controls consisted of 10 serum and saliva samples from PCM patients and 10 serum samples from healthy individuals previously selected from an urban area were used as negative controls. Immunoplates were coated with *P. brasiliensis* exoantigen, incubated with saliva (1/10) or serum (1/200), followed by incubation with mouse anti-human IgG labelled with peroxidase. A total of 1/39 (2.6%) from group I, 1/41 (2.4%) from group II, 1/31 (3.2%) from group III, 1/16 (6.3%) from group IV and 1/25 (4.0%) from group V of saliva samples and 17/75 (22.7%) serum samples reacted against exoantigens. In the control groups, 10 (100%) out of 10 PCM patients, 0 (0%) from 10 negative serum samples and 7 (70%) out of 10 PCM saliva samples were reactive to exoantigens These preliminary results suggest the possibility of using saliva samples as an epidemiological parameter in children, although better results can be obtained with serum samples.

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02-09

IDENTIFICATION OF SPECIFIC B-CELL EPITOPE FROM HSP60 TO IMPROVE THE DIFFERENTIAL DIAGNOSIS OF PARACOCCIDIOIDOMYCOSIS, HISTOPLASMOSIS AND COCCIDIOIDOMYCOSIS

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Members of the heat shock protein (HSP) family participate in several cellular processes and are essential for cell survival. HSP60 has critical cellular functions, including transport of proteins and promotion of folding and assembly of polypeptides. HPS60 also is a major immunodominant antigen in microorganisms and a target of the cell mediated and humoral immune responses to infection. Recently, our group evaluated the immunological reactivity of recombinant HSP60 from *Paracoccidioides brasiliensis* by Western blot. Although HSP60 of *Paracoccidioides brasiliensis*, *Histoplasma capsulatum* and *Coccidioides immitis* showed 90% of homology to each other, the sensitivity and specificity of the test was 97.3 and 92.5%, respectively. The antibodies were likely directed against non-homologous epitopes. Mapping of specific B-cell epitope (s) of HSP60 from three etiologic agents of systemic mycoses would permit selected peptides to develop immunoassays and improve the diagnosis. HSP60 sequence motifs from *P. brasiliensis* (GenBank AF059523), *H. capsulatum* (LI1390) and *C. immitis* (U81786) were aligned by FASTA Program and searched by similarity. A peptide domain comprised of amino acid residues 476 through 485 (LRRISSLVS) presented low homology, and could be an important, specific B epitope. This hypothesis was confirmed by analyses of antigenic index, hydrophobicity, flexibility, surface probability, polarity plots and molecular modeling. Studies are underway to determine the antigenic sites and the applicability on immunoassays.

02-10

THE ROLE OF IMMUNOENZYMATIC ASSAY IN THE FOLLOW-UP OF PARACOCCIDIOIDOMYCOSIS AFTER NEGATIVE RESULTS BY IMMUNODIFFUSION TEST

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The aim of this study was to evaluated the reliability of the double immunodiffusion test (ID), indirect immunofluorescence (IFI) and immunoenzymatic assay (ELISA) in the periods of pre-treatment, during treatment and serological cure of patients with acute and chronic forms of paracoccidioidomycosis (PCM). For each test the following parameters were evaluated: Sensitivity (SE), Specificity (SP), Positive and Negative Predictive Values (PPV and NPV) and Efficiency (EF). In the pre-treatment period these values for IFI were SE=92.3%, PPV=87.5%, NPV=92% and EF=88.6%. For ID: SE=94.9%, PPV=97.4%, NPV=95% EF=96.2%. For ELISA: SE=100%, PPV=95%, NPV=100% and EF=97.4%. In the normal blood control, the values for IFI, ID and ELISA the SP were 93.3%, 100% and 100%, respectively. In patients with other systemic mycosis the SP for these tests were respectively 60%, 90% and 80%. During the period of treatment, the highest correlation was observed between ID and ELISA ($r= 0.61591$, $p<0.05$), and the lowest correlation was observed with IFI. Since the IFI was positive for long periods after treatment (serological scar), we concluded that IFI should be excluded from the serologic monitoring of PCM. ELISA presented negative results in a larger period (Md=20 months) than ID (Md= 13.5 meses). In overall, the ELISA still detected antibody nine months after ID. These results suggest that ELISA could be a referential test to evaluate the time to interrupt PCM treatment, what putatively would prevent relapses. Moreover, its high values of SE, SP, PPV, NPV and EF indicate that the ELISA should be included in the serological routine tests, being an additional criteria of cure of PCM.

02-11

RAPD AS A VALUABLE TOOL FOR IDENTIFYING A SUBGROUP OF Paracoccidioides brasiliensis PRESENTING CLINICAL TRIMETHOPRIM/SULPHAMETHOXAZOLE RESISTAMCE

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Twenty and nine isolates of *Paracoccidioides brasiliensis* obtained from different geographic regions of Brazil (MT, MG, SP, RS states), Peru, Colombia, Venezuela and Argentine were investigated in relation to clinical and *in vitro* trimethoprim/sulphamethoxazole susceptibility. Furthermore DNA profiling of the same strains was done by 05 arbitrary primers (OPA-1, OPA-2, OPA-3, OPA-4 and OPG-14). The DNA amplification patterns obtained allowed the differentiation of all 29 analyzed isolates. The RAPD data were used in a phenetic approach constructed by UPGMA., which showed two major clusters named group I and group II. The first one encompassed only isolates from MT state of Brazil (07 from 11 analyzed) and another grouped the remainder strains including the other isolates from MT (04), the isolates from the other geographic areas of Brazil (13) as well the strains obtained from the distinct South Americas countries (05). These last strains were subgrouped in a specific branch within group II. No correlation between the RAPD patterns and pathological features of the disease, geographical regions or period of isolation of strain was observed. However we detected a interesting association between the strains belonging to group I, wich were isolated from chronic clinical cases never treated before and a high susceptibility to trimethoprim/sulphametoxazole verified *in vitro* and *in vivo*. These results seem to indicate the existence of genetically more related groups of *Paracoccidioides brasiliensis* more susceptible to the sulphas.

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02-12

PARACOCCIDIOIDOMYCOSIS IN PERNAMBUCO, BRAZIL.

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In the Medical Mycology Laboratory, Depto. Mycology, CCB, UFPE, from 1994 to 2002, cases of paracoccidioidomycosis were identified through analysis of clinical specimens sputum, secretion, scarified from mucous membrane and of skin, liquor, bronchial wash, blood, ganglion fragments and the large intestine. Paracoccidioidomycose porter, with 04 to 65 years of age, from male and female subjects. As for their professional fielas it was found that most of them were rural workers, bricrlayers, watchmen and drivers. Mycological diagnosis was called for at detection of *Paracoccidioides brasiliensis* through direct examination and or culture. With regard to the diagnosis preceding the mycological diagnosis, the patients were without diagnosis, diagnosed for tuberculosis or cancer. Referral for mycological examination resulting from mainly to slow or no clinical evolution, in the treetmonts of other ethiologies. The Pbmycosis carrier individual were residents in the state of Pernambuco, Northeastern of Brasil.

02-13

PRESUMPTIVE DIAGNOSTIC OF PARACOCCIDOIDOMYCOSIS IN PERNAMBUCO, BRAZIL

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Serum samples of patients suspected of micosis infection were analysed in the Immunodiagnosis Laboratory of Sistemic Mycosis, Depto. de Micologia, CCB, UFPE, Recife. These individuals had been referred to: Hospital das Clínicas, CCS, UFPE; Hospital Oswoldo Cruz, UPE; Hospital Otávio de Freitas, all the city of Recife. Serum sample residents from the rural area of the municipality of Nazaré da Mata, Pernambuco, were analysed to. Double immunodifusion (ID) and Counter-Immunoelectrophoretic (CIE) techniques were utilized. Antibodies anti-*Paracoccidioides brasiliensis* were found in serum samples by ID and/or CIE. Serum positive cases of Paracoccidioidomycosis was ascertained through direct examination and/or culture. Serology positive cases without confirmation through direct examination and/or culture were regarded as preventive diagnosis for Pbmycosis. The patients analyzed were all residents in the state of Pernambuco, Northeastern of Brazil.

02-14

DETECTION OF SPECIFIC ANTIBODIES TO A 63-kDa COMPONENT OF Paracoccidioides brasiliensis IN INFECTED PATIENTS

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In the last decade a variety of antigens from *Paracoccidioides brasiliensis* (PB) have been characterized and used for the development of different methods for the serological diagnosis of the infection. These methods are mainly based on the employment of purified or recombinant antigens or specific monoclonal antibodies (Mabs). In our laboratory we have obtained a Mab (PB-2C3) specific to a cytoplasmic component of 63 kDa from PB that does not react with an extract from *Histoplasma capsulatum*. Our main goal was to analyze the reactivity of sera from patients with paracoccidioidomycosis (PCM) or other related and unrelated mycosis to this antigen. To this end we employed a ELISA with Mab PB-2C3 capturing its specific antigen from a crude extract from PB. We studied sera from 47 PCM patients (40 with chronic disseminated and 7 with acute PCM), 24 *Histoplama capsulatum* infected patients (HP), 3 suffering aspergillosis (ASP) and 2 with candidiasis (Cd). To establish the cut-off value of the ELISA sera from 20 healthy subjects were used. Results show that serum from 33/47 of PCM patients and 7/24 from HP, were able to recognize the 63 kDa antigen while the rest of sera analyzed were negative. No differential degree of reactivity was observed between chronic and acute PCM subjects. By indirect ELISA with a PB crude extract we observed that 45/47 of PCM, 18/24 of HP and 2/3 of ASP patients were positive. On the basis of these results we are trying to purify and identify this 63 kDa component that could be better than the total extract from PB in the development of serological methods or the diagnosis of PCM.

02-15

CO-INFECTION HIV AND Paracoccidioides brasiliensis: NEW CASES FROM 1998 TO 2001

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Five male patients from 28 to 47 years old, infected by HIV and *Paracoccidioides brasiliensis* were treated in Infectious Parasitic Clinical of Hospital das Clínicas of Faculdade de Medicina of Universidade de São Paulo from 1998 to 2001. Diagnosis of paracocidioidomycosis was made simultaneously to HIV infection in one case, and opportunistic infections were reported in four cases (tuberculosis in two patients, neurotoxoplasmosis in one patient and cryptococosis in another patient). In the first patient, lesions were found in the upper respiratory tract mucosa; in the second in the lungs, lymph node and mucosa; in the third and fourth they were disseminated on skin; in the fifth, in cervical lymph nodes. The fungus was identified in the lesions through mycological or histopathological examinations. CD4 level was decreased in all cases (6, 8, 36, 54 and 125μl) simultaneously to viral load of 350 to 110 000/ml. Anti *P. brasiliensis* antibody levels measured by contraimunoelectrophoresis reaction were low in three cases and high in two other cases. Paracoccidioidomycosis was treated with Amphotericin B in three cases, with sulfadiazine (or cotrimoxazole) in another and with itraconazole in one case, and was followed by improvement and decreasing of the lesions in all cases; in one case the patient was followed during only four months. For maintenance therapy fluconazole or cotrimoxazole or itraconazole was prescribed on four cases. New cases of this above mentioned association have been diagnosed eventhough Brazilian Antiretroviral Program has been successful. Probably these cases occurred in patients without previous diagnosis of HIV or without cotrimoxazole prophylaxis for pneumocystosis. Paracocidioidomycosis should included as a criteria for diagnosis of aids and alternative techniques should be sought for pos-therapeutic control of this co-infection.

02-16

DETECTION OF ANTIBODY TO Paracoccidioides brasiliensis IN DOGS OF A VETERINARY TEACHING HOSPITAL FROM PARANÁ STATE, BRAZIL

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Paracoccidioidomycosis (PCM) is a systemic mycosis that causes granulomatous lesions in several organs as lungs, spleen, liver, skin. Although much progress have been achieved in diagnosis and pathogenesis of PCM, the ecoepidemiology of the ethiological agent, *P. brasiliensis*, is poorly understood. The role of animals in the ecology of *P. brasiliensis* is inconsistent since, except for armadillos, isolations from animals were not reproducible. In a recent study a high positivity to *P. brasiliensis* infection in dogs was shown although attempts to isolate *P. brasiliensis* from seropositive dogs were unsuccessful. In order to evaluate the infection of dogs attended at the State University of Londrina Veterinary Teaching Hospital, in this work one hundred and eight serum samples were analyzed by indirect ELISA and Immunodiffusion test, using gp43 and exoantigen, respectively. The positivity observed in this study was of 25.9% by indirect ELISA although no positive sample was observed by Immunodiffusion test. No significant difference for sex was observed. Considering that Paraná State is an endemic area for PCM and the high positivity to *P. brasiliensis* mainly in dogs from rural areas it is possible that canine PCM may be occurring but are not being diagnosed.

02-17

SEROEPIDEMIOLOGICAL SURVEY OF PARACOCCIDIOIDOMYCOSIS IN DOGS OF RURAL AREAS OF NORTHERN PARANÁ STATE

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Paracoccidioidomycosis (PCM) is the most prevalent systemic mycosis in Latin America, specially in Brazil, that shows the higher percentage of PCM patients. The infection takes place in the lungs and can spread to other organs of the body. Although much progress has been achieved in studies on the diagnosis and pathogenesis of this mycosis, little is known about the ecology of *Paracoccidioides brasiliensis*. In this study 126 serum samples from dogs were analyzed by ELISA and Immunodiffusion test, using gp43 and exoantigen, respectively. Serum specimens of 72 dogs were sampled from three rural areas of Northern Region of Paraná State: Londrina (n = 38), Cambé (n = 21) and Rolândia (n = 13), and as control, 54 serum samples were obtained from Londrina urban dogs with little contact with rural areas. The positivity by ELISA for the rural dogs from Londrina, Cambé and Rolândia was 89.5%, 76.2% and 69.2%, respectively. The control group showed positivity of 13.6%. All serum samples were negative by Immunodiffusion test. These results indicate that *P. brasiliensis* is widely distributed in rural areas from Northern Region of Paraná State and reinforce that dogs could be sensitive epidemiological markers of *P. brasiliensis* in the environment.

02-18

EPIDEMIOLOGICAL SURVEY WITH PARACOCCIDIOIDIN E HISTOPLASMIN IN RURAL AREA OF MUNICIPAL DISTRICT OF NAZARÉ DA MATA, PERNAMBUCO, BRAZIL

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The present work was realized with the purpose of discovering risk factors that favor the infection by *Paracoccidioides brasiliensis* and *Histoplasma capsulatum* in 168 individuals in the rural population of the Municipal district of Nazaré da Mata, Pernambuco, Brazil, through the sensibility test with paracoccidioidin and histoplamin, among 02 to 90 years old. The positivity reactors to paracoccidioidin and histoplasmin were respectivy of 32.7% and 18.7%. Was observed in the factores such sex, habit of chewing small sticks, to do anal toilet with leaves of vegetables, to create dog, cat an fowl in their homes and the kind of occupation had not a significant influence in the positivity to paracoccidiodin and histoplasmin. About the age there was a little predominance in individuals over 30 years old. Was not possible to isolate the *P. brasiliensis* and the *H. capsulatum* of the 12 soil samples and of the 10 samples of excrements of fowls analyzed. The positivity indexes found with paracoccidioidin and histoplasmin suggests exposition of the individuals to the existing fungus in the studied region.

02-19

ECOLOGICAL FEATURES OF THE NINE-BANDED ARMADILLO AND ITS RELATIONSHIP WITH THE PATHOGENIC FUNGUS Paracoccidioides brasiliensis

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Dasypus novemcinctus, commonly designated as nine-banded armadillo, is one of the species of the Family Dasipodidae, (Class Mammalia, Order Xenarthra), that displays the highest geographic distribution, inside the order, from Northern of United State of America, to Tierra del Fuego, Southern of South America. It is a very important biological model to the biomedical research, since it is a reservoir and/or host of numerous infectious agents, e.g. *Micobacterium leprae*. At the end of 1980's *Paracoccidoides brasiliensis*, a pathogenic fungus, was found in some specimens of *D. novemcinctus* opening the possibility of understanding the Paracoccidioidomycosis. In our study, we obtained the spatial distribution of nine-banded armadillo, using the GIS (Geographic Information System) in an endemic area of Paracoccidioidomycosis, located in the Lageado's Farm, countryside of Botucatu – SP - Brazil. Ecological data were obtained by the Transect Method in an area of 10 ha, within a riparian forest, exposed fields, swampy fields, and rural built areas. Number and position of burrows, presence of foraging and the animal trails, in transect line was also obtained. Each animal burrow was geolocated by a Global Positioning System. The preferential habitat of the nine-banded armadillos was studied through the analysis of the concomitant presence of burrows and foraging activities in the different habitats, by X^2 Test. The results showed that *D. novemcinctus* have ecological affinity for riparian forest instead of other habitats ($p < 0.001$). The knowledge obtained from the spatial distribution of these animals, will help to the elucidation the ecological niche of *P. brasiliensis*, as well as in the adoption of preventive measures to avoid infection.

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02-20

USE OF THE RECOMBINANT Paracoccidioides brasiliensis P27 PROTEIN AS AN ANTIGEN IN A DOT BLOT ASSAY USE IN PARACOCCIDIOIDOMICOSIS DIAGNOSIS

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Paracoccidioidomycosis (PCM), is produced by the dimorphic fungus *P. brasiliensis,* and represents one of the most important systemic mycosis in Latin-America. This mycosis preferentially affects working-age farmers, affecting primarily the lungs and disseminating to other organs. PCM diagnosis has been based on microbiological and immunological methods; one of the difficulties of the later is the high level of cross reactivity found among patients with histoplasmosis, partially due to the use of crude antigens, as well as on their high variability. These circumstances makes it difficult to standardize diagnostic techniques in different laboratories. We used a recombinant protein in our search for more reproducible results, based on the recombinant p27 the antigen in a dot blot assay and evaluated its usefulness in diagnosis. In these assays, antibodies present in the sera of patients with PCM, when used pooled or individually, recognized the recombinant protein. No cross-reaction was observed when sera from patients with the following mycoses: hitoplasmosis, aspergilosis, sporotrichosis, cryptococosis and chromoblastomycosis. These results encouraged us to continue evaluating this recombinant protein with a large number of sera from patients affected by other pathologies.

02-21

IMMUNODIAGNOSIS OF PARACOCCIDIOIDOMYCOSIS: EVALUATION OF DIFFERENT ANTIGENIC PREPARATIONS

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Characterization of the antigenic components of pathogenic fungi is of great importance for evaluation of the host immune response. The purpose of this work was to evaluate the reactivity of some yeast-like forms of *Paracoccidioidides brasiliensis* antigens, metabolic (AgM), somatic (AgSO) and soluble (AgS) in serodiagnosis. Three antigenic preparations were obtained from isolates Pb 113 and 339 cultivated in NGTA liquid medium for 20 days and in Fava-Netto's agar for 3 and 7 days respectively. Antigens reactivity were analyzed against 90 paracoccidioidomycosis (PCM) serum samples by immunodiffusion (ID) assay. Reactivity of AgM, AgSO and AgS were 81.1%, 95.5% and 78.9%, respectively. Negative PCM sera were also analyzed employing other *P. brasiliensis* antigenic preparations: 2 culture filtrates from Pb113, one cultivated in NGTA and the other in modified Negroni´s media, both at 36°C for 20 days, as well as soluble component of the cell wall outer surface of *P. brasiliensis* (SCCWOS) from Pb113 cultivated at 36°C in Fava-Netto´s agar for 5, 10, 15 and 20 days. These antigens were obtained 15 years ago. Positivity percentages were 83.3%, 83.3%, 90%, 90%, 93.3% and 90%, respectively. The results with indicate a level of sensitivity and specificity of AgSO and SCCWOS obtained at the 15th day were greater than or similar to those reported with other antigenic preparations of *P. brasiliensis* used for serodiagnosis. In the other hand, the results obtained with antigenic preparations from NGTA, Negroni´s modified medium and SCCWOS attest the enhanced stability property of these biological reagents.

02-22

EXOANTIGENS OF Paracoccidioides brasiliensis

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Paracoccidioidomycosis (PCM) is a systemic mycosis caused by *P. brasiliensis* (Pb) fungus. This study aims to characterize *P. brasiliensis* (Pb) exoantigens and find out their applicability in PCM immunodiagnosis. Exoantigens were obtained from isolates 113 and 339 cultivated for 20 days in NGTA liquid medium at 36°C. Specificity of antigenic preparations was evaluated against sera of patients with different clinical forms of PCM; histoplasmosis and aspergillosis patients sera; sera of healthy individuals as well as *Histoplasma capsulatum*, *Aspergillus fumigatus*, Pb, and anti-gp43 rabitt antisera by immunodiffusion (ID) and immunoblotting. By ID assay we observe that the reactivity pattern of exoantigens from isolates Pb 113 and 339 was 100% against sera of patients with chronic multifocal form. In relation to chronic unifocal form, positivity percentage of exoantigens was 80% and 70%, respectively. Severe form showed 90% specificity. We have not observed antigen cross-reactivity against the studied heterologous sera and antisera. Exoantigen eletrophoretic analysis by SDS-PAGE showed high complexity of protein fractions (>25 to >100 kDa), with large quantities of gp43 and gp70. Immunoblotting showed that sera of patients with different clinical forms of PCM recognized mainly the 43, 50, 60, and 70 kDa antigenic fractions. Our results showed that antigens from isolates Pb 113 and Pb 339 presented high specificity against all clinical forms of PCM. We have concluded that antigens from a single isolate showed the same discriminatory efficacy as the antigenic pool commonly used by different laboratories. One of the explanations for this high discriminatory efficacy is that these isolates present large quantities of glycoproteins gp43 and gp70.

02-23

SOLUBLE ANTIGENS FOR THE SERODIAGNOSIS OF PARACOCCIDIOIDOMYCOSIS

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Paracocidioides brasiliensis (Pb) is a dimorphic fungus responsible for the paracoccidioidomycosis (PCM), one of the most important systemic mycosis in Latin America. The purpose of this work was to study the soluble antigens (AgS) of Pb to be used in serodiagnosis. Strains of Pb 113, 339 and 265 were cultivated at 36°C, using different incubation times (3 and 7 days) as well as agar culture mediums [Fava-Netto's (FN), Sabouraud's (SAB), Sabourad-tiamina-asparagina (STA) and NGTA]. The antigens specificity was evaluated by immunodifusion (ID) and immunoblotting assays against pool of patients sera with multifocal and unifocal chronic forms as well as severe form of PCM. By ID, we observe that reactivity pattern of AgS from isolate Pb113 was 100% against sera of individuals with multifocal and unifocal chronic forms and 50% for severe form. AgS from isolates Pb339 and 265 showed similar reactivity profile against sera of patients with multifocal chronic form (90% and 87%). In relation to unifocal chronic form, positivity percentage of AgS was 70% and 60%, respectively and 50% for severe form. AgS obtained using different incubation times and culture mediums showed distinct pattern of recognition. AgS from isolates Pb 113, 339 and 265 cultivated by 3 days in FN, SAB and STA mediums demonstrated high reactivity against all sera from patients with PCM analyzed. SDS-PAGE analysis revealed high complexity of proteins fractions (25 to >170kDa), showing large quantities of gp43 and gp70. By immunoblotting, we observe that sera of patients with different clinical forms of this mycosis recognized a lot of antigenic fractions besides gp43 and 70. The results obtained by SDS-PAGE and immunoblotting permitted verify the immunogenic capability, sensitivity and specificity of various antigenic fractions. In conclusion, of the three isolates evaluated Pb 113 was found to be excellent for AgS preparation.

02-24

CANINE PARACOCCIDIOIDOMYCOSIS: REPORT ON THE FIRST CASE OF THE LITERATURE

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Paracoccidioidomycosis (PCM) is considered one of the most important systemic mycosis which causes granulomatous lesions in the lungs. It is highly endemic in Brazil and in some Latin American countries. The etiologic agent *Paracoccidioides brasiliesis* (Pb) is a dimorfic fungus which habitat is presently unknown although even it has been isolated in the soil in Brazil, Argentina and Venezuela. The frequency of the infection or disease in domestic or wild animals is rare. The mycosis has been reported, by natural infection, in armadillos and maybe in bats. Some investigations showed positivity for the paracoccidioidin skin test in domestic and wild animals, but without the presence of the disease. We report on a case of a female dog, Doberman, who showed enlargement of the cervical lymph nodes. It is important to stress that the dog had an urban life, she went very rarely to the countryside. The house where it lived, in a hyperendemic area of PCM, had a garden with usual plants, i.g., flowers, grass, and some fruit trees. It is important to point out that the other dog who lived in the same house and shared the same place did not develop the disease and niether the people. After ressection of a superficial lymph node, the diagnosis of PCM was established and the dog was treated with ketoconazol and the nodes disappeared. There was an apparent recurrence, without histopathological confirmation 2 years later, when the dog was sacrificed and not autopsied. The histopathology of the excised lymph node showed tipical alterations related to PCM epithelioid granuloma and great number of multiple budding yeast cells, viable and non viable. The fungal identification was confirmed by imunohistochemistry using a specific polyclonal antibody specific anti-Pb. As in North American blastomycosis, dogs maybe an important protagonist of the natural history of PCM.

02-25

DETECTION BY POLYMERASE CHAIN REACTION OF Paracoccidioides brasiliensis IN LESIONS FROM PATIENTS WITH PARACOCCIDIOIDOMYCOSIS

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Paracoccidioides brasiliensis (Pb) is a dimorphic fungus, whose growth occurs in two different forms in two different temperatures. It is the etiological agent of paracoccidioidomycosis (PCM), a deep mycosis limited to Latin America. In Brazil the states with highest incidence are: Sao Paulo, Bahia, Minas Gerais, Rio de Janeiro and Parana. The PCM is a systemic, granulomatous, chronic and recurrent disease. The Polymerase Chain Reaction (PCR) is a modern technique with relevant application in health care and it has been used with success in analysis of several microorganisms. The aim of this project was to standardize a PCR technique to detect the presence of the fungus in paraffin embedded tissue using primers based on the gp43 gene and to compare our methodology with another that uses a primer based on the ITS (Internal Transcribe Space) region. Then we correlated the results with the lesion caused by Pb, regarding some histopathologic features and also the fungal borden. Our main objective was to optimize the diagnosis of this important endemic disease in our country. Our preliminary results were encouranging in the detection of Pb in lesions using PCR with primers based on gp43. Our main difficulty was related to the DNA extraction once the samples (about 125 biopsies) were probably not fixed and processed in such way that DNA could be always recovered in adequate amount.

02-26

PARACOCCIDIOIDOMYCOSIS CLINICAL AND EPIDEMIOLOGICAL PROFILE IN UBERABA, MG 1990-2000

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INTRODUCTION: Paracoccidioidomycosis is endemic in Latin America, mainly in Brazil, where 80% of the cases of this disease that attacks principally individuals in the productive period of life are registered. In South, Southeast and Center-West regions a greater number of cases is recorded. This report describes the epidemiological, clinical and evolutive features of patients with this mycosis in Uberaba. METHODS: Medical prontuaries, records from pathology and microbiology laboratory were reviewed in order to identify the patients diagnosed with paracoccidioidomycosis from 1990 up to 2000 in the Hospital Escola of Faculdade de Medicina do Triângulo Mineiro. RESULTS: In this period,104 patients were diagnosed. Of these, 91 (87.5%) were male, aged between 25 and 54 years. Agricultural workers were identified in 40 (38.4%) cases. Regarding the origin, 76 (73.0%) patients were from Uberaba and Triângulo Mineiro, MG. Among the 104 cases, 10 (10.4%) positive for HIV, and six of these died. The signs and symptoms time of evolution was =B3 6 months in 62 (59.6%) of the patients. Hyporexy, ponderal loss, fever, cough, dyspnea and lymphadenomegaly were the clinical findings more frequently observed. The diagnosis was performed through histopathology in 90 (86.5%) of the cases. The adult chronical phase was identified in 86 (82.6%) of the patients. Sulphonamide and imidazolic by-products were the therapies more used. Thirty-nine (37.5%) were treated in < six months and 38 (36.5%) between 6 and 24 months. Clinical healing was verified in 68 (65.3%) and 22 (21.1%) relapsed. COMMENTS: The features described above are similar to those of other small centers that treat these patients. The high percentage of diagnosis of this mycosis through histopathology, the regular adhesion to the treatment and the relapse high rate of this disease are emphazised.

02-27

SEROLOGICAL DETECTION OF PARACOCCIDIOIDOMYCOSIS IN DOGS FROM THE ENDEMIC AREA OF BOTUCATU SP, BRAZIL

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The natural occurrence of Paracoccidiodomycosis (PCM) infection in animals has not been properly studied despite of its importance in the disease eco-epidemiology. Dogs have been considered harbinger animals of Blastomycosis, caused by *Blastomyces dermatitidis*, a dimorphic pathogenic fungus closely related to *Paracoccidioides brasiliensis* (Pb). Dogs are the closer animals in contact with man in daily activities and furthermore have smelling and digging habits. However, a possible involvement of dogs in PCM infection had not been well analysed. This study aimed to evaluate the presence of PCM infection, using a serological approach (ELISA), in dogs from the rural area of the Botucatu, SP, Brazil - a "Cuesta" region, with distinct ground planes (600 and 800 m of medium altitudes). Tests were standardized in order to obtain the optimal concentration and/or dilution of conjugate, antigen and serum, as well as the cut-off determination, which was considered as the mean Optic Density (O.D.) plus three standard deviation of a group of healthy urban animals. Briefly, plates were sensitized with 20 microgram/ml of soluble antigen pool of Pb (isolated from armadillos) and the optimal dilution of serum and conjugate were 1/200 and 1/2000, respectively. Each serum was evaluated in quadruplicate, and data were expressed as P/S values, which means the relation between the sample and two known controls, a positive (an immunized dog) and a negative one. Among 275 evaluated animals, 73 (26.5%) were PCM-infection positive. PCM infection in males (30%) was higher than in females (19%), and also higher in animals from the lowest area (29.9%) than in the highest one (22.6%) of the "Cuesta". Our data contribute with further information to a better understanding of the importance of this pathogen in Veterinary Medicine and to elucidate the places of environmental occurrence of *Paracoccidioides brasiliensis*.

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