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INVITED SPEAKER

Strategies of *Trypanosoma rangeli* for survival and transmission in invertebrate hosts

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Trypanosoma rangeli is a protozoan that infects triatomines and mammals in Central and South America. Although it does not cause disease in humans, this parasite induces varying levels of pathogenicity in its invertebrate host, primarily in species of the genus *Rhodnius*. Several pathological effects caused by *T. rangeli* have been reported, including prolongation of the intermolt period or interruption of the molting process, defective ecdysis, increased mortality rates, and reduced reproductive success. The parasite also reduces the microbiota diversity of *Rhodnius prolixus*, likely affecting the insect's fitness. Systemic infections are also associated with alterations in lipid metabolism and transport. These metabolic changes trigger behavioral modifications that, in turn, enhance parasite transmission. The strategies employed by the parasite to ensure transmission are discussed in the context of the pathological effects it exerts on the insect host.

Giardia intestinalis-induced Type 2 mucosal immunity attenuates bystander intestinal Inflammation

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Diarrheal diseases are the second leading cause of death in children worldwide. Epidemiological studies show that co-infection with *Giardia intestinalis* decreases the severity of diarrhea in school-aged children. Here, we show that *Giardia* is highly prevalent in the stools of asymptomatic children in Nigeria. Using a mouse model, we demonstrate that *Giardia* orchestrates a Type 2 mucosal immune response, characterized by high levels of antigen-specific Th2 cells, IL-25, Type 2-associated cytokines, and goblet cell hyperplasia. Specifically, *Giardia* infection expanded IL-10-producing Th2 and GATA3+ Treg cells that promoted parasite colonization, life cycle transmission and conferred protection against inflammatory-driven diseases including *Toxoplasma gondii*-induced lethal ileitis and DSS-driven colitis. Protection was dependent on STAT6 signaling, as *Giardia*-infected STAT6-/- mice failed to expand IL-10+ Th2 cells. Instead, STAT6-/- mice developed Th1/Th17 responses that cleared infection, but resulted in collateral tissue damage associated with high levels of IFN-g and IL-17A. Similar results were observed during the blockage of IL-4R and IL-10R. Our findings demonstrate that *Giardia* infection reshapes mucosal immunity toward a Type 2 response, which facilitates parasitism, confers a mutualistic protection against inflammatory disease processes and identifies a critical role for protists in regulating mucosal defenses.

Living in Endemic Area for Infectious Diseases is Associated to Differences in Immunosenescence and Inflammatory Signatures

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Research on aged individuals from developed countries show that lifestyle factors such as diet, physical activity, stress, smoking, and sleep quality impact aging. However, other relevant factors may influence aging in less-studied populations, such as Brazilian cohorts. This study aimed to analyze immunosenescence profile of individuals living in an endemic area for several infectious diseases in Brazil (schistosomiasis, leishmaniasis, hanseniasis). We showed that these individuals exhibited accelerated epigenetic aging and increased production of IL-12p70, IL-17A, and IL-9. Production of inflammatory mediators IL-12p70, IL-6, IL-1β, IL-2, and IL-1ra in individuals with flu-like symptoms and those with COVID-19 was higher among residents in endemic areas than in residents from a control non-endemic area. Furthermore, residents of the endemic area had a more prominent inflammatory profile during viral infection and a different pattern of plasma mediators when compared to residents of a non-endemic area. Our data suggests that these two cohorts had specific immune signatures regardless of the presence or the type of infection at study. Therefore, we demonstrated that there were distinct patterns of immune responses and epigenetic aging depending on the environment the individuals live in. These observations add a layer of diversity to the studies of human aging by including individuals from less represented regions.

Keywords: endemic area, infectious diseases, immunosenescence, epigenetic clocks, environment

Financial support: Merck Sharp & Dohme - MSD (Investigator Initiated Studies Program), CNPq, CAPES.

Applying Bioinformatics to Study Leishmania

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Leishmania parasites are the causative agents of leishmaniasis, a neglected tropical disease with global health implications. These parasites need to adapt to diverse environments within mammalian hosts and insect vectors, leading to rapid physiological and metabolic changes. In the context of genomics, our studies on the genetic diversity of *Leishmania braziliensis* isolates from Pernambuco, Brazil, revealed distinct evolutionary groups and substantial genetic variability. This high level of diversity underscores the complexity of *Leishmania* transmission and highlights the need for a more precise definition of its eco-epidemiology. By investigating RNA modification mechanisms, we identified candidates of enzymes responsible for N1-methyladenosine (m1A) and 5-methylcytosine (m5C) modifications. Interestingly, despite evidence of N6-methyladenosine (m6A) modification in *Trypanosoma brucei* mRNA, homologs of m6A writers have not been identified in *Trypanosomatids*. These RNA modifications are believed to be crucial for parasite survival and may serve as potential therapeutic targets. We have applied computational methods to assess the predicted *Leishmania* proteomes, integrating functional and structural information to identify potential drug targets and small molecules for further experimental validation. Based on single-cell RNA sequencing and computational analysis, we have gained deeper insights into the immunological mechanisms underlying leishmaniasis chronicity in HIV-coinfected patients. Bioinformatics has become indispensable for advancing our understanding of *Leishmania* biology and pathogenesis. It facilitates the identification of novel drug targets by focusing on the parasite's unique biochemical pathways and RNA modification machinery. Moreover, genomic characterization enables improved disease tracking and control. The integration of these approaches is essential for developing effective strategies for diagnosing, treating, and preventing leishmaniasis.

EMPOWERING PEOPLE WITH CUTANEOUS LEISHMANIASIS (ECLIPSE): THE BRAZILIAN EXPERIENCE WITH IN-FORMATION AND KNOWLEDGE EXCHANGE TRAINING COURSES TO PRIMARY CARE AND EDUCATIONAL PROFESSIONALS FROM ENDEMIC AREA IN SOUTHERN BAHIA, BRAZIL

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We present a qualitative research-intervention based on the education and professional training practices built with ECLIPSE - Empowering people with cutaneous leishmaniasis: Intervention programme to improve patient journey and reduce stigma via community education - an interdisciplinary applied health program based in Brazil, Ethiopia, Sri Lanka, and the United Kingdom. It is a strategy of community education based on the dialogue between different knowledge and experiences to enhance professional awareness and to guarantee community engagement and participation in the prevention of cutaneous leishmaniasis (CL). It was agreed to assume a Problem-based Learning pedagogic model, promoting dialogue and interaction through triggering questions, activities, or problem situations related to the CL training courses' program themes with a total 20-hour workload. Therefore, pre-established working groups can discuss internally, based on consultation of the didactic-informative material available. Subsequently, all teams will bring their topics to discuss with the entire class collectively led by researchers from the ECLIPSE/BRAZIL team. Community engagement activities were affected by the COVID-19 pandemic, which brought the imposition of social distancing. Even so, our methodology was approved for the development of CL knowledge, and participants evaluated the CL information provided as useful for their professional activities in rural communities, reflected by high satisfaction with the quality of the ECLIPSE - CL training courses. A positive evaluative aspect was observed when the participants were protagonists in the actions to multiply the information about CL. This is a strategy for the sustainability of our health interventions. The focus on community engagement in a health project represents one avenue through which we can decolonize research practice and knowledge production, and steer away from top-down modes of engagement in a collective health.

Supported by the UK National Institute for Health Research (NIHR200135).

Keywords: Cutaneous Leishmaniasis; Community Engagement; Health Education.

Innovative Helmintex modifications as reference method and for late stages of schistosomiasis transmission interruption

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Helmintex (HTX) isolate *Schistosoma mansoni* eggs from a larve fecal sample, using their magnetic interaction with paramagnetic beads in a magnetic field. Sensitivity is estimated as 100% for egg burdens higher than 1.3 eggs per gram. It is not meant to be used in routine diagnosis, but as a reference method to correctly classify samples to test other methods in accuracy studies. It is also a laborious and time-consuming method. Several innovative improvements are underway. Besides the large fecal volume, the concentration HTX steps faces a challenge: the huge number and diversity of fecal debris, as well as their tendency to aggregate. Clustering also occurs with *S.mansoni* eggs, with yet unknown consequences to the ability of parasitological methods to detect them. HTX comprises two main stages: 1) concentration and cleaning; 2) detection at the microscope. For stage 1, prototypes of tridimensional filter (3D printing) have been successfully tested. For stage 2: automated microscopy with IA-algorithms, egg staining with fluorescent markers and egg sorting in a flow large particle system. Abbreviated HTX is another interesting development under evaluation, both with the examination of fecal volumes smaller than 30g, and modelling the probability of distribution of eggs in sediments placed onto microscope slides. Taken together, these improvements may help consolidate HTX as a reference method, as well as expand its role as an additional tool for schistosomiasis interruption certification.

The depths of kala-azar clinical presentation

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The roots of the clinical presentation will be discussed from the moment of infection to the unlikely disease and then to complications and death. The main signs and symptoms, such as fever, wasting, paleness, hepatosplenomegaly, jaundice, and edema, together with the central laboratory findings of pancytopenia and albumin-globulin inversion, will also be analyzed. Finally, the most typical causes of death, bacterial infections, and hemorrhages will be explained with promises to reduce disease mortality.

DEVELOPING RNA VACCINE STRATEGIES FOR DISEASES CAUSED BY TRYPANOSOMATIDS

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Trypanosomatids are intracellular protozoan parasites that cause neglected diseases with a major impact on public health, such as Chagas disease (*Trypanosoma cruzi*) and leishmaniasis (*Leishmania* spp.). Despite the urgent need for effective preventive measures, the development of vaccines against these pathogens faces several challenges including the large genetic variability of the parasite population and immune evasion mechanisms developed by these pathogens. Our group has previously described several recombinant antigens from *T. cruzi* and *Leishmania* spp. They were able to generate robust cellular and humoral responses that result in partial protection against challenge with different parasite species as well as parasite strains. Since RNA vaccines are based on intracellular antigen synthesis and, therefore, induces a potent Th1 response, which is essential for intracellular pathogen infection control, we decided to compare immunization protocols based on recombinant protein and the corresponding mRNA encapsulated into lipid nanoparticles (LNPs). Sequences from different antigens, cloned into a mammalian expression vector, were used for mRNA synthesis using in vitro transcription protocols. After cell transfection, the capacity of these mRNAs to produce the antigens were evaluated by western blot using antibodies generated against the recombinant proteins. Mice immunization with the RNA/LNP formulation encoding a *Leishmania* antigen resulted in a more prominent Th1 response and similar cellular response as shown by IFN-γ production, compared to immunization with the recombinant protein. Challenge of the immunized mice with *Leishmania amazonensis* showed that both vaccines result in similar protection. Immunization with RNA/LNP formulations containing sequences from *T. cruzi* Trans-sialidases induces similar humoral and cellular immune responses compared to immunization with recombinant proteins, as well as similar protection against challenge with a virulent *T. cruzi* strain, as shown by parasitemia and tissue parasitism. We are currently testing different prime-boost heterologous protocols in which immunization with recombinant protein and RNA/LNP formulations are combined.

Supported by: CNPq, CAPES, INCTV

Keywords: RNA vaccine, *T. cruzi*, *Leishmania* spp.

Single-cell atlas of *Leishmania* in the sand fly vector during development and transmission

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Sand fly vectors transmit *Leishmania* by egesting parasites into the host's skin. The transmissible dose is shaped by *Leishmania*'s development within the sand fly gut, characterized by a sequential differentiation of promastigote morphotypes. Although the transcriptional programs and molecular markers of mammal-infective metacyclic promastigotes are well-documented, there is limited knowledge about other coexisting stages in the midgut during mature infections that are also transmitted by the sand fly bite. In this study, we elucidate the single-cell transcriptomic complexity of *Leishmania major* colonizing its natural vector, *Phlebotomus duboscqi*, at early and late infection stages, identifying markers for three transmissible stages. Our analyses reveal a non-linear developmental progression, bifurcating into either replicating early metacyclics or attached and detached haptomonads. We demonstrate that haptomonads are a key component of the transmitted dose and, along with non-dividing late metacyclics, are infectious and exacerbate pathology in mice. Furthermore, we validate these developmental markers in *Leishmania infantum*, a visceralizing species, within its natural vector, *Lutzomyia longipalpis*, revealing conserved and species-specific aspects of differentiation and transmission. Our single-cell analysis resolves discrete subpopulations within mixed stages, providing valuable insights into *Leishmania* life cycle progression and its implications for vector-to-host transmission. These validated markers will be instrumental in future research on *Leishmania* surveillance, transmission dynamics, and potential intervention strategies.

The role of the retromer complex Vps5 in the biology of *Trypanosoma cruzi*

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The retromer complex (RC) is a set of proteins involved in the retrograde transport of intracellular proteins from endosomes/lysosomes to the Golgi. Previous studies have shown that the RC is important for viability, protein trafficking in the endocytic/secretory pathways and autophagy of *Trypanosoma brucei*. However, the role of the RC in *Trypanosoma cruzi* is still unknown. This work aims to characterize the role of Vps5 in the secretory/endocytic pathways of *T. cruzi*. The components of the RC in *T. cruzi* were identified by comparative genomics using orthologous genes of *T. brucei*, yeast and humans. The candidate *TcVps5* is a single copy gene and the encoded protein showed 54.65% identity with TbVps5. The relative expression of the *TcVps5* and *Tcgapdh* genes by RT-qPCR among the evolutionary forms of *T. cruzi* strain Dm28c revealed that TcVps5 is more expressed in epimastigote forms compared to amastigotes and tissue culture trypomastigotes. The functional characterization of the TcVps5 was made by CRISPR/Cas9 and the knockout (KO) parasites, in comparison to the control expressing *sp*Cas9, were analyzed for proliferation capacity and protein trafficking in the secretory/endocytic pathways, and the results were: i) reduction in the proliferation rate, mainly in the KO clones; ii) increased activity and processing of cruzipain in the KO clones; iii) slight increase of the Golgi by MET, iv) increased labeling of the WGA, GSIB4 and tomato lectins on the surface of the KO clones. Also, the KO clones had an impaired capacity to differentiate into metacyclic trypomastigotes, and, apparently, had a slower amastigotes infection index compared to control. These results suggest that TcVps5 interferes with protein trafficking in the secretory/endocytic pathways, and this interference impairs the proliferative capacity of *T. cruzi* epimastigote forms, compromises the parasite differentiation capacity and may play a role in the formation of mammalian *T. cruzi* evolutionary forms.

Supported by: FAPERJ, UB-SUNY

Key words: Vps5 retromer complex, cruzipain, *T. cruzi*

Parasitology and Ethnic-Racial Issues: Integrating Teaching and Research in Education

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This study aimed to train higher education teachers in the health sciences with a focus on integrating human parasitology content and ethnic-racial issues. We observed disharmonious ethnic-racial relations, marked by tensions arising from oppression affecting indigenous and Black populations in Brazil. We developed and implemented a teaching sequence for undergraduate learning about malaria, designed to foster dialogue between these áreas, and analyzed data obtained from its implementation with students. We conducted discussions that addressed conceptual and socio-scientific content related to malaria. As a result, some students revised their opinions and developed more informed arguments on the topic. This implies that the sequence successfully engaged undergraduate students, as it offered a model of socio-scientific issue oriented malaria teaching. Historical disputes in ethnic-racial relations are frequently translated into the poor valorization of knowledge and epistemes from oppressed groups, as indigenous and Black communities. The multi-(inter-) and transdisciplinary nature of this teaching proposal is crucial to address antiracism strategy in science education.

EXPLORING TRICHURIS INFECTION WITH ADVANCED MICROSCOPY

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Trichuriasis, a neglected tropical disease caused by *Trichuris trichiura*, primarily affects children and impoverished populations. Its zoonotic potential is evidenced by infections in nonhuman primates. Chronic infections lead to mucosal damage, bacterial translocation, and inflammatory infiltration; however, the progression of these events remains unclear. Our projects investigate *Trichuris muris* eggs, adult worm structures, and different phases of experimental trichuriasis, detailing nematode migration, immune responses, and bacterial translocation using a multidimensional imaging approach. Our findings reveal structural and chemical details of the egg, the ultrastructure of adult worms, and immune responses initiated by larval-induced lesions in the intestinal mucosa, which are further modulated by L4 larvae and adult worms in the cecum. A 3D animation illustrates how the worm's attachment mechanism facilitates bacterial translocation. To further elucidate the Trichuris egg, adult worm, and attachment process, we employed HPF/FS and multimodal imaging techniques, including X-ray microtomography (µCT), fluorescence stereomicroscopy, light-sheet microscopy, TEM, FIB-EDS, and FIB-SEM tomography. These techniques mapped detailed structures of the egg/L1 larvae, the nematode, including the bacillary band, composed of stichocytes and bacillary glands, which play a role in immunomodulation, as well as host tissues. Rendered 3D models and animations demonstrate how parasite movement and attachment promote bacterial translocation and secondary infections. Our approach integrates microscopy, physiological assessments, and computational modeling to provide new insights, from egg and larval stages before hatching to host-parasite interactions, particularly regarding parasite-microbiota dynamics. This study underscores the value of advanced imaging tools for understanding helminth infections, contributing to translational biology in helminthology, including egg ultrastructure, physiopathology characterization, and future therapeutic development.

Supported By: FAPERJ-JCNE, CNPq-Universal, FINEP.

Keywords: Helminth; Infection; Nematode; Bacterial translocation; Immune response; host-parasite interaction

DIAGNOSIS OF CUTANEOUS AND MUCOSAL LEISHMANIASIS: INNOVATIONS FROM PARASITOLOGICAL TO MOLECULAR APPROACHES

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Between 2001 and 2023, 17 countries in the Americas reported more than 1 million cases of cutaneous (CL) and mucosal (ML) leishmaniasis, with an annual average exceeding 50,000 cases. Brazil, Colombia, and Peru, along with seven countries from other continents, accounted for 91% of the global cases. CL is the most prevalent form of leishmaniasis and exhibits significant clinical diversity. In general, 3–5% of asymptomatic individuals or those who have recovered from CL develop ML, a disfiguring and stigmatizing condition with a strong psychosocial and economic impact on affected communities. Diagnosis remains one of the main challenges in controlling the disease as a public health issue. While clinical diagnosis is essential, laboratory confirmation is crucial. The use of deep learning techniques may facilitate access to diagnosis, but reliance on laboratory tests remains critical. In 2023, there was an improvement in CL and ML diagnosis across the Americas, with 86.2% of cases confirmed through laboratory methods, a 7% increase compared to the previous year. Direct parasitological examination (DP) is the most specific and of low complexity, but it requires well-trained professionals for slide preparation and reading, as well as access to a quality microscope. Continuous training of these professionals, along with monitoring their performance, directly impacts results in different regions. Complex CL cases, with low parasite loads in the lesion—also observed in ML—can result in false negatives in DP. In the absence of rapid tests for detecting antibodies or antigens, molecular tools based on PCR represent promising alternatives for molecular parasitological diagnosis, parasite load determination, and identification of the etiological agent. This study will present the contributions and advances of parasitological tests, both direct and indirect, as essential tools for diagnosing CL and ML in the Americas.

Supported by: DNDi, PAHO, CNPq, FAPERJ, Fiocruz

Keywords: cutaneous leishmaniasis, mucosal leishmaniasis, diagnosis

Vertical Distribution of Phlebotominae (Diptera: Psychodidae) in the Riparian Forest of the Paraná River Floodplain, Argentina

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Tegumentary leishmaniasis (TL) in Argentina has multiple hotspots and sporadic outbreaks, particularly in the northern regions where TL is endemic, including different provinces of the Chaco Bioregion. This study investigates the vertical distribution of Phlebotominae sandflies in the riparian forest of the Paraná River floodplain in Argentina, focusing on how environmental changes—particularly flooding—affect their population dynamics and behavior. The research was conducted between September 2011 and December 2012 across two ecologically distinct sites with different levels of human modification. Sandflies were sampled monthly using CDC light traps placed at ground (1.5 m) and canopy (10 m) levels in domiciliary, peridomiciliary, and forest environments. A total of 28,462 Phlebotominae were captured, with the majority found in forested areas. The most abundant species was *Nyssomyia neivai*, especially in forest and ground-level traps, although notable species diversity and evenness were observed in the canopy. Seasonal variations showed peak sandfly abundance in the warm and rainy months, with the highest ground-level activity in spring and canopy activity in summer. Statistical models indicated that minimum temperature positively influenced sandfly abundance, while river height and precipitation had negative effects, particularly at ground level. The study confirms that environmental factors, including climate and vertical stratification, significantly influence sandfly distribution. The presence of sandflies in the canopy suggests potential arboreal transmission cycles for Leishmania, especially relevant during flood events when ground habitats become unsuitable. These findings highlight the importance of considering vertical ecological dimensions in vector surveillance and disease control strategies in changing landscapes.

