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PCM

International Conference
on Paracoccidioidomycosis

Challenges & Milestones in the 21st Century

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International Conference on Paracoccidioidomycosis: Challenges and Milestones in the 21st Century, from December 10-13, 2024, in Campo Grande, Mato Grosso do Sul.

Paracoccidioidomycosis (PCM) is a neglected disease that was recently formally recognized by the World Health Organization (WHO). As we move into the 21st century, we continue to face challenges that require innovative solutions.

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PCM 001- MITOCHONDRIAL GENOMA OF THE HUMAN PATHOGENIC FUNGUS *PARACOCCIDIODES RESTREPIENSIS*.

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ABSTRACT-Introduction. *Paracoccidioides restrepiensis* is a thermodimorphic fungus, which know etiologic agent of paracoccidioidomycosis, a systemic mycosis endemic in Latin America. Different assemblies of the other species within the genus have been reported. Although its nuclear genome has been studied in depth, updates on its mitochondrial genome mtDNA are less frequent despite its significance in cellular energy production, apoptosis and pathogenicity. However, in *P. restrepiensis*, this study has not yet been conducted. **Objective.** This study aims to provide obtain an assembly for comprehensive data on the structure, gene content, and potential functional implications of the mitochondrial genome of *P. restrepiensis*. **Methodology.** A DNA was extracted from clinical isolates (Strains Pb339 and ATCC 60855) was carried out and sequenced with the Oxford Nanopore technology MinION (Flow Cell FLO-MIN114). Subsequently, a BLASTn was run and a hybrid assembled of Pb339 was performed with Spades v3.13.0 to obtain the number of contigs of mitochondrial DNA, bioinformatics analyses was performed to annotate the mitochondrial genes and compare them to mtDNA sequences. **Results.** The mitochondrial genome of Pb339 was found, containing two contigs of length 84703 bp and 122449 bp with a coverage of 2511x and 607x respectively, including protein-coding genes, tRNAs and rRNAs. The structure showed high similarity to *Paracoccidioides brasiliensis* and the strain ATCC 60855 it's in process of analysis. **Conclusions.** This new mitochondrial genome data for *P. restrepiensis* enhances our understanding of its biology, opening avenues for exploring its role in pathogenicity, potentially generate alternative treatment options.

Keywords: Mitogenome, mitochondria, *Paracoccidioides, restrepiensis*, NSG

PCM 002 - WHOLE GENOME SEQUENCING OF *PARACOCCIDIOIDES RESTREPIENSIS* ISOLATES USING THIRD-GENERATION SEQUENCING TECHNOLOGY

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ABSTRACT-Introduction. *Paracoccidioides* spp. is a thermomorphing fungus responsible for Paracoccidioidomycosis, a mycosis restricted to Latin America. The species *P. restrepiensis* has been found almost exclusively in Colombia. Genomes have enabled a deeper understanding of the structure, function, and taxonomy of the genus *Paracoccidioides*; however, the genome of *P. restrepiensis* remains incomplete due to technical limitations of the initial sequencing with Illumina technology. Objective. The aim of this study is to evaluate the complete genome of *P. restrepiensis* isolates using Oxford Nanopore (ONT) sequencing technology. Methodology. Three *P. restrepiensis* isolates (Pb339, ATCC60855, PbCNH) were selected, and DNA extraction was performed using the Phenol:Chloroform:Isoamyl Alcohol method. Libraries were prepared with the SQK LSK109 kit and sequenced on an ONT MinION (R10.4.1 flow cell). The raw reads were analyzed with FastQC and assembled de novo with Flye. The assembly quality control was performed using Quast. Results. In *P. restrepiensis* Pb339, 390,163 raw reads were obtained with a median Q score of 16, producing 1,183 fragments; the largest was 338,261 bp, with a genome size of 28.8 Mb. The N50 was 54,521 bp, and the coverage was 15X. For isolate ATCC60855, 250,688 raw reads (median Q score of 27) generated 250 fragments; the largest was 1,118,864 bp, with a genome size of 30.02 Mb. The N50 reached 294,824 bp with an 18X coverage. For PbCNH, 426,624 raw reads (median Q score of 26) yielded 90 fragments; the longest was 4,926,958 bp, with a genome size of 29.8 Mb. The N50 was 1,870,312 bp and coverage 33X. ONT-sequenced isolates are being polished with Illumina reads to improve quality. Conclusions. The reference genome of *P. restrepiensis* can be updated using the sequences obtained with ONT. These assemblies will help to complete missing or fragmented information from the reference genome of this fungal pathogen.

Keywords. *Paracoccidioides*, *restrepiensis*, sequencing, genome, NanoPore.

PCM 003- THE EPIDEMIOLOGY OF HISTOPLASMOSIS IN THE CAPITAL OF BRAZIL, BRASÍLIA-DF

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Abstract - 1. Introduction. Histoplasmosis is an endemic mycosis in Brazil with a high prevalence among people living with HIV/AIDS. Despite its high mortality among immunocompromised patients, the disease is underrecognized by healthcare authorities and is only notifiable in the state of Goiás. Additionally, the epidemiology of histoplasmosis is unknown in Distrito Federal. Since the 1970's, two outbreaks have been reported in this area. Along with autochthonous cases, this suggests that *Histoplasma* sp. is endemic to this region. Moreover, as no genotyping has been completed on isolates circulating in Distrito Federal, it is unknown which species of *Histoplasma* exist in this region. **2. Objective(s).** Our study aims to perform a molecular epidemiology study of histoplasmosis in Distrito Federal and surroundings. **3. Methodology.** We carried out a retrospective study on histoplasmosis cases in Distrito Federal to gather clinical and sociodemographic data from medical records in the Hospital Universitário de Brasília and Laboratório Central de Saúde Pública do Distrito Federal. Demographic data were used to map histoplasmosis cases through the ArcGIS software. Three clinical isolates were sequenced on Illumina HiSeq 2500 and NextSeq 550 platforms. The NASP pipeline was used to call SNPs and a Maximum Likelihood (ML) phylogenetic tree was constructed using IQ-TREE v2.1.1. **4. Results.** We identified 34 cases of the disease in Distrito Federal and surrounding areas spanning 1994 to 2022. Additionally, we observed a lethality rate of 43% among people living with HIV/AIDS. Lastly, phylogenetic approaches revealed that two isolates nested within the *H. suramericanum* Northeast BR clade, while the other clustered within the LAmB1 clade. **5. Conclusions.** This work represents the first contribution towards understanding the epidemiology of histoplasmosis in Distrito Federal and surroundings.

Keywords: histoplasmosis, Distrito Federal, *Histoplasma*, epidemiology, HIV.

PCM 004 - MOLECULAR DIAGNOSIS OF PARACOCCIDIOIDOMYCOSIS IN FFPE SAMPLES: ADVANCES IN SURVEILLANCE AND CONTROL WITHIN THE BRAZILIAN HEALTHCARE SYSTEM

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ABSTRACT - 1. Introduction. Paracoccidioidomycosis (PCM), caused by *Paracoccidioides* spp., is the most prevalent systemic mycosis in Brazil. Its diagnosis typically involves correlating clinical manifestations with histopathological identification of fungal structures. However, morphological similarity between makes it challenging to differentiate between several fungal species based solely on histopathological features, leading to potential misdiagnosis or delayed treatment. **2. Objective.** We propose the use of a fiable conventional polymerase chain reaction (cPCR) to detect species-specific agents, *Paracoccidioides brasiliensis* and *Paracoccidioides lutzii*, in formalin-fixed paraffin-embedded (FFPE) tissue samples. **3. Methodology.** For assay standardization, we used two reference strains (*P. brasiliensis* - 1320 and *P. lutzii* - 8334) and a clinical FFPE sample (ID: 1093227) from a cervical lymphadenectomy of a PCM patient. Two primer sets were tested: one targeting exon-2 of the *gp43* gene (Plu-F/Plu-R) and the other targeting the ITS2/28S rDNA region (Paracoco-F/Paracoco-R). cPCR products were sequenced (Sanger) and aligned with sequences in GenBank and MycoBank. **4. Results.** The Paracoco-F/Paracoco-R primers generated a 143 bp amplicon in all samples, while Plu-F/Plu-R produced a 142 bp amplicon exclusively for the *P. lutzii* strain. Sequencing of the ITS/28S rDNA region from *P. brasiliensis* and the clinical sample showed 99% similarity with *P. brasiliensis* sequences. The *P. lutzii* strain showed 100% similarity with *P. lutzii* sequences and 99% similarity with the exon-2 region of the *gp43* gene. **5. Conclusions.** PCM was added to Brazil's notifiable disease list in April 2024 due to its significant mortality from fungal infections. This cPCR assay shows potential to improve diagnosis, especially in cases with negative serology but positive direct examination. Further validation of this method is ongoing to confirm its effectiveness as a complementary tool to histopathology, enhancing PCM surveillance and control in the Brazilian Unified Health System (SUS).

Keywords: Paraffin Embedding, Paracoccidioidomycosis, Molecular Diagnostic Techniques, Polymerase Chain Reaction.

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PCM 005 - CECAL APPENDICITIS AS A RARE MANIFESTATION OF PARACOCCIDIOIDOMYCOSIS: A CASE REPORT AND SYSTEMATIC REVIEW OF THE LITERATURE

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ABSTRACT - Introduction: Paracoccidioidomycosis (PCM) is a systemic mycosis endemic to Latin America, with the acute/subacute form predominantly affecting children and young adults. It involves the mononuclear phagocyte system, including gut-associated lymphoid tissues. However, cecal appendicitis caused by *Paracoccidioides* spp. is rarely reported. To describe the clinical manifestations and evolution of a case of cecal appendicitis due to PCM and to conduct a systematic literature review on the topic.

Case: A case report and systematic literature review were conducted using Embase, Web of Science, LILACS, MEDLINE, LIEPCS, PubMed, SciELO, and gray literature using PRISMA as writing guide. Articles describing confirmed cases of appendicitis due to PCM were selected. **Discuss:** We present the case of a 20-year-old male with generalized lymphadenopathy who was diagnosed with PCM and treated with oral trimethoprim-sulfamethoxazole. After the initial improvement, the patient returned with clinical deterioration. The treatment was changed to liposomal amphotericin B. Six days later, the patient developed an acute abdomen and underwent exploratory laparotomy with appendectomy. Histopathological examination confirmed acute granulomatous appendicitis due to PCM, and the patient showed postoperative clinical improvement. Of the 11 identified articles included in the systematic review, most case reports with a low risk of bias were found in countries as Brazil, Peru and Venezuela. Five patients had confirmed appendicitis due to PCM through biopsy, while others had confirmed PCM at another site. Two patients were initially misdiagnosed with Crohn's. Most studies have reported favorable outcomes after adequate treatment. Appendicitis caused by PCM is rare, even in endemic countries. It has a benign course when properly treated with both clinical and surgical management. This should be considered in the differential diagnosis of acute abdomen with lymphadenopathy. Furthermore, the importance of the study to alert the occurrence of appendicitis due to PCM, especially in young patients in endemic countries.

Keywords: Paracoccidioidomycosis, *Paracoccidioides brasiliensis*, appendicitis, appendectomy, acute abdomen.

PCM 006-EPIDEMIOLOGICAL, CLINICAL, AND MICROBIOLOGICAL ASPECTS OF RARE FORMS OF PARACOCCIDIOIDOMYCOSES

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ABSTRACT - 1. Introduction. Paracoccidioidomycosis (PCM) is an endemic systemic fungal disease in Latin America caused by the *Paracoccidioides* spp. The most common form is chronic, typically presenting with exuberant lung involvement and skin and mucocutaneous lesions. However, the involvement of some other systems has been described, such as the eyelid, genital, and osteoarticular systems. **2. Objective.** Evaluate rare forms' epidemiological, clinical, and microbiological aspects of PCM. **3. Methodology.** This investigation is a retrospective cross-sectional study, including patients with rare forms of PCM) assisted at the Hospital Cassiano Antônio de Moraes of the Federal University of Espírito Santo between January 1987 and December 2020. A confirmed PCM presented clinical manifestations compatible with *Paracoccidioides* spp. in secretions or lesions. The laboratorial diagnostic was performed by direct mycological examination, double immunodiffusion, histopathological examination, or culture. **4. Results.** Thirty-three patients with rare forms of PCM (eyelid, genital, and osteoarticular) were included in this study. Ten patients were affected by the genital form, eleven by the eyelid form, and twelve by the osteoarticular form. All patients with eyelid or genital involvement had the chronic form, while in the osteoarticular involvement, eight patients (66.6%) had the acute/subacute form. Smoking (60.60%) and alcohol intake (60.60%) were quite frequent. For the cases of osteoarticular PCM, the preferred site of involvement was the vertebrae, followed by the knees and scapulae. For genital PCM, epididymis, testicles, prostate, penis, and scrotal pouch were all affected. **5. Conclusions** Know the aspects of rare forms of PCM that can improve the clinical management of this disease. These results indicate the importance of PCM as a differential diagnosis in endemic areas of other diseases, such as leishmaniosis, sporotrichosis, skin neoplasms, and tuberculosis. Such analyses can indicate trends in how the disease manifests in practice, guiding clinical reasoning for a more accurate diagnosis.

Keywords: Rare forms of Paracoccidioidomycosis, Eyelid Paracoccidioidomycosis, Genital Paracoccidioidomycosis, Osteoarticular Paracoccidioidomycosis.

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PCM 007 - PATIENTS WITH ACUTE/SUBACUTE PARACOCCIDIOIDOMYCOSIS IN AN ENDEMIC REGION OF SOUTHEASTERN BRAZIL: APPROACH EPIDEMIOLOGICAL AND CLINICAL

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ABSTRACT – 1. Introduction. The literature holds few descriptions of acute/subacute paracoccidioidomycosis (PCM). **2. Objective(s).** We aimed to synthesize epidemiological, clinical, laboratorial, and therapeutic data of patients with acute/subacute PCM assisted by a reference center for PCM care to improve our understanding of this disease. **3. Methodology.** This descriptive study reports a series of cases of acute/subacute PCM, consisting of patients treated at HUCAM/UFES from 1978 to 2020 who were diagnosed based on clinical-epidemiological history and confirmed by laboratorial tests. Clinical and epidemiological data were subsequently collected from the patient's medical records. We statistically analyzed the data by tracing the frequencies and distributions of the main variables used to characterize the sample. **4. Results.** We included 86 cases of acute/subacute PCM. The median age was 22 years, predominantly male, with a male-female ratio of 4.06:1. Smoking (33%) and alcohol intake (31%) were infrequent. The most common acute/subacute manifestation was adenomegaly (86/86, 100%). Other common manifestations were weight loss, fever, hepatomegaly, skin lesions, and splenomegaly. Laboratorial presentation included anemia (60/79, 76%) and eosinophilia (27/79, 35%). The majority of patients presented positive results in histopathology (78/86, 91%) and serology (47/63, 75%) but not on direct microscopic examination (37/86, 43%). The main options for treatment were Amphotericin-B (AmB) alone and a combination of sulfamethoxazole-trimethoprim (SMT-TMP) with AmB. Data on the regularity of treatment were obtained from 47 patients (55%), 35 (74%) reported regular treatment, and 14 were treated for >18 months. Eighteen patients (37%) progressed with total regression of diseases. **5. Conclusions.** Acute/subacute PCM usually presents in young adults with adenomegaly, weight loss, and fever. Positive results were commonly found in histopathology and serology. Mortality was high, and treatment with AmB followed by an antifungal agent such as SMT-TMP or itraconazole may decrease mortality.

Keywords: Acute paracoccidioidomycosis, paracoccidioidomycosis, endemic fungi

PCM 008- MAPPING OF NEW CASES OF PARACOCCIDIOIDOMYCOSIS IN THE STATE OF SÃO PAULO BY THE REFERENCE LABORATORY

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ABSTRACT- Introduction: Paracoccidioidomycosis (PCM) is a endemic systemic mycosis that affects the lungs and can spread through the lymphatic and hematogenous routes to adjacent organs and tissues. Its etiological agents are fungi belonging to the genus *Paracoccidioides*. Despite its high endemicity in the state of São Paulo, it was only in April 2024 that PCM was included in the state's list of notifiable diseases. **Objective:** From January 2020 to July 2024, the Adolfo Lutz Institute in São Paulo mapped new cases of PCM. **Methodology:** Information's were obtained through secondary data. **Results:** PCM's new cases were defined as any patient with serological reactivity to *P. brasiliensis* antigens for the first time by the double immunodiffusion technique in the Mycoses Immunodiagnosis Laboratory. We recorded 497 new cases in the state, with the regions of Campinas and Sorocaba having the highest numbers (124/497 and 101/497, respectively), establishing themselves as major endemic areas. There was a higher incidence among males (400/497) than females (97/447), ages ranging from 3 to 86 years. Of this total, 20 individuals were aged 15 years or under, 14 were males, and 6 were females. The majority of individuals were white, followed by brown and black people. The titer of circulating anti-*Paracoccidioides* spp. antibodies ranged from undetectable to 1024. **Conclusion:** This survey does not represent the total number of new cases in the state, because some public universities and private laboratories also offer the test. However the identification of new PCM cases by Adolfo Lutz Institute, the LACEN of São Paulo state, combined with sociodemographic data, will assist Epidemiological Surveillance in identifying areas with endemic potential. This will also aid in defining and implementing prevention and control measures for this mycosis, which predominantly affects rural workers, there by contributing to the reduction of morbidity, mortality, and sequelae caused by the disease.

Keywords: Paracoccidioidomycosis, Paracoccidioides, Disease notifications

PCM 009 - ANALYSIS OF VIRULENCE FACTORS IN EXTRACELLULAR VESICLES RELEASED BY *PARACOCCIDIOIDES BRASILIENSIS* PB18 AND ITS ATTENUATED VARIANT

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ABSTRACT – 1. Introduction. Extracellular vesicles (EVs) are structures involved in cargo delivery to the extracellular environment, protected by a bi-layered membrane. They can play key roles in signaling between pathogens and hosts, as well as between microorganisms. Previously, we reported that vEVs from *Paracoccidioides brasiliensis* vPb18 isolate, originally virulent, could restore the expression of virulence traits related to oxidative and nitrosative stress in attenuated aPb18 upon aPb18/vEV co-incubation. In parallel, vEVs and aEVs (from aPb18) differentially stimulated murine macrophages in vitro, but both exacerbated the mice infection under our prophylactic vaccination model both exacerbated the mice infection. **2. Objectives.** This study aimed to compare the vEV and aEV proteomes with focus on potential virulence factors. **3. Methodology.** EVs were isolated by ultracentrifugation after other steps of differential centrifugation (Octaviano et al., 2022). Proteins were trypsin-digested and the resulting peptides were analyzed via nano LC-ESI-MS/MS. Protein analysis used the *P. brasiliensis* Pb18 database (UP000001628) and label-free quantification. Differential abundance was determined using a p-value < 0.05 and a log₂ fold-change threshold of ≤ -0.38 or ≥ 0.38. **4. Results.** Among the 870-900 validated EV proteins, 121 are potential virulence regulators, with 57% detected uniquely or more abundantly in vEVs. Compared to aEV proteins, the differentially represented vEV sequences formed a far greater number of interactions in the interaction network analysis. Moreover, enrichment analysis (FDR ≤ 0.05) highlighted 22 proteins involved in cellular stress responses (including 14 related to oxidative stress and six heat-shock-protein homologs) and 21 related to signal transduction (11 of the MAPK-signaling-pathway); the ABC multidrug transporter SidT detox stress protein was unique to vEV. **5. Conclusions.** Using the virulent/attenuated *P. brasiliensis* model, our results expanded the concept that virulence factor transport may be another mechanism by which EVs contribute to pathogenicity and might induce phenotypic changes in recipient fungal cells.

Keywords: virulent/attenuated *Paracoccidioides brasiliensis*, extracellular vesicles, proteome, virulence factors.

Support: CAPES, CNPq (Universal 408843/2021-7), and FAPESP (2022/11123-5)

PCM 010 - HETEROLOGOUS EXPRESSION OF THIOREDOXIN REDUCTASE FROM *PARACOCCIDIOIDES LUTZII* AS A TARGET FOR NOVEL THERAPEUTICS

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ABSTRACT – 1. Introduction. Thioredoxin system is composed of two principal proteins: thioredoxin (Trx) and thioredoxin reductase (TrxR). This system is tasked with the control of oxidative stress and the maintenance of intracellular redox balance. In fungi, this system serves as a virulence factor, enhancing the ability of these microorganisms to survive under stress conditions. A comprehensive understanding of these proteins' structure and function is essential for developing new therapeutic targets, especially against *Paracoccidioides* spp. **2. Objective(s).** The objective of this study was to establish a standardized protocol for the production and purification of a high-quality recombinant TrxR protein from *Paracoccidioides lutzii*, with the aim of conducting studies to elucidate the three-dimensional structure of this protein in complex with inhibitors. **3. Methodology.** Competent *E. coli* BL21 DE3 and BL21 DE3 pLysE cells transformed with the pet21a(+) plasmid containing the *Paracoccidioides lutzii* TrxR sequence were induced with IPTG (Isopropyl-β-D-1-thiogalactopyranoside) for approximately 6 hours. Samples were collected from different clones, and the most productive ones were purified by nickel affinity chromatography, employing varying imidazole concentrations and optimized wash steps. **4. Results.** The *E. coli* BL21 DE3 clone was selected for TrxR production, with induction carried out for 6 hours using IPTG (1 mM). Optimal purification results were obtained with four washes using a wash buffer containing 80 mM imidazole. The resulting protein concentration was 2.12 μM from a single purification. SDS-PAGE analysis of the purified samples showed a prominent target band, free from contaminants. **5. Conclusions.** The production of *Paracoccidioides lutzii* TrxR through heterologous expression is a significant step toward elucidating this protein's native structure. This advance will facilitate studies on its interaction with promising antifungal molecules, contributing to the design and refinement of new specific antifungal drugs.

Keywords: Thioredoxin system, protein purification, *Paracoccidioides lutzii*, recombinant protein

Support: CAPES, CNPq, FINEP and FAPDF.

PCM 011 - ASSESSMENT OF ENZYMATIC HYDROLYSIS OF THE GP43 GLYCOPROTEIN BY ELASTASE

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1. Introduction: Glycoprotein GP43 is an important component of the cell wall in the *Paracoccidioides* spp. fungal complex, playing a crucial role in the interaction between the fungus and the immune system. It has been shown that GP43 induces NETs, and that P4 and P10 induce the formation of amyloid fibrils, which are implicated in NETs induction (unpublished data). By understanding the enzymatic breakdown of GP43, we can gain valuable insights into how this fungus interacts with different cells, potentially leading to new treatment strategies. **2. Objective:** The primary objective of the study was to identify the formation of peptide fragments (P4 and P10) resulting from the enzymatic action of human elastase on GP43, one of the most important enzymatic components in NETs. **3. Methodology:** The study employed several methodologies, including nanodrop spectrophotometry for protein quantification, gel electrophoresis, and liquid chromatography-tandem mass spectrometry (LC-MS/MS) to analyze the resulting peptide fragments after GP43 enzymatic digestion. **4. Results:** Key findings indicated that elastase effectively hydrolyzed GP43, producing specific peptide fragments and cleaving P4 and P10. In contrast, the enzyme myeloperoxidase showed no activity on GP43, serving as a control. The results suggest that elastase's action on GP43 induces the cleavage of P4 and P10, which may contribute to amyloid fibril formation. These fibrils are known to trigger the release of neutrophil extracellular traps (NETs), possibly explaining the mechanism of NETs induction in paracoccidioidomycosis. **5. Conclusions:** The research provides significant insights into the enzymatic interactions with GP43, enhancing our understanding of the immune response to *Paracoccidioides*. This study may guide future therapeutic approaches for infections caused by this pathogenic fungus.

Keywords: *Paracoccidioides* ssp, GP43, Human Elastase, Myeloperoxidase, Peptides.

Support: PIBIC - CNPq.

PCM 012- NEW CLINICAL AND GENOMIC INSIGHTS INTO PARACOCCIDIOIDOMYCOSIS IN PARAGUAY: A NEGLECTED ENDEMIC AREA

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ABSTRACT – 1. Introduction. Paracoccidioidomycosis (PCM) is a prevalent systemic mycosis in Latin America, with a high incidence in Brazil, Argentina, Venezuela, and Colombia. Despite its geographical proximity to these endemic areas, PCM in Paraguay is often overlooked, with over 400 cases reported but limited information on circulating *Paracoccidioides* species. **2. Objective(s).** This study describes the epidemiological and molecular aspects of PCM in Paraguay from 2014 to 2024. **3. Methodology.** A retrospective analysis was conducted at the Center for Dermatological Specialties (CED) in San Lorenzo, Central state. Clinical and epidemiological data were collected from the Mycology Section's database. Whole-genome sequencing of nine clinical isolates was performed, with single nucleotide polymorphisms (SNPs) identified using the NASP pipeline. A Maximum Likelihood phylogenetic tree was constructed with IQ-TREE v2.1.1 to examine evolutionary relationships. **4. Results.** From 2014 to 2024, 62 confirmed PCM cases were identified at the CED, with a predominance in males and chronic forms. The majority (69%) were agricultural workers, indicating high occupational risk. Pulmonary and mucosal involvement was common, especially in men aged 40-60. Only two female cases were reported. Molecular analysis revealed a predominance of S1b genotypes and the first identification of S1a genotypes in Paraguay. One isolate may represent an evolutionary link between these subgroups, suggesting a unique regional genetic variant. **5. Conclusions** This study provides the first comprehensive epidemiological and molecular characterization of PCM in Paraguay, highlighting a predominance of chronic cases, significant agricultural occupational risk, and previously undocumented genetic diversity among isolates.

Keywords: paracoccidioidomycosis, Paraguay, phylogenomics, epidemiology.

Support: CAPES [concession number 8887.843704/2023-00].

PCM013- IMPACT OF AREA SILENCING ON NITROGEN SCAVENGING AND GROWTH IN *P. LUTZII*

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ABSTRACT – 1. Introduction. Nitrogen is an essential macronutrient, playing a role in the biosynthesis of proteins and nucleic acids, thus supporting fungal budding and tissue invasion. The Nitrogen Catabolite Repression (NCR) is a mechanism that allows the fungus to assimilate nitrogen from non-preferential sources in conditions of depletion of the primary ones. The transcription factor GATA *AreA* is described as the main regulator of this process in the *Paracoccidioides* genus. Its activity modulates a wide set of genes for the scavenging, uptake, and metabolism of non-preferential nitrogen sources under NCR. **2.Objective.** The aim of this work was to assess the growth, viability, cell wall remodeling and gene expression of *AreA*-silenced and wild type strains of *P. lutzii* conditioned to starvation and nitrogen sufficiency conditions. **3.Methodology.** The strains were cultivated in minimal medium supplemented with glutamine and proline. Viability and Cell wall composition were estimated by propidium iodide and calcofluor white staining. Additionally, the expression of *AreA* gene was evaluated by RT-qPCR. **4. Results.** Our findings demonstrated a decreased ability of the *AreA*-silenced strain to grow when cultivated in proline compared to the wild-type. Furthermore, we detected evidence of cell wall changes occurred mainly in the silenced strain in both sources. Additionally, we observed that the *AreA* expression in silenced strain was elevated in non-preferential source. **5. Conclusion.** We propose that the *AreA* transcription factor serves as a regulator of nitrogen scavenging in *P. lutzii*, influencing cell-wall dynamics and yeast growth. Proteomics and transcriptomics' studies are currently underway with the aim of conducting a comprehensive analysis of the role of *AreA* in nitrogen metabolism.

Keywords: Paracoccidioidomycosis; Nitrogen Metabolism; *AreA*.

Support: CNPQ, CAPES, FAPEG.

PCM 014- ACUTE PARACOCCIDIOIDOMYCOSIS APPROACH IN A CHILD, DIAGNOSE AND TREATMENT CHALLENGES: A CASE REPORT

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ABSTRACT – 1. Introduction. Paracoccidioidomycosis is a systemic mycotic disease caused by fungi of the genus *Paracoccidioides*, prevalent in South and Central America. Studies have divided the infirmity in two types, acute/subacute and chronic forms. Approximately ten percent of all cases are included in the former and its representatives are composed of children, teenagers and young adults, therefore it is called juvenile paracoccidioidomycosis. **2. Case.** ALCO, female, 9 years, admitted in Maria Aparecida Pedrossian University Hospital (HUMAP), complaining of fatigue for 15 days and showing fever, hepatosplenomegaly and anemia, without previous comorbidities or pathologic backgrounds. Submitted to antibiotic therapy with no clinical response. After 10 days of hospitalization, putting away other infections and rheumatological diseases, a cervical lymph node biopsy was performed, due to necessity of a different approach, with positive results for *P. brasiliensis*, besides serology test with 1:128 of titration. Liposomal amphotericin B was administered and the patient evolved with hypoalbuminemia and drug associated nephropathy (creatinine raise and hydroelectrolyte disorder). After adverse reaction, sulfamethoxazole-trimethoprim was initiated and clinical improvement and hospital discharge was achieved within 3 months of hospitalization. The patient has been followed up by HUMAP outpatient service ever since. **3. Discussion.** From this case, it should be noticed that paracoccidioidomycosis, even despite the low incidence of its acute form in young population, must be present among the differential diagnoses of diseases that can cause lymph node enlargement and hepatosplenomegaly, such as leukemias and exanthematous diseases, especially in children, because this clinical presentation is frequent, and paracoccidioidomycosis may be present in immunocompetent children. Furthermore, attention should be paid to the possibility of adverse events during or after the administration of amphotericin B, because of its high toxicity rate, as such cases can worsen the condition and result in serious sequelae.

Keywords: paracoccidioidomycosis, children, hepatosplenomegaly, amphotericin B, adverse events.

PCM 016- TRACHEAL INVOLVEMENT IN PARACOCCIDIOIDOMYCOSIS

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ABSTRACT – 1. Introduction: The tracheal involvement in paracoccidioidomycosis (PCM) is rarely observed, with publications as case reports. **2. Objectives:** Evaluation of the prevalence, diagnosis and management of the tracheal paracoccidioidal involvement. **3. Methodology:** PCM patients with tracheal involvement were evaluated based on data from their medical records, and clinically classified according to Mendes et al. (2017). All the patients were submitted to the simultaneous evaluation of the Thoracic Surgery Service. A systematic review was performed using the databases Lilacs, PubMed, Web of Science, Cochrane, Scopus, Embase, and Cinahl,. The findings of our patients were compared with those from the cases reported, using Fisher's exact test and significance at $p \leq 0.05$. **4. Results:** 12 patients from IS were included in the study – all were male, median age of 48.5 years; eight presented the chronic moderate form and the other four, the chronic severe form. The diagnosis of tracheal involvement by PCM was confirmed by histopathological evaluation. The prevalence was 1.9%. Oral mucous membrane (9.1%), larynx (45.5%), lungs (100.0%), skin (18.2%), adrenal glands (36.4%) and lymph nodes (9.1%) were also involved. The characteristics of the 26 patients identified in nine articles from the systematic review were very similar to those of the present study, except by oral lesions - more prevalent and adrenal glands involvement - less prevalent in the published cases. All the patients received antifungal compounds. Six of them, with stenosis <50%, received conservative treatment, 4 received the Montgomery tube, 1 was submitted to laryngotracheoplasty with a 6cm resection, and 1 tracheal orthosis (Dumon). **5. Conclusions:** Tracheal involvement in PCM is observed in the chronic form, has low prevalence and should be treated by infectious diseases specialists associated with thoracic surgeons. In some cases an emergency tracheostomy has to be performed, and others should be submitted to complex mediastinal procedures.

Keywords: Paracoccidioidomycosis, trachea, *Paracoccidioides spp.*, upper airways.

Support: PIBIC – PROPe funding.

PCM 017- PARACOCCIDIOIDOMYCOSIS IN SOUTHERN BRAZIL: REPORT OF TWO CASES

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ABSTRACT – 1. Introduction. Paracoccidioidomycosis (PCM) is a systemic fungal infection with high morbidity due to the prevalence of chronic sequelae, even after treatment. Due to this, *Paracoccidioides* spp. are listed as medium-priority pathogens in the first list of fungal priority pathogens published in 2022 by the World Health Organization. Therefore, aiming to add to data, we reported two cases of PCM diagnosed between 2022 and 2023 at a tertiary hospital in Southern Brazil. **2. Cases.** Patient 1: a 51-year-old male farmer showing dyspnea for the last 6 months, hemoptysis, night sweats, anorexia and a skin lesion on the lower lip presented a history of tuberculosis diagnosis in 2019 with unfinished treatment. Serum and bronchoalveolar samples were collected and PCM was confirmed through mycology culture and IDGA. Patient 2: a 25-year-old male farmer showing lymphadenomegaly, daily fever and weight loss (13 kg) for the last month, in addition to erythematous-violaceous papular skin lesions with central ulceration on the back, abdomen, axilla and temporal region. He presented as history a cervical adenopathy diagnosed as a dental abscess 6 months before. PCM was diagnosed through isolation of *Paracoccidioides* sp. from a skin sample in mycologic culture. Both patients were smokers and tested negative for HIV and syphilis serology. Antifungal therapy was prescribed but patients did not come to the follow-up visits, thus data of disease progression and treatment response were lost. **3. Discussion.** In one year of study, two patients were diagnosed in a hospital previously without any case of PCM reported. This fact, in addition to a long-time patients remained with clinical symptoms without clinical suspicion proves the negligence status of this disease. Our reports are a highlight of the need for continuous education on health measures to foment clinical suspicion, early diagnosis and correct treatment aiming the better prognosis of the patients.

Keywords: Systemic fungal infection, Paracoccidioidomycosis, *Paracoccidioides* spp.

Support: None.

PCM 018- IMPACT OF PARACOCCIDIOIDOMYCOSIS SEQUELAE IN ORAL HEALTH: A CROSS-SECTIONAL STUDY.

Realization:



Supporters:



PCM 019- EFFICACY, EFFECTIVENESS AND SAFETY OF ITRACONAZOLE VERSUS COTRIMOXAZOLE IN THE TREATMENT OF PARACOCCIDIOIDOMYCOSIS

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ABSTRACT – 1. Introduction. Itraconazole (ITC) and cotrimoxazole (CMX) are the primary drugs used to treat Paracoccidioidomycosis (PCM), but their efficacy has been underexplored. **2. Objective.** To compare the efficacy, effectiveness and safety of ITC versus CMX in the treatment of PCM. **3. Methodology.** This quasi-experimental study occurred at the University Hospital/UFMS from Jan/2000 to Dec/2021. Patients with PCM, treatment-naïve or untreated for over six months, receiving either ITC or CMX were included. Those with systemic comorbidities or on drugs that interfere with ITC or CMX kinetics were excluded. The decision to treat with ITC or CMX depended on their availability in the local pharmacy. Treatment used: stage initial and complementary, ITC 200mg 24/24h, CMX 800/160mg 12/12h. **4. Results:** Were included 259 patients: 66 in the ITC group and 193 in the CMX group, with no differences in age, gender or disease form. Initial stage: efficacy was 97.8% (95% CI: 93.6%-100%) for ITC and 96.5% (95% CI: 93.1%-99.9%) for CMX.; and the effectiveness was 68.2% (95% CI: 56.9%-79.4%) for ITC and 58.5% (95% CI: 51.6%-65.5%) for CMX. Median time to clinical cure was 6 months for ITC, 7.8 months for CMX ($p = 0.124$). Complementary stage: efficacy was 86.7% (95% CI: 76.7%-96.6%) for ITC, 95.6% (95% CI: 91.8%-99.4%) for CMX, while effectiveness was 51.1% (95% CI: 37.0%-65.0%) for ITC, 62.8% (95% CI: 53.6%-71.2%) for CMX. Patients on ITC achieved serological cure faster ($p < 0.001$). ITC was safer overall. **5. Conclusions:** ITC and CMX demonstrate similar efficacy and effectiveness in treating PCM, however ITC shows shorter treatment time and a better safety profile.

Keywords: Paracoccidioidomycosis. Treatment. Efficacy. Effectiveness. Safety.

Support: PMA (public policy, humanities, health). FIOCRUZ Mato Grosso do Sul. UFMS.CNPq. CAPES.

PCM 021- ATYPICAL CHRONIC PARACOCCIDIOIDOMYCOSIS. REPORT OF THREE CASES IN PARAGUAY

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ABSTRACT- Introduction. Paracoccidioidomycosis (PCM) is an endemic mycosis in Latin America, caused by dimorphic fungi *Paracoccidioides*. The most common clinical form is chronic multifocal, mainly affecting the lungs in individuals exposed to rural environments. **Case 1.** A 51-year-old male, farmer from San Pedro, presented a one-month history of ulcerative lesions in oral cavity and submandibular adenopathy. A simple CT scan showed a right paracapsular hypodensity and bilateral pulmonary infiltrates. Brain MRI revealed ring-like structures in deep ganglia and the cortico-subcortical junction. Scraping and biopsy of oral mucosa revealed multibudding yeasts, and positive culture for *Paracoccidioides* sp. Diagnosis: chronic PCM with pulmonary, mucocutaneous, and central nervous system involvement. Treatment: Amphotericin B deoxycholate 0.7 mg/kg, total dose 1650 mg, with clinical improvement and discharge with itraconazole 200 mg/day. **Case 2.** A 62-year-old male, a brickmaker from Coronel Oviedo, with a history of myelodysplastic syndrome, two months of fever, and hepatosplenomegaly. RK39, blood cultures, and febrile antigens were negative. No skin lesions or respiratory involvement. During hospitalization, he developed an oral lesion; scraping and serology for *P. brasiliensis* were positive (dilution 1:1). Treatment: Amphotericin B deoxycholate. The patient had poor prognosis, with death after three days of treatment. **Case 3.** A 62-year-old male, farmer from Cordillera, with four months of disseminated cutaneous lesions associated with fever. Viral serology was negative, and there was no respiratory involvement. Scraping showed multibudding yeasts compatible with *Paracoccidioides* sp., confirmed by real-time PCR with pb27 gene amplification. Treatment: Amphotericin B deoxycholate, total dose 1100 mg, with renal and infectious complications, leading to death. **Discussion.** Three cases described in men, with higher incidence in rural areas. Chronic PCM with atypical presentations, with no lung involvement in two of the cases. Of the three patients treated with Amphotericin B, only one showed clinical improvement, highlighting the need for early diagnosis and appropriate management.

Keywords: Paracoccidioidomycosis, Chronic PCM, Amphotericin B

PCM 023- IDENTIFICAÇÃO MOLECULAR E MODELAGEM DE NICHOS ECOLÓGICO DE *Paracoccidioides* sp. E *Coccidioides* sp. NO SEMIÁRIDO BRASILEIRO

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ABSTRACT – 1. Introduction: Paracoccidioidomycosis (PCM) and coccidioidomycosis (CM) are endemic mycoses caused by thermophilic fungi of the genera *Paracoccidioides* and *Coccidioides*, respectively. PCM occurs exclusively in Latin America, from Mexico to Argentina. CM, on the other hand, is endemic to arid and semiarid areas of the American continent. **2. Objective(s):** To identify the presence of *Coccidioides* and *Paracoccidioides* DNA in soil samples collected from armadillo burrows in northeastern Brazil and to understand the geographical limits of both agents. **3. Methodology:** Soil samples were collected from armadillo burrows in 20 cities across northeastern Brazil. Samples were taken from approximately 30 cm inside the burrow entrance, located in native forest areas. DNA was extracted using a commercial soil-specific kit, and molecular identification of the pathogens was performed via nested PCR targeting the ITS region. In the first round of PCR, ITS4-5 primers at the species level were used, while the second round utilized specific internal primers. Ten positive samples for *Coccidioides* and *Paracoccidioides* were sequenced using the Sanger method, and phylogenetic analyses were conducted using the Maximum Likelihood method. **4. Results:** Out of the 296 soil samples collected, 46 (15.5%) tested positive for *Coccidioides* and 87 (29.4%) for *Paracoccidioides*, with 13 samples (4.4%) showing the presence of both fungi. Phylogenetic analysis revealed that eight *Paracoccidioides* samples clustered with soil sequences, while three grouped with clinical samples, all aligning with *P. brasiliensis*. All positive soil samples for *Coccidioides* grouped with clinical sequences of *C. posadasii*. **5. Conclusions:** The molecular detection of these fungi in environmental samples demonstrated high efficacy in identifying their presence, confirming the coexistence of *Coccidioides* and *Paracoccidioides* in northeastern Brazil. This highlights the potential overlap and geographic limits of both pathogens, emphasizing the need for further studies to map their distribution and understand the environmental and epidemiological factors influencing their spread.

Keywords: Paracoccidioidomycosis, Coccidioidomycosis, Molecular Detection, Brazilian semiarid

Support: CNPq

PCM 024- DEVELOPMENT OF A THREE-DIMENSIONAL CELL CULTURE MODEL FOR HOST-FUNGUS INTERACTION OF *Paracoccidioides brasiliensis*.

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ABSTRACT - Monolayer culture has been a well-established standard for in vitro cellular analyses such as cytotoxicity, mutagenicity, genotoxicity, and microorganism infections in disease studies. However, this method presents limitations due to biological discrepancies between monolayer cultures and three-dimensional (3D) living organisms. Studies demonstrate the high similarity between 3D cultures and living organisms, showing their potential for generating complementary data thanks to the increased complexity observed in these models. This study aims to develop a 3D cell culture model encapsulated in a sodium alginate matrix to facilitate host-fungus interaction analyses using *Paracoccidioides brasiliensis* (Pb18) as the infecting organism, previously studied only in monolayer. To provide an advanced 3D model that allows for the investigation of host-fungus interactions and enhances understanding of infectious processes. A suspension of cells was mixed with sodium alginate and subsequently dripped into a calcium chloride solution, creating microencapsulated cell beads. These were monitored daily under phase-contrast and fluorescence microscopy. Parameters such as cell viability (cytotoxicity), metabolic activity, and cell proliferation were assessed using various methods for both cell lines. Following initial characterization, the model was infected with *P. brasiliensis*, and the beads were split for two experiments: i) confirming fungal presence within the matrix and potential cell interaction via confocal microscopy; ii) isolating the fungus from within the beads for culture on an appropriate medium. The proposed model showed low cytotoxicity to A549 cells, increased cell density (indicative of cell proliferation), and maintained viability across most of the culture period. Infection analysis through confocal microscopy confirmed fungal presence within the matrix; the fungus was successfully isolated and cultivated, indicating that extracellular matrix components influence the infection process. The A549 line proved suitable for interaction studies in both 3D and monolayer models. 5. Conclusions. The developed 3D model successfully replicates key aspects of host-fungus interactions, providing an advanced alternative to monolayer cultures.

Keywords: 3D culture, sodium alginate, host-fungus interaction, alginate beads.

Support: CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) and FAPESP (Fundação de Amparo a Pesquisa).

**PCM 025- COMPARISON BETWEEN PCR-RFLP AND SEQUENCING
TECHNIQUES IN THE ANALYSIS OF *PARACOCCIDIOIDES* SPP.
BIODIVERSITY: LIMITATIONS AND INSIGHTS INTO SPECIES AND VARIANT
DIFFERENTIATION**

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ABSTRACT –The study of *Paracoccidioides* spp. faces significant challenges due to limitations inherent in the molecular biology techniques employed. Recently, new species were described whose geographical and genetic distributions were investigated. The phylogenetic studies have revealed that genotypes originally thought to be exclusive in specific regions from South American countries are now being found in other areas of the continent. This finding indicates a broader geographic distribution of these genotypes than previously recognized. To evaluate two molecular biology techniques employed to identify genotypes of *Paracoccidioides* spp. strains from a Brazilian culture collection previously identified only by mycological methods. DNA samples from 35 *Paracoccidioides* spp. strains maintained in a Brazilian culture collection were subjected to amplification and enzymatic digestion with PCR-RFLP of *tub1* gene, followed by sequencing of *gp43* Exon 2 loci. Strains with species identification discrepancies had their *tub1* sequences determined to verify possible nucleotide mutations. The genotypic characterization of *Paracoccidioides* spp. using PCR-RFLP of the *tub1* gene identified 22 isolates as *P. brasiliensis* sensu stricto, two as *P. americana*, four as *P. restrepiensis*, and eight as *P. lutzii*. Sequencing of the *gp43* Exon 2 loci revealed discrepancies in the identification of four *P. venezuelensis* isolates, previously characterized as *P. brasiliensis* sensu stricto by PCR-RFLP of *tub1*. The sequencing of *tub1* from *P. brasiliensis* sensu stricto and *P. venezuelensis* isolates revealed nucleotide differences in the pyrimidine class (C and T) in their sequences, specifically at the position 176 bp. These molecular tools were able to establish the genetic diversity within the *Paracoccidioides* genus, crucial for taxonomy and epidemiology studies. The discovery of *P. venezuelensis* in Brazil, previously thought to be exclusive to Venezuela, highlights genetic connections and evolutionary divergences within the genus. While the PCR-RFLP of *tub1* technique showed limitations in identifying *P. venezuelensis*, sequencing of the *gp43* Exon 2 loci was able to accurately identify this genotype. Thus, our findings contribute to the understanding of the molecular epidemiology of PCM and emphasize the need for precise species characterization in mycological research.

Keywords: Paracoccidioides species, Restriction Fragment Length Polymorphism, DNA Sequence Analysis, Biodiversity, Molecular Biology.

PCM 026- IRON METABOLISM AND HEMATOLOGICAL CELLS IN CHRONIC PULMONARY ASPERGILLOSIS

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ABSTRACT – 1. Introduction. Chronic Pulmonary Aspergillosis (CPA) is a progressive fungal infection that affects lungs with architecture altered by previous diseases. The chronic inflammation caused by the infection releases cytokines that inhibit erythrocyte production and affect iron metabolism, resulting in anemia of inflammation. Additionally, CPA can present with hemoptysis, which may lead to iron-deficiency anemia. The study aims to investigate hematological cells and iron metabolism in patients with CPA. **2. Methodology.** Blood cell counts and iron metabolism parameters were studied in patients with APC treated at HUMAP between March 2023 and July 2024, in two stages of the disease. Patients over 18 years old with a diagnosis of APC, according to Ocansey's criteria, were included. Statistical analysis was performed using Jamovi software, employing Student's t-tests or Wilcoxon tests, depending on the distribution, with a $p \leq 0.05$ considered significant. **3. Results.** The present study includes 15 patients, predominantly men (53.3%), non-white (66.6%), with a median age of 56 years. The most common symptoms observed were cough (93.3%). Most had the cavitory form of APC (60%). In the initial analysis, anemia was the most common finding (53.3%), being 44.4% microcytic and hypochromic and 44.4% normocytic and normochromic, followed by lymphopenia (73%), leukocytosis (33.3%), thrombocytosis (26.6%), and neutrophilia (20%). After 6 months of treatment, anemia was present in 60%, being 83.3% microcytic and hypochromic, followed by lymphopenia (50%), leukocytosis (40%), thrombocytosis (40%), and neutrophilia (30%). After 6 months of treatment, none of the variables showed significant changes ($p < 0.05$). **4. Conclusion.** The described changes and the mean C-reactive protein levels after 6 months of treatment lead us to observe that the patients, even after the initiation of antifungal treatment, still exhibited inflammation and significant alterations.

Keywords: hematology, persistent infection, tuberculosis

Support: Capes

PCM 028- DEVELOPMENT OF PULMONARY FIBROSIS IN EXPERIMENTAL PULMONARY PARACOCCIDIOIDOMYCOSIS DURING PROLONGED COTRIMOXAZOLE TREATMENT

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1. Introduction. The antifungal treatment for paracoccidioidomycosis (PCM) is prolonged, with itraconazole typically being the first choice, except in cases involving the central nervous system, malabsorption syndrome, among other conditions. Sulfamethoxazole-trimethoprim (Cotrimoxazole, CMX) is widely used due to its efficacy, extensive tissue distribution, fewer side effects, and accessibility. Most patients with the chronic form of PCM, even after effective treatment, experience sequelae, such as pulmonary fibrosis (PF). Given its potential to cause disability, PCM represents a significant public health issue, but often underestimated. Although fibrogenesis in PCM is recognized as an early process, its mechanisms remain poorly understood, especially during and after extended antifungal therapy. **2. Objectives.** This study aimed to evaluate the changes in lung tissue and the associated immune response related to the development of (PF) in mice infected with *Paracoccidioides brasiliensis* (*Pb*) during prolonged CMX treatment. **3. Methodology.** Male BALB/c mice were inoculated intratracheally with *Pb* yeast. CMX (Bactrim[®], Roche) treatment was initiated four weeks post-infection and administered once daily via gavage. At 4, 10, and 20 weeks of treatment, assessments included lung fungal load, lung histopathology, inflammatory cytokine production by alveolar macrophages (AM), total leukocyte distribution, and subpopulations of blood monocytes and splenic dendritic cells. **4. Results.** Our results demonstrated that, at 4 weeks of treatment CMX enhanced H₂O₂ and TNF- α production by AM, and increased tissue expression of F4/80, Collagen I, and III in the lungs, with well-defined granulomas surrounded by collagen fibers. After 20 weeks of treatment, we observed fungal clearance, normalized H₂O₂ and TNF- α levels, and tiny, residual granulomas; however, there was intense collagen deposition in the alveolar septa and in areas distant from the residual granulomas. Even after fungal clearance, mice displayed a high percentage of inflammatory blood monocytes and increased MHC-II expression in myeloid dendritic cells. **5. Conclusion.** Our results suggest that CMX enhances the immune response against the fungus during the initial treatment phase. However, this immune profile appears to contribute to an imbalance between immune response and tissue repair over the course of treatment, leading to the establishment of pulmonary fibrosis with systemic repercussions.

Keywords: Paracoccidioidomycosis, Pulmonary Fibrosis, Cotrimoxazole, Tissue Repair.

PCM 029- IMMUNOMODULATION BY BIOACTIVE SUBSTANCES FROM *PIPER TUBERCULATUM* JACQ. IN THE CONTEXT OF PARACOCCIDIOIDOMYCOSIS: FOCUSING ON INFLAMMASOME ACTIVATION

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1. Introduction. Patients with paracoccidioidomycosis during the active disease present intense local and systemic production of pro-inflammatory cytokines, including IL-6, TNF- α and IL-1 β . While nuclear factor κ B (NF- κ B) is a transcription factor that regulates the expression of numerous genes involved in the production of pro-inflammatory response, IL-1 β is uniquely produced through the activation of a multimolecular complex called the inflammasome, which responds to pathogenic agents and host damage signals. In a previous study, we showed that monocytes isolated from PCM patients exhibit increased expression of NLRP3 inflammasome components. In addition, we also have demonstrated that crude plant extracts from species of the *Piper* genus modulate cytokine production, including IL-1 β , indicating potential interference with inflammasome activation. **2. Objectives.** To evaluate the immunomodulatory potential of the crude extract, fractions, and the isolated compound piplartine from *Piper tuberculatum* Jacq. on inflammasome activation. **3. Methodology.** THP-1 cell-derived macrophages were *in vitro* exposed to bioactive substances (crude extract, fractions, and piplartine), along with positive (lipopolysaccharide) and negative (caspase-1 inhibitor) controls. Supernatants were then collected for IL-1 β measurement using an ELISA assay. **4. Results.** Our results showed that the crude extract, fractions, and the isolated compound piplartine from *P. tuberculatum* decreased the production of IL-1 β by macrophages. **5. Conclusion.** our findings indicate a promising immunomodulatory potential for these substances, particularly piplartine, suggesting its possible role as an immunomodulatory agent in inflammatory infectious diseases, including PCM.

Keywords: Bioactive substances, *Piper tuberculatum*, Inflammasome, IL-1 β .

Support: The São Paulo Research Foundation - FAPESP

PCM 030 - NUCLEASE PRODUCTION BY THE *PARACOCCIDIOIDES SPP.* FUNGAL COMPLEX

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Abstract- Evolutionary molecular studies suggested different mechanisms of pathogenicity and intracellular survival for the *Paracoccidioides spp.*, demonstrating that this genus presents relevant differences regarding its metabolic profiles, and which should play a critical role during the host-pathogen interaction. Since the production of nuclease molecules like the DNase II is an important virulence factor already observed in several pathogens, we aimed to search for this protein in different strains of the *Paracoccidioides spp.* species complex. For this, Pb18, Pb265, Pb192, Pb339, *P. lutzii* and Bt85 were analysed by DNase test agar, RT-qPCR and Proteomic Analysis. The strains Pb18 and *P. lutzii* induced DNA degradation on the DNase Test Agar plates while the other strains were not able to do so. The RT-qPCR assay indicated that there was an expression of the sequences corresponding to hypothetical proteins with DNase activity, such as PADG_11161, PADG_08285, and PAAG_07101 genes, mainly in Pb18 and *P. lutzii*, in different periods. However, the proteomic analyses did not indicate the presence of those proteins induced probably by these genes, and then we searched for the most suspect hypothetical proteins that may have a nuclease activity on a list. The Pb18 cell lysate sample contained another hypothetical protein that integrates the cell membrane, and it too may be a candidate responsible for the DNase activity observed. Furthermore, *P. lutzii* had 14 suspects of hypothetical proteins highlighted. This was the highest number registered among all the strains and also had a greater variety of functions. Hypothetical proteins like ACO22_06935, ACO22_01582, and ACO22_03440 with the function of DNA binding, hydrolase activity, and catalytic activity, respectively, were selected because these are characteristics that a nuclease would have and because there was no other information that would exclude them as possible DNA cleaving molecules.

PCM 031- EPIDEMIOLOGICAL EVALUATION OF PARACOCCIDIOIDOMYCOSIS IN PONTAL DO PARANAPANEMA: A REGION OF VULNERABILITY IN THE STATE OF SÃO PAULO

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ABSTRACT – 1. Introduction. Paracoccidioidomycosis (PCM) is a systemic mycosis caused by fungi of the Paracoccidioides genus. Although the disease is endemic in several regions of Brazil, data on its incidence in Pontal do Paranapanema, an area of vulnerability, are scarce. In light of this gap, we conducted a study to analyze the cases of PCM recorded in the pathology laboratory database of Universidade do Oeste Paulista (UNOESTE), located in Presidente Prudente/SP, from 1984 to 2024. **2. Objective(s).** The aim of this study is to evaluate the clinical-epidemiological profile of PCM cases diagnosed in Pontal do Paranapanema region from 1984 to 2024, analyzing the characteristics and trends of the disease over the past four decades. **3. Methodology.** Data on the patients diagnosed with PCM were collected from reports of the pathology laboratory of UNOESTE. The study was approved by the Research Ethics Committee of UNOESTE. **4. Results.** We evaluated 69 reports of patients diagnosed with PCM. Most cases occurred in males (83%) compared to the females (17%). The average age of the patients was 52 years old. In relation to race, 62% declared themselves white, 25% black and 13% brown. The involvement was predominant in the oral cavity (74%), followed by the lower and upper respiratory tract. The decade of 2000 presented the biggest number of cases, with 2003 being the year of greatest incidence. As to the provenance, 34% of the patients were from Presidente Prudente. **5. Conclusions.** The results suggest the implementation of measures aimed at increased reporting and monitoring of PCM cases, with the objective of improving early diagnosis and management of the disease. From this analysis, it is possible to identify areas of higher risk and propose more effective strategies for the control of PCM, especially in endemic regions such as Pontal do Paranapanema.

Keywords: Paracoccidioidomycosis, Epidemiology, Pontal do Paranapanema

PCM 032- CONTRIBUTION OF INFLAMMASOME GENE POLYMORPHISMS TO SUSCEPTIBILITY AND RESISTANCE IN PARACOCCIDIOIDOMYCOSIS

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ABSTRACT – 1. Introduction. Genetic susceptibility to infectious diseases is crucial for understanding disease mechanisms and potential therapeutic targets. In Paracoccidiodomycosis (PCM), host genetic factors may influence the immune response to *Paracoccidoides* spp. Inflammasomes are cytoplasmic multiprotein complexes activated by recognizing specific pathogen components or cellular damage. The complex includes a pathogen recognition receptor (e.g., a member of the Nod-Likereceptor family), an adaptor protein (e.g., Apoptosis-associated Speck-like Protein Containing a CARD), and an effector protein (e.g., Caspase-1), which converts inactive IL-1 β or IL-18 into their active forms, thereby triggering a local and systemic inflammatory response. **2. Objective.** To identify single nucleotide polymorphisms (SNPs) in genes involved in the inflammasome pathways that are associated with: (1) susceptibility to PCM, (2) distinct clinical manifestations of PCM, and (3) disease severity. **3. Methodology.** The study included a total of 266 individuals, comprising 124 patients diagnosed with PCM from Botucatu, São Paulo, and 142 healthy individuals, rural workers, with a positive paracoccidiodin test from Mato Grosso do Sul. Related individuals were excluded from the study. Blood samples were collected for genomic DNA extraction. Clinical and demographic data were recorded. Seven genetic markers from the following genes were analyzed: *NLRP1*, *NLRP3*, *CASP1*, *CARD8*, and *IL1B*. Genotyping was performed using qPCR with specific probes for each SNP. Allelic and genotypic frequencies were compared using the chi-square test, and logistic regression was used to evaluate associations, with significance set at $p < 0.05$. **4. Results.** *CASP1* and *CARD8* were not in Hardy-Weinberg equilibrium and were therefore excluded from the analyses. No SNP showed an association with the development of PCM or its clinical forms (acute/subacute or chronic). On the other hand, only *NLRP1* showed an association with protection against developing the severe form of PCM (Dominant model, OR = 0.43 [0.19 - 0.97], $p_{FRD} = 0.050$). **5. Conclusions.** These findings suggest that genetic variations in the *NLRP1* gene may play a critical role in modulating the host's immune response to *Paracoccidoides* spp., highlighting its potential as a therapeutic target in preventing severe PCM. Further research is warranted to explore the mechanisms behind this protective association and to confirm these findings in larger, diverse populations.

Keywords: Immune Response, Inflammasome, single nucleotide polymorphisms.

PCM 033- DESIGN AND SYNTHESIS OF PEPTIDES FOR SEROLOGICAL DIAGNOSIS OF PARACOCCIDIOIDOMYCOSIS

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ABSTRACT – 1. Introduction. Paracoccidioidomycosis (PCM) is a systemic mycosis caused by fungi of the *Paracoccidioides* genus, with a high incidence in Brazil and a need for new biomarkers for disease detection and monitoring. Immunoinformatics and immunoproteomics tools allow for the mapping of antigens to identify epitopes with highest antigenic potential to diagnosis. *In silico* analyses are fast, cost-effective and enable the identify protein antigens that are not expressed *in vitro*. **2. Objective(s).** This study aimed to design and synthesize selected epitopes using bioinformatics and immunoproteomics tools to design a multi-epitope molecule. **3. Methodology.** The selection of peptides consisted of *in silico* approaches for predicting B cell epitopes, antigenicity, localization, function, and protein characterization. The design of antigens was based on unique *Paracoccidioides* peptides previously described. Subsequently, the synthesis was performed using a peptide synthesizer, and purification was carried out through chromatography techniques. **4. Results.** So far, three low-molecular-weight epitopes (antigens) have been selected and synthesized (2.40 kDa, 2.34 kDa, and 2.27 kDa), all with hydrophilic characteristics and twenty-amino-acid sequences, with two being acidic and one basic. Besides, a multi-epitope molecule was proposed, containing 62 amino acids and molecular weight of 8.15 kDa. **5. Conclusions.** The selected antigens are promising as biomarkers for the development of a rapid test kit, with wide application, which does not depend on complex laboratory infrastructure, favoring epidemiological screening, diagnosis and monitoring of patients with PCM. Afterwards, as next steps, sera from patients with systemic mycoses, including PCM, and from patients without any previous or current fungal disease will be tested against the synthetic peptide using the ELISA (Enzyme-Linked Immunosorbent Assay) method, and parameters such as accuracy, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) will be determined.

Keywords: Bioinformatics, epitopes, *in silico* analyses, immunoproteomics, *Paracoccidioides*.

Support: CAPES, CNPq, FAPEG, Fundect, LabDIP.

PCM 034- CHARACTERIZATION OF MIXED BIOFILMS OF *Paracoccidioides brasiliensis* AND *Candida albicans* ON NITROCELLULOSE MEMBRANE SURFACE AND HOST-PATHOGEN INTERACTION IN THREE-DIMENSIONAL ORAL MUCOSA MODEL

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ABSTRACT – 1. Introduction. Paracoccidioidomycosis (PCM) is a systemic mycosis that primarily affects the lungs, with potential involvement of the oral cavity, where *Candida albicans* exist as a commensal. **2. Objective.** This study aims to structurally characterize mixed biofilms of *Paracoccidioides brasiliensis* and *C. albicans* on a three-dimensional oral mucosa model and nitrocellulose membrane surface. **3. Methodology.** Mixed biofilms of *P. brasiliensis* (Pb18) and *C. albicans* (ATCC 90028) were formed on nitrocellulose membrane, either simultaneously or after 12 h of preformed *C. albicans* biofilm, and evaluated from 24 to 168 hours. Metabolic activity was assessed by XTT assay, and biomass was quantified using crystal violet. Biofilm and spheroid structure were analyzed by scanning electron microscope (SEM); spheroids formed with oral keratinocytes (NOK) were evaluated for sphericity, solidity, diameter, and viability using resazurin reduction. **4. Results.** The *P. brasiliensis* biofilm showed increased metabolic activity from 48 h to 120 h, with a decline at 144 h, while the simultaneous and preformed mixed biofilms exhibited similar trends, with activity rising within the first 24 h. All biofilms exhibited increased biomass from 24 to 72 h, extending to 120 h for *C. albicans* and dual-species biofilms, and a decrease at 96 h for *P. brasiliensis*. The optimal cell concentration for NOK was 6×10^4 cells/well, with sphericity and solidity close to 1 and a diameter of 456.2 μm . The cellular model maintained viability above 70% for up to 168 hours. SEM images revealed dense, adherent biofilms composed of a complex network of cells and extracellular matrix, including in the spheroids. **6. Conclusions.** Mixed biofilms of pre-cultured *C. albicans* and *P. brasiliensis* exhibited greater biomass and metabolic activity, suggesting an additive effect. The micrographs revealed cell interactions and structural architecture typical of multicellular communities. The NOK cell model effectively assesses pathogen-host interactions.

Keywords: *Paracoccidioides brasiliensis*, *Candida albicans*, Mixed biofilms, 3D Model, NOK cells.

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PCM 035- CLINICAL-EPIDEMIOLOGICAL ASPECTS OF PARACOCCIDIOIDOMYCOSIS IN A REFERENCE CENTER IN PARAGUAY

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ABSTRACT

1. Introduction: Paracoccidioidomycosis (PCM) is a prevalent invasive fungal infection in tropical and subtropical areas of Latin America and represents a significant public health problem in Paraguay. It is caused by the dimorphic fungus *Paracoccidioides brasiliensis*, with acute/subacute and chronic clinical presentations, the latter being the most common. **Objective:** To describe the clinical, epidemiological, and diagnostic characteristics of patients diagnosed with PCM between 2020 and 2024 at the Institute of Tropical Medicine in Asunción (IMT). **Methodology:** This is a retrospective and descriptive study based on clinical records of patients diagnosed with PCM and treated at the IMT. **Results:** A total of 15 cases of PCM were identified, with 4 cases (26.7%) from the San Pedro Department. The median age was 57 years, 14 (93.3%) male and 10 (66.7%) working as farmers. Concerning personal history, 12 patients (80%) reported alcohol use, 10 (66.7%) were smokers, 5 (33.3%) had a BMI <18.5 kg/m², 2 (13.3%) had type 2 diabetes, 2 (13.3%) had hypertension, and 3 (20%) had chronic obstructive pulmonary disease (COPD). The chronic clinical presentation was observed in 14 patients (93.3%), with a median duration of symptoms of 4 months. The disease primarily affected the skin and mucosa (predominantly nasopharyngeal cavity) in 11 cases (73.3%), pulmonary involvement in 4 cases (26.7%), and 1 case (6.7%) involved the central nervous system (CNS). Diagnosis was confirmed in all cases (100%) through histopathology, and 14 patients (93.3%) received treatment with amphotericin. All patients were hospitalized, with a mortality rate of 1 case (6.7%). **Conclusions:** PCM is an endemic disease in Paraguay characterized by its clinical pleomorphism, which can resemble other diseases and may progress into severe and disabling forms. This necessitates the expansion of diagnostic capacity and the dissemination of knowledge at the national and regional level.

Keywords: paracoccidioidomycosis, Paraguay, epidemiology, clinical, diagnosis.

**PCM 036- POLYMICROBIAL BIOFILMS OF *PARACOCCIDIOIDES BRASILIENSIS* AND *KLEBSIELLA PNEUMONIAE*:
ANTIMICROBIAL ACTIVITY OF CHITOSAN NANOPARTICLES LOADED WITH
BENZOFUROXANE**

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Introduction: Paracoccidioidomycosis (PCM) is caused by *Paracoccidioides* spp and *Klebsiella pneumoniae* (Kp) is a bacterium associated with infections such as pneumonia, cystic fibrosis, and severe systemic pulmonary diseases. The association of these agents has been described and potentially lead to fatal outcomes. **Objective:** Demonstrate the potential formation of a polymicrobial biofilm by both microorganisms (Pb18) and (Kp) and propose the use of chitosan nanoparticles loaded with benzofuroxan (NQBZ) in combination with Imipenem (IMP). **Methods:** We used Pb18 (ATCC 32069) and Kp (ATCC 700603). Polymicrobial biofilm was conducted for pre-formed (Pb18+Kp) and simultaneous (Kp and Pb18) formation. The metabolic activity of the biofilm was assessed using XTT and the biomass was by crystal violet and scanning electron microscopy (SEM). All evaluations were read by a spectrophotometer (iMark™; BIO-RAD), with the treatment of NQBZ and IMP. Statistical analyses were conducted with a significance of $P < 0.05$, in independent experiments. **Results:** The formation of polymicrobial biofilm was demonstrated in both conditions (Pb18+Kp) and (Kp and Pb18). No significant difference ($p < 0.05$) was observed when compared to the Pb18 monospecies biofilm, but a significant difference ($p < 0.01$) was observed in the Kp biofilm, suggesting that Kp biofilm is affected by the interaction with Pb18 in the mixed biofilm. The inhibition of the biofilm metabolic activity of BNZ and NQBZ varied by 34 to 52% ((Pb18+Kp) and 39 to 55% (Kp and Pb18). It was unable to eliminate the biofilm due to lower results. Therefore, the combinatorial activity of NPBNZ+IMP showed synergistic activity. 70% inhibition was obtained in mixed biofilms (Pb18+Kp) with MIC of 62.5 µg/mL and 68% with MIC of 31.5 µg/mL in the case of Kp and Pb18. **Conclusions:** The results showed great potential for NPBNZ+IMP in mixed biofilms, which may contribute significantly to the therapeutic arsenal under these conditions.

Keywords: antibiofilm, *Paracoccidioides brasiliensis*, *Klebsiella pneumoniae*

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PCM 037- EVALUATION OF THE BINDING CAPACITY OF Ptr-FUNCTIONALIZED GOLD NANORODS TO PARACOCCIDIOIDOMYCOSIS-SPECIFIC ANTIBODIES

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ABSTRACT – Introduction. Paracoccidioidomycosis (PCM) is a prevalent systemic mycosis in Latin America caused by *Paracoccidioides* spp. Early detection of PCM is critical for improving clinical outcomes. Current serological diagnostic methods are limited in sensitivity and specificity, highlighting the need for innovative biosensor platforms. Functionalizing gold nanorods (GNRs) with recombinant protein Ptr offers a promising approach for detecting PCM-specific antibodies. **Objective(s).** Herein, we evaluated the binding affinity between Ptr-functionalized GNRs and PCM-specific antibodies, aiming to optimize this platform for sensitive PCM diagnostics. **Methodology.** Ptr was attached to the GNR surface through covalent bonding using carbodiimide chemistry. The nanoplatform (GNR-Ptr) was confirmed using ultraviolet-visible (UV-Vis) spectroscopy and transmission electron microscopy (TEM). Zeta potential measurements assessed the nanoplatform's stability and aggregation state. The purified polyclonal anti-Ptr antibody was used to evaluate its interaction with GNR-Ptr. The antibody was serially diluted (10 µg to 1 fg) and incubated with GNR-Ptr. **Results.** Changes in the longitudinal plasmon absorption peak were observed by UV-Vis/NIR spectroscopy, confirming the formation of the GNR-Ptr platform. TEM analysis revealed Ptr on the GNR surface as a thin, homogeneous film. The resulting nanoplatform was positively charged, well-dispersed, and stable. GNR-Ptr recognized the specific antibodies, showing shifts in the longitudinal plasmon absorption peak at dilutions ranging from 100 ng to 1 ng. A linear regression ($R^2 = 0.9718$) was generated from the plasmon absorption peak changes, demonstrating strong interaction between GNR-Ptr and the specific antibodies. **Conclusions** These findings show that Ptr-functionalized GNRs are a promising platform for sensitive detection of PCM-specific antibodies, representing potential for developing innovative diagnostic tools for PCM.

Keywords: Paracoccidioidomycosis, recombinant protein, gold nanorods, antibody detection, optical immunosensors.

Support: FINEP, FAPEMIG, CNPq.

PCM 038- ASSOCIATION BETWEEN PARACOCCIDIOIDOMYCOSIS AND SOLID TUMORS. EVALUATION OF PATIENTS FROM A UNIVERSITY HOSPITAL AND SYSTEMATIC REVIEW

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ABSTRACT

Background. The association of paracoccidioidomycosis (PCM) with solid tumors (ST) has been few times analysed, constituting the objective of this study. **Methods.** PCM cases, defined by mycological methods or serological immunodiffusion tests, associated with solid tumors, diagnosed by histopathological examination of clinical specimens, were studied. PCM was classified as to Mendes et al. (2017). A systematic review was performed in seven databases. Our findings were compared with those from the literature using the SAS program – version 9.4. **Results.** In the period 2013 – 2022, 154 PCM patients were admitted, 15 (9.7%) of them presented ST associated. The increased survey identified a total of 32 patients, included in the study. The chronic form predominated - 96.9%, as well as the males (87.5%), ages between 40 and 69 years old, and smokers (87.5%). PCM was diagnosed before the solid tumor in 21 patients, and simultaneously in the other 11. Lungs (35.5%), larynx (19.4%) and oral cavity (9.7%) were involved with higher frequency. Spinocellular carcinoma (37.5%), adenocarcinoma (15.6%) and neuroendocrine carcinoma (12.5%) were the predominant histological type of tumor. The prevalence of the different histological types of tumor were not different as to involved organs. Our results [A] on site of tumor were compared with those of Shikanai-Yasuda et al. (2008) [B] and data of Rodrigues et al. (2010) – patients [C] and literature review [D] showing differences only in the lungs – higher prevalence in C (62.5%) than B (32.5%) and D (29.8%), and an intermediate frequency for A (35.5%). **Conclusions.** Our findings showed that *i*) is relevant the frequency of association PCM-ST; *ii*) the previous or simultaneous diagnosis of PCM suggest a possible role of paracoccidioidal antigens in ST pathogeny; *iii*) lungs, larynx and oral cavity were the main sites of tumor, and *iv*) spinocellular carcinoma was the most prevalent histological type of ST.

Keywords: Paracoccidioidomycosis, solid tumors, association.

PCM 039- PATHOGENICITY AND IMMUNOGENICITY OF CLINICAL ISOLATES OF THE *PARACOCCIDIOIDES* GENUS AND ITS ASSOCIATION WITH PATIENTS' SEVERITY

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ABSTRACT

Background. Influence of the *Paracoccidioides* virulence on the patients severity remains poorly demonstrated. **Objective.** To evaluate the association between severity of paracoccidioidomycosis (PCM) patients and pathogenicity and immunogenicity of the corresponding isolates. **Methods.** Four PCM patients recently hospitalized were evaluated, and their clinical isolates - Pb234 and Pb417 from patients with the chronic moderate form, Pb326 from the severe acute/subacute form, and Pb531 from the severe chronic form were identified by sequencing the Exon 2 region of gp43. Pathogenicity was evaluated by the lethal dose 50% determination, and by counting the number of colony forming units in infected BALB/c mice sacrificed at the 2nd, 4th and 6th week of infection. Immunogenicity was evaluated by the IL-2, IL-10, IFN- γ , TNF- α , and VEGF concentrations in pulmonary tissue. The Spearman correlation test, at a $p \leq 0,05$, was used to associate patients severity and laboratory findings. **Results.** Pb417 and Pb326 were identified as *P. brasiliensis* S1a, Pb531 as *P. brasiliensis* S1b, and Pb234 as *P. restrepiensis* (PS3). A direct correlation between pathogenicity of the isolates and patients' severity was demonstrated. The virulence was high - Pb531, intermediate - Pb326, or low – Pb417 and Pb234. The pulmonary cytokines concentrations revealed a balance at the 2nd and 4th weeks of infection, and a predominance of IL-10 at the 6th week, but in infection with all the isolates, with few variations among them. **Conclusions.** Our findings showed that: a) *P. restrepiensis* is present in Botucatu region (São Paulo state, Brazil); b) there is an association between patients severity and virulence of the respective isolates; c) infection of BALB/c mice, easily available, and the fungal load count only at the 2nd week of infection are enough for evaluating virulence; d) in the six weeks of infection, cytokines concentration in pulmonary tissue did not discriminate the immunogenicity of the isolates.

Keywords: Paracoccidioidomycosis, pathogenicity, immunogenicity

PCM 040- PARACOCCIDIOIDOMYCOSIS: MORTALITY IN RONDÔNIA FROM 1997 TO 2024

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Introduction. Paracoccidioidomycosis (PCM) is a systemic granulomatous fungal disease, classified among the medically significant fungal infections, caused by fungi of the *Paracoccidioides* spp. complex and is closely associated with agricultural activities. The annual incidence in Brazil ranges from 0.71 to 3.70 cases per 100,000 inhabitants. PCM ranks among the top ten causes of death due to chronic and recurrent infectious and parasitic diseases. In Rondônia, diagnoses were based on direct mycological examination (41.6%), histopathology (14.4%), serology (5.2%), or culture (3%), and in 35.8% of cases, on clinical, epidemiological, and radiological data. Recent reports indicate epidemic peaks of up to 15.3 cases per 100,000 inhabitants and a lethality rate of up to 22.7. **2. Objective.** This study aimed to analyze PCM-related mortality in Rondônia since the disease became a notifiable condition in the state, 28 years ago. **3. Materials and Methods.** This descriptive study analyzed person, time, and place attributes using data from the Notifiable Diseases Information System (SINAN) and the Mortality Information System (SIM) from 1997 to 2023. Deaths with PCM (B41) and Blastomycosis (B40) as the underlying cause were considered, according to the 10th revision of the International Classification of Diseases (ICD-10). Additionally, a literature review was conducted in major health databases (PubMed, SciELO, Virtual Health Library). **4. Results and Discussion.** The general mortality coefficient for PCM was 0.11 cases per 100,000 inhabitants between 1997 and 2022, with an average annual mortality rate of 6.57 across temporal intervals. It is worth noting that data for 2023 and 2024 are still under consolidation, so the database contains partial results that may change. **5. Conclusion.** Regarding PCM-related mortality, Rondônia accounted for 178 deaths during the study period, with a higher prevalence among males in the productive age range. Variations in reported deaths were observed over the 28 years analyzed.

Keywords: Paracoccidioidomycosis, Epidemiology, Mortality, Rondônia

PCM 041- DEVELOPMENT AND ANALYTICAL PERFORMANCE OF AN ELECTROCHEMICAL IMMUNOSENSOR FOR PARACOCCIDIOIDOMYCOSIS DETECTION

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ABSTRACT – 1. Introduction. Paracoccidioidomycosis (PCM) is a neglected occupational disease and the most prevalent systemic mycosis in South America. Current diagnostic methods exhibit limitations, including low reproducibility, accessibility, and selectivity, as well as long turnaround times. In this context, immunosensors have been explored as alternative diagnostic tools, offering faster, more sensitive, and accessible solutions for infectious disease detection, and could be an alternative to overcome current PCM diagnostic limitations. **2. Objective(s).** In this study, an electrochemical immunosensing platform was developed using a recombinant *Paracoccidioides* antigen. The aim was to evaluate its analytical performance in detecting anti-*Paracoccidioides* immunoglobulins and assess its potential as a serological test for PCM. **3. Methodology.** The immunosensor was fabricated using mechanically assisted chemically exfoliated graphene and a synthesized semiconducting polymer. The recombinant antigen was conjugated to the platform to enable specific interactions with antibodies. Platform characterization was performed via microscopy, spectroscopy and particle analysis techniques. The sensor detection was analysed with Electrochemical Impedance Spectroscopy (EIS) **4. Results.** EIS analyses revealed that the sensor's charge transfer resistance (R_{ct}) was sensitive to the presence of anti-*Paracoccidioides* immunoglobulins. The immunosensor demonstrated a linear response range from 100 to 500 ng/mL of IgG, a detection limit of 92.07 ng/mL, low response variability (5.2%), and high recovery rates (80–110%). Non-specific antibodies/sera elicited significantly lower responses compared to specific anti-*Paracoccidioides* antibodies or infected sera. Moreover, the tests with infected mouse sera showed an increase in R_{ct} of 33.5% and 85% at 15 and 60 days post-infection, respectively. **5. Conclusions.** The immunosensors were successfully constructed and characterized, and presented satisfactory analytical performance for PCM detection, such as low detection limit and good selectivity. These findings suggest the potential of this platform for future applications in PCM diagnostic methods.

Keywords: Paracoccidioidomycosis, biosensor, nanomaterials, recombinant protein, impedance spectroscopy.

Support: FINEP, FAPEMIG, CNPq, CAPES.

PCM 042- CRYPTOCOCCOSIS: DEATHS IN THE STATE OF MATO GROSSO

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Introduction: The systemic mycoses are severe infections caused by the inhalation of fungal spores present in the environment, such as in soil and decomposing organic materials, including excreta from birds and mammals like bats. Cryptococcosis, caused by the fungus *Cryptococcus neoformans*, is a potentially fatal disease that primarily affects individuals with compromised immune systems, such as those who have undergone organ transplants or are undergoing immunosuppressive treatment. The increasing incidence of this infection in immunocompromised patients has made cryptococcosis a growing concern for public health, being considered an emerging disease. **Objective:** To investigate the number of deaths caused by cryptococcosis in the state of Mato Grosso between 2013 and 2023. **Methodology:** Ecological study, with data collection performed from the Mortality Information System (SIM). The sociodemographic variables used were: age group and sex during the period from 2013 to 2023. **Results:** During the period from 2013 to 2023, 41 deaths due to cryptococcosis were reported in Mato Grosso. Of these, 22% were in individuals aged 30 to 39 years and 4.9% in individuals aged 1 to 4 years. Among the reported cases, 68% were male and 32% were female. **Conclusion:** Cryptococcosis is a disease difficult to diagnose, as its fungal manifestations are varied and often hard to identify, leading to underdiagnosis and underreporting. It is a potentially fatal condition, especially for individuals with compromised immune systems. By better understanding the disease, health services will be more prepared to adopt specific strategies and measures that can enhance care for the affected population.

Keywords: *Cryptococcus neoformans*; Systemic mycosis; Deaths.

PCM 043- PARACOCCIDIOIDOMYCOSIS: DEATHS IN THE STATE OF MATO GROSSO

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Introduction: Paracoccidioidomycosis, also known as Lutz-Splendore-Almeida disease, is a systemic mycoses caused by the dimorphic fungus *Paracoccidioides brasiliensis*, primarily acquired through the inhalation of spores present in the soil. Considered the most important fungal disease in Latin America, it has a high prevalence in Brazil, especially among rural workers and individuals exposed to the soil. The hot and humid climate creates a favorable environment for its development. As it is not a mandatory reportable disease, epidemiological data are less accurate and complete. Estimates of prevalence, incidence, and morbidity for this disease generally rely on various sources. Although data are limited, reports help provide an overview of the disease's distribution and impact in affected regions. Without a formal reporting system, we heavily depend on epidemiological studies, case series, hospitalization records, and mortality data to gain a clear understanding of paracoccidioidomycosis. **Objective:** To investigate the number of deaths caused by paracoccidioidomycosis in the state of Mato Grosso between 2013 and 2023. **Methodology:** Ecological study in which data were collected from the Mortality Information System (SIM). The sociodemographic variables used were: age group, sex, and education level from 2013 to 2023. **Results:** During the period from 2013 to 2023, 44 deaths due to paracoccidioidomycosis were reported, with 29.5% of people in the 50 to 69 age group. No cases were recorded in people under 20 years of age. Among the reported cases, 88,6% were male and 11,4% were female. A low level of education was observed among the victims of the disease, with 84.1% of those affected having completed 7 years or fewer of schooling. **Conclusion:** The collection of data and information is essential for building a solid clinical hypothesis and selecting appropriate diagnostic tests. This ensures that the proposed treatments are based on solid evidence and can be applied with greater safety and effectiveness. Understanding the epidemiology, clinical manifestations, and treatment response of diseases like paracoccidioidomycosis, even with limited data, is crucial for improving patient care. It also contributes to the development of more effective clinical protocols and the reduction of complications.

Keywords: *Paracoccidioides brasiliensis*; Systemic mycosis; Deaths.

PCM 044- DELAY IN DIAGNOSING A TYPICAL CASE OF PARACOCCIDIOIDOMYCOSIS: HOW MUCH LONGER?

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Abstract: Introduction. Paracoccidioidomycosis (PCM) is a systemic fungal infection endemic to Latin America, with higher incidence among populations exposed to rural environments. **2. Case.** A 52-year-old farmer sought dental care for oral mucosal lesions persisting for over a year. Additional symptoms included cervical lymphadenopathy, productive cough, and weight loss of less than 10% of body weight. Throughout the past year, the patient visited medical facilities multiple times but had not received a definitive diagnosis. The dentist performed an incisional biopsy of the oral lesion, which histopathology revealed as pseudoepitheliomatous hyperplasia and microabscesses, some containing large yeast cells with birefringent membranes and multiple budding—characteristic of *Paracoccidioides*. Subsequently, the dentist referred the patient to an infectious disease specialist. A chest computed tomography scan showed diffuse pulmonary lesions with a random distribution pattern, an inverted halo sign, and some lesions with small internal cavitations. The patient began treatment with itraconazole 200 mg daily for moderate chronic PCM. At the first follow-up visit, no adverse effects from the medication were reported, and the patient demonstrated clinical improvement and weight gain. **3. Discussion.** This case highlights the delay in diagnosis of a typical presentation of PCM in a rural worker from an endemic area of the disease. In addition, the crucial role of dental professionals in identifying the disease. Increasing clinical suspicion and developing health care networks targeted at rural populations are essential to improve the management and outcomes of PCM.

Keywords: Palavras-chave: *Paracoccidioidomycoses, diagnosis, dentist, dental care.*

PCM 045-EPIDEMIOLOGICAL SURVEILLANCE OF SYSTEMIC MYCOSES IN THE STATE OF RIO DE JANEIRO.

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ABSTRACT-Introduction: Histoplasmosis, Paracoccidioidomycosis, Coccidioidomycosis and Cryptococcosis, are not included in the list of diseases subject to mandatory notification in Brazil by the Ministry of Health. Consolidated data on these mycoses and their occurrence in the national territory are not yet known. Classified as neglected diseases by the World Health Organization, they have contributed to unfavorable outcomes, with disabling sequelae and premature deaths, mainly due to the difficulty in diagnosis and timely access to treatment. The State of Rio de Janeiro, through State Health Department Resolution No. 2.485 from 10/18/21, made the notification of these mycoses mandatory. This initiative has contributed to the planning and implementation of more efficient public policies for the prevention, diagnosis and immediate treatment of these diseases in the state. **Objective(s):** Determine the magnitude of these diseases in the state of Rio de Janeiro; know the epidemiological, social and clinical profile to support the implementation of health policies in the state of Rio de Janeiro. **Methodology:** The cases were monitored through the Notifiable Diseases Information System (SINAN) from Oct/21-Sep/24. **Results:** 207 Paracoccidioidomycosis cases were reported/131(63%) confirmed, 354cases of histoplasmosis reported and 179(50%) confirmed 106 cases of Cryptococcosis reported and 67(63%) confirmed, 01 case of Coccidioidomycosis were reported but not confirmed. The highest prevalence of these mycoses was found in the metropolitan region of the city. **Conclusion:** The objective is to raise awareness among health professionals of the suspected diagnosis, promoting timely diagnosis and treatment, avoiding severity disability and premature deaths. More epidemiological data are needed, considering that the incidence of these diseases in the state may be higher than currently estimated, especially in patients with co-infections in which there is still failure/lack of knowledge of notification by health professionals

Keywords: Neglected, Histoplasmosis, Paracoccidioidomycosis, Coccidioidomycosis, Cryptococcosis.

Support: Rio de Janeiro State Health Department (SES)

PCM 046- INVESTIGATING THE ANTIFUNGAL EFFICACY OF LMM6 ON DIMORPHIC TRANSITION IN *Paracoccidioides brasiliensis*

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Introduction: In the World Health Organization's priority list of pathogens, *Paracoccidioides* spp. are classified as a medium-priority pathogen. Despite ranking last on this list, this neglected disease has received international attention for the first time, emphasizing the necessity to consider neglected tropical mycoses. LMM6 is an inhibitor of thioredoxin reductase (Trr1) with promising antifungal activity. Given the evidence that alterations in the control of oxidative stress may modulate the virulence factors of several fungi, this study was focused on the key virulence factor of thermodimorphic fungi.

Objective: To evaluate the effect of LMM6, a thioredoxin reductase inhibitor on the mycelium-to-yeast (M-to-Y) transition of *P. brasiliensis*. **Methodology:** *P. brasiliensis* strain Pb18 was cultured in mYPD broth at 25°C with agitation to induce the filamentous form. Transition to yeast was evaluated following treatment with LMM6 (0.5-2 µg/mL) at 37°C in duplicate, with controls. Optical microscopy assessed fungal morphology up to 96 hours, with LMM6 replenished every 24 hours. **Results:** Control samples displayed early yeast formation at 48 hours, with a clear transition to predominantly yeast cells by 96 hours. In contrast, LMM6-treated samples showed an extended presence of hyphal structures, especially at the 2 µg/mL dose, where yeast formation was notably delayed until the experiment's end. These findings suggest that LMM6 inhibits the M-to-Y transition in a dose-dependent manner. **Conclusions:** The LMM6 treatment effectively delayed the dimorphic transition in *P. brasiliensis*, demonstrating a dose-dependent effect. These results align with previous studies connecting the oxidative stress to fungal differentiation and reinforcing the potential of LMM6 as a therapeutic agent targeting fungal virulence by interfering with critical adaptive mechanisms. Further studies will clarify LMM6's exact mechanisms and validate its application in antifungal therapy.

Keywords: *Paracoccidioides brasiliensis*, dimorphic transition, oxidative stress, antifungal therapy

Support: CAPES (Coordination for the Improvement of Higher Education Personnel), CNPq (National Council for Scientific and Technological Development), FINEP (Funding Authority for Studies and Projects).

PCM 047- TOXICITY OF INHIBITORY CONCENTRATIONS OF 2-HYDROXYCHALCONE AGAINST *Paracoccidioides brasiliensis* IN *Galleria mellonella* e *Caenorhabditis elegans*.

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Abstract – Chalcones are precursor compounds of flavonoid biosynthesis that demonstrate several pharmacological properties, including antimicrobial activities.

Objectives: To determine the minimum inhibitory concentration (MIC) of 2-hydroxychalcone in planktonic cells of *P. brasiliensis* and to evaluate the toxicity of the concentrations in larvae of *G. mellonella* and *C. elegans*. **Material and Methods:** The MIC was determined by serial microdilution (0.12–62.5 µL/mL), according to the M37-A2 protocol (CLSI, 2008), in RPMI medium, using the *P. brasiliensis* S1 strain isolate 18 (Pb18). The General Health Index (GHI) of *G. mellonella* was: Health (no injection), PBS group (diluent control), Amphotericin B (AmB) group (drug control – 8 µg/mL) and 2-Hydroxychalcone group (0.24 to 1.92 µg/mL). After injection of the substances, groups of 20 *G. mellonella* larvae were monitored daily for: activity, cocoon formation, melanization and survival, with a score being provided for each of these. The density of *G. mellonella* hemocytes was verified after 24 h of treatment with 2-hydroxychalcone and AmB, performing hemolymph collection and dilution in ice-cold PBS (1:20) and counting in a hemacytometer. *C. elegans* (N2 and AU37) (n=20 each) were evaluated for survival rate, in a concentration range of 0.12 to 61.4 µg/mL. **Results:** The MIC of 2-hydroxychalcone against Pb18 was 0.48 µg/mL and of AmB was 0.25 µg/mL. Regarding the *G. mellonella* IGS, the Health and PBS groups presented scores of 8.3 to 9 points and the group with the lowest score was with 2-hydroxychalcone (1.92 µg/mL): 6 points. The hemocyte count was 9.28x10⁷ hemocytes/mL for AmB and 12.4x10⁷ hemocytes/mL on average for 2-chalcone. As for *C. elegans*, the AU37 strain demonstrated a higher survival rate for 2-hydroxychalcone (96%), while N2 presented a frequency of 92.6% of live larvae. **Conclusion:** The results demonstrate that *G. mellonella* and *C. elegans* are rapid and useful models to evaluate the toxicity of substances that can be used in the treatment of fungal diseases.

Keywords: *Caenorhabditis elegans*; Chalcon; *E. Galleria mellonella*; *Paracoccidioides*.

Support: This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001. CNPq, process 310524/2018-0 e 431455/2019-0. FAPESP

PCM 048 - LMM2 A NEW ANTIFUNGAL MOLECULE AGAINST PARACOCCIDIIDIOMYCOSIS WITH LOW CYTOTOXICITY PROFILE

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ABSTRACT – 1. Introduction. Paracoccidioidomycosis (PCM) is a systemic infection endemic to Latin America, with the main clinical sign being the formation of granulomas in the lung tissue, which can lead to fibrosis. The treatment is limited to two classes of antifungals: polyenes and azoles. Additionally, the use of high doses and prolonged treatments results in adverse effects, hepato- and nephrotoxicity. Nanoformulated treatments reduce these problems but come at a higher cost. Therefore, the need for new antifungals is urgent. Our group has been addressing this issue for over 10 years. In previous studies, four essential genes were identified, as sterol C-24 methyltransferase (Erg6). A virtual screening was conducted to select molecules that interact with this key enzymatic ergosterol biosynthesis. The molecule LMM2 exhibited antifungal activity against paracoccidioidomycosis, showing a fungicidal profile. **2. Objective.** To evaluate the cytotoxic effects of LMM2 in different cell lines. **3. Methodology.** The assay was conducted on five distinct cell lines: HeLa, HaCaT, Caco-2, Vero and L929. The CellTiter 96 assay based on the reduction of MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium], was used to evaluate the effect of LMM2 after 24 hours. Controls with the diluent (Dimethylsulfoxide; DMSO) were also performed. In the calculation of the selectivity index (SI = CC50 / IC50), SI values > 20 indicate toxicity to fungi, while SI values < 20 demonstrate toxicity to mammalian cells. **4. Results.** The highest concentration tested, 256 µg/mL, demonstrated significant toxicity in the different cell lines. However, at concentrations below 128 µg/mL, all cell lines showed approximately 100% viability, resulting in a selectivity index of > 20 (HeLa), < 20 (HaCaT), > 20 (Caco-2), > 20 (Vero) and < 20 (L929) **5. Conclusions.** LMM2 may be a promising candidate for the development of new antifungals, as it shows an acceptable safety profile at concentrations relevant to treatment.

Keywords: Paracoccidioidomycosis; toxicity tests; cell line; antifungal agents; biotechnology.

Support: CAPES; CNPq; FINEP; FAPDF and Fundação Araucária.

PCM 050- ASSOCIATION OF PARACOCCIDIOIDOMYCOSIS AND TUBERCULOSIS IN A BRAZILIAN RECYCLING WORKER: A CASE REPORT AND OCCUPATIONAL RISK CONSIDERATIONS

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Introduction: Paracoccidioidomycosis (PCM) is a systemic fungal infection endemic in Latin America, particularly Brazil, with clinical presentations that often overlap with tuberculosis (TB), especially in cases involving pulmonary symptoms. Co-infection with TB has been reported in approximately 10% of PCM cases, making clinical suspicion essential for accurate diagnosis. **Case:** A 47-year-old male from Campo Grande, MS, employed in recycling, presented with a three-month history of cough, hemoptysis, weight loss, anorexia, fatigue, and afternoon fevers. His history included alcohol use, smoking, cocaine paste use, and recent incarceration. TB was diagnosed via GeneXpert molecular testing of sputum, which showed a medium bacillary load. The HIV serology resulted negative. He began treatment with rifampicin and isoniazid, showing good adherence. However, he subsequently developed oropharyngeal lesions, including ulcers on the palate and lips, along with dysphagia and hoarseness. These symptoms raised suspicion for PCM. Ultrasound revealed multiple hypoechoic cervical lymph nodes and atypical cervical lymphadenopathy. The thoracic computed tomography revealed suggestive changes of PCM. Direct mycological examination confirmed PCM with the presence of *Paracoccidioides* spp. Antibodies anti-*Paracoccidioides* were detected by double radial immunodiffusion. The patient was discharged with combined treatment for PCM (sulfamethoxazole-trimethoprim) and TB, with outpatient follow-up planned. **Discuss:** The concurrent presence of PCM and TB in patients suggests a potential immunological link. While PCM commonly affects agricultural workers, recycling work may similarly increase risk, given the fungus's role as a soil saprophyte. Therefore, in endemic areas, recycling should be recognized as an occupational risk factor for PCM.

Keywords: Paracoccidioidomycosis; tuberculosis; co-infection

PCM 051- EFFICACY OF COTRIMOXAZOLE IN THE PARACOCCIDIOIDOMYCOSIS TREATMENT USING SERUM SULFAMETHOXAZOLE DOSAGE

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Introduction. Paracoccidioidomycosis (PCM) is an endemic mycosis in Latin America. Although itraconazole (ITC) is the drug of choice for its treatment, the co-trimoxazole (CMX) remains widely used, especially in Brazil, where it is more accessible due to its free distribution in throughout the country. However, CMX is less potent than ITC, requiring months to years of treatment. **Objective.** This study aimed to evaluate the association between serum levels of sulfamethoxazole (SSL) and the time to achieve clinical and serological cure in PCM treatment. **Methodology.** A non-concurrent cohort of 106 PCM patients treated with CMX was studied and evaluated monthly until clinical cure and quarterly until serological cure regarding clinical, laboratory, and SSL dosage parameters. A Cox proportional hazards regression model was performed to determine which SSL levels were associated with a faster therapeutic response. **Results.** The median of age was 46 years [31 – 54] and 93 (86.9%) of patients were male. The most prevalent clinical form was moderate chronic (59.8%), followed by severe acute (29.0%) and severe chronic (11.2%). Patients with SSL above 70 µg/mL had earlier time to clinical cure ($p < 0.01$) and those ones with SSL above 50 µg/mL had a shorter time to reach serological cure ($p = 0.01$). The range of 71–90 µg/mL of SSL [HR=1.93 (1.02–3.65); $p < 0.01$] and above 90 µg/mL [HR=2.98 (1.72–5.17); $p < 0.01$] were independently associated with earlier clinical cure. The range of 50 – 70 µg/mL of SSL [HR=2.52 (1.17–5.44); $p < 0.01$], 71–90 µg/mL [HR=4.77 (2.00–11.36); $p < 0.01$], and above 90 µg/mL [HR = 4.23 (2.11–8.50); $p < 0.01$] were independently associated with earlier serological cure. **Conclusions.** This study demonstrates that the use of SSL dosage is associated with the time to cure of PCM and can be used for treatment monitoring, reducing the duration of CMX therapy.

Keywords: Paracoccidioidomycosis, trimethoprim-sulfamethoxazole, serum levels, treatment outcomes.

Support: This study has no support.

PCM 053- PARACOCIDIOIDOMYCOSIS EPIDEMIOLOGICAL PROFILE IN RONDÔNIA, BRAZIL, 2014 TO 2024: PRELIMINARY RESULTS

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1. Introduction. Paracoccidioidomycosis (PCM) is the most important systemic mycosis in Brazil. The country represents the largest endemic area in the world. It is caused by a dimorphic fungus (*Paracoccidioides* spp.) encountered in rural areas. The most common lesions frequently occur in the lungs, buccopharynx mucosa and lymph nodes. It is not a disease of national mandatory notification, what doesn't allow the real knowledge of the magnitude of problem. **2. Objective.** Conduct a retrospective assessment of patients diagnosed with PCM over the past ten years, treated at CEMETRON. **3. Methodology.** Patients treated from 2014 to 2024 with diagnosis of PCM were included. Demographic and clinical variables were assessed. Patient information's were accomplished in standardized protocol. The data were organized and analyzed using Epi Info, Tabwin and Excel. **4. Results.** The incidence was more important in central and southern state (83% of PCM cases) and 79% of patients related rural work. More than 99% were adults and 95% were male, average age 53 years old (range 15-79). The clinical chronic presentation was present in 96,4%, with lungs 90%, mucosa 57%, lymph nodes 30% and skin 22%. The diagnosis was confirmed in 44% of PCM cases by direct examination, 40% histopathologic, 9% culture, 1,4% clinical and epidemiological and 0,7% serology. More than 90% received itraconazole for treatment. **5. Conclusions** The males of productive age in southern rural areas in Rondônia were the most affected. The local PCM incidence increased during the last years, and PCM is considered as an emerging disease. The local compulsory notification allowed this epidemiological analysis. The availability of the drug has been an important factor for therapeutic management and clinical monitoring. There is need of training more health professionals in clinical diagnosis, laboratory, treatment and epidemiological surveillance in order to control program sustainability, promotion, prevention and assistance to the PCM patients.

Keywords: Paracoccidioidomycosis, Epidemiology, Rondonia.

**PCM 054- DIFFERENT TYPES OF PROPOLIS IMMUNOMODULATE
NEUTROPHILRESPONSES AND HAVE ANTIMICROBIAL EFFECTS ON
*Paracoccidioides brasiliensis***

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ABSTRACT-Introduction. The fungi of the complex *Paracoccidioides spp* are responsible for causing Paracoccidioidomycosis (PCM), a systemic mycosis that requires long-term treatment. Different types of propolis-(PBGR), identified according to their color (green, brown and red) have great economic and medicinal importance. **2. Objective(s).** The ability of PBGR to activate PMNs and its antifungal activity on Pb18 was evaluated. **3. Methodology.** Antifungal activity of PBGR at a concentration of 500mg/mL was tested against a virulent *P. brasiliensis* isolate (Pb18), using the macrodilution technique. Mice were inoculated via subcutaneous air-pouch with Pb18. On the fifth day of infection, treatment with 500mg/mL PBGR via air-pouch was initiated and maintained for 3 days until the collection of the PMNs, at 8 days of infection and treatment. The following parameters were analyzed: absolute number of cells at the air-pouch, mitochondrial activity, levels of ROS, total proteins production, as well as the number of viable fungi. **4. Results.** In vitro experiments showed remarkable direct antifungal activity of PBGR. PBGR reduced the number of viable fungi in relation to the original inoculum after 72h of incubation. *Ex vivo* experiments showed that PBGR caused increased mitochondrial activity and decrease in ROS production and in the influx of PMNs in relation to untreated Pb18 infected controls. PBGR was able to markedly reduce the number of viable Pb18 as compared to Pb18-infected controls. **5. Conclusions** Our results show that PBGR has a direct antifungal effect and is able to prevent fungal growth and increase PMNs activation. This data allows us to suggest that PBGR can be a new natural complementation to the existing therapeutic options in PCM treatment.

Keywords: Polymorphonuclear; Propolis; *Paracoccidioides brasiliensis*

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L.A.Santos

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PCM 055 - IMMUNOMODULATORY AND ANTIFUNGAL ACTIVITY OF FLUCONAZOLE IN *P.*

lutzii AND *P. brasiliensis* INFECTIONS

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ABSTRACT -Introduction. Recently many cases of paracoccidioidomycosis (PCM) can be attributed to *Paracoccidioides lutzii* in addition to those caused by *P. brasiliensis*. Fluconazole (FLU) is a triazole antifungal routinely used in PCM, but its interaction with the immune response has never been studied. **2. Objective(s).** To evaluate the *in vitro* and *ex vivo* antifungal activity of FLU on *P.lutzii* (PI) and *P.brasiliensis* (Pb18), as well as its influence on immunological parameters. **3. Methodology.** Antifungal susceptibility to 1, 2 and 3 mg/mL FLU was tested *in vitro* using the macrodilution technique. FLU cytotoxicity was assessed against splenocytes cultures. Female Swiss mice were infected via subcutaneous air pouch in an *ex vivo* model. The material obtained was used to evaluate immunological parameters, such as absolute and relative cell counts, cellular activation parameters, content of nitric oxide, hydrogen peroxide, catalase, ROS, and of cytokines. **4. Results.** Cellular influx to air pouch was altered by the presence of FLU, resulting in reduction in neutrophils numbers, in parallel to an increase in lymphocytes. All concentrations of FLU showed antifungal activity against Pb18 and PI. FLU stimulated the cells present in the exudate. There was an increase in the peroxide content in the PI infection with treatment with 2mg/mL. Furthermore, there was an increase in catalase in the PI-infected group. In both infections there was an increase in ROS. However we observed no change in NO synthesis with the treatment. FLU stimulated the production of Th1-associated cytokines and suppressed Th2 cytokines in infections with either fungal strain. **5. Conclusions** FLU presents marked antifungal effect against both PI and Pb18. The increase in lymphocytes in the infection by either strain indicates an anti-inflammatory role of FLU, as well as activation of cells present in the air pouch, demonstrating its interaction with the immune response.

Keywords: *Paracoccidioides lutzii*; *Paracoccidioides brasiliensis*; Fluconazole; Air pouch

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PCM 056- PREVALENCE AND CLINICAL EVOLUTION OF CHRONIC PULMONARY ASPERGILLOSIS IN PATIENTS WITH TUBERCULOSIS

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ABSTRACT – 1. Introduction. Chronic pulmonary aspergillosis (CPA) is a slow and progressive lung disease that develops in patients with chronic respiratory disease, with previously treated pulmonary tuberculosis (PTB) being the main predisposing factor. The prevalence of CPA in patients with previously treated PTB is known to be around 11%; however, the coexistence of the two diseases has been little studied. **2. Objective(s).** To estimate the prevalence and evaluate the clinical and tomographic evolution of CPA in patients with active PTB. **3. Methodology.** An epidemiological study was conducted with 85 patients diagnosed with PTB for less than 4 months, consecutively attended at the Hospital Universitário Maria Aparecida Pedrossian, between June 2022 and June 2023. All participants were investigated for CPA, including clinical, tomographic, and serological evaluations. **4. Results.** Among the 85 participants, three were diagnosed with CPA, yielding a prevalence of 3.5% (95% Confidence Interval: 1.2%-9.9%). All three patients were male, with a median age of 54 years (minimum 40; maximum 58 years). Additionally, two were people living with HIV/AIDS (PLWHA) with a CD4+ cell count lower than 300. The patients presented weight loss (n=3), cough (n=2), chest pain (n=1), and hemoptysis (n=1). All three were treated concomitantly for TB and CPA. Regarding the findings observed on the first computed tomography (CT), all three patients had more than one cavity, as well as pleural thickening and nodules. The cavities were bilaterally located in the apical region (n=3). Two patients had a fungal ball. In the follow-up CT evaluation, one patient showed disappearance of the fungal ball and cavities, with residual dense stripes remaining. One patient exhibited progression with persistence of the fungal ball and cavities. One of the patients lost follow-up. **5. Conclusions.** These findings demonstrate the importance of systematic investigation of CPA in patients with active PTB, especially in those with cavities observed on CT.

Keywords: Chronic pulmonary aspergillosis, Prevalence, Pulmonary tuberculosis, Clinical Evolution.

PCM 057- INVESTIGATION OF THE IMMUNE RESPONSE OF THE ALTERNATIVE MODEL *GALLERIA MELLONELLA* TO MONOCLONAL ANTIBODIES AGAINST *PARACOCCIDIODES LUTZII* OBTAINED THROUGH SUBTRACTIVE IMMUNIZATION

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ABSTRACT – 1. Introduction. Paracoccidioidomycosis is an endemic mycosis in South and Latin America caused by fungi of the genus *Paracoccidioides*, mainly by species *P. brasiliensis* and *P. lutzii*. Larvae of *Galleria mellonella* serves as an alternative model to mammalian studies due to their similar innate immune system. Subtractive immunization and monoclonal antibodies (mAbs) are employed to enhance the understanding of the immune response against pathogens. **2. Objective.** Investigate the immune response of *G. mellonella* against *P. lutzii* using mAbs produced through subtractive immunization. **3. Methodology.** To assess the survival of *G. mellonella* infected with *P. lutzii* and *P. brasiliensis*, strains Pb18 and PL01 were cultured in YPD medium and adjusted to 6×10^6 cells/mL. Five groups of 10 larvae were incubated at 37°C: (1) unmanipulated; (2) injected with 10 μ L of PBS; (3) injected with commercial IgG; (4) infected with Pb18; and (5) infected with PL01. Survival, capsule formation, mobility, and melanization were monitored. For mAb production, hybridomas (PL01S5C92-H10) were cultured in RPMI medium with additives and fetal bovine serum (FBS). After confirming viability, FBS was gradually reduced, and mAb production was induced by medium acidification over 14 days. The supernatant was purified; SDS-Page electrophoresis confirmed the presence of antibodies. **4. Results.** Larvae infected with Pb18 and PL01 showed a survival of 5 days, while controls and the IgG-treated group survived, indicating immunoglobulin safety. PL01 exhibited faster lethality than Pb18. The H10 hybridoma maintained viability with low FBS concentrations, revealing a 150 kDa band under non-reducing conditions and light and heavy chains under reducing conditions. Western blotting confirmed H10 reactivity with a 50 kDa band, not previously described. **5. Conclusion.** MAbs against *P. lutzii* were produced using subtractive immunization. H10 demonstrated high specificity, offering potential for studies on *G. mellonella* immune responses, possibly advancing understanding of paracoccidioidomycosis immunopathogenesis and host resistance mechanisms.

Keywords: *Paracoccidioides lutzii*, Monoclonal antibodies, Subtractive immunization, *Galleria mellonella*

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PCM 058- TEN YEARS OF PARACOCCIDIOIDOMYCOSIS NOTIFICATION IN MATO GROSSO DO SUL, BRAZIL: INSIGHTS AND CHALLENGES

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Abstract- Introduction: Paracoccidioidomycosis (PCM) is a systemic fungal disease caused by fungi of the *Paracoccidioides* genus, predominantly found in Latin America, with Brazil accounting for the highest prevalence. Mato Grosso do Sul is a predominantly agricultural state with a history of reported PCM cases and was one of the first in Brazil to adopt compulsory notification of PCM, starting in 2005. **Objective:** To analyze the experience of ten years of compulsory PCM notification in Mato Grosso do Sul. **Methodology:** Data were retrieved from the Notification of Diseases Information System (SINAN) for the period between 2014 and 2024. The analysis included the number of reported cases and sociodemographic variables. **Results:** Between 2014 and 2024, 177 PCM cases were reported, averaging 17 cases per year. Notably, only pulmonary disease cases were included in the notification form. Men accounted for 85.4% of cases, with 89% occurring in individuals over 40 years old. Women represented 14.6% of cases, predominantly in the age group over 45 years (60%). Cases were distributed across 31 municipalities, primarily concentrated in the central-southern region of the state, with the capital, Campo Grande, responsible for the majority of notifications (48%). **Conclusion:** Over the ten-year period, an average prevalence of approximately six cases of pulmonary PCM per 100,000 inhabitants was observed, reinforcing the endemic nature of the disease. Cases were primarily distributed in regions with lower temperatures (22°C and 24°C). However, the information collected in the notification system was limited, lacking critical data such as clinical forms, diagnostic methods, risk factors, and mortality, which are essential for a comprehensive understanding of the disease.

Keywords: Paracoccidioidomycosis; Mato Grosso do Sul, cases

PCM 059- ATYPICAL PARACOCCIDIOIDOMYCOSIS IN A PATIENT WITH CARD9 HETEROZYGOUS MUTATIONS

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CASE REPORT - 1. Introduction. Paracoccidioidomycosis (PCM) is a systemic mycosis caused by thermotolerant fungi of the genus *Paracoccidioides*. In Latin America, PCM is endemic, with Brazil reporting the highest number of cases. Atypical presentations of PCM are rare and may be associated with Inborn Errors of Immunity (IEI). The connection between paracoccidioidomycosis (PCM) and IEI remains poorly understood. This is due to the limited number of reports documenting associations with CD40 ligand deficiency, IL12R β 1 deficiency, and heterozygous STAT4 mutations in the literature. **2. Case Report.** We present the case of a 45-year-old man from Campo Grande, MS, Brazil, single, with a complete vaccination history. In 2001, he was diagnosed with PCM, presenting with an acute-subacute form, involving the larynx and cervical lymph nodes but sparing the lungs. In 2006, he experienced a reactivation, this time involving pulmonary compromise, characteristic of the chronic form of PCM. In 2010, chest imaging findings suggested pulmonary tuberculosis (TB); however, sputum smear testing for acid-fast bacilli (AFB) was negative, and treatment for TB was initiated. In 2011, he underwent treatment for visceral leishmaniasis. Between 2006 and 2024, the patient exhibited irregular compliance with antifungal therapy (sulfamethoxazole + trimethoprim), resulting in chronic hypoxemia-induced pulmonary fibrosis, secondary polycythemia, Addison's disease, and hypoalbuminemia due to protein-losing enteropathy identified by scintigraphy. Additionally, he developed thrombosis in the artery of his right lower limb and sustained a traumatic brain injury due to a pedestrian-vehicle collision in 2019. In 2024, investigations for IEI revealed a reduced CD4+ T cell count (237 cells/mm³). Whole-exome sequencing identified two heterozygous mutations in the CARD9 gene: c.302G>T and c.951+9_951+17 delinsA. The patient has achieved clinical and serological cure since October 2021 and remains under follow-up awaiting confirmation of apparent cure. **3. Discussion.** CARD9 plays a pivotal role in intracellular signaling via C-type lectin receptors, which recognize fungal structures and activate downstream immune responses. Deficiency or mutations in CARD9 have been implicated in severe fungal infections, including Aspergillosis and Candidiasis. Our data suggest that CARD9-related deficiencies shed light on the immunopathogenesis of PCM and other granulomatous infections.

Keywords: Paracoccidioidomycosis, CARD9, Inborn Errors of Immunity.

PCM 060- CHRONICLES OF RESILIENCE: GIVING VOICE AND SUPPORT TO PATIENTS WITH PARACOCCIDIOIDOMYCOSIS

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ABSTRACT – 1. Introduction. Paracoccidioidomycosis (PCM) is the leading endemic systemic mycosis in Latin America, characterized by high morbidity and mortality, primarily affecting rural workers. In addition to physical limitations, patients face social stigma that impairs their quality of life. The expression of personal experiences in therapeutic settings can foster support and resilience while also providing valuable insights into psychosocial experiences. **2. Objective(s).** To investigate the psychosocial impacts of PCM using "escrevivência" (life writing) as a method to document, analyze, and understand life experiences in contexts of stigma and illness. **3. Methodology.** This is an exploratory qualitative study using "escrevivência" (life writing) as a method for data collection and analysis. Patients with a confirmed diagnosis of PCM were recruited to participate in group sessions conducted by two occupational therapists. During the sessions, participants shared their experiences, which were documented in the form of chronicles. The texts were presented to the group, collectively revised, and thematically analyzed to identify patterns and common experiences. Clinical and demographic data were collected to characterize the sample and provide context for the narratives. **4. Results.** The group consisted of 8 patients (mean age: 60 years; 4 mixed-race and 4 white). Seven presented the chronic form of PCM, and one had the acute-subacute form. Five cases were of moderate severity, and three were severe, with the time since diagnosis ranging from 1 to 8 years. The thematic analysis of the 8 chronicles revealed key categories such as fear of death, loneliness, distress related to delayed diagnoses, physical limitations, and the impact of social stereotypes. **5. Conclusions.** Life writing proved to be an effective method for uncovering psychosocial aspects associated with PCM, highlighting the importance of humanized care. The results emphasize the need for strategies to mitigate the impact of stigma and strengthen emotional support.

Keywords: Paracoccidioidomycosis, life writing, care, humanization.

Support: Programa Inova Fiocruz.

PCM 061- ENHANCING PARACOCCIDIOIDOMYCOSIS DIAGNOSIS: A 10-YEAR INTEGRATION OF CONVENTIONAL AND MOLECULAR TECHNIQUES IN A NON-ENDEMIC AREA

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Introduction Paracoccidioidomycosis (PCM) is a systemic endemic mycosis in Latin America. The increasing migration of populations and the impact of climate change necessitate increase diagnostic capabilities for PCM in clinical mycology laboratories, particularly in non-endemic areas. **Objective** This study aims to report a series of proven and probable cases of PCM diagnosed at a public university hospital in Buenos Aires, Argentina. **Materials and methods** A retrospective observational analysis was conducted on patients diagnosed with PCM from January 2015 to October 2024 at the hospital. Cases were classified as **confirmed** when patients with clinical and epidemiological criteria showed microbiological (direct examination of clinical specimens or recovery of fungus in culture) or histopathological criteria, and as **probable** when patient showed positive specific antibodies (Ab) by immunodiffusion test and DNA detection using a nested PCR. The following data were collected: age, sex, place of birth and residence at the time of admission. **Results** A total of 26 cases with clinical suspicion of PCM were identified. Among these, 17 were confirmed PCM cases (89.5%) and 2 were probable PCM cases. These 19 patients were adult males with an average age of 54.4 years (range 23-72 years). Of these, 15 patients were born and currently inhabit in endemic areas, while 4 were born in endemic areas but reside in non-endemic areas. A total of 44 samples were processed (oral mucosa, laryngeal, nasal and skin biopsies, respiratory samples, lymph node biopsies, adrenal gland fluid). The direct microscopic examination was the method that allowed us to make 89.5% of cases diagnoses. Culture was positive in 31.5% of cases, while specific Ab and DNA detection of *Paracoccidioides* was positive in 63.1% of the patients. **Conclusion** The data suggest that increased migration patterns may contribute to the emergence of PCM cases in non-endemic areas and highlight the importance of training healthcare professionals in recognizing this mycosis. This report shows the presence of PCM in a non-endemic setting, emphasizing the need for ongoing surveillance and improved diagnostic protocols in response to demographic shifts and environmental changes. Enhanced understanding and recognition of PCM will facilitate timely intervention and better patient outcomes.

Keywords: PCM, non-endemic area, laboratory diagnosis, molecular diagnosis

PCM 062- PRELIMINARY RESULTS ON THE STIMULATION OF HUMAN NEUTROPHILS AND RECOVERY OF FUNGAL TRAITS BY EXTRACELLULAR VESICLES USING THE VIRULENT/ATTENUATED PB18 *PARACOCCIDIOIDES BRASILIENSIS*

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ABSTRACT – 1. Introduction. Extracellular vesicles (EVs) are by-layered membrane cellular round structures involved in cargo delivery. Previously, our group reported that vEVs from the virulent vPb18 *Paracoccidioides brasiliensis* isolate could, upon co-incubation, revert the expression of oxidative/nitrosative stress traits of attenuated aPb18. Additionally, vEVs and aEVs differentially stimulated murine macrophages *in vitro* and exacerbated murine infection. **2. Objectives.** To investigate the differential effects of vEVs and aEVs in aPb18 growing under cell wall stress conditions and on human neutrophil stimulation. **3. Methodology.** EVs were isolated by serial centrifugations and filtrations, with a final 1-h 100,000-g ultracentrifugation. 4-h EV co-incubations with fungal cells or neutrophils were standard. Fungal viability was visually observed in serial dilution spot tests on mYPD plates. Neutrophil stimulation was estimated by extracellular DNA released on NETs. **4. Results.** In a preliminary experiment, we observed a similar increase in human neutrophil extracellular DNA when the cells were co-incubated with the highest concentration (5×10^8) of both aEVs and vEVs. DNA was detected by fluorescence and DNA quantification. We also observed that pre-incubation of vPb18 and aPb18 with both vEV and aPb18 visually increased resistance to sorbitol and Congo red. **5. Conclusion:** Preliminary results showed that both vEVs and aEVs might contribute to human neutrophils NET formation. Further replicates are needed to demonstrate that the extracellular DNA presently measured is due to NETs release and to investigate ROS production. On the other hand, our preliminary results on the fungal recovery of resistance to stress agents, as mediated by EVs, suggest that it is due to common components transported by both vEVs and aEVs, but that assumption needs further investigation.

Keywords: *Paracoccidioides brasiliensis*; extracellular vesicles; attenuated/virulent Pb18; NETs; neutrophils.

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