Supported by: CONICET

Keywords: Vertical stratification, Zoonotic disease, Vector

LIPID FLOW FROM HOSTS TO PARASITES: AN UNEXPLORED CASCADE OF BIOCHEMICAL MECHANISMS

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Parasites have evolved sophisticated strategies to manipulate the lipid metabolism of their hosts, ensuring their survival and replication. Our laboratory investigates how parasites alter lipid uptake, storage, and biosynthesis pathways, creating a favorable environment for their development. We have demonstrated that *Plasmodium*, *Trypanosoma cruzi*, and *Leishmania amazonensis* incorporate and metabolize host lipoproteins in a time- and dose-dependent manner, utilizing these lipids to organize membranes or store them in lipid droplets. For instance, *L. amazonensis* uptakes LDL in lipid rafts located in the flagellar pocket, while *Plasmodium* utilizes lipoproteins for crucial life cycle transitions, such as zygote-to-ookinete and oocyst-to-sporozoite transformation. Lipid depletion prevents these transitions, highlighting the parasite's dependence on host lipids. Moreover, parasites modulate host metabolism. In *Plasmodium chabaudi*-infected mice, we observed plasma dyslipidemia, with increased levels of triacylglycerols, free cholesterol, and fatty acids in the liver, as well as altered expression of key enzymes and transcription factors involved in lipid metabolism. Phosphorylation of AMPK, a central regulator of energy metabolism, was significantly reduced, promoting lipogenic pathways and inhibiting lipolytic pathways. Pre-treatment with metformin, an AMPK activator, protected the liver against these alterations and reduced parasitemia. These findings underscore the importance of parasite-induced metabolic reprogramming in disease progression and chronic infection. Understanding these mechanisms is crucial for developing novel therapeutic strategies targeting parasite-induced metabolic alterations.

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DRUG REPURPOSING FOR THE TREATMENT OF LARVAL CESTODIASES

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Larval cestodiases, caused by parasitic flatworms of the class Cestoda, are debilitating diseases that affect hundreds of millions of people worldwide. These parasites can also infect domestic animals, facilitating their transmission to humans and causing significant economic losses in livestock. Infections by larval cestodes are of difficult treatment, largely due to the limited availability and efficacy of currently available anthelmintic drugs. For example, the number of drugs for treating echinococcoses and cysticercoses is small, and their efficacy is limited, often providing only parasitostatic rather than parasiticidal effects. Additionally, there are already cases of emerging parasite resistance to existing drugs. In the search for new therapeutic targets and drugs for larval cestodiases, we have used *in silico* approaches to prospect available genomic, transcriptomic, and proteomic data for cestodes and other helminth parasites. The analyses performed allowed for the identification of proteins and cellular pathways associated with differential metabolic and developmental processes in parasites compared to their hosts. Components of these differential pathways have been further evaluated *in silico* for their druggability potential and to define possible inhibitory compounds for their predicted activities. Among the druggable target proteins, those targeted by compounds already approved for human and/or animal use in the treatment of other diseases have been prioritized, aiming for their repurposing as novel anthelmintics. The most promising drugs identified through *in silico* analyses are being tested *in vitro* against cestodes, using larval (tetrathyridia) cultures of the model cestode *Mesocestoides corti* and protoscoleces (pre-adults) of *Echinococcus* spp., as well as *in vivo* in mice experimentally infected with *M. corti*. Furthermore, proteomic studies are being carried out to globally assess direct or indirect effects of treatment with each candidate drug.

Supported by: CNPq, CAPES and FAPERGS.

Keywords: drug repurposing, echinococcosis, cysticercosis.

UNDERSTANDING THE MUCOSAL LEISHMANIASIS THROUGH AN EXPERIMENTAL MICE MODEL

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Leishmaniasis is a neglected tropical disease caused by Leishmania parasites, with mucosal leishmaniasis (ML) leading to severe destruction of oral and nasal tissues and presenting treatment challenges. To better understand ML pathogenesis, we established a murine model using BALB/c mice inoculated intranasally with *Leishmania amazonensis* promastigotes. The infection resulted in progressive edema in the nasal and premaxillary regions, with a higher parasite load compared to ear epidermal infections. Micro-CT scans revealed that the infection primarily affected the nasal vestibule, while histopathology showed epithelial disturbances and inflammatory infiltrates with infected macrophages and surrounding neutrophils in squamous and transitional tissues. These effects were dependent on the mucosal inoculation site. FACS analysis revealed that, unlike ear infections, mucosal infections maintained steady frequencies of CD4+ and CD8+ T cells but exhibited reduced IL-10+ cells and increased cytotoxic mediators (granzyme B, perforin, and CD107a). Systemically, nasal infections triggered higher IgM and IgG antibody production, with IgG isotype profiles dependent on the specific mucosal inoculation site. Our findings suggest that *Leishmania amazonensis* adapts more effectively to mucosal tissues, leading to impaired immune regulation. This murine model closely resembles natural disseminated mucosal leishmaniasis and provides valuable insights into the disease's immunopathology, potentially guiding the development of improved treatments.

Supported by: CNPq; CAPES; FAPERJ

Keywords: Mucosal Leishmaniasis; Experimental; Immunology

Elucidating the function of the "dark genome" in *Leishmania* using genome-scale life cycle fitness phenotyping and subcellular protein localization.

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During their life cycle *Leishmania* must migrate through the sandfly (promastigotes), pre-adapt for mammalian infectivity (metacyclic promastigotes) and proliferate inside macrophages (amastigotes), while withstanding host immune defences. Adaptation to these radically different environments involves many changes, including metabolic and cell structure specialisations, ensuring survival and transmission. However, current understanding of these processes is disjointed and biased towards predicted/expected pathways. Half of *Leishmania's* protein-coding genes, including most unique to *Leishmania,* have no known or predicted function, (the "dark genome"), leaving potentially exploitable parasite biochemistry untapped in the search for new therapeutics. Since the early 1990s, the assessment of gene function in *Leishmania* has been possible though genetic manipulation utilising homologous recombination (HR), usually on a gene-by-gene basis. More recently, CRISPR-Cas9 mediated HR has improved efficiency, allowing barseq analysis of pooled barcoded gene deletion mutants. In the LeishGEM project we aim to identify all important parasite pathogenicity factors by assessing the fitness cost of gene deletion, using barcoded libraries, in each life cycle stage. We are also determining the subcellular localisation of *Leishmania* proteins using LOPIT spatial proteomics and mNeonGreen tagging and fluorescence microscopy. The fitness phenotyping and localisation data generated by the LeishGEM project is available as we generate it at browse.leishgem.org/ and the concept of the work is published in Trends in Parasitology PMID: 39030136. As of Jan 2025, this database contains fitness data for 3,150 gene deletion mutants, subcellular localisation for 1,642 tagged cell lines where we have completed localisation annotation, and 3,782 proteins for which LOPIT-DC fractionation and mass spectroscopy yielded information on their subcellular localisation. Integrative analysis of this deep dataset is identifying key pathways in organelle adaptation for an intracellular parasitic lifestyle and in host-parasite interaction. We are identifying virulence factors on a genome scale, determining the most important pathways required for parasitism and illuminating the 'dark genome'.

Novel genomics and deep learning tools to study host-parasite interactions in the *Plasmodium*-infected liver

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Malaria transmission includes obligate transition of the parasite between the mosquito and the vertebrate host. Upon infecting its vertebrate host, the malaria parasite initially invades the liver where it undergoes massive replication, whilst remaining clinically silent. The coordination of host responses across the complex liver tissue during malaria infection remains largely unexplored. We have optimized spatial transcriptomics for analyses of liver tissue, and more recently, for liver tissues from mice infected with *Plasmodium berghei*. Our data reveals significant changes in spatial gene expression in the malaria-infected tissues, including changes related to lipid metabolism in parasite neighborhoods during late infection, distinct inflammation programs between lobular zones and the formation of immune cell aggregates, particularly during early infection. In our current efforts to study the coordination of the immune response against *Plasmodium* in the liver, we are establishing additional tools, including an *ex vivo* liver infection model and a data-driven computational resource, with the ambition to uncover genes and pathways without being restricted by a biased lens. Most common methods used for transcriptome analyses are limited by their reliance on a framework of our current knowledge of gene functions and pathways. Our resource, Deep Integrated Network Analysis (DINA), encompasses a broad range of nearly 700 manually curated, published transcriptomic data sets, including: human, monkey, mouse, rat and other organisms. DINA uses statistically conserved gene-gene “interactions” to construct unbiased liver-specific cellular pathways or "modules”. The resource contains over 80,000 interactions between 19,105 genes in a single network. These are not biased in favour of well-studied genes, and encompass connections that have not previously been described. The genes are organised into a single network, where the structure is represented by “modules” of genes that are co-regulated in similar contexts. We aim to provide novel, highly informative strategies for investigating host-parasite interactions in tissues in order to provide insights regarding *in vivo* study design linked to infection and offer useful tools for the discovery and validation of *de novo* intervention strategies aimed at malaria liver stage infection, but which can be translated to study any parasite infected tissue.

Dynamics of infestation of the primary vectors of *Trypanosoma cruzi* in Brazil

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Among the approximately 160 species of triatomines described in Brazil, the most important are undoubtedly *Triatoma brasiliensis* and *Panstrongylus megistus*, due to their high domiciliation capacity, and *Triatoma sordida*, as it is the triatomine most frequently captured in artificial environments. Chemical control by insecticide spraying is admittedly limited, since there is persistence of bugs in the Domiciliary Units (DU), with rapid recovery of triatomine densities. Our study confirms, through microsatellite marking, that these three species can persist in the DU by the survival of triatomines after spraying or by invasion of insects from the wild environment. However, this dynamic is different among species. Monitoring the infestation by *T. sordida* in northern Minas Gerais before and at 7 and 13 months after spraying, insects captured in the different periods were not identified grouped in any location. It suggests that the reinfestation processes are occurring from a new invasion, and not for the presence of insects that survived the spraying. Both reinfestation processes were observed with *T. brasiliensis* associated with rocky outcrops in the backlands of Ceará, also in a temporal sequence (2009, 2010, 2015), revealed by the low genetic variability of the triatomines, suggesting the existence of a panmictic unit, with free gene flow between the samples. The same pattern was not observed near the coast of Ceará, characterized by vast palm groves and predominance of the xique-xique cactus, to which wild triatomines are associated. A study comparing wild and domestic *P. megistus* in Jabotictubas, MG, revealed an excess of homozygotes and an absence of flow between environments. These results highlight particularities of the species, with important adaptations to environmental conditions. The population dynamics of these and other Brazilian triatomines must be better understood, which would help the election of adequate methodologies of control.

USE OF REAL-TIME PCR WITH HIGH RESOLUTION MELTING AS A GENOTYPING STRATEGY FOR *Giardia lamblia*

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Giardia lamblia is an intestinal parasite with zoonotic potential, phylogenetically divided into assemblages A-H according to host affinity. Assemblages A and B are the most frequently reported in humans and exhibit high anthropozoonotic potential. The other assemblages are still considered host-specific. Therefore, understanding the circulating *G. lamblia* assemblages can be an important tool for control, as it helps identify potential hosts involved in transmission cycles. Currently, genotyping of *G. lamblia* is performed using *multilocus* genetic sequencing, which is an expensive technique, particularly in developing countries where the highest infection frequencies are found. In this context, our group has standardized and validated real-time PCR with High Resolution Melting (qPCR-HRM) as a new tool for differentiating *G. lamblia* assemblages. The qPCR-HRM is an easy-to-perform technique, provides rapid results, and is up to 20 times less costly than genetic sequencing. The use of qPCR-HRM will enable the expansion of genotyping studies and guide more rational giardiasis control strategies.

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Keywords: *Giardia lamblia*, genotyping, assemblages, qPCR-HRM.

LEISHMANIA AMAZONENSIS AQUAGLYCEROPORIN 1 IS A GLYCOSOMAL PROTEIN INVOLVED IN ANTIMONIAL RESISTANCE, IRON HOMEOSTASIS AND PARASITE VIRULENCE

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Leishmania faces critical conditions within macrophages, notably a scarcity of essential nutrients such as iron, which triggers the differential expression of various genes. *In silico* analyses identified *aquaglyceroporin 1* (*aqp1*) as a conserved gene modulated by iron availability, containing predicted transmembrane domains and a glycosomal targeting sequence in *L. amazonensis*. Glycosomes are unique trypanosomatid organelles that compartmentalize glycolysis, β-oxidation of fatty acids, and several essential enzymes, including the iron-dependent superoxide dismutase. AQP1 was previously characterized as a porin involved in the transport of trivalent antimony (SbIII), water, glycerol, urea, dihydroxyacetone, methylglyoxal, and polyols. Additionally, AQP1 has been detected in distinct subcellular compartments in *L. major* and *L. donovani*. Given the central role of glycosomes in *Leishmania* metabolism and the species-specific subcellular localization of AQP1, we sought to investigate its function in *L. amazonensis*. Using immunofluorescence, we confirmed AQP1´s glycosomal localization in this species. Employing the CRISPR/Cas9 strategy, we generated full knockout and add-back strains, which were validated by PCR analysis. The knockout of *aqp1* impaired promastigote *in vitro* growth, particularly under heme-depleted conditions, induced resistance to SbIII, altered SbIII uptake and iron intracellular levels, and increased superoxide dismutase activity. Furthermore, AQP1 deficiency significantly reduced both *in vitro* and *in vivo* virulence in murine host. These findings establish AQP1 as a player in *Leishmania* iron homeostasis, drug susceptibility, and pathogenesis, providing new insights into parasite metabolism and potential therapeutic targets.

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Keywords: CRISPR/Cas9, membrane transport, virulence

Leishmania Extracellular Vesicles: Their Role in Host-Leishmania Interaction, Infectious Process and Beyond

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Leishmaniasis, a complex pattern of diseases caused by sand fly-transmitted *Leishmania* sp. causes over 2 million new infections and 30,000 deaths each year. In mammals, *Leishmania* parasites establish a persistent infection by inducing MØ dysfunction through direct manipulation of MØ signaling. We have deciphered the mechanisms whereby *Leishmania* exploits MØ signaling pathways to block microbicidal functions and innate inflammatory responses during infection. Work stemming from my lab was the first to show that *Leishmania major* GP63 was enriched in *Leishmania* exosomes/extracellular vesicles (EVs), and to play a pivotal role in those deactivation process of MØ responses. We were the first to report that *Leishmania* EVs are released in the gut of its sand fly vector and to be co-inoculated with *Leishmania* promastigotes during blood meals. Co-egested *Leishmania* exosomes were found to favor myeloid cells infection and to exacerbate cutaneous leishmaniasis skin lesions by overproducing inflammatory cytokines fueling Th17 immune response. More recently, *Leishmania* RNA virus 1 (LRV1) infecting certain *Leishmania* species was found to be associated with aggressive mucocutaneous disease triggered in response to this dsRNA virus. However, it was unclear how LRV1 is exposed to the mammalian host cells. In higher eukaryotes, some viruses are known to utilize the host exosome pathway for their formation and cell-to-cell spread. As a result, exosomes derived from infected cells contain viral material or particles. Recently, we found that LRV1 exploits the *Leishmania* exosome pathway to reach the extracellular environment. Biochemical and electron microscopy analyses of exosomes derived from LRV1-infected *Leishmania* revealed that most dsRNA LRV1 co-fractionated with exosomes, and that a portion of viral particles was surrounded by EVs. Transfer assays of LRV1-containing exosome preparations showed that a significant number of parasites were rapidly infected by LRV1. Remarkably, *Leishmania* becoming infected by LRV1 sees their survival fitness augmented explaining in part, how LRV1-containing *Leishmania* confers a stronger infection and pathology development. Collectively, our work provides evidence that *Leishmania* EVs are key player in *Leishmania* infection, as well as to modify the level of infectivity depending on the exosome cargo such as to contain whole virus, facilitating LRV1 transmission to naïve *Leishmania* increasing their infectivity in the mammalian host.

Challenges for Immunodiagnosis and Control of *Stongyloides stercoralis* Infection

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Strongyloides stercoralis is the main etiological agent of human strongyloidiasis and can be found in several regions of the planet, infecting around 600 million people. Recently, the strongyloidiasis was added to the 2021-2030 roadmap of the World Health Organization (WHO), for the control of Neglected Tropical Diseases. In Brazil, the prevalence of infection by *S. stercoralis* is greater than 5.5%, being considered a hyperendemic area. Diagnosis is performed by searching for larvae in feces, which has low sensitivity and serological methods are still limited, mainly due to the difficulty in producing antigens and false positive results due to the presence of cross-reactions. The lack of reliable diagnostic methods also affects treatment monitoring and infection control. Currently, two recombinant antigens are being tested for the immunodiagnosis of strongyloidiasis, rSs-NIE-1 and rSs-IR. Despite their high sensitivity, they present cross-reactions, mainly with *S. mansoni* and geohelminths. In Brazilian regions with low socioeconomic conditions, several species of helminths coexist in the same areas. Therefore, the selection of molecules specific *to Strongyloides* and not shared with other parasites is essential for the development of an effective test for the immunodiagnosis and S. stercoralis infection control.

AcetyLeish: Uncovering the impact of protein acetylation in *Leishmania mexica*na stage differentiation

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Protein acetylation has been implicated in the regulation of essential cellular processes in diverse prokaryotes and eukaryotes, including protozoan parasites. Previous proteomic analysis from our group revealed differential protein acetylation among the three main *Leishmania mexicana* stages (procyclic, metacyclic and amastigote), suggesting a central role for this modification during parasite differentiation. Lysine acetylation is regulated by lysine acetyltransferases (KATs), which add acetyl groups to lysines, and lysine deacetylases (KDACs) which remove the acetyl groups. The KDACs are divided into two classes: Zn-dependent (DACs) and NAD^+-dependent. To expand our knowledge on how changes in protein acetylation affect *Leishmania* stage differentiation, we characterized the four DACs (DAC1, 3, 4 and 5) of *L. mexicana*. Using the CRISPR-Cas9 we obtained DAC knockout mutant parasites and found that DAC1 and 3 are essential for procyclic parasite stage, while DAC4 and 5 are dispensable. Using fluorescent-tagged DAC-expressing parasites, we demonstrated that DAC1 and 5 are cytoplasmic, while DAC3 and 4 have nuclear localization in the three parasite stages. Phenotype screening assays using the knockout parasites showed that DAC1, 3 and 5 are involved in procyclic multiplication, while DAC1 and 5 impairs differentiation from procyclic to infective metacyclics *in vitro*. Experimental *in vivo* infection of *Lutzomyia longipalpis* further confirmed the importance of DAC5 for parasite differentiation by revealing impaired metacyclogenesis. In addition, we found that the absence of DAC5 significantly affects procyclic differentiation to axenic amastigotes and vice versa. Finally, we evaluated the impact of DACs in the progression of host infection *in vitro and in vivo*. We found a significant reduction in intracellular amastigote multiplication of DAC5 null mutants *in vitro* compared to the parental cell line. A similar scenario was observed in the mouse *in vivo* infection assays for DAC5 mutants, with no apparent lesion development compared to parental parasites, indicating that DAC5 gene disruption leads to an attenuated parasite. Altogether, these results suggest that regulation of protein acetylation levels is important for *Leishmania* stage differentiation.

NEW SEROLOGICAL TESTS FOR THE DIAGNOSIS OF VISCERAL LEISHMANIASIS BASED ON RECOMBINANT CHIMERIC PROTEINS: A HISTORICAL AND BIOTECHNOLOGICAL PERSPECTIVE

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Visceral Leishmaniasis (VL) remains a public health problem in Brazil and elsewhere, with its diagnosis in both humans and animals hosts still presenting challenges leading to underdiagnosed cases and maintenance of disease transmission. Serological methods are the most effective for VL diagnosis, with multiple ELISA and rapid tests (RDT) being currently available but with issues regarding efficiency and/or costs. To address these issues, new chimeric recombinant proteins are being proposed as the basis for ELISA and RDT assays to be used for the diagnosis of both human and canine VL. These are based on native “Leishmania infantum” antigens previously identified through multiple serological screenings of bacterial expression libraries, most of them found to mainly consist of repetitive regions which might be associated with their enhanced antigenicity. When expressed as recombinant proteins, and evaluated for their potential use for VL diagnosis, several of these antigens were shown to be efficient for the diagnosis of either human or canine VL, but not both. Fragments encoding repetitive and non-repetitive regions from a selection of these antigens were then joined in “in silico” designed chimeric genes, which were optimized for expression in “Escherichia coli” as his-tagged proteins. Multiple rounds of gene design, expression and serological evaluation of the corresponding proteins were performed through ELISA, with increasingly better performance for the diagnosis of both human and canine VL. Two new chimeric protein, respectively with fragments from three and five native “L. infantum” antigens, were selected for further evaluation using both ELISA and RDT. Both are efficiently expressed and purified and show great potential for use in the serodiagnosis of VL in humans and dogs as the basis for new low-cost tests, possibly applicable for the hard to diagnose cases of VL/HIV co-infection, and amenable to further improvements through changes in protein design.

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Keywords- Leishmaniasis; Immunodiagnosis; Recombinant antigens

Evaluation of the response to treatment based on immunochemotherapy in dogs naturally infected with *Leishmania infantum*

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The aim of this study is to monitor dogs with Canine Visceral Leishmaniasis (CVL) treated with chemotherapy and chemoimmunotherapy. These animals will be evaluated regarding their clinical condition, parasite load, and immune response over the course of one year, with the goal of enhancing knowledge about the effectiveness of treatment using miltefosine in combination with immunotherapeutic formulations. "Avaliação da resposta ao tratamento baseado em imunoquimioterapia em cães naturalmente infectados por *Leishmania infantum"* O objetivo deste estudo é acompanhar cães portadores de Leishmaniose Visceral Canina (LVC) tratados por quimioterapia e por quimioimunoterapia. Esses animais serão avaliados quanto ao estado clínico, carga parasitária e resposta imune ao longo de um ano visando aperfeiçoar os conhecimentos acerca da efetividade do tratamento utilizando miltefosina associada a formulações imunoterápicas.

IDENTIFYING PARASITIC DISEASE VECTORS IN THE AGE OF ARTIFICIAL INTELLIGENCE

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We are living in the age of digital technologies and artificial intelligence (AI), which are impacting various fields of knowledge, including medicine. Chagas disease, leishmaniasis, arboviruses and spotted fever are neglected tropical diseases transmitted by vectors with a major impact on public health. Innovations to identify vectors (kissing bugs, mosquitoes and ticks) are needed to strengthen surveillance and control. Here, I show how artificial intelligence can help identify vectors to encourage health professionals to use the tool and strengthen disease surveillance and control services. My research team organized image banks of vectors (bugs, mosquitoes and ticks) and processed them with artificial intelligence algorithms based on a convolutional neural network (AlexNet), three boosting-based classifiers (AdaBoost, AB; Gradient Boosting, GB; and Histogram-based Gradient Boosting, HB); and a linear discriminant model (LD). The images were processed and used to train and test the models. We measured the accuracy of the AI networks in identifying the vectors. We obtained the following accuracies: 1) 3 genera of ticks, *Triatoma*, *Rhodnius* and *Panstrongylus*, 100%, highest for AlexNet, the network selected for other experiments, 2) 65 species of ticks from 27 countries: ~93% (95% CI: 0.91-0.94), 3) 48 species of bugs reported in houses or infected with *Trypanosoma cruzi*: 95% (CI: 93-96), 4) mosquitoes, 4 species, vectors of yellow fever (*Aedes*, *Sabethes* and *Haemagogus*): 95% (CI: 98-79). 3) Ticks, spotted fever vectors, 6 species of *Amblyomma*: 89% (CI: 89-89). These results show that artificial intelligence is promising for vector identification, and that these databases and algorithms can be incorporated into applications that can be made available to the community, further empowering citizen science.

Supported By: CNPq, CAPES

Keywords: neglected tropical diseases, vectors; public health; artificial intelligence.

Soil-transmitted helminthiasis. How long will it persist?

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Soil-transmitted helminthiasis remains a significant public health problem in various parts of the world, especially in low- and middle-income countries, where environmental, socioeconomic, and sanitary factors contribute to its persistence. In Brazil, these parasitic infections are endemic in many regions, primarily affecting vulnerable populations living in poor sanitation and hygiene conditions. The main etiological agents, such as *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworms, have life cycles closely linked to the environment, being transmitted through contact with soil contaminated by human feces containing infective eggs and larvae. This transmission dynamic contributes to a high morbidity rate, associated with malnutrition, anemia, impaired child development, and compromised school performance. Although Brazil has made progress in control policies, such as school-based chemoprophylaxis programs and improvements in access to basic sanitation, the eradication of soil-transmitted helminthiasis still faces significant challenges. Epidemiological data indicate that, despite a reduction in parasitic load in some regions, transmission continues in rural and peri urban communities, where access to sanitary infrastructure remains inadequate. The country's tropical climate favors the survival of eggs and larvae in the environment, while socioeconomic inequalities hinder the adoption of effective preventive measures. This lecture will address the main ecological and social determinants of the persistence of soil-transmitted helminthiasis in Brazil, analyzing the effectiveness of current interventions and the need for complementary strategies for sustainable control. Innovative initiatives will be discussed, such as intersectoral approaches that combine health education, environmental improvements, and new technologies for diagnosis and monitoring. In light of this scenario, the question arises: how long will these neglected infections continue to impact vulnerable populations, and what paths should be taken to achieve their effective eradication in the country?

CONTROL AND DIAGNOSIS OF INTESTINAL HELMINTH INFECTIONS

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During the most recent World Health Assembly, Brazil has signed the reformulated Millenium Goals for the control of the most common Neglected Tropical Diseases (NTD), which are sought to be controlled and/or eliminated as a public health problem until 2030. For schistosomiasis and intestinal helminth infections, considerable progress has been achieved during the last decades through individual diagnosis and treatment campaigns, conducted especially within the Brazilian Schistosomiasis Control Program (BSCP). However, since reinfection in endemic areas is very common and fast and in order to achieve sustainable and long-lasting effects, new, integrated approaches have to be implemented in endemic areas. Here, we report on the use of copro-parasitological techniques to detect schistosomiasis and soil-transmitted helminth infections and present and discuss data on parasitological, molecular, and serological alternatives. Furthermore, we discuss the need for mass-treatment campaigns and for integrated control measures with respect to WASH (water, sanitation, hygiene) and to 'One Health'.

Supported by: FAPEMIG, CNPq, CAPES

Keywords: *Schistosoma mansoni*, soil-transmitted helminths, epidemiology, control, one health

PURINERGIC SIGNALING IN *TRICHOMONAS VAGINALIS*-HOST INTERACTION

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Trichomonas vaginalis is the etiologic agent of trichomoniasis, the most prevalent non-viral sexually transmitted infection worldwide. The infection is associated to infertility, cervical and prostate cancers, low-weight and premature birth, and increase in HIV transmission. The investigation of biochemical aspects of the parasite and the relation with the host is important to understand trichomoniasis's pathogenesis. ATP can act as damage-associated molecular pattern (DAMPs) with proinflammatory function. In the other hand, adenosine may revert some of effects induced by extracellular ATP through immunosuppressive modulation. Both ATP and adenosine play their effects by binding to specific receptors. The regulation of this cell signaling can be attributed to enzymes called ectonucleoside triphosphate diphosphohydrolase (NTPDase). In sequence, adenosine deaminase (ADA) catalyzes the conversion of adenosine to inosine. In parasites the purinergic system represents an important mechanism of escape of host immune response by ATP degradation and adenosine production. Moreover, pathogens may scavenge extracellular adenosine through nucleoside transporters since these organisms lack the ability to synthesize the purine ring *de novo*. NTPDase, and ADA activities have already been characterized in *T. vaginalis* trophozoites by our group. Adenosine and inosine decreased the reactive oxygen species, nitric oxide, and IL-8 levels, revealing the immunosuppressive effect of adenosine. Moreover, adenosine, but not guanosine, protects vaginal epithelial cells from *T. vaginalis* cytotoxicity. The enzyme hydrolyzes ATP producing adenosine uptake by the parasite for survival additionally to the anti-inflammatory effects of adenosine that contribute to the establishment of infection. In this context, NTPDase and adenosine transporter may be considered interesting therapeutic targets in trichomoniasis.

Key words: *Trichomonas vaginalis,* purinergic signaling, adenosine.

FIOCRUZ BAHIA'S SCIENCE OUTREACH INITIATIVES IN PARASITOLOGY

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The Oswaldo Cruz Foundation (Fiocruz) in Bahia, Brazil, has an institutional science outreach program that focuses on priority scientific areas, including neglected tropical diseases (NTDs). Fiocruz Bahia's science outreach initiatives aim to inspire curiosity and interest in scientific knowledge among children and youth, contributing to the popularization of science. Covering diverse topics such as health education, parasites, disease vectors, and insect biodiversity, these initiatives raise awareness and support efforts to prevent, control, and eliminate diseases like leishmaniasis, Chagas disease, and schistosomiasis. Through these efforts, Fiocruz Bahia demonstrates its commitment to science communication and its active role in combating NTDs, ultimately contributing to public health improvements.

Supported by: Fiocruz Bahia
Keywords: Science outreach, Neglected tropical diseases, Science communication, Popularization of science.

POSTGRADUATE PROGRAMS AT FIOCRUZ BAHIA: CONNECTING KNOWLEDGE IN PARASITOLOGY TO CONTRIBUTE TO THE DISSEMINATION OF KNOWLEDGE AND HEALTH EDUCATION IN THE MUNICIPALITIES OF SALVADOR AND CACHOEIRA

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Over the past two years, the graduate Program in Human Pathology (PGPAT) at the Federal University of Bahia (UFBA), in partnership with the Oswaldo Cruz Foundation (Fiocruz) in Bahia and the graduate Program in Biotechnology in Health and Investigative Medicine (PGBSMI) at Fiocruz Bahia (both CAPES level 6), has implemented educational strategies to train teachers and students in science and health projects, particularly in parasitology. Participants received training through an Institutional Program of Continuing Education in Active Teaching Methodologies, enabling them to apply their knowledge in various health-related undergraduate programs, as well as in elementary and high schools. Activities included lectures, workshops, science fairs, graffiti, and interactive games. Addressing topics ranging from health education to the exploration of parasites, vectors, and insect biodiversity, these initiatives aim to raise awareness and support efforts to prevent, control, and eliminate diseases such as leishmaniasis, Chagas disease, and schistosomiasis. Through this project, we strive to integrate parasitology knowledge into society while promoting cultural diversity and addressing ethnic-racial issues in public schools in Salvador and quilombola communities in Cachoeira, Bahia.

Supported by: Fiocruz Bahia, UFBA, CAPES, FAPESB

Keywords: Graduate Program, Parasitology, Education, Science

The Use of Artificial Intelligence in Systematic Reviews and Evidence Classification in Health: Where We Are and What to Expect in the Future

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Systematic reviews play a critical role in evidence-based health decision-making, providing high-quality syntheses of scientific knowledge in the field of parasitology. However, conducting systematic reviews -especially those involving a large number of studies- is a time-consuming and labor-intensive task. This presentation will explore how artificial intelligence (AI) can be used in the development of systematic reviews, offering opportunities for semi-automation and efficiency gains. Initially, key concepts will be introduced, including systematic reviews, evidence-based health approaches, the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) classification system, and AI concepts. The increasing volume of published research has driven interest in AI-powered tools that support systematic reviews, from study selection and data extraction to quality assessment and evidence synthesis. The presentation will highlight the evolution of scientific publications on this topic, identify specific review stages where AI can be applied, and present some existing AI-based tools designed to facilitate the process, including how ChatGPT can be used in this context. While AI can significantly enhance efficiency of conducting systematic reviews, its implementation faces challenges. Therefore, the presentation will also address the limitations of current developments and the challenges for broader adoption. The discussion will conclude with insights into future trends.

Supported by: CNPq

Keywords: Artificial Intelligence; Systematic Reviews; Evidence-Based Health

HOW CAN YOU MEND A BROKEN HEART? INSIGHTS FROM CHAGAS DISEASE CARDIOMYOPATHY

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Human infection with *Trypanosoma cruzi* leads to Chagas disease and induces profound changes in the immune response across different immune cell subsets, influencing parasite control and disease pathology. Dissecting the functional characteristics of distinct immune cells in patients with the asymptomatic (indeterminate – IND) or with the cardiac (CCC) clinical forms is crucial for unveiling mechanisms of disease progression and pathology and identifying disease markers. Here, single-cell RNA sequencing was applied to peripheral blood mononuclear cells (PBMCs) obtained from IND and CCC patients to unravel the immune landscape in this polar, well-characterized, clinical groups. Our findings revealed different myeloid and lymphoid cell clusters in both cohorts, each exhibiting unique gene expression patterns. CCC was characterized by an increased frequency of KLRB1+CD4+, TBX21+CD8+ T cells, and NK cells, which exhibited upregulation of genes associated with inflammatory, cytotoxic and apoptotic responses. Further cellular analysis demonstrated that *in vitro* infection with *Trypanosoma cruzi* leds to an intense activation of T cells bearing the TCR gamma delta, with cytotoxic and inflammatory characteristics, especially in CCC. Translational studies demonstrate that this cytotoxic and inflammatory profiles can be modulated using pentoxifylline. The nuanced transcriptional changes indicate an immune signature linked to the severity of chronic Chagas disease, identifying cell populations as targets to control the establishment or the development of Chagas disease pathology.

Supported by: CNPq, INCT-DT, NIH

Keywords: Chagas disease, cardiomyopathy, inflammation, immunotherapy.

IDENTIFICATION AND TESTING OF CANDIDATES FOR THE DEVELOPMENT OF LEISHMANIASIS TRANSMISSION BLOCKING VACCINES

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American visceral leishmaniasis (AVL) is considered a major neglected public health problem. There has been a significant increase in the number of human cases of visceral leishmaniasis in recent years, which in Brazil is caused by *Leishmania infantum chagasi*, transmitted by *Lutzomyia longipalpis.* There is a demand for new approaches in the control of leishmaniasis due to the lack of vaccines and limitations in the use of traditional vector control methods, such as insecticide resistance, costs, etc. An alternative strategy for controlling this disease is the use of transmissionblocking vaccines (TBVs), which consist of immunizing vertebrate hosts with vector or parasite antigens responsible for establishing the pathogen in the vector, thus stimulating the production of neutralizing antibodies that will interrupt the development of the parasite inside the vector, blocking transmission. We are working with several promising candidates in the parasite, both by testing insect infection with mutant parasites (either obtained through collaborations or by creating mutants through CRISPR) and performing artificial infections in the presence of antibodies against proteins of interest. Our group identified potential targets for TBVs in *Leishmania*, which may be essential for its development in the insect. Among them are LFR1 and LIT (related to iron metabolism), ATP11 and ABC (transporters of amino acids and other molecules, respectively) and a chitinase, which appears to play an important role in the transmission of Leishmania by the vector. We produced *Leishmania* chitinase mutants by CRISPR that were 100 times less efficient than controls in artificial transmission assays. From the identified targets, synthetic peptides were designed and inoculated into mice or rabbits, in the presence of different adjuvants. Finally, artificial infections of *L. longipalpis* with *L. i.chagasi* were performed in the presence of the sera. Initial results show promising results with the decrease of parasite load in insects infected in the presence of the immune sera.

EPIDEMIOLOGY/ EDUCATION/ COMMUNICATION

DIDACTIC MODELS OF PARASITOLOGY AS AN INCLUSION IN THE TEACHING OF THE VISUALLY IMPAIRED AND AS A PEDAGOGICAL AND SCIENTIFIC DISSEMINATION TOOL IN NON-FORMAL TEACHING SPACES

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The aim of this work was to create reliable three-dimensional models of parasitology using recyclable materials (*Ascaris lumbricoides*, *Taenia saginata*, *Taenia solium* and *Schistosoma mansoni) in order to* promote the inclusion of visually impaired people in the teaching of parasitology and to explore an innovative approach to disseminating scientific content to children at the university museum.

The models were made from simple materials such as EVA putty, durepox, PET bottles, scrap metal such as bicycle chains and sprockets, foam, spaghetti floats, toilet paper rolls, plastic rings and pieces of hose. The pieces were attached with Loctite Super Bonder glue or hot glue and the models were painted with latex paint.

For the visually impaired, the didactic models made it possible to follow the content taught alongside the sighted students, filling a significant gap in terms of accessibility. According to a visually impaired student who used the material, the diversity of textures and shapes made it easier to interpret and understand the content studied.

At the museum, the models promoted a new form of teaching, allowing visitors to interact directly with the content of the exhibition, drawing attention to the subject and helping to stimulate children's scientific curiosity. The models were also presented in parasitology workshops, impacting the children and enabling playful and interactive learning.

In summary, the models made allowed visually impaired students to build appropriate mental images of the content, promoting their academic inclusion. And in the museum, they provided a more engaging and participatory approach, helping to disseminate science.

Keywords: Museum, Teaching models, Inclusion

Analysis of the spatial-temporal risk of dengue occurrence in the Metropolitan Region of Belo Horizonte in Minas Gerais, Brazil, 2007-2022

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Dengue is one of the most important arboviruses for global public health. The pathogen is mainly transmitted by female *Aedes aegypti* mosquitoes, and the incidence of the disease is linked to environmental and socioeconomic variables. It is widespread in countries with tropical and subtropical climates, in regions where the lack of adequate waste disposal in urban areas provides a favorable environment for the development of the vector. In the Americas, Brazil is one of the countries with the highest rates of the disease. The metropolitan region of Belo Horizonte - RMBH (Núcleo e Colar), the third largest urban agglomeration in the country and with a large number of dengue cases in the past, was defined as the scenario for conducting the dengue occurrence risk analysis. The objective of this study was to assess the risk of dengue occurrence in the RMBH (Núcleo e Colar) in Minas Gerais, Brazil, from 2007 to 2022. The spatial, temporal and spatiotemporal scan statistics software SatScan™ version 9.4.4 was used. A discrete Poisson model ($p < 0.05$) was used to define the spatio-temporal clusters. It was found that the clusters with a high relative risk (RR) occurred mainly in the first half of each year (January to May). The clusters that had a low RR for dengue occurrence of dengue mainly started in the months of May, June and July. The year 2013 was the year in which the highest RR values occurred: Cluster 02 (RR: 20.72), Cluster 06 (RR: 16.49) and Cluster 11 (RR: 15.89). The years 2013, 2016 and 2019 were the years with the highest number of municipalities with high RRs for dengue, with all 50 municipalities of the RMBH (Núcleo and colar)

having high RRs during this period. The data presented shows the patterns of occurrence of the dengue epidemic in the study area.

Supported By: Coordination Foundation for the Improvement of Higher Education Personnel - CAPES

Keywords: Dengue, Epidemiology, Spatial analysis

SEROPREVALENCE AND RISK FACTORS FOR TOXOCARIASIS IN PEOPLE OVER 50 YEARS OLD IN WESTERN SÃO PAULO, BRAZIL

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Toxocariasis is a geohelminthiasis with worldwide seroprevalence estimated in 19%. The disease is caused by the nematoda *Toxocara canis* (of dogs) and *T. cati* (of cats). Although children have been considered more prone to infection, adults are also considered as being at risk. Despite the importance of toxocariasis, there is a scarcity regard to the investigation in people over 50 years old. The aim of this study was to evaluate the risk factors for seropositivity for *Toxocara* spp. in a population over 50 years old attended by the Public Health System in the West region of the São Paulo state. A total of 290 individuals were included in the study. Socioepidemiological information were gathered via a semi-structured questionnaire and blood samples were collected to obtain serum samples. Presence of anti-*Toxocara* spp. antibodies was screened by ELISA test. Uni and multivariate analyses were performed using the statistical software R to evaluate the relationship between seropositivity and associated variables. The presence of anti-*Toxocara* spp. antibodies was observed in 89/290 (30.7%; 95% CI: 25.7-36.2%) individuals. The multivariate analysis revealed that individuals who raised both dog and cat had three times higher odds for IgG seropositivity compared to those who declared not having any pet, while a higher educational level was a protective factor. No influence on the seropositivity was observed considering the other variables (age, gender, monthly income, consumption of drinkable water or raw/undercooked meat, having dirty floor at home, contact with soil, washing vegetables and hands before meals, onychophagy). In conclusion, toxocariasis is likely underreported and neglected in elderly population. Considering the seroprevalence observed herein in a group at risk of immunosenescence, this age group should be monitored. Furthermore, proper hygiene and regularly deworming of pets owned by elderly people need to be considered to reduce the risk of toxocariasis.

Supported by: Not applicable

Keywords: *Toxocara* spp., epidemiology, old age

EPIDEMIOLOGY AND DIAGNOSIS OF INTESTINAL PARASITIC INFECTIONS ACROSS DIFFERENT AGE GROUPS: A STUDY IN TERESINA, BRAZIL

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Intestinal parasitic infections are highly prevalent, with approximately 3 million people worldwide infected by some type of parasite. In developing countries with socioeconomic and environmental deficiencies, prevalence is notably high; however, parasitic infections are often underreported. In Brazil, the Northeast stands out as one of the regions with the highest rates of enteroparasitoses. This study aimed to provide health access to socially vulnerable areas through fecal sample analysis and the characterization of the epidemiological and socioeconomic profile of children, adolescents, and adults. Fecal examinations and socioeconomic-epidemiological questionnaires were conducted with participants from two regions in Teresina: CETI José Pereira da Silva school in the urban area and Soin Municipal School in the rural area. Fecal sample analysis was performed at the Zoology and Parasitology Laboratory of the State University of Piauí using the modified Ritchie and modified Willis-Mollay techniques. In the urban area, among 45 participants, *Entamoeba coli* was identified in 11% of samples. In the rural area, 35% of the 37 participants were infected, with 12 cases of *E. coli* and one case of co-infection with *E. coli* and hookworm. Additionally, it was noted that a significant portion of participants, 85%, used untreated running water for food hygiene, while others used a bleach solution. At the end of the study, a “Day D” event was held at both schools, featuring lectures on parasitosis prevention, medical and nutritional consultations, and the distribution of personal hygiene kits and clay water filters. This study highlights the importance of access to essential services such as sanitation and clean water, as these are critical factors in reducing the prevalence and transmission of intestinal parasitic infections.

Supported by: FAPEPI

Keywords: Enteroparasitoses, Public Health, Health Education

HEALTH AND EDUCATION: PARASITOLOGICAL SURVEY AS A TOOL FOR INTERVENTION AND HEALTH PROMOTION IN RURAL AREAS OF BAHIA

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Parasitosis are neglected diseases with high morbidity and mortality rates in peripheral urban and rural areas of the country. The objective was to determine the parasitic and epidemiological profile of rural communities, identify associated risk factors and carry out health education actions for their control and prevention. It was developed in Várzea da Casa and Várzea Nova II, Santo Estevão, Bahia. After signing the terms of consents, the collection of samples of feces, soil and vegetables was carried out for examination and the mapping of the socioeconomic and sanitary conditions of the population. Data stored on the Vicon Saga platform and analyzed in the Epi Info software. Positive individuals were referred for treatment by the Family Health Unit. We examined 172 stool samples and positivity was 16.3%. In Várzea da Casa were identified protozoa (Entamoeba histolytica/dispar, Giardia. Duodenalis and Entamoeba. coli) and helminths (S..stercolaris, S. mansoni and Ancylostomides) In Varzea Nova II, E. histolytica/dispar, E. coli and E. nana. The majority of the population had elementary school (46.2%), represented by farmers (40%) and retirees (21.7%), and income from 1 minimum wage (53.3%). The households were supplied by the public network (90.0%) and used the pit (94%) for the destination of the waste. Analysis of soil samples detected anyclostomides larvae and vegetables, amoeba cysts. No statistically significant association was observed between parasite carriers and the variables studied. Health education activities were directed to the use of gloves, footwear and care in the hygiene of food. The forms of water treatment were presented, importance of using the clay filter and practical demonstration of candle hygiene and hand washing. The implementation of public policies on basic and environmental sanitation and health education practices are essential to reduce the occurrence of these diseases and improve the quality of life of rural communities.

SOURCE OF FINANCING: PROEX/UEFS

KEYWORDS: Parasitological survey, Health education, Rural communities.

MOLECULAR DIAGNOSIS OF SYPHILIS: APPLICATION OF ANCIENT DNA PROTOCOLS FOR THE DETECTION OF *Treponema pallidum* SUBSP. *pallidum* IN SEROLOGICAL SAMPLES

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Syphilis is an ancient, congenital, and sexually transmitted infectious disease that remains a major public health challenge, with an increasing incidence in Brazil and worldwide. Although traditional diagnostic methods, such as serological tests like VDRL and TPHA, are widely employed, they have limitations, mainly in the early stages of infection, due to a high rate of false positives. The detection of *Treponema pallidum* subsp. *pallidum*, the causative bacterium of syphilis, is hindered by the low recovery of the spirochetes in relation to the different phase of disease. However, some biological samples with minimal bacterial concentration, such as serum, could be an alternative source for highly sensitive molecular diagnosis. To address this issue, an ancient DNA (aDNA) protocol was applied to optimize the molecular diagnosis of syphilis from serological samples. DNA extraction and molecular reconstruction aDNA protocols were performed, followed by PCR and Sanger sequencing for detecting *T. pallidum* subsp. *pallidum* in 18 patients from healthcare units specializing in sexually transmitted infections, Rio de Janeiro. The selected serum samples showed different positivity titers for VDRL test of 1:2, 1:4, 1:8 and non-reactive as negative controls. Enzymatic amplifications were conducted using *tpp*15, *tpp*47, and *pol*A *T. pallidum* markers. PCR results revealed positivity in 13/18 patients and preliminary nucleotide sequencing analysis confirmed *T. pallidum.* subsp. *pallidum* identification in 10/13. The results demonstrated the effectiveness of the applied aDNA methodology for precise bacterial identification in serological samples. Despite the challenge of molecular detection on serum, the aDNA approach employed allowed a confirmatory specific diagnosis of syphilis. Integrating molecular techniques, including aDNA-specific protocols, may enhance the diagnostic accuracy and help with disease control strategies, contributing to improved public health outcomes in Brazil and globally.

SUPPORT: Capes; Faperj; CNPq; LPIP-IOC/FIOCRUZ

KEYWORDS: Syphilis Diagnosis, *Treponema pallidum*, Molecular Detection

ANALYSIS OF ENTEROPARASITOSES IN VULNERABLE COMMUNITIES OF TIMON-MA

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Text: Enteroparasitoses, classified as neglected diseases, still significantly affect various regions of Brazil, being associated with poor sanitation infrastructure. This study aimed to analyze fecal sam ples and describe the socio-epidemiological profile of three vulnerable communities in Timon-MA, two in rural areas and one in an urban area. Serial fecal samples were collected and analyzed us ing the Hoffman and modified Willis-Mollay techniques. A socio-epidemiological questionnaire was applied, followed by lectures, distribution of educational booklets, personal hygiene kits, clay filters, and recreational activities to prevent intestinal parasitoses. A total of 192 individuals participated, of whom 118 returned fecal samples. Among the 118 tests performed, 24 showed positivity for enteroparasites, with protozoa and helminths identified. Cases of polyparasitism were recorded in 16.6% (4/24) of the positives. The detected parasites were: *Entamoeba dispar/histolytica* (71%), Ancylostomatidae (21%), *Ascaris lumbricoides* (13%), *Iodamoeba butschlii* (13%), *Giardia lamblia* (5%), and *Enterobius vermicularis* (5%). The questionnaire revealed that 52% (100/192) of partic ipants used filtered water, 82.8% (159/192) washed their hands before meals and after using the bathroom, 30.2% (58/192) had piped water, 19.2% (37/192) reported having basic sanitation, and only 17.1% (33/192) sanitized fruits and vegetables with chlorinated solution. These data reinforce that the prevalence of enteroparasitoses is directly related to socioeconomic conditions, exacerbated by the lack of basic sanitation and limited access to treated water. It is concluded that access to potable water and basic sanitation is not only a matter of comfort but an essential right to ensure quality of life and health for the population.

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Keywords: Intestinal parasitoses, sanitation infrastructure, community epidemiology

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UNDER THE MICROSCOPE'S LENSES: DEMOCRATIZING PARASITOLOGY FOR HEATLH AGENTS IN PONTAL OF TRIÂNGULO MINEIRO, MINAS GERAIS

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Abstract

Parasitic diseases disproportionately affect vulnerable populations, often causing debilitating comorbidities. These diseases are frequently neglected in research, funding, and public awareness. Thus, educational events are crucial for disseminating preventative knowledge. In August 2024, we conducted an educational update for 55 community health workers and endemic disease control agents in Capinópolis, Minas Gerais, Brazil. The event included theoretical explanations on intestinal, tissue, and blood parasites, followed by viewing some of them under a microscope or a stereomicroscope. Post-intervention knowledge gain was assessed via pre- (55 respondents) and post-event questionnaires (51 respondents), focusing on parasitic disease definitions, enteroparasitosis (prevention, diagnosis, and symptoms), toxoplasmosis prevention in pregnant women, and triatomine bug management. When asked about prior training on parasitic diseases, 36.4% of participants reported never having attended any event. Participants demonstrated improved knowledge post-event, correctly identifying parasitic diseases as caused by protozoa and worms (70.9% pre-event, 86.9% post-event correct response rates), recognizing enteroparasitosis prevention (74.5% pre-event and 98% post-event) and diagnosis (92.7% pre-event and 96.1% post-event), toxoplasmosis prevention (81.8% pre-event and 96.1% post-event), and demonstrating understanding the importance of sending of triatomine bug to the Center for Zoonosis Control (78.2% pre-event and 96.1% post-event). However, knowledge gaps still remained regarding enteroparasitosis symptoms (40% pre-event, 50% post-event correct response rates). In fact, the symptoms of enteroparasitosis are nonspecific, but clinical suspicion is essential for correct referral. These findings underscore the importance of ongoing training for these professionals, given their critical role in community health.

Supported by: Institutional Program of Support Extension Activities at the State University of Minas Gerais.

Keywords: Enteroparasitosis; Toxoplasmosis; Chagas Disease; Prevention.

HEALTH EDUCATION: INTESTINAL PARASITOSIS IN HUMANS AND ANIMALS

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Abstract

Intestinal parasites represent a public health problem, especially in regions with poor basic sanitation, affecting humans and animals, and compromising the quality of life of communities. In this context, the sub-project “Health Education: Intestinal Parasitosis in Humans and Animals” was developed within the Extension Program “Health and Well-Being: UENP in the Community”, which aims to tackle this problem through educational strategies and preventive actions in schools, promoting awareness of the risks, prevention and control of parasitosis. The aim was to raise awareness among the school community about the prevention and control of intestinal parasites, emphasizing the importance of personal hygiene, proper handling of pets and the implementation of basic sanitation. The subproject's actions were carried out in a municipal early childhood education school in the municipality of Bandeirantes-PR, involving the school community, 75 schoolchildren aged between three and four, eight teachers and four cooks. Workshops and interactive theater presentations were held, covering topics such as: modes of transmission, clinical signs and preventive measures related to intestinal parasitosis, correct hand washing, safe food preparation, and care in handling domestic animals. In addition to the school, the sub-project was presented at an event such as the “UENP in the Community Meeting”, held in the Bandeirantes-PR sports hall, where more than a thousand visitors from schools in the region and the local community were able to gain an understanding of parasite prevention. Educational materials were handed out to the parents and/or guardians of the schoolchildren, teachers, cooks and the community attending the event in the sports hall. It can therefore be concluded that the sub-project helped to increase knowledge about parasites and raise awareness of ways of preventing them by developing health education on intestinal parasites in the participating community.

Supported by: Fundação Araucária, PIBEX-UENP

Keywords: schoolchildren, teaching, parasites.

Fuzzy logic as a tool for taxonomic identification of protozoa from the Kinetoplastea class

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Kinetoplastea class exhibits remarkable diversity, including free-living protozoa and parasites capable of infecting vertebrates, invertebrates, and plants. The organisms' taxonomic classification is currently being extensively revised due to new features obtained from DNA sequencing and phylogenetic studies. Parasitic species are in constant evolution, resulting in individuals from closely related species having in common different proportions of genetic material. In this study a mixed clustering approach was adopted in that Fuzzy C-Means, which captures uncertainties with greater flexibility compared to traditional methods, was combined with Hierarchical Clustering, which provides a hierarchical view of data relationships to classify kinetoplastids. Unaligned kinetoplastid sequences were obtained through next-generation sequencing (18S rDNA gene, Illumina platform) from different hosts and Brazilian biomes to form clusters, and then, validated the groups based on total inertia to classify Kinetoplastea species. Sequences from species such as *Trypanosoma cruzi* (DTUs TcI, TcII, TcIV, and Tcbat), *T. cruzi marinkellei*, *T. dionisii*, *T. rangeli*, *T. janseni*, *T. sp.* Neobat, *T. lewisi*, *T. cascavelli*, *T. minasense*, *Crithidia mellificae*, *Leishmania infantum*, *Neobodo*, *Parabodo*, and representatives of the Bodonidae family were analyzed. The results suggested 4 to 6 clusters. *T. cascavelli*, *T. cruzi*, and *T. rangeli* formed unique groups, with *T. cascavelli* being the most homogeneous. Free-living protozoa formed a separated group only as the number of clusters increased. Other species exhibited heterogeneous classifications, reinforcing the potential of fuzzy logic in evolutionary analysis. The combination of Fuzzy C-Means and Hierarchical Clustering to analyze Kinetoplastea revealed homogeneity in *T. cascavelli* and heterogeneity in free-living species. Fuzzy logic proved effective in capturing genetic uncertainties, advancing the taxonomic and evolutionary understanding of these organisms.

Supported by: Fiocruz, Ufrj, CAPES, Faperj
Keywords: Kinetoplastid, taxonomy, fuzzy logic

EDUCATIONAL MATERIALS ON MEDICALLY IMPORTANT MOLLUSKS IN SERGIPE: A DIDACTIC PROPOSAL FOR HEALTH EDUCATION

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Sergipe ranks as the state with the highest prevalence of schistosomiasis in Brazil relative to its population size. However, the intermediate host of the parasite responsible for the disease is often unrecognized or mistaken for other gastropod species. This study highlights the importance of health education in accurately identifying the species *Biomphalaria glabrata*, which is associated with the transmission of *Schistosoma mansoni*, the causative agent of this endemic disease in the state. Additionally, it addresses *Achatina fulica*, a species linked to the transmission of *Angiostrongylus costaricensis*, the causative agent of abdominal angiostrongyliasis, and *A. cantonensis*, responsible for eosinophilic meningitis. The objective of this study was to develop accessible and straightforward educational material to disseminate information about these species, which play critical roles as intermediate hosts of helminths with significant local medical relevance. A qualitative bibliographic research approach was adopted, drawing on reference works on the topic and analyses by health education experts, addressing key gaps in existing materials. The outcome is an informational brochure on these mollusks and the helminthiases they transmit. The material uses simple, direct, and interactive language in a digital format, with strong potential to disseminate knowledge about the morphology and habitat of these animals. The conclusion emphasizes the importance of creating innovative educational materials and the need to implement them in diverse educational settings for evaluation by professionals and educators. This initiative seeks to strengthen knowledge about these animals and the neglected diseases with epidemic potential they are associated with, thereby fostering health promotion.

Keywords: Health Education; Schistosomiasis; Angiostrongyliasis.

FROM THEORY TO PRACTICE: EDUCATING STUDENTS ON *Schistosoma mansoni* THROUGH DIDACTIC MODELS

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The use of didactic models is highly relevant for teaching and learning, especially in the field of Parasitology, which encompasses a range of complex concepts that are often difficult to understand and not always properly addressed. This study aims to evaluate whether didactic models can facilitate the teaching and learning process of the *Schistosoma mansoni* life cycle for second-year high school students at a public state school in the municipality of Queimados, Rio de Janeiro. To achieve this, a pre-questionnaire was administered to assess students' prior knowledge. Subsequently, the actual effectiveness of the model in the teaching and learning process was analyzed through a post-questionnaire. The model was constructed using the following materials: white biscuit clay, pink and burgundy dyes, styrofoam (50 cm x 50 cm), colored cardstock, hot glue, office paper, toothbrush bristles, figurines, Velcro, and modeling tools. After implementing the didactic model in the classroom, a 92% increase in correct answers was observed, demonstrating its effectiveness in the learning process. Within this percentage, students showed a clear understanding of schistosomiasis, correctly identifying the characteristics of the definitive and intermediate hosts of *S. mansoni*, the main symptoms of the disease, the most effective preventive measures, and the specifics of its treatment. We conclude that the use of didactic models in education facilitates the comprehension of both basic and complex topics, such as those covered in parasitology. This approach makes learning more dynamic and effective, standing out as a valuable pedagogical tool for enhancing students' knowledge acquisition. Additionally, it fosters critical thinking, promotes inclusion, and stimulates scientific curiosity.

Keywords: Pedagogical Resources. Parasitology Education. Health Education.

CONTAMINATION OF DOG HAIR BY *Toxocara* spp. EGGS IN A DISADVANTAGED POPULATION OF SOUTHEAST BRAZIL: A ZOONOTIC RISK

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Toxocariasis is considered a worldwide geohelminthiasis, particularly in disadvantaged populations. Transmission of toxocariasis to humans occurs mainly by accidental ingestion of soil containing infective *Toxocara* spp. eggs, shed by dogs and cats (definitive hosts). Other routes of transmission have been postulated, including the direct contact with dogs or cats harbouring infective eggs in their hair. The aim of the present study was to evaluate the frequency and the factors associated with the presence of *Toxocara s*pp. eggs in the hair of dogs raised by disadvantaged population in Presidente Prudente, southeast Brazil. Hair samples were collected (perineal, tail and dorsal regions) from 100 dogs, processed (rinsing in Tween 80 followed by sieving: 300µm, 212µm, and 38µm) and analysed under microscope (10 and 40X). Association between the presence of eggs and some variables (age, sex, dirty floor at household, cohabitation with other pets, free-roaming on the streets, and deworming) were evaluated. Presence of *Toxocara* spp. eggs was observed in the hair of 8/100 dogs (8%; IC 95%: 4.1-15.0), mainly in male (5/8), dogs aging over 1-year (6/8), living in a house with dirty floor (5/8), living with other pets (6/8), and having access to street (7/8). Lack of deworming was observed in all positive dogs. No variables influenced the presence of eggs in the hair samples ($p>0.05$). Excepting one, all the *Toxocara* spp. eggs retrieved were classified as viable. The number of eggs ranged from 1 to 6 per dog. Our results show that the presence of *Toxocara* spp. eggs in dogs' hair may predispose disadvantaged population at risk of toxocariasis. Therefore, educative programs should be considered for enhancing hygienic habits and prophylactic anthelmintic treatment of dogs to mitigate the risk of toxocariasis transmission.

Supported by: FAPESP (2024/02836-3)

Keywords: infection, toxocariasis, zoonosis

PREVALENCE OF AMEBIASIS AND GIARDIASIS IN RIVERSIDE COMMUNITIES OF THE PARAGUAY RIVER

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Amebiasis and giardiasis are Socially Determined Diseases (SDD) and represent a challenge for vulnerable populations, including riverside communities. We carried out a cross-sectional study to investigate the prevalence of SDD caused by intestinal parasites in riverside communities in the South and North Sections of the Paraguay River. Two parasitological examination techniques were employed: Hoffman-Pons-Janer (HPJ) and Willis. The HPJ method, also known as the Lutz method, is a versatile method based on spontaneous sedimentation, allowing the observation of helminth eggs and larvae in addition to protozoan cysts and trophozoites. The Willis method is based on the difference in specific density between worm eggs and protozoan cysts and oocysts, allowing these organisms to float on the surface of a NaCl-concentrated solution or sucrose. To carry out the research, 787 fecal collection containers were distributed to the population and 227 returned (28.84%). We found 10,13 and 60,79 cases per 100 inhabitants, respectively, of *Entamoeba histolytica / Entamoeba dispar* and *Giardia duodenalis*. The prevalence of amebiasis and giardiasis in the assisted riverside communities was 1.76% and 21,58%, respectively. Moreover, the prevalence of individuals presenting co-infection with other protozoa and/or helminths was 8,37% for amebiasis and 39.20% for giardiasis. Our results highlight the high prevalence of amebiasis and giardiasis in the riverside community population of the Paraguay River, reflecting the unfavorable socioeconomic conditions and the lack of adequate infrastructure. The implementation of epidemiological surveillance programs, health education, and improvement in sanitary conditions is essential to reduce the impact of these parasites and promote the health of the population.

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Keywords: Parasitic Intestinal Diseases; Prevalence; Cross-sectional studies

BOOKLET “INSECTS OF MEDICAL INTEREST”: A SCIENTIFIC DISSEMINATION TOOL

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Insects of medical interest include the group of vectors and invertebrates that can transmit pathogens that cause diseases in humans and animals. The objective of this work was to produce a booklet with accessible information about the habits of insect vectors. The booklet “Insects of medical interest” is one of the products of the One Health Project for the “Igarapés” of Porto Velho – SUIgPVH and was aimed at residents near riparian vegetation areas and public schools located in the neighborhoods where the project was developed. To produce the booklet, we included original illustrations and language that were easy for the local population to understand. The booklet has seven distinct sections, classified by the common and scientific name of each insect (Dengue mosquito - *Aedes aegypti*; Common mosquito - *Culex quinquefasciatus*; Nail mosquito - *Anopheles darlingi*; Straw mosquito - *Psychodopygus davisi* and *Nyssomyia antunesi*; Meruim - *Culicoides paraensis*; Pium - *Simulium argentiscutum* and *Simulium pertinax*; Barber - *Rhodnius robustus* and *Panstrongylus geniculatus*). For each vector, the participation in disease transmission cycles, preferred breeding and blood-feeding habits and main prevention measures were highlighted. To date, 266 booklets have been distributed to residents of homes adjacent to the riparian vegetation and 576 booklets for schools, covering grades from the 3rd year of elementary school to the 1st year of high school. The booklet was also made available in digital format (https://www.rondonia.fiocruz.br/wp-content/uploads/2024/04/CARTILHA-DIGITAL.pdf) via QR code and has been distributed at Fiocruz Rondônia events. Scientific dissemination and popularization of science are initiatives that help prevent and control insect vectors of diseases and have an important social impact, increasing community involvement with science.

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Keywords: Entomology, Booklet, Scientific dissemination.

PRACTICE AS A CURRICULAR COMPONENT IN THE TEACHING OF PARASITOLOGY FOR THE DEGREE COURSE IN BIOLOGICAL SCIENCES

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Practices as a Curricular Component (PCC) are activities that allow reflection on the content learned during the undergraduate course and didactic transposition in their professional work as teachers. Specifically in the degree course in Biological Sciences, the PCC must articulate biological knowledge with conditioning factors, particularities and objectives in formal basic education and in other non-school educational spaces. Following discussions on the approach to health education in basic education, students on the Biological Sciences degree course at UFCAT proposed the development of the “Parasito-on” game. The game was inspired by “Pokemon Hunt” and aimed to find evolutionary forms of parasites on UFCAT Campus I. An avatar of each parasitic evolutionary form was created using the “IbisPaint” application, purchased free from Google Play. The avatars were also accompanied by descriptions of the species such as biology, evolutionary cycle, pathogenesis and prevention. A total of 15 avatars were created: Leishmania sp (promastigote, amastigote and vector); Trypanosoma cruzi (trypomastigote, amastigote and vector); Giardia lamblia (trophozoite, cyst); Trichomonas vaginalis (trophozoite); Entamoeba histolytica (trophozoite, cyst); Plasmodium sp (merozoite and sporozoite); Toxoplasma gondii (tachyzoite and bradyzoite). The students mapped the campus using the “Google Maps” application to establish the hiding places of the “Parasito-nos” (parasitic evolutionary forms). To ensure that the game wasn't just virtual and had concrete material, the third group was responsible for making didactic models of the parasitic evolutionary forms out of biscuit. A hiding place was mapped out for each species. After finding the first point, accessing the QR-code, observing the avatar, reading the characteristics and analyzing the biscuit model, it was time to move on to the next point.

Keywords: Practices as a Curricular Component, Parasitology, Health Education

INTEGRATED APPROACH TO PARASITOLOGICAL SURVEILLANCE: THE USE OF eDNA IN HELMINT MONITORING IN ENDEMIC AREAS

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The One Health approach, which promotes the integration of multiple sectors, is essential in addressing Neglected Tropical Diseases (NTDs). The NTDs are endemic diseases caused by various infectious or parasitic agents that affect vulnerable populations living in poverty and with limited access to basic health services. Helminth parasites are especially relevant in this context, as they are directly related to poor sanitation infrastructure, which favors their spread through contact with contaminated soil, water and food. Although traditional microscopy is the standard method for identifying these parasites, molecular methods can offer greater sensitivity and specificity. The use of environmental DNA (eDNA) in parasitology is relatively recent but promising for understanding the dynamics of transmission and identifying sources of infection, which can contribute to early diagnosis and control, especially in endemic areas. The present study proposes the development of an auxiliary method to the traditional one based on eDNA extraction to evaluate environmental contamination by parasites and its impact on the health of residents of Brejo do Amparo, Januária - MG. To investigate the association between soil contamination and human infection, biological samples (faeces) were collected from 265 residents and 10 soil samples for standardization of the eDNA technique by Multiparallel qPCR. Of the biological samples, 81 (30.5%) were positive for helminths, in which the majority were positive for *Schistosoma mansoni* (26%), followed by *Strongyloides stercoralis* (3%), hookworms (0.75%), *Enterobius vermicularis* (0.38%), and *Hymenolepis* spp. (0.38%). In the soil, *Ascaris lumbricoides* (20%), *Toxocara cati* (30%), and *Taenia solium* (30%) were the most commonly detected species. These findings reinforce the potential of eDNA in analyzing environmental parasite burden, helping to assess risk of infection and targeting strategies for the control and prevention of NTDs.

Supported by CAPES and CNPq

Keywords: One Health, Neglected Tropical Diseases, Multiparallel qPCR

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EVALUATION OF INTESTINAL PARASITOSIS, EOSINOPHILIA AND NUTRITIONAL STATUS IN RIVERSIDE INDIVIDUALS OF THE RECÔNCAVO OF BAHIA

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Abstract

The aim of this study was to evaluate the frequency of intestinal parasites in riverside dwellers and to associate them with nutritional status, presence of anemia and eosinophilia. Fecal and blood samples were collected from individuals from Santiago do Iguape, Cachoeira, and Cabaceiras do Paraguaçu, in the Recôncavo region of Bahia. Feces were examined using the Baermann, Lutz and Kato-Katz methods, and hemoglobin and eosinophil levels were assessed by blood count. Weight, height and abdominal circumference were used to assess nutritional status. Of the 267 participants, 194 (72.7%) delivered fecal samples, and of these, 80 (41.2%) were infected with intestinal parasites, with 73 (37.6%) positive for protozoa and 12 (6.2%) for helminths. The main protozoa diagnosed were *Endolimax nana* (24.7%), *Entamoeba coli* (21.6%), *Entamoeba histolytica/dispar* (9.8%) and *Giardia duodenalis* (1.6%). Among the helminths, there were identified *Schistosoma mansoni* (2.6%), *Enterobius vermicularis* (1.6%), *Ascaris lumbricoides* (1.0%), *Trichuris trichiura* (0.5%) and *Strongyloides stercoralis* (0.5%). The highest parasite loads were found in 3 of the 5 individuals infected with *S. mansoni*, with 1,776, 792 and 272 eggs/gram of feces, also presenting the highest rates of eosinophilia, 15%, 13% and 12%, respectively. No significant changes were observed in the hemoglobin levels of the studied population, regardless of the presence of parasites. Furthermore, between 80-90% of the individuals analyzed were considered eutrophic, and no association was found between parasitic infections and changes in the Body Mass Index. The occurrence of *Schistosoma mansoni* suggests contact of the population with transmission areas in the Paraguaçu River. On the other hand, access to treated water, even with occasional interruptions in the supply, in addition to the sewage system in some locations, must have influenced in the low frequency for other helminths observed.

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Keywords: Parasitic infections; riverside populations; eosinophilia.

PARASITODEX: A USER-FRIENDLY APPLICATION FOR THE IDENTIFICATION OF PROTOZOAN AND HELMINTH SPECIES IN MEDICAL PARASITOLOGY

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Parasite identification is a challenge in teaching medical parasitology. Traditionally, practical classes are based on microscopic observation of protozoans and helminths. Therefore, it is important to develop applications for parasite identification. We have developed an application to facilitate the recognition of protozoan and helminth species of interest in medical parasitology. A database of parasite morphological information was organized in Excel. Images were photographed at the Laboratory of Medical Parasitology and Vector Biology, Universidade de Brasília; other images were taken from books and identification guides. App development followed the method described for VetorDex. The database contained 15 species of helminths and 17 species of protozoa. The first question determines the origin of the sample (fecal, blood or urogenital). If fecal samples are selected, the app directs you to protozoa or helminths. The shape, size and number of nuclei of the structures are displayed to determine the protozoan species (*Entamoeba* and other amoebas, *Cystoisospora*, *Balantidium* and *Giardia lamblia*). For helminths, the app first separates eggs and larvae. Eggs are differentiated by shape, size and membrane characteristics (*Ascaris*, *Trichuris*, *Enterobius*), while larvae are differentiated by buccal vestibule and tail (*Strongyloides* and Ancylostomidae). Intracellular blood parasites (*Toxoplasma*, *Leishmania/Trypanosoma*, and *Plasmodium*) were classified according to organelle and nuclear characteristics. Extracellular blood parasites were classified according to the position of the nucleus/kinetoplast (*T. cruzi*, *T. rangeli*, *Leishmania*) and the presence of a sheath (*Wuchereria bancrofti*). For urogenital specimens, the system offers a single option (*Trichomonas vaginalis*). Once validated, ParasitoDex will be able to assist in the morphological identification of protozoa and helminths, making it easier for students to identify these parasites.

Supported By: CNPq, CAPES

Keywords: app, protozoa, helminth

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POSTGRADUATE PROGRAMS AT FIOCRUZ BAHIA: CONNECTING KNOWLEDGE IN PARASITOLOGY TO CONTRIBUTE TO THE DISSEMINATION OF KNOWLEDGE AND HEALTH EDUCATION IN THE MUNICIPALITIES OF SALVADOR AND CACHOEIRA

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Abstract

Over the past two years, the graduate Program in Human Pathology (PGPAT) at the Federal University of Bahia (UFBA), in partnership with the Oswaldo Cruz Foundation (Fiocruz) in Bahia and the graduate Program in Biotechnology in Health and Investigative Medicine (PGBSMI) at Fiocruz Bahia (both CAPES level 6), has implemented educational strategies to train teachers and students in science and health projects, particularly in parasitology. Participants received training through an Institutional Program of Continuing Education in Active Teaching Methodologies, enabling them to apply their knowledge in various health-related undergraduate programs, as well as in elementary and high schools. Activities included lectures, workshops, science fairs, graffiti, and interactive games. Addressing topics ranging from health education to the exploration of parasites, vectors, and insect biodiversity, these initiatives aim to raise awareness and support efforts to prevent, control, and eliminate diseases such as leishmaniasis, Chagas disease, and schistosomiasis. Through this project, we strive to integrate parasitology knowledge into society while promoting cultural diversity and addressing ethnic-racial issues in public schools in Salvador and quilombola communities in Cachoeira, Bahia.

Supported by: Fiocruz Bahia, UFBA, CAPES, FAPESB
Keywords: Graduate Program, Parasitology, Education, Science

EVALUATION OF THE EFFICACY OF SURVEILLANCE, MONITORING AND PUBLIC MANAGEMENT INTERVENTIONS FOR THE CONTROL AND ELIMINATION OF SOIL-TRANSMITTED HELMINTHS AS A PUBLIC HEALTH PROBLEM: A LITERATURE REVIEW

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The global estimates from the World Health Organization (WHO) indicate that more than 970 million people are infected with one or more of the major soil-transmitted helminths (STH), which represents 64% of the total prevalence of major neglected tropical diseases. Moreover, a global burden of STH infections is estimated to be 3394 million disability-adjusted life years. Considering the global burden of STH and, in line with the goal of the World Health Organization (WHO) 2030 Agenda to eliminate such diseases as a health problem, the synthesis of the best current evidence from the scientific literature on diagnosis, treatment, prevention and health education related to the control, surveillance and monitoring of STH infections becomes an important tool to support managers in the elaboration of public health policies. In this rapid review we are investigating which of these interventions are most effective for the control and elimination of soil-transmitted helminths as a public health problem. The acronym PICO was used to create the question and a systematic search was conducted in Embase, Lilacs, Pubmed, Scielo, Scopus, and Web of Science databases, identifying 1558 studies from 2000 to 2024. Systematic reviews were selected according to inclusion criteria: studies published in English, Portuguese or Spanish in which participants were human populations infected with Ascaris, hookworms, Trichuris or Strongyloides. Interventions were any type of diagnosis, treatment, water, sanitization, hygiene, or health education actions. Outcome measures were changes in prevalence, incidence, mortality, morbidities or parasite load. A total of 37 studies were included for in-depth analysis. Data extraction and evaluation of data quality is ongoing. The results will allow us to synthesize evidence on the best interventions in control, surveillance and monitoring to support the creation of public policies aimed at eliminating STH infections as a public health problem.

Supported By: Brazilian Ministry of Health

Keywords: Ascariasis, Hookworm, Trichuriasis.

HYPOALLERGENIC MOLECULE OF *Blomia tropicalis* AND THE POLARIZATION OF MACROPHAGES IN DIFFERENT ASTHMATIC PHENOTYPES

Exposure to the house dust mite *Blomia tropicalis i*s one of the main risk factors for the development of asthma in tropical and subtropical climates. Advances in molecular biology have allowed the development of hypoallergenic hybrid proteins. Our research group used fragments of the Blo t 5 and Blo t 21 proteins, the main allergens of *Blomia tropicalis*, to construct the hypoallergenic protein - BTH2. Recent studies have shown that the interaction of T, B, dendritic, epithelial cells, and macrophages individually contributes to clinical tolerance. It is believed that macrophage polarization may help to reduce the pathogenesis of asthma. Therefore, the purpose of this study was to characterize the effects of the hypoallergenic recombinant protein on the activation profile of macrophages among the different asthmatic endophenotypes. For this purpose, the study population of this project consisted of 30 individuals classified into 3 groups: severe asthma, mild/moderate asthma, and control (without asthma). Sociodemographic data were collected, vital signs were measured, anthropometry was performed and allergy tests were performed. Monocyte-derived macrophages were challenged with crude extract of *Blomia tropicalis*, recombinant proteins Blo t 5 and Blo t 21, and the hypoallergenic molecule - BTH2. The immunophenotyping of macrophages was evaluated by flow cytometry through surface markers that corresponded to each phenotype (CD14, CD80, MHCII, CD163, and CD206). There was a high expression of the CD14 co-receptor in the severe asthma group. The CD80 and MHCII markers were similar between the groups tested. The CD163 molecule was more observed in cultures challenged by BTH2 from the severe asthma group. The CD206 molecule showed lower expression in macrophages stimulated by BTH2 in the severe asthma group. The BTH2 molecule was able to induce an immunoregulatory macrophage profile, enabling the prevention of asthma onset in its early stages. It is believed that it may promote immunomodulation of the disease by directing the immune response to the specific antigen, modifying its mechanisms and consequently biomarkers, resulting in a lasting and even lifelong reduction in symptoms.

CASES OF ATYPICAL CUTANEOUS LEISHMANIASIS CAUSED BY *LEISHMANIA INFANTUM*: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Clinical forms of leishmaniasis are related to *Leishmania* species enrolled in the infection and host immunological background. Cases of leishmaniasis caused by atypical etiological agents have been widely reported in the literature. However, there is no consensus regarding the clinical profile of these patients or the therapeutic approaches to be adopted. This systematic review and meta-analysis characterizes the clinical profile of patients with leishmaniasis caused by unexpected species of *Leishmania*. For this, we developed a study following PRISMA guidelines with focus on case reports of leishmaniasis caused by atypical agents published in the indexed journals on the Scielo, Cochrane, LILACS, PROSPERO and PUBMED. The search was conducted from July 19, 2024 to November 19, 2024. The following descriptors used “Leishmaniasis, Cutaneous” or “Leishmaniasis, Mucocutaneous” or “Leishmaniasis, Visceral” and “*Leishmania*” and “Atypical.” Then, we check the quality of the studies using the CARE protocol by two independent reviewers. A total of 115 articles were found, of which only 43 were eligible as case studies. To meta-analysis, we included a total of 50 participants. The total cases of atypical cutaneous leishmaniasis were caused by *Leishmania infantum* and the cases were not restricted to the disease endemic areas. The mean of the age was 46,5 years and women composed 32% (16/50) of the patients. Most of the patients were immunocompetent and 48% (24/50) presented ulcer lesions without clinical symptoms of visceral leishmaniasis. In addition, 34% (17/50) the patients were immunosuppressed, 18% (9/50) cases presented non-leishmania coinfections and 38% (19/50) cases presented therapeutic failure. In conclusion, the data indicate that the reported cases of atypical leishmaniasis are essentially caused by *L. infantum* and they differ from the dermatological disease described in the literature as post-kala azar dermal leishmaniasis.

Keywords: Atypical Leishmaniasis; Leishmania species; Meta-Analysis.

TERMINOLOGICAL GLOSSARY IN BRAZILIAN SIGN LANGUAGE (LIBRAS) ON NEGLECTED TROPICAL DISEASES (NTDS) AS A TOOL FOR PROMOTING COMMUNICATIONAL ACCESSIBILITY IN HEALTH AND EDUCATION

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Abstract

Inclusive education requires an environment that reflects human diversity, promoting democratic coexistence and accessibility. However, deaf individuals face communication barriers in public spaces, especially in healthcare services, which limit access to essential information, such as that related to Neglected Diseases (NDs), health conditions prevalent among vulnerable populations. This study aimed to identify the sign-terms used by deaf individuals to refer to NDs and develop a glossary in Brazilian Sign Language (Libras) designed for deaf students and healthcare professionals. The glossary addresses technical terms about NDs to facilitate understanding, raise awareness, and assist in the training of healthcare professionals to better diagnose and treat deaf patients with these diseases. A qualitative exploratory research approach was adopted as the methodological framework. During the data collection phase, the study included the identification of Libras signs, the provision of these signs, and their validation by the deaf community through the Content Validity Index (CVI) in healthcare settings. A total of 17 disease-related expressions caused by parasites, viruses, and disease vectors were evaluated, significantly contributing to the cataloging of Libras signs for NDs. The results indicate that the Libras glossary significantly contributes to inclusive access to health and science, empowering the deaf community and facilitating the dissemination of information about NDs. However, it is crucial to understand the urgent need to expand this approach to other areas of knowledge, ensuring the inclusion of the deaf community in the context of public health.

Supported by: CAPES/Fiocruz.
Keywords: Health Education. Neglected Diseases. Glossary. Libras.

SURVEILLANCE OF *Leishmania infantum* IN THE MUNICIPALITY OF NATAL/RN

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Leishmania infantum, the main etiologic agent of visceral leishmaniasis (VL) in Brazil, is primarily transmitted by the sandfly *Lutzomyia longipalpis*. Humans and dogs are susceptible to developing VL and dogs are the main domestic reservoirs, playing a crucial role in parasite transmission. Therefore, this study aimed to determine the status of *L. infantum* infection in dogs around households of cases of human VL in the municipality of Natal/RN. Between 2003 and 2023 a surveys were conducted around recent human VL cases, with a 300-meter radius for canine serology. Overlapping radii expanded the coverage area. Rapid testing (TR DPP) was used, followed by ELISA for confirmation. A total of 69,798 dogs were tested and 8.84% were seropositive (n=6,179) and the peak occurred in 2017 (reaching 21.4% of all seropositive). The majority of dogs surveyed were from the North district, representing 82.7%, followed by the West district with 14.3%. East and South districts accounted for 0.3% and 2.7%, respectively. Dogs from the North district were 2.8 times more likely to be seropositive ($p<0.0001$). Within north zone, Nossa Senhora da Apresentação neighborhood presented the higher seroprevalence reaching the peak in 2014 (19.2%).The finds highlights the hole of high dogs seroprevalence that may sustain the *L. infantum* transmission in the North Zone of Natal. Similarly, a VL endemic area in Piauí presented 12,28% of dogs' seropositivity. In contrast , a study developed in Sergipe reported a higher seroprevalence (34.69%), reinforcing the importance of dogs in *L. infantum* cycle maintenance. Finally, continuous surveillance aiming to determine the status of *L. infantum* infection in dogs signal for potential cycles of *L. infantum* infection at a population level, with in infection in the more affected areas varying from 10% to 32% indicating the need of measures to decrease burden including use of collars and determining the magnitude of *L. infantum* vertical transmission.

Supported by: Municipal Health Department.

Keywords: Seroprevalence. Leishmaniasis. Dogs.

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USE OF PLAYFUL ACTIVITIES AS A TEACHING STRATEGY FOR CHILDREN ABOUT INTESTINAL PARASITOSIS: AN EXPERIENCE REPORT

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Abstract

Parasitic infections are more prevalent among school-age children due to inadequate hygiene habits. In rural environments, with poor sanitation and water treatment, the risk of transmission increases among individuals and makes children the main target of health education activities. In this context, a playful activity among 68 elementary school students, aged 8 to 11 years, was carried out to promote health education, focusing on the main intestinal parasitic infections. A questionnaire adapted to the age groups was applied before and after the interventions to assess the acquisition of knowledge. The questionnaire was designed with five questions about concept, transmission, symptoms, prophylaxis and treatment. Four activities were developed: 1) conversation circle and discussion about the content of the questionnaire; 2) interactive demonstrations with the participation of children explaining the life cycle of Schistosoma mansoni and Ascaris lumbricoides. Educational materials prepared manually about each phase of the cycles were used; 3) a large-scale board game, consisting of fifteen squares of paper and a large die constructed from recycled bottles. The students were divided into groups, with one member from each team representing the pawns. The students answered questions about the topics discussed in the previous activities; 4) painting of hands with fluorescent paint, followed by washing of hands. After washing, they were asked to place their hands in a box prepared to create a dark environment and finally a black light flashlight was used to reveal any remaining paint. The activities emphasized the importance of proper hand washing. There was great involvement of the children and it was possible to promote an educational environment with positive repercussions in the responses after the intervention.

Supported by: FAPEMAT, CNPq
Keywords: Playful activities, intestinal parasitic disease, health education.

HEALTH EDUCATION AS A MEASURE ESSENTIAL PROPHYLACTIC TO SUPPORT THE PREVENTION OF SCHISTOSOMIASIS MANSONI

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Introduction:Schistosomiasis mansoni (MS) is a parasitic disease caused by Schistosoma mansoni associated with numerous social problems. Among the most common prophylactic measures for MS are: I) avoiding bathing in rivers with a history of transmission and with the presence of the host mollusc; II) adequate treatment of parasitized individuals; III) not defecating near rivers; IV) control of the Biomphalaria mollusc and V) health education. **Objective:** To examine the impact of education on the prevention of MS.
Methodology: Integrative literature review was conducted, including data collection, between November 2024 and January 2025 in the Virtual Health Library (VHL), using descriptors such as “Health Education”, “Schistosomiasis” and “Public Health”. Studies published between 2019 and 2024, in Portuguese, English or Spanish, and with free access were included. Duplicate records and those without an approach to health education and/or MS were excluded.
Results and Discussion: Of the 52 studies evaluated, 13 were selected after applying the inclusion and exclusion criteria. The analysis indicated that 75% of the studies focused on MS, notably epidemiological, pharmacological and transmission pattern research. In only 25% of the articles was there an association between MS and health education. In these studies, in particular, the authors pointed out that education measures should not be applied in isolation, and that actions to combat MS should be integrated and multi-professional. They stressed the importance of more investment in infrastructure, treatment and control. In order to effectively combat MS during medium and long term, education must not be neglected. **Conclusion:** The study highlights the lack of research on the role of health education in preventing MS. The analyses collected point to the need to improve educational measures in the fight against MS, in line with the common sense that education can interrupt and/or minimize countless problems.

Keywords: Health Education, Schistosomiasis, Actions

TEACHING PARASITOLOGY IN DIFFERENT UNDERGRADUATE PROGRAMS

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Prevention and control of parasitic diseases in humans and animals are related to education, that is why the study of Parasitology is important. This work aims to report on the teaching of Parasitology to different undergraduate programs: Bachelor's and Teaching degrees. The Instituto Federal Baiano (Federal Institute) – Santa Inês Campus offers a Bachelor's Degree in Animal Science and a Teaching in Biological Sciences programs, both including Parasitology subjects with different teaching approaches. In the Bachelor's in Animal Science program, students are encouraged to interact with small-scale farmers of various production animal species, where they learn about different parasites and associated problems to them, the locations where they are found, and their impacts to humans and animals as well. Discussions also cover preventive measures, the use of different antiparasitic products, highlighting their purposes, withdrawal periods, methods of application, and biological risks. In the Teaching degree in Biological Sciences, each student is responsible for a specific parasite and its associated diseases. Through playful language, they become multipliers of information on the topic in educational units at different levels of education, from elementary to higher education. Explanatory posters and a playful activity are used for each topic, as an information tool to students from several, different schools that were visited. Students in both Bachelor's in Animal Science and Teaching degree in Biological Sciences experience Parasitology in an applied manner, demonstrating teaching strategies that go beyond the traditional methods, while also stimulating public knowledge in general.

Keyword: Parasite; Learning; Parasitic Diseases

STRATEGIES FOR DISSEMINATING INFORMATION ABOUT TICKS AND THEIR IMPORTANCE

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The use of active methodologies in Biology teaching enhances learning, especially in complex subjects such as Parasitology. This study aimed to report a playful teaching proposal about ticks and their importance. Undergraduate students of Biological Sciences at Instituto Federal Baiano – *Campus* Santa Inês, using illustrative posters about ticks and a quiz-style educational activity with true or false answers, visited schools from different educational levels, including Elementary School (Stages I and II), High School, Higher Education, and Youth and Adult Education. During these visits, they interacted with students from various schools in different cities of the Vale do Jiquiriçá region, Bahia. A total of 10 schools across six municipalities (Cravolândia, Itaquara, Jiquiriçá, Mutuípe, Santa Inês, and Ubaíra) were visited between August and September 2024. It was observed that students from rural areas demonstrated better basic knowledge due to their experiences with ticks in animal handling activities. In contrast, among urban students, the approach fostered a collaborative learning environment with mutual contributions among students, enriching the educational process and strengthening the effectiveness of playful methodologies in basic education. This experience highlighted the importance of active strategies and methodologies in the education of Biological Sciences undergraduate students, who will become future Biology teachers, thus enhancing their knowledge of Parasitology.

Keywords: Active methodology; Parasitology; Learning.

PERCEPTION OF THE POPULATION ABOUT WORMS

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Worm infestations negatively impact the physical and cognitive development of children and adolescents, as well as reducing the work performance of adults. One of the main ways to interrupt this chain of transmission is through adequate basic hygiene habits. Thus, we aimed to assess the population's knowledge about worm infestations, seeking to understand which information is well-established and which is deficient and requires government measures in health education. We applied an online questionnaire with questions about helminths that addressed: identification; evolutionary cycle; prevention measures and epidemiology; diagnosis; symptomatology. The target population was users and non-users of Family Clinics (CF). In the first stage with non-users, we had 65 participants. Of these, 93.8% lived in areas with sanitation and 56.9% had completed higher education. Regarding diseases caused by helminths, the most cited were: taeniasis (75.4%), ascariasis (61.5%), giardiasis (56.9% - protozoan disease). Regarding the forms of contagion, contamination by water (98.5%), food (90.8%) and soil (81.5%) was indicated. 89.2% of the participants knew the prophylactic measures, with the most prevalent being “washing hands before eating and after going to the bathroom” (95.4%) and “preventing insects from landing on food” (92.3%). This allows us to infer that there is no full knowledge regarding the identification of worm infestations and their forms of transmission. However, the main prophylactic measures were present in the daily lives of the participants, as well as an understanding of the main forms of contamination. The follow-up of this project will make it possible to compare the results obtained in the two groups and verify whether the activities developed in the CF contribute to expanding the population's knowledge about the health area. Furthermore, printed educational material will be given to the participant addressing the main points discussed in the questionnaire.

Keywords: Helminthiasis; Knowledge; Education
Funding Agency: Scientific Initiation Program (PIC) of the medicine course at Faculdade Souza Marques

A PLAYFUL TEACHING EXPERIENCE ABOUT LEISHMANIASIS

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The importance of playful teaching on Parasitology facilitates the understanding of parasitic diseases for different school levels. The objective was to report a playful teaching experience on leishmaniasis. In an activity developed in the discipline of Parasitology by the students of the Degree in Biological Sciences of the Instituto Federal Baiano - *Campus* Santa Inês, on leishmaniasis, the content was presented to students from different Schools in the Jiquiriçá Valley, southwestern region of Bahia. The activity was developed through illustrative posters, containing a playful activity where spectators interacted and fixed knowledge. In 2024, 10 schools were visited in 6 Municipalities (Cravolândia, Itaquara, Jiquiriçá, Mutuípe, Santa Inês and Ubaíra). Since the beginning of this extension activity in 2013, it is estimated that 15,000 students have participated in the activity, including students from Elementary School (stages I and II), High School, Youth and Adult Education (EJA) and Undergraduate courses. The interest and participation of the students who showed enthusiasm, motivation and curiosity of the information presented about leishmaniasis was notorious. Although the Jiquiriçá Valley is an endemic area for leishmaniasis, many spectators were unaware of the diseases, preventive measures and symptoms, understanding, learning and fixation to content was facilitated with the use of playfulness popularizing the teaching of Parasitology.

Keywords: Active education, Learning, Parasitology.

TEACHING PROPOSAL ABOUT SCHISTOSOMIASIS MANSONIS IN CRAVOLÂNDIA- BA

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Schistosomiasis mansoni is a parasitic disease endemic to the state of Bahia, where it causes several health problems and death. This paper aims to present a proposal for teaching about schistosomiasis mansoni in an endemic area. Students of the Bachelor's Degree in Biological Sciences (LCB) of the Instituto Federal Baiano Campus Santa Inês, with the help of an illustrative poster and a playful activity on the subject, visited elementary schools in the city of Cravolândia-Bahia, located in the Jiquiriçá Valley, southwest of Bahia. This is an endemic area for schistosomiasis mansoni. The activity was carried out in public municipal schools, for children in Elementary School I and adults in the Education for Young People and Adults (EJA) in Elementary School II. The posters and playful activities were the same, adjusting the language of the LCB students, so that each audience could understand. The lack of knowledge of some basic scientific concepts about the form of infection, symptoms and preventive measures of the disease was evident. Children, in Elementary School I, were very curious and interacted with the exhibitors. As for the adults (EJA students), in Elementary School II, it was noticed that there was popular knowledge and they reported some myths, which were duly clarified. This teaching proposal, called “PARASITOLOGICAL SHOW: Promoting Education and Health”, favors the training of future Biology teachers (LCB students), while disseminating correct knowledge to students of different ages and teaching modalities, popularizing Parasitology.

Keywords: *Schistosoma mansoni*, playful teaching, health education.

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SEROPREVALENCE OF TOXOPLASMOSIS IN RIVERSIDE POPULATIONS FROM TWO CITIES IN THE RECÔNCAVO OF BAHIA, BRAZIL

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Abstract

Toxoplasmosis is a parasitic zoonosis with an impact on public health, especially in regions with unfavourable socioeconomic conditions. Riverside populations may be more exposed to infectious agents due to precarious environmental and sanitary conditions and low access to health services. This study aimed to investigate the frequency of IgG and IgM antibodies against *Toxoplasma gondii* in riverside populations of the Recôncavo da Bahia. A cross-sectional and descriptive study was carried out between April/2022 and August/2024, involving the population of the cities of Cabaceiras do Paraguaçu and Cachoeira, Bahia, Brazil. Sociodemographic data were collected and serological tests were performed to evaluate anti-*T. gondii* IgM and IgG. In Cabaceiras do Paraguaçu, 74 individuals were screened, with a mean age of 33.84 (± 20.92). The majority were female and over 31 years old, with an income lower than the minimum wage, self-declared black and working as family farmers. In Cachoeira, the mean age was 21.45 (± 13.78), with the majority within the 0-14 years age range and female, with an income lower than the minimum wage, students and self-declared black. In Cabaceiras do Paraguaçu, 40 (54.1%) presented reactive anti-*T. gondii* IgG, while one (1.4%) was reactive for IgM. In the city of Cachoeira, 80 (49.1%) were reactive for anti-*T. gondii* IgG, while 6 (3.7%) presented positivity for IgM. These data showed that approximately 49-54% of the individuals studied had previous or chronic infection by *T. gondii* and 7 cases suggestive of acute infection were identified. It is important to emphasize that the population of Cachoeira was composed mainly of young individuals. An assessment of the association of seroprevalence with sociodemographic factors will be further analysed. Assessing the seroprevalence of this zoonosis in the riverside regions of Recôncavo Baiano is essential to understand the level of exposure and guide targeted prevention and control measures.

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Keywords: Toxoplasmosis, Riverside populations, Seroprevalence.

PLAYFUL INTERVENTION IN THE TEACHING OF PARASITIC DISEASES: A UNIQUE HEALTH APPROACH

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One Health recognizes the interdependence between human, animal, and environmental health in controlling zoonotic and vector-borne parasitic diseases. However, the assimilation of these concepts may be limited by traditional approaches, which do not always promote an integrated and practical understanding. Interactive methods are effective in stimulating engagement and facilitating learning. This study evaluated the impact of an educational intervention using playful strategies to consolidate knowledge about One Health and parasitic and vector-borne diseases among first-year Veterinary Medicine students. Knowledge was assessed before and after the intervention through a structured questionnaire covering zoonoses, transmission, prevention, and control of diseases such as Schistosomiasis, Chagas Disease, and Leishmaniasis. The study was approved by the Ethics Committee (CEP/UFOB), approval number 6.033.386. Statistical analysis was performed using McNemar's test, with a significance level of $p<0.05$. The sample was predominantly female (75%), reflecting the growing presence of women in Veterinary Medicine, linked to the increasing participation of women in biological sciences and health-related fields. Before the intervention, all students scored above 50%, with 85.7% classified as Excellent and 14.3% as Good. After the intervention, 92.9% reached the Excellent level, while 7.1% remained at the Good level. No student was classified at Regular or Poor levels, indicating a high baseline knowledge of the topic. Statistical analysis revealed a significant improvement in performance, confirming the positive impact of the playful approach. The increase in students at the Excellent level reinforces the effectiveness of active methodologies in veterinary education, enhancing students' understanding and refining their knowledge. Future studies may explore knowledge retention and its practical application in disease prevention.

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Keywords: Higher Education, Vector-borne Diseases, Zoonoses.

EXPERIENCE OF UNIVERSITY EXTENSION CURRICULARIZATION IN THE PREVENTION OF GIARDIASIS IN A RURAL COMMUNITY OF BAHIA

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University extension strengthens the relationship between university and community by providing a space for dialogue and shared knowledge. This study aims to report experiences of undergraduate students in carrying out extension activities in a rural community in municipality of Santo Estevão, Bahia. These activities are part of the activities of a curricular component, with support from the Parasitological Studies Extension Program of UEFS, which defines the parasite profile and promotes health education actions in the community. The methodology consisted of presenting the Program to the community and a conversation circle on intestinal parasites. Stool samples were collected for parasitological examination at the Clinical and Parasitology Analysis Laboratory of UEFS, following the signing of informed consent forms. After the results were delivered, the positive individuals were referred to treatment at the local Health Unit. Subsequently, an intervention proposal was developed and implemented based on identified parasitic profile. A total of 66 families participated in the study, and 216 stool samples were analyzed. The overall positivity rate was 31.5%, with 5.6% for helminths and 25.0% for protozoa (including both commensal amoebas and parasites such as *Giardia duodenalis*. These results guided the intervention proposal, which consisted of holding a dialogue lecture, presenting a video on Giardiasis, and producing informative material on transmission mechanisms and prevention measures. The importance of treated water was emphasized, and a practical demonstration was given on the care needed when using a clay filter. By developing these activities, students demonstrated technical-scientific knowledge obtained in the classroom and applied in practice, in a community. We conclude that the carried-out extension activities strengthen the relationship between the university and the community and contribute to the prevention of parasitic diseases, such as giardiasis.

Funding Source: PROEX/UEFS
Keywords: Extension curricularization; Health education; Parasitic diseases.

THE IMPORTANCE OF ACADEMIC MONITORING IN PARASITOLOGY – EXPERIENCE REPORT

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Academic monitoring is one of the various programs present in universities. The monitor plays a role that spans different levels, from supporting the professor in teaching activities, deepening theoretical knowledge of course content, and developing methodological teaching strategies, which help establish connections among students. In addition to these standard activities, the monitor can also contribute to the preparation of materials for practical classes. Thus, this study aims to describe the experiences gained during the monitoring of the Veterinary Parasitology course at the Federal University of Bahia (UFBA), with emphasis on the preparation of *Sarcoptes scabiei* mite slides. In general, the monitoring experience proved to be important, as it revealed the difficulties students face in Veterinary Parasitology due to the large number of concepts and the high level of detail in its content. The experience also allowed the monitor to deepen their knowledge of parasitology. The slide preparation activity involved selecting *Sarcoptes scabiei* mites from the collection of the Veterinary Parasitology Laboratory, located at the Institute of Health Sciences – UFBA. The mites were selected using a stereoscopic microscope (magnifying glass) with the aid of a scalpel and mounted on microscope slides using Hoyer's medium (Flechtmann, 1975). After assembly, the slides were placed in an incubator at 60°C for up to three days for medium drying, clarification, and mite expansion. A total of 100 slides were prepared. This report concludes that academic monitoring provides significant benefits for the professional development of students engaged in parasitology laboratory practices. Furthermore, these monitoring activities encourage students to actively seek specific knowledge in their field, facilitating their learning process.

Keywords: monitoring, mites, *Sarcoptes*

GASTROINTESTINAL PARASITES OF PIGS AND THEIR PRODUCERS: ASSOCIATING SCIENTIFIC RESEARCH AND RURAL EXTENSION

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Brazil stands out in pig production, with properties characterized as industrial and family-run. In these properties, parasitic diseases represent obstacles to production. In addition, there are zoonotic parasites, with pigs, such as *Balantioides coli*, being the main reservoir. This study aimed to estimate the prevalence and risk factors inherent to gastrointestinal parasites in pigs and their producers, as well as to mediate information. Visits were made to 15 pig farms in cities in Rio de Janeiro and Minas Gerais. Feces were collected from 1,148 pigs and 47 from producers of these animals. Forms were applied, and extension activities were conducted. These fecal samples were subjected to direct examination, sedimentation, flotation, FLOTAC techniques and molecular tools for research on *B. coli*. Parasites were detected in 69.9% of the pigs, with emphasis on Grupo Ciliophora (50%), coccidia (37.3%), *Trichuris suis* (19.7%) and strongyles (19%). Microscopy detected 2 individuals with hookworms and 1 with *Blastocystis* spp.. The circulation of *B. coli* in feces of pigs from all properties and in 12 rural producers was molecularly confirmed. Statistical differences were obtained when comparing the helminth egg count values between the types of properties. Several factors were associated with the frequency of parasites in pigs, such as the arrangement of drinkers on the floors of the stalls and the type of antiparasitic provided to the animals. Among the extension activities, the following stood out: “Happy Pig and Sad Pig”, a dynamic of self-recognition of animal management and “Correction of homework” to remember the mediated information. The results generated highlighted the need for improvements in national production, including the creation of programs that provide assistance and training for producers to invest in the control of these parasites, valuing animal welfare and producer health.

Keywords: Diagnosis, Prevalence, Zoonosis

Supported By: Capes, CNPq, FAPERJ

PARASITIC DISEASES AND HEALTH EDUCATION ACTIONS IN A RURAL SCHOOL IN BAHIA: PROMOTING KNOWLEDGE AND PREVENTING PARASITIC INFECTIONS

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Parasitic diseases have a significant impact on growth and development, especially among children, interfering with school performance, and they also affect adults, influencing work activities and social interactions. In this context, children become vulnerable due to a lack of knowledge about basic hygiene principles and the forms of transmission of parasites. Therefore, educators play a fundamental role in promoting knowledge about parasitic diseases and addressing this issue in schools is essential to prevent new cases. The aim of this study was to conduct a parasitological survey in the community of Colégio Estadual do Campo Maria Quitéria in Feira de Santana, Bahia, and based on the results, implement educational actions to promote health. The methodology included parasitological examinations of feces, delivery of results, referrals of positive cases for treatment at the local health unit and carrying out health education activities. Participants signed the Free and Informed Consent Forms (CAAE 58923722.8.0000.005). The survey identified 21.7% of samples positive (parasites and commensals). Among the commensals, *Entamoeba coli, Iodamoeba butschlii* and *Endolimax nana*; among the parasites, *Giardia duodenalis*. These findings guided activities in elementary school II and high school classes, with lectures, board games and practical microscopy classes. An illustrated booklet was created with information, word searches and interesting facts about the parasites studied. To enhance learning, students produced posters, and these were presented at the 8th UEFS Graduation Fair. These approaches proved to be relevant in stimulating student protagonism and participation, contributing to the perception and awareness of measures to prevent and control parasites. These strategies reinforced the importance of health education for learning and improving the community's quality of life.

Funding source: PROEX/UEFS
Keywords: Parasitic diseases; Parasitological survey; Health education.

SURVEY AND CHARACTERIZATION OF CASES OF HUMAN TEGUMENTARY LEISHMANIASIS: HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL ANALYSIS

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Cutaneous leishmaniasis (TL) is a dermatological infection caused by the multiplication of amastigote forms of *Leishmania* in cells of the mononuclear phagocytic system, affecting the skin and mucous membranes, usually with a benign evolution. In this sense, early and accurate diagnosis is crucial, but it faces challenges, such as low sensitivity, high cost and requirement of advanced infrastructure. Therefore, this study aims to characterize and analyze the cases of TL diagnosed in the Neumann anatomopathological laboratory between 2019 and 2024, as well as to evaluate the relevance of the immunohistochemistry technique in the diagnosis. Of the 57 cases analyzed, approximately 37 (64.9%) were men and 20 (35%) were women, with approximately 57.89% coming from private health insurance and 42.1% from the public service. The predominant age group was 41 to 60 years, with a total of 20 patients, 16 (80%) male and 4 (20%) female. The year 2021 had the highest number of notifications in the reference laboratory. Among the clinical findings described, a spectrum of chronic and ulcerated skin lesions were found, often confused in the clinic with leprosy and other skin alterations. This demonstrates the importance of histopathological analysis in the differential diagnosis, even in endemic areas. Histopathology and immunohistochemistry are essential for a more accurate diagnosis and targeted treatment, allowing for more assertive clinical and treatment conduct.

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Ethics Committee: 4.563.832, Universidade Federal do Oeste da Bahia, Barreiras-BA

Keywords: Cutaneous leishmaniasis, immunohistochemistry, histopathology

Monitoring waterborne pathogenic protozoa in Conceição Lagoon, Florianópolis-SC, Brazil: Implications for recreational water quality and public health

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Conceição Lagoon in Florianópolis, SC, has suffered environmental degradation due to unplanned urban growth, intense tourism, wastewater discharge, and accidents, compromising marine life as well as food and water security. Pathogenic protozoa such as *Giardia duodenalis*, *Cryptosporidium* spp., *Toxoplasma gondii*, and *Blastocystis* sp. exhibit high environmental resistance and a strong potential for transmission in recreational waters. Additionally, pathogenic free-living amoebae (FLA), including *Acanthamoeba* and *Naegleria*, pose further public health risks as agents of severe diseases and carriers/reservoirs of pathogenic microorganisms. This study assessed the lagoon's recreational suitability and environmental quality through comprehensive pathogen monitoring. Bimonthly surface water samples were collected from February 2023 to February 2024 at eight georeferenced sites and analyzed for physicochemical parameters. FLA were isolated by culture, followed by morphological and genetic characterization. *Giardia*, *Cryptosporidium*, and *T. gondii* were detected by immunofluorescence and *nested*-PCR, while *Blastocystis* was identified by *nested*-PCR. Of the 56 samples, all tested positive for at least one pathogen, with prevalence rates of *Blastocystis* (85.4%), *Giardia* (55.4%), *T. gondii* (23.2%), and *Cryptosporidium* (12.5%). Among the amoebae, 25 isolates were obtained: 52% were *Acanthamoeba*, and one isolate exhibited exflagellation, suggesting *Naegleria*. Areas with higher urbanization showed elevated contamination, highlighting the impact of human activities on pathogen spread. These findings emphasize the need for environmental surveillance and targeted interventions to improve recreational water quality and reduce the risk of diarrheal outbreaks and severe infections. In conclusion, integrated monitoring is vital for situational diagnosis and effective public health management, guiding mitigation and conservation policies for sustainable resource use.

Supported By FAPESC and CAPES

Key words Conceição Lagoon, Pathogenic protozoa, Free-living amoebae (FLA).

OCCURRENCE OF INTESTINAL PARASITES IN PRESCHOOL-AGED CHILDREN IN MUNICIPAL EARLY CHILDHOOD EDUCATION CENTERS IN NATAL-RN

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Intestinal parasites represent a serious public health problem, especially in preschool-aged children, due to their impact on physical and cognitive development. This study aimed to identify the frequency of intestinal parasites in children aged 1 to 6 years enrolled in Municipal Early Childhood Education Centers (CMEIs) in Natal-RN, during the years 2022-2023. Stool samples, subungual content, and perianal region imprints were collected from 81 children, with 44 in the first year and 37 in the second. Parasitological analyses were performed using the main methods. In the first study (2022), 61% of the children were parasitized, with a remarkable presence of *Ascaris lumbricoides* (44%), followed by Ancylostomatidae (33%) and *Entamoeba coli* (26%). Additionally, 40% of the children exhibited biparasitism or polyparasitism, with the most frequent association between *A. lumbricoides* and *E. coli.* In the second study (2023), 21% of the children were positive for parasites, with a predominance of *E. coli* (18.9%), followed by *A. lumbricoides* (8.1%) and *Entamoeba histolytica/dispar* (2.7%).Biparasitism was observed in 62.5% of the positive samples, with the most common association between *A. lumbricoides* and *E. coli*. The higher prevalence of helminths in the first study may be related to the socioeconomic and basic sanitation conditions in the northern region of Natal, while the predominance of protozoa in the second study may reflect better living conditions in the southern region. The results highlight the importance of continuous epidemiological studies to understand the dynamics of parasitic infections in preschool children, as well as the need for prevention, diagnosis, and treatment actions to reduce the impacts of these infections on child development. The high frequency of biparasitism underscores the complexity of treatment and the need for integrated public health interventions.

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Keywords: helminths, protozoa, socioeconomic disparity.

EPIDEMIOLOGIC FEATURES OF PATIENTS WITH CHRONIC CHAGAS DISEASE IN BARREIRAS-BA: ANALYSIS OF FILES REGISTERED IN THE E-SUS NOTIFICATION SYSTEM.

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The Chagas disease (ChD) is an anthropozoonosis of high prevalence, morbidity and mortality. It's considered a neglected tropical disease and, as a resut, a global public health problem. Prior to 2023 there was no mandatory notification of ChD, with the compulsory notification being established by the Ministry of Health only in January of that year, leading to an important lack of information on the epidemiology of chronic ChD patients. This research aims to analyze the clinical and epidemiological features of patients notified through the standard notification form established by the Ministry of Health, with the information being filed in the "E-sus Notifica" system between the years 2023–2024 in the city of Barreiras-BA. The analyses were performed using the quantitative cross-sectional method with an exploratory approach. According to data consolidation from notifications for chronic Chagas disease, between January 2023 and November 2024, 690 patients were registered, of which 489 (70.86%) were female and 201 (29.13%) were male. Regarding the race-ethnicity variable, 42 individuals (6.08%) were described as East Asian, 114 (16.53%) as white, 377 (54.64%) as mixed, 149 (21.59%) as black and 08 records (1.16%) did not include this information. There was no mention of individuals living in traditional indigenous communities or quilombos. Regarding age, notifications of chronic cases varied in individuals from 14 to 92 years, with a mean age of 54.9 years and a median of 54 years, with most cases in the age group of 50 to 59 years (N=188, 40.35). Therefore, carrying out additional investigations on the prevalence of Chagas disease in the chronic phase is crucial to promoting the development of public policies aimed at the prevention and control of this disease.

Keywords: Chagas disease. Epidemiology. Prevalence. Health profile.

SANITARY QUALITY OF FRESH FRUITS: STANDARDIZATION OF A METHOD FOR THE MONITORING AND DETECTION OF HELMINTH EGGS IN ORGANIC STRAWBERRIES

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Parasitic diseases acquired by the ingestion of fresh fruits are frequently neglected. Strawberry is the most consumed berry in Brazil, and it is often consumed raw or with inadequate sanitation methods that may fail to remove parasites. The main goal of the present study was to develop a methodology for the detection of helminth eggs in strawberries, for further evaluation of this fruit sold in open markets in Curitiba, PR. For this, artificial contamination assays were performed with two known doses of *Toxocara canis* eggs (33 and 103) and *Ascaris suum* eggs (71 and 139). To estimate the sensitivity recovery rate, 6 protocols per model / dose were analyzed in triplicate, with 72 experiments in total. The contaminated strawberries were subjected to two washing procedures (manual agitation and brushing), and different variables were analyzed: dissociation solution (Glycine 1M, Alconox® 0.1%, or Tween® 20 0.1%) and solution temperature (room temperature or heated at 37 °C). All protocols included a common step of sedimentation of the liquid from the two washing procedures separately, followed by centrifugation and microscopic examination of the entire pellets obtained. After standardization, a total of 70 units (± 940 g) of organic strawberries were obtained and divided into 16 pools for analysis. The results showed that regardless of the solution, the best recovery rates was attained when heated solutions were used. The Protocol 3 (heated Alconox® solution) yielded the best recovery rates for both types of eggs, being elected to search for helminths contamination at commercialization stage, where 6,25% of pool harbored *Ascaris* sp., eggs. This standardization may contribute to the sanitary surveillance sector, since there is no ISO for the detection of helminth eggs in berries. The assessment of contamination at the marketing stage further strengthens the standardized method highlighting the potential risk of acquiring parasites through the ingestion of this fruit.

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Keywords: *Fragaria*; *Ascaris suum*; *Toxocara canis*.

NEGLECTED WATERBORNE AND FOODBORNE PARASITES: EVALUATION OF SANITARY QUALITY OF ALTERNATIVE WATER SUPPLY SOLUTIONS AND FRESH VEGETABLES DESTINED TO SCHOOL-AGE CHILDREN IN A LOW-INCOME RURAL AREA

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Children constitute a specific vulnerable group to the acquisition of parasitic infection by food or waterborne routes. This study aimed to investigate the sanitary quality concerning parasitological contamination of alternative water supply solutions (AWSS) and raw vegetables used for child feeding in a rural area of the metropolitan region of Curitiba, with a low score within the Sustainable Development Goals. A cooperative of rural producers provided 140 samples of leafy vegetables, such as lettuce, red and green cabbage, escarole, parsley and chives for the analysis of helminths and protozoa. For this, 70 samples (30g each) were washed with glycine solution (1M), decanted, centrifuged, and all the sediment was examined under microscopy for the research of helminth eggs. Another 70 samples, each with three 30g replicates (90g/sample), were washed with 250mL of glycine, resulting in 750mL/sample. The eluate was concentrated by membrane filtration followed by elution and centrifugation, and the sediment was used for DNA extraction and PCR or qPCR analysis to detect *Cryptosporidium* spp. (*18S*), *Giardia* spp. (*bg*), and *Toxoplasma gondii* (*REP-529*). For AWSS, 20L of water from 12 sites (8 wells and 4 springs) were collected. The samples were filtered through a membrane and sediment analyzed using the aforementioned molecular methods. Direct immunofluorescence assay was also performed to detected (oo)cysts. Contamination by helminth eggs or larvae was identified in 41.4% of vegetables: hookworm and *Toxocara* sp., were detected more frequently. Pathogenic protozoa were identified in 16 vegetables samples: 47.5% harbored *Giardia*, 43.7% *Cryptosporidium* and 6.25% both. *Cryptosporidium* spp. were detected in 8.33% of AWSS. The results indicate widespread circulation of enteroparasites in vegetables and water used in meals for pediatric populations, highlighting the need to adopt control, management and sanitation measures to safeguard children's health.

Supported by: FEDERAL UNIVERSITY OF PARANÁ (UFPR).

Keywords: Childhood Nutrition; Protozoa; Helminths.

EPIDEMIOLOGICAL ASPECTS OF HOSPITALIZED PATIENTS DUE TO VISCERAL LEISHMANIASIS IN SOBRAL, CEARÁ, BRAZIL

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The SUS Hospital Information System (SIH/SUS) is essential to the epidemiologic surveillance of visceral leishmaniasis, as it provides refined data regarding aspects such as hospitalization, hospitalization costs, mortality rates, and spatial distribution of a certain disease. We aimed to evaluate the epidemiological profile of patients hospitalized due to visceral leishmaniasis, from 2014 to 2023, in Sobral/Ceará. This study is a cross-sectional, retrospective, and descriptive analysis of secondary data from the Department of Information Technology of Unified Health System (DATASUS), specifically in the SIH/SUS. We selected the section “Epidemiology and Morbidity”, and the sub-section “Hospital Morbidity” and applied the filter “General, by the city of hospitalization from 2008”. All data was restricted to the municipality of Sobral in the TabNet tool. We evaluated sex, age, race, hospital admissions per year, and mortality rate. Sobral registered 515 cases of human visceral leishmaniasis, 265 hospital admissions, and 15 deaths as an outcome of the disease. Among the hospitalized patients, 60% were male and 34,3% were between 1 and 4 years old. The hospitalization rate was 130,52/100000 inhabitants, and the average time patients stayed at the hospital was 18,7 days; the average cost per patient hospitalized was 904,79 reais. Visceral leishmaniasis is an endemic disease in Sobral, and we observed an upward trend in the number of confirmed cases, especially from 2020 to 2023. Considering the data retrieved from SIH/SUS, health authorities can decide the better use of financial resources, especially for drug supply and patient management at public health facilities. Therefore, an efficient surveillance system is essential for the early detection of visceral leishmaniasis cases, allowing a fast response and contributing to reducing hospitalizations and mortality rates by visceral leishmaniasis.

Financial support: not applicable.

Keywords: Visceral leishmaniasis; epidemiological aspects; prevention.

CANINE LEPTOSPIROSIS AND ZOONOTIC TRANSMISSION: CASE REPORT OF HUMAN INFECTION DURING PROFESSIONAL ACTIVITY

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Abstract

Leptospirosis is a zoonosis of major public health concern, with the potential to significantly impact both human and animal health. *Leptospira* spp. affect various animal species, including rodents, which are the main urban reservoirs, as these bacteria are continuously excreted in the urine of these hosts. However, dogs can also be carriers of the disease, and in close contact with humans, they may contribute to disease transmission. Transmission occurs through direct contact with animals or contaminated water and soil. Symptoms range from asymptomatic to severe systemic manifestations. Factors such as seasonal variation and a lack of information on diagnosis and treatment increase the risks and complications of the disease. This study presents a case of fatal canine leptospirosis and occupational zoonotic transmission. A 35-year-old immunocompetent veterinarian treated a one-year-old male mixed-breed dog, which had an up-to-date vaccination protocol, and presented with anuria, jaundice, prostration, and abdominal distension three days after ingesting a rat. The animal was hospitalized, and laboratory tests revealed elevated levels of urea, creatinine, aspartate aminotransferase, and alanine aminotransferase. The dog succumbed 72 hours after treatment, and serological testing confirmed the presence of two Leptospira serovars: *L. copenhageni* and *L. castellonis*. In this context, seven days after the consultation, the veterinarian underwent serological testing for leptospirosis, which returned positive for *L. shermani*. On the twelfth day, the veterinarian developed nausea, arthralgia, myalgia, and fever. He was hospitalized, and additional tests confirmed leptospirosis. Treatment with ceftriaxone was effective, resulting in a favorable clinical outcome. This case highlights the importance of prophylaxis and early diagnosis for the prevention or favorable progression of leptospirosis, alerting animal health professionals to the risks of zoonotic transmission, especially for those working in high-exposure environments.

Supported by: CNPq, Newton Paiva Wyden.

Keywords: risk factors, occupational leptospirosis, dog.

Development of an application for notification and spatial analysis of positive cases of Canine Visceral Leishmaniasis in the municipality of Araçatuba, state of São Paulo, Brazil

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The city of Araçatuba, São Paulo, is endemic for Visceral Leishmaniasis (VL), which affects mainly dogs and humans. Despite being a mandatory notifiable disease, it is suspected that there is an underreporting of cases in dogs to the Zoonosis Surveillance Unit by veterinarians, which makes it difficult to understand the epidemiological situation of Canine Visceral Leishmaniasis (CVL). Therefore, the objective of this project was to create an application (app) for these professionals to notify this disease in the city of Araçatuba and, thus, compare the occurrence of CVL, before and after the development and validation of this technological tool. The mobile app was developed using the Scrum methodology for software development and is compatible with Android and iOS devices. In addition to notification, this app also allows the collection of data on the geographic distribution of positive cases, generating a map of canine positivity for this disease. Furthermore, the diagnostic techniques used by professionals will be verified, as well as the presence of clinical signs observed in dogs. After conducting continuing education workshops for veterinarians from public and private institutions in Araçatuba, with the production of educational material, the application will be validated within a period of one year. Thus, the intention of this research will be to incorporate the use of the application into the city's zoonosis notification system and, based on the results obtained, to elucidate the epidemiological panorama, which may lead to the creation of public policies to control and combat LVC.

Supported by: Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)

Keywords: *Leishmania* spp., Epidemiological Surveillance, technological tool

Wild boars as a reservoir of parasites with zoonotic potential in the state of São Paulo, Brazil

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In this study, we investigated the occurrence of parasites with zoonotic potential in slaughtered wild boars in the northwest region of the state of São Paulo, Brazil. A total of 40 animals of different sexes and ages were examined. To investigate enteroparasites, fecal samples were collected directly from the rectal ampulla of all animals and submitted to coproparasitological techniques (Willis–Mollay, Faust and Hoffman Pons and Janer) and an aliquot was subjected to extraction of genetic material for molecular detection of *Cryptosporidium* spp. and *Giardia* spp. by nested PCR. Fragments of liver, spleen, heart, tongue and lymph nodes of the 40 animals were subjected to genomic DNA extraction and subsequently nested PCR and conventional PCR were performed for molecular detection of *Toxoplasma gondii* and *Leishmania infantum*, respectively. The epidermal region of 30 wild boars was also analyzed for ectoparasites. In our study, we observed an occurrence of 100% (40/40) and 43.33% (13/30) of endo and ectoparasites, respectively, with the identification of eggs of *Metastrongylus* spp. (15% [6/40]), *Strongyloides ransomi* (25% [10/40]), *Trichuris suis* (7.5% [3/40]) and nematodes of the order Strongylida (100% [40/40]), as well as oocysts of protozoan Eimeriidae (50% [20/40]). Infestations by ectoparasites were mainly by ixodids of the genus *Amblyomma* spp. (43.33% [13/30]) and by *Cochliomyia hominivorax* larvae (10% [3/30]). Regarding molecular detection, we identified *T. gondii*, *L. infantum* and *Cryptosporidium scrofarum*, respectively, in eight (20%), four (10%) and three (7.5%) of the 40 wild boars. Thus, several agents with zoonotic potential were identified in this study, such as *T. gondii*, *L. infantum*, *C. scrofarum* and infestations by *Amblyomma* spp. and *C. hominivorax*, agents with relevance in Public Health.

Palavras-chave: helminths, protozoosis, suids.

PARASITO2025
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RISK ASSESSMENT FOR A VISCERAL LEISHMANIASIS OUTBREAK IN BIGUAÇU (SANTA CATARINA, BRAZIL)

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Abstract

Leishmaniasis is a neglected tropical disease caused by protozoa of the genus *Leishmania*, transmitted by infected female sandflies. Santa Catarina (SC) was the last state in Brazil to report autochthonous cases of Visceral Leishmaniasis (VL), with the first case of Canine Visceral Leishmaniasis (CVL) in Florianópolis in 2010. To date, it remains the only municipality in the State with autochthonous cases in dogs and humans. The main vector of *Leishmania infantum*, *Lutzomyia longipalpis*, has not been detected in SC. However, there is limited data on sandfly fauna and CVL cases in the Metropolitan Region of Florianópolis. This study aims to identify the sandfly fauna of the Municipality of Biguaçu (SC, Brazil), a neighboring municipality of Florianópolis, and assess the natural infection by *L. infantum*, while also investigating CVL occurrence using non-invasive canine conjunctival swab (CS) samples. Twelve monthly sandfly collections were carried out with CDC light traps in three locations of the municipality. The presence of *L. infantum* was assessed by conventional PCR using primers designed to target kDNA and tested for specificity using *L. infantum* and *L. braziliensis* DNA. Additionally, sensitivity was tested using a 10-fold dilution of *L. infantum* DNA. CS were collected from 217 dogs at Departamento de Bem-Estar Animal (DIBEA) in Biguaçu. In total, 367 sandfly specimens were captured. Among the ten species identified, three have potential vector capacity, *Migonemyia migonei*, *Nyssomyia neivai,* and *Pintomyia fischeri*, and it is the first record of *Psychodopygus geniculatus* in SC. PCR assay detected only *L. infantum*, with concentrations up to 0.1 picogram. One dog from DIBEA tested positive, and *L. infantum* infection was confirmed by DNA sequencing. Until now, 50 female sandflies have been analyzed by PCR, with none testing positive. These findings indicate a potential VL outbreak risk in Biguaçu, highlighting the need for continuous surveillance and control measures.

Supported by: CAPES, IOC/Fiocruz, CNPq, INCT-EM

Keywords: PCR, sandfly, canine visceral leishmaniasis

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SCHOOL VISITS TO THE MICROBIOLOGY AND PARASITOLOGY LABORATORIES AT THE FEDERAL UNIVERSITY OF UBERLÂNDIA AS A STRATEGY FOR PROMOTING HEALTH EDUCATION

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Abstract

Parasitic and infectious diseases are topics related to Health Education, taught in Basic Education within Science and Biology curricula. For effective learning, it is essential to associate theoretical content with practical activities. However, many schools unfortunately lack the infrastructure for practical lessons. Our project aims to bridge this gap and democratize access to Microbiology and Parasitology laboratories through guided visits focused on promoting Health Education. In 2023/2024, seven visits were conducted, involving 160 students and 10 teachers aged between 12 and 60. The guided tours included explanations and presentations of parasites (*Ascaris lumbricoides*, *Enterobius vermicularis*, *Taenia solium*, *Taenia saginata*, *Schistosoma mansoni*, *Trypanosoma cruzi*, *Giardia duodenalis*, *Pediculus humanus*, *Pthirus pubis*, *Rhipicephalus sanguineus*, *Tunga penetrans*, *Aedes aegypti*, *Triatoma infestans*, and various flies), bacteria (*Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* spp.), and fungi (*Aspergillus* spp. and *Candida albicans*) under a microscope. Parasites preserved in formalin, insect collections, and 3D models of bacteria and fungi were also observed. At the end of the visits, a discussion session was held, followed by a questionnaire. The results indicated that 55.9% of the students had previously participated in similar activities, while 44.9% had not; 47.4% reported having practical Science/Biology classes, while 52.5% did not. Moreover, 93.2% acknowledged knowing that parasites are harmful to health, while 6.7% did not. Regarding student perceptions of the activity, 68.6% rated it as "loved it," 29.6% as "liked it," 0.84% as "somewhat liked it," and 0% as "disliked it." In conclusion, the project contributed to democratizing access to knowledge and the university, fostering students' interest in the covered topics and encouraging them to become knowledge multipliers, thereby contributing to a healthier society.

Supported by: UFU

Keywords: university extension, dialogic interaction, democratization of knowledge.

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VISCERAL LEISHMANIASIS IN BRAZIL FROM 2007 TO 2022: ECOLOGICAL TIME SERIES ASSESSMENT AND SPATIAL MAPPING OF EPIDEMIOLOGICAL AND OPERATIONAL INDICATORS

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Abstract

Visceral leishmaniasis (VL) is a neglected zoonotic disease caused by protozoa of the genus *Leishmania* that are transmitted to humans by sand flie (*Psychodidae* family) bites. If left untreated, VL can be fatal in approximately 90% of cases, with clinical manifestations including fever, hepatosplenomegaly, and weight loss. The disease is endemic in over 70 countries, with Brazil among the most affected nations. This study aims to describe the epidemiological profile of VL cases reported in Brazil from 2007 to 2022 using data from the National System for Notifiable Diseases (SINAN). We conducted an ecological time-series analysis, calculating incidence and case-fatality rates across different regions. The study also examined sociodemographic and clinical characteristics, including diagnostic criteria, HIV coinfection, and treatment outcomes. Trends were analyzed using linear regression models to determine annual percentage variations. The incidence rate of visceral leishmaniasis per 100,000 population over time, with a relatively stable trend until 2018, followed by a sharp decline from 2019 onwards. The lethality rate of VL, with a general increase until around 2018, followed by a sharp decline from 2019 onwards. The highest number of VL cases in Brazil (2000–2022) occurred in the central and northeastern regions. The Local Moran's I analysis identifies clusters of high and low incidence of visceral leishmaniasis in Brazil, with high-risk areas in central and northeastern states. Our findings provide a comprehensive assessment of VL in Brazil, highlighting regional disparities and contributing to improved surveillance and control strategies.

Supported by: Coordination for the Improvement of Higher Education Personnel (CAPES); National Council for Scientific Research and Development (CNPq), Fundação de Apoio a Fiocruz (FIOTEC)

Keywords: Leishmaniasis; Visceral leishmaniasis; Epidemiological.

TEACHING OF MEDICAL PARASITOLOGY IN BRAZIL: PROFILE OF MEDICAL SCHOOLS THAT INCLUDE THE SUBJECT IN THEIR CURRICULA

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Abstract

The objective of this study was to map the teaching of Parasitology in medical schools in Brazil. This descriptive study analyzed secondary data from the web data using the descriptors “medical schools, parasitology, curriculum, Brazil”. Of the 447 mapped medical schools, 32.2% were public institutions, 36% were located in the Southeast region, and 9.1% in the North. Among the 201 curricula accessed, 53.7% included the Parasitology course, with 36.1% in public institutions and 63.8% in private ones. Additionally, 58.3% of the institutions offered the course within a single academic term. Teaching methodology, 38.5% delivered the content through theoretical lectures and 19.4% with theoretical-practical approach. Information on the class workload was available for 65 institutions, with a median of 102 class hours and a mode of 80 class hours; for practical lessons, both the median and mode were 40 hours. This study provided insight into how Parasitology is approached in Brazilian medical schools. A greater number of private institutions were observed, with concentrations in the Southeast and South regions of the country. It was found that 53.7% of the analyzed schools offered the Parasitology course; however, workload and teaching methodologies varied significantly. Teaching Parasitology in medical education effectively contributes to combating parasitic diseases by equipping future doctors with the ability to diagnose early, treat, and understand risk factors, transmission mechanisms, and clinical manifestations of parasitic diseases. This study highlighted the need for standardizing both the teaching methodology and workload of Parasitology courses in medical education, as well as the need for curricular revisions to strengthen this subject in Brazilian medical programs. Given the impact of parasitic diseases as a public health issue, such improvements are essential.

Supported by: Secretariat of Health and Environment, Ministry of Health

Keywords: Parasitology, Medicine, Brazil

AVALIAÇÃO DE INTERVENÇÃO EDUCATIVA NO CONHECIMENTO DE TUTORES DE CÃES SOBRE A LEISHMANIOSE VISCERAL EM IGARITÉ, MUNICÍPIO DE BARRA, BAHIA

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A leishmaniose visceral (LV) é uma doença de ocorrência mundial que quando não tratada, pode levar a óbito em até 90% dos casos. O presente estudo foi desenvolvido na Comunidade Quilombola Igarité, município de Barra, Bahia, onde há confirmação da presença do vetor e de casos positivos de LV em humanos e cães. O objetivo foi avaliar o nível de conhecimento da população de tutores de cães antes e após uma intervenção educativa. Foi avaliado o conhecimento prévio dos tutores de cães sobre a LV canina e humana através da aplicação de questionário epidemiológico, logo após foi realizada uma intervenção educativa utilizando comparativamente um vídeo e um folder, após sete dias, o questionário foi reaplicado com o objetivo de avaliar o impacto da intervenção. A análise estatística foi efetuada pelo teste de McNemar, adotando um nível de significância de $p<0,05$. Houve maior participação (77,8%) de mulheres, 79,4% dos entrevistados relataram receber até um salário mínimo e a maioria (48,1%) não concluíram o ensino básico. Foi verificado que havia uma carência de informação dos entrevistados sobre o conceito de zoonose, transmissão, sintomas e vetor da LV. A intervenção educativa promovida pelo trabalho melhorou o conhecimento dos tutores visto que houve um aumento de 37,1% (grupo folder) e 59,2% (grupo vídeo) entre tutores que acertaram mais da metade das questões após a realização da intervenção educativa, porém essa diferença não foi estatisticamente significativa. Essa doença atinge as regiões mais pobres e pessoas com baixo nível de escolaridade têm mais chances de ser infectadas, por isso, há a necessidade de democratização do conhecimento entre a população em condições socioeconômicas vulneráveis. Além disso, o uso de ferramentas como o vídeo e folder podem ser usados para aumentar o conhecimento da população e consequentemente maior prevenção e controle da LV. Mais estudos devem ser realizados para determinar o impacto dessa intervenção a médio e longo prazo.

Palavras chave: Leishmaniose, Educação em Saúde, Zoonoses.

EVALUATION OF EDUCATIONAL INTERVENTION ON DOG OWNERS' KNOWLEDGE ABOUT VISCERAL LEISHMANIASIS IN IGARITÉ, MUNICIPALITY OF BARRA, BAHIA

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Visceral leishmaniasis (VL) is a worldwide disease that, when left untreated, can lead to death in up to 90% of cases. The present study was conducted in the Quilombola Community of Igarité, municipality of Barra, Bahia, where the presence of the vector and positive cases of VL in humans and dogs have been confirmed. The objective was to assess the level of knowledge among dog owners before and after an educational intervention. The prior knowledge of dog owners about canine and human VL was assessed through the application of an epidemiological questionnaire. Immediately after, an educational intervention was conducted using a video and a folder for comparison. After seven days, the questionnaire was reapplied to evaluate the impact of the intervention. Statistical analysis was performed using the McNemar test, adopting a significance level of $p<0.05$. There was a higher participation rate (77.8%) of women, 79.4% of respondents reported earning up to one minimum wage, and the majority (48.1%) had not completed basic education. It was found that there was a lack of information among respondents regarding the concept of zoonosis, transmission, symptoms, and VL vector. The educational intervention improved dog owners' knowledge, as there was an increase of 37.1% (folder group) and 59.2% (video group) in the number of owners who answered more than half of the questions correctly after the intervention. However, this difference was not statistically significant. This disease affects poorer regions, and individuals with a low level of education are more likely to be infected, highlighting the need to democratize knowledge among socioeconomically vulnerable populations. Additionally, tools such as videos and folders can be used to increase public knowledge and, consequently, enhance VL prevention and control. Further studies should be conducted to determine the medium- and long-term impact of this intervention.

Keywords: Leishmaniasis, Health Education, Zoonoses.

Comparative Analysis of Congenital Toxoplasmosis Notifications in the Northeast Region Between 2020 and 2024

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Toxoplasmosis, a widespread zoonosis, is caused by Toxoplasma gondii. Congenital Toxoplasmosis (CT), acquired during pregnancy or reactivated in immunosuppressed individuals, leads to ocular and neurological damage or fetal death. In Brazil, its impact is linked to socioeconomic inequalities, poor sanitation, and limited healthcare access. This study examines CT distribution in Brazil, particularly in Bahia, assessing epidemiological data, confirmation rates, and sociodemographics.
A retrospective longitudinal study analyzed SINAN/SUS data (2020–2024). Cases were grouped by macro-region, focusing on the Northeast and Bahia. Microsoft® Excel® (2019) was used. From 2020 to 2024, 23,193 CT cases were reported: North (2,173), Northeast (6,722), Southeast (7,822), South (3,902), and Central-West (2,574). In the Northeast,
Pernambuco (1,325), Ceará (1,166), and Bahia (1,126) had the most cases. In Salvador, cases increased from 2020 to 2022, then declined in 2023.
Of the cases, 74.91% were in mixed-race individuals, followed by white (6.81%), black (6.36%), and Asian (0.54%). In Bahia, 57.17% of cases were confirmed. CT cases were concentrated in the Northeast, especially Bahia. Salvador had the highest notifications, followed by Vitória da Conquista and Ilhéus, suggesting greater surveillance. The data highlight the link between social vulnerability and incidence, revealing inequalities in diagnosis and prenatal care. The predominance in mixed-race individuals may reflect socioeconomic barriers. The lack of confirmation in some cases suggests the need for improved diagnostics and surveillance. Early detection and adequate care are essential to reducing CT morbidity and mortality.

Keywords: Congenital toxoplasmosis, Epidemiological surveillance, Health disparities

PARASITOLOGICAL SURVEILLANCE IN WHITE-FACED SPIDER MONKEYS (*ATELES MARGINATUS*) IN THE MUNICIPAL NATURAL PARK OF THE MATO GROSSO FOREST

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Abstract

Non-human primates (NHPs) inhabiting natural environments can serve as hosts for various enteroparasites with zoonotic potential, posing a significant risk to both wildlife and human health. These parasites can be transmitted between animals and humans, causing gastrointestinal infections, nutritional disorders, and even more severe diseases, especially in vulnerable populations.

Infection in these animals by protozoa and helminths may occur through the ingestion of contaminated water, the consumption of small arthropods such as cockroaches and crickets, or the accidental ingestion of soil and fruits contaminated with parasite eggs, cysts, or larvae.

In this study, fecal samples from white-faced spider monkeys (*Ateles marginatus*) were analyzed in the Municipal Natural Forest Park (PNMF) in Sinop, Mato Grosso. A total of 22 fecal samples were collected and processed using the coproparasitological techniques of spontaneous sedimentation (Hoffman) and flotation (Sheather). The results indicated that ten samples tested positive and twelve negative for intestinal parasites.

The analysis of the 10 positive fecal samples revealed the presence of intestinal protozoa and helminths with the following absolute and relative frequencies: *Blastocystis sp.* (27.2%), *Endolimax nana* (4.5%), and *Entamoeba sp.* (4.5%). Among the helminths, hookworms (13.6%) and *Toxocara sp.* (9.09%) were identified.

Additionally, polyparasitism was observed, with some samples containing more than one parasite species simultaneously. These findings reinforce the importance of parasitological surveillance in wildlife for the early detection of zoonotic pathogens. The implementation of preventive measures, combined with interdisciplinary research, contributes to biodiversity conservation and the reduction of public health threats.

Keywords: Parasitology, Zoonoses, Atelidae

One Health: SummerClass in Field Epidemiology as a Tool for Continuous Training in Geohelminth Diagnosis and Control

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[1] GRUPO DE PESQUISA EM ZOONOSES E SAÚDE PÚBLICA, UNIVERSIDADE ESTADUAL DE FEIRA DE SANTANA (UEFS), BAHIA, BRAZIL, [2] PROGRAMA DE PÓS-GRADUAÇÃO EM MODELAGEM EM CIÊNCIAS DA TERRA E DO AMBIENTE (PPGM, UEFS)

The SummerClass in Field Epidemiology aimed to promote the continuous training of professionals and students in Health and related fields through an integrated and innovative pedagogical approach. The course, divided into field and laboratory activities with data analysis between December 2024 and January 2025, combined theoretical classes, practical workshops, and debates, emphasizing active and collaborative learning. In the field phase, participants were trained to plan and carry out sample collection in two urban areas, obtaining fecal samples for the diagnosis of geohelminth infections and protozoa. Ovitraps were distributed to collect culicid eggs, especially from the *Aedes* genus, reinforcing the importance of vector surveillance for arboviruses. The participatory georeferencing platform “+Lugar” stood out as an innovative resource, enabling the precise localization of collection points and fostering collective participation in the construction of epidemiological maps. This tool, combined with the spatial analysis of collected data, allowed for a deeper interpretation of environmental and parasitological risks. The pedagogical aspect of the course was highlighted through scientific writing workshops, which equipped participants with the skills to communicate their results clearly and objectively. The adopted methodology, integrating theory and practice, facilitated the understanding of data collection, analysis, and interpretation processes, demonstrating the effectiveness of this educational approach for field learning. The results showed significant progress in participants' competencies, both in performing field epidemiology techniques and in critically analyzing data, reinforcing the importance of training that integrates interdisciplinary knowledge and collaborative practices. The SummerClass proved to be a transformative experience, preparing professionals to face the challenges of surveillance and disease control in an integrated and innovative manner.

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Keyword: Technology and Health, Integrated Education, Collaborative Learning

PARASITO2025
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KNOWLEDGE ABOUT TOXOPLASMOSIS AMONG HEALTHCARE PROFESSIONALS IN PRIMARY HEALTHCARE UNITS IN THE CITY OF RIO DE JANEIRO IN AREAS WITH OCCURRENCES OF ANIMAL TOXOPLASMOSIS

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Abstract

Toxoplasmosis is a zoonosis caused by the protozoan *Toxoplasma gondii*, with cats as its main definitive host in the urban environment. There may be shared risk factors for its transmission between humans and animals, which can be sentinels for the presence of *T. gondii* in the environment. The present study aimed to identify the knowledge of primary health care professionals about toxoplasmosis in two neighborhoods of the Rio de Janeiro city (Tijuca and Maré), where seropositivity was found in cats and dogs, laboratory-confirmed by the Municipal Institute of Sanitary Surveillance, Zoonoses Surveillance, and Agricultural Inspection of Rio de Janeiro (IVISA-RIO). For this purpose, two structured questionnaires were applied, one directed to mid-level professionals (group 1), and another to higher education professionals (group 2). The study demonstrated that higher education professionals tend to provide guidance to patients about this parasitosis in their routine, while mid-level professionals do not, for the most part, with a tendency for patient guidance to increase with the longer working time of professionals in group 1 ($p=0.0186$). Regarding the biological cycle of the parasite, 30.4% (7/23) of the physicians and nurses answered erroneously that animals release oocysts throughout their lives, and 39.1% (9/23) stated that the oocysts are infectious immediately after their release. A significant difference was observed between the responses of groups 1 and 2 concerning the possible transmission routes of *T. gondii* ($p<0.001$), regarding the animal capable of releasing oocysts in feces ($p=0.0432$), as well as the control of insects in the household environment and daily use of repellent as prophylactic measures against toxoplasmosis ($p<0.05$). Overall, both groups had partial knowledge, with a need for greater understanding of biological cycle and transmission. This highlights the necessity for health education interventions for these professionals focusing on zoonosis.

Keywords: Parasite; One Health; Primary Care

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Leishmaniases in the Municipality of Diamantina (MG): Spatial and Epidemiological Analysis from 2022 to 2024

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Abstract

Leishmaniases are endemic zoonotic parasitic diseases in Brazil, caused by *Leishmania* protozoa. They manifest as American cutaneous leishmaniasis (ACL), with cutaneous and mucosal lesions, and visceral leishmaniasis (VL), affecting internal organs such as the liver and spleen. VL also impacts dogs (*Canis familiaris*), causing a chronic, systemic disease. The prevalence of these diseases in both urban and rural areas is linked to factors like population density, environmental degradation, lack of sanitation, and vector adaptation to urbanized areas. This descriptive study aimed to analyze the epidemiological profile and georeference cases of ACL, human VL, and canine VL in Diamantina between 2022 and 2024. Secondary data from the Diamantina Municipal Health Department and the Notifiable Diseases Information System (SINAN) were examined, focusing on age range, sex, area of residence, case status, and disease progression. Georeferencing was conducted using QGIS® software. Ten cases of ACL were confirmed, with no human VL cases reported, and 292 dogs were seropositive for canine VL, yielding a prevalence rate of 12.19%. Canine serological testing is done on demand, which may lead to overreporting. ACL primarily affected individuals aged 50-59, with 80% of cases in men and 70% in rural areas. Most cases (70%) were new, and 90% recovered after treatment. These findings suggest ongoing transmission of both ACL and VL in Diamantina. Dogs, as main reservoirs, play a crucial role in maintaining the transmission cycle. Despite the rural prevalence of ACL, the presence of canine VL and ACL in urban areas highlights issues such as lack sanitation. The coexistence of ACL and canine VL, despite the lack of recent human VL cases, emphasizes the importance of continuous surveillance and integrated monitoring of these diseases. Spatial analysis identified priority areas for intervention, underscoring the need for further research on environmental and ecological factors.

Supported by: CNPq

Keywords: Leishmaniasis, Spatial analysis, epidemiology.

CO-ENDEMICITY OF SCHISTOSOMIASIS AND TEGUMENTARY LEISHMANIASIS: SPATIAL CO-CLUSTERING IN ENDEMIC AREAS

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Schistosomiasis and tegumentary leishmaniasis simultaneously affect areas in tropical and subtropical regions. Co-infected individuals show a less-than-optimal response to treatment and increased regulatory immune responses. However, no study has determined where *Schistosoma-Leishmania* co-infections are more likely to occur. To determine co-endemic areas at a local level, an ecological study was conducted on confirmed cases of American tegumentary leishmaniasis (ATL) and schistosomiasis in the State of Minas Gerais, Brazil, between 2013 and 2017. Autochthonous cases of ATL were retrieved from the Information System for Notifiable Diseases (SINAN), while cases of schistosomiasis were recovered from both SINAN and the Information System of the Schistosomiasis Surveillance and Control Program. Local Indicators of Spatial Association (LISA) analyses were used to search for co-endemic hotspots, using a queen contiguity spatial weight matrix. Univariate LISA indicated 13 municipalities as high-high clusters for both ATL and schistosomiasis in Minas Gerais. Furthermore, bivariate LISA analyses identified 61 municipalities as high-high clusters, grouped in seven co-endemic hotspots, distributed among the mesoregions of Norte de Minas, Jequitinhonha, Metropolitana de Belo Horizonte, Vale do Mucuri, Vale do Rio Doce, and Zona da Mata. LISA analyses are a useful tool for identifying areas where co-infection cases are more likely to occur. Similar analyses will assist authorities and healthcare providers when formulating policies and treating *Schistosoma-Leishmania* co-infected patients and will provide valuable data to enable researchers to explore the impact of this and other co-infections.

Supported by: CAPES, Fundep, PPSUS

Keywords: Spatial correlation, *Schistosoma*, *Leishmania*.

PREVALENCE OF CHAGAS DISEASE BEFORE, DURING AND AFTER THE COVID-19 PANDEMIC IN THE DEPARTMENT OF COCHABAMBA-BOLIVIA.

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Abstract

Chagas disease, caused by *Trypanosoma cruzi*, is widespread in Bolivia, affecting between 20% and 40% of the population, especially in rural areas. This study analyzed the seroprevalence of Chagas disease and *Triatoma infestans* infestation in three periods: before the pandemic (2019), during the pandemic (2020-2022), and after the pandemic (2023-2024). Before the pandemic, the overall prevalence was 16%, which increased to 19% during the pandemic due to interruptions in control programs, and then decreased to 15% in the post-pandemic period. By age group, in 2019, the positivity rates were 0.9% for children aged 1-4 years, 2.8% for those aged 5-14 years, and 16.6% for adults aged 15-90 years. During the pandemic, these rates increased to 3.1%, 2.9%, and 14.5%, respectively, while in the post-pandemic period, they stabilized at 2.56%, 2.57%, and 13.54%. The percentage of infected triatomines also varied, going from 40.7% before the pandemic to 42.3% during the pandemic, and then stabilizing at 40% afterward.

These changes reflect alterations in transmission dynamics linked to interruptions in control programs. The increase in young children suggests recent exposure, while the high seroprevalence in adults indicates sustained transmission. Triatomine infestation remained high, highlighting the reduction in surveillance and insecticide application.

Although no significant changes were observed, specific variations emerged. The impact of COVID-19 highlighted vulnerabilities in health infrastructure and how reduced monitoring can reverse previous progress. The post-pandemic prevalence reflects the risk of persistent transmission, particularly in rural areas. Although there has been some recovery in control strategies, challenges remain. Epidemiological surveillance, vector control, and timely access to diagnosis and treatment remain essential to mitigating the impact of the disease in Cochabamba and Bolivia.

Keywords: prevalence, Covid-19, Trypanosomiasis

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SOCIAL MEDIA IN SCIENTIFIC DISSEMINATION OF PROPHYLACTIC MEASURES FOR THE CONTROL OF PARASITIC DISEASES

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Abstract

The advancement of communication technologies has made social media an integral part of everyday life, serving both as a platform for disseminating reliable information and for spreading opinions lacking a theoretical basis. The unsupervised and inappropriate use of social media in so-called scientific dissemination can hinder efforts to combat and control parasitic diseases, representing a setback in this field. Understanding how this topic is addressed online is crucial for ensuring high-quality scientific communication. This study analyzed how social media has been used either to enhance or to undermine knowledge about personal hygiene as a prophylactic measure against parasitic diseases. To achieve this, the term “personal hygiene” was searched on Instagram and TikTok, two of the largest social media platforms. The first 20 posts with a comedic nature—known for attracting more views—were selected. Among the analyzed videos and photos, only four had an educational approach supported by scientific evidence, either presented throughout the content or in the post caption. The remaining sixteen posts were purely playful, relying on common sense opinions rather than scientific accuracy. These posts either reinforced aesthetic standards of hygiene or served as entertainment aimed at gaining views and attracting potential customers for advertised products. Additionally, some posts addressed so-called “unhygienic” behaviors, yet they lacked reliable sources to explain why these behaviors were considered incorrect. The findings highlight the need for a more critical approach to using social media as a tool for disseminating information on preventive measures against parasitic diseases. Without proper evaluation, social media content may reinforce misleading behaviors that fail to contribute to health education and promotion.

Supported by: CNPq.
Keywords: parasite, communication, public health.

TEACHER TRAINING AS A SUPPORT STRATEGY IN THE PREVENTION OF INTESTINAL PARASITIC INFECTIONS IN PRESCHOOL-AGED CHILDREN

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Preschool-aged children are prone to intestinal parasitic infections, and in this context, it is important to highlight the role of teachers in promoting and maintaining hygiene and prophylactic habits to prevent the spread of these diseases. However, teachers are not always prepared for such responsibilities. In this scenario, the extension project "Teach and Learn: Educational Actions for the Prevention of Parasitic Diseases," in its 9th edition, aimed to develop educational actions with teachers from a Municipal Center for Early Childhood Education located in the city of Natal. The project focused on the characteristics of the main parasitic diseases, their modes of transmission, and prophylactic measures. Initially, a digital questionnaire was administered to assess the teachers' prior knowledge of parasitology. Subsequently, through playful and digital resources, as well as specimens of parasites (slides or preserved samples), diseases such as schistosomiasis, taeniasis-cysticercosis complex, ascariasis, enterobiasis, and cutaneous larva migrans were presented, covering aspects such as transmission, signs and symptoms, and prophylactic measures. Finally, a discussion circle was held to address doubts and share experiences. Upon analyzing the initial questionnaires, it was found that none of the teachers knew more than 30% of the parasitic diseases presented during the activity. Although 58.8% of them had been working in early childhood education for over 5 years and 94% had completed higher education or were currently pursuing a degree, none of the teachers were familiar with enterobiasis, and only 59% had a superficial knowledge of cutaneous larva migrans and schistosomiasis. At the end of the activity, the teachers were awarded 4-hour certificates for the continuing education workshop they attended. Additionally, the educational action was evaluated by the teachers as a positive and necessary intervention.

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Keywords: knowledge in parasitology. continuing education. child health.

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ANALYSIS OF THE RATE OF HOSPITALIZATIONS FOR AMOEBIASIS IN THE STATE OF CEARÁ BETWEEN 2008 AND 2023

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Abstract

Intestinal amoebiasis is a disease caused by *Entamoeba* spp., and has a major impact on human morbidity and mortality. Its incidence is highest in tropical, subtropical and hot climate areas, where hygiene and health education conditions are often inadequate. In view of the above, the present study aimed to provide an updated statistical overview of the statewide hospitalization rate due to amoebiasis in the state of Ceará over the last 15 years. The epidemiological parameters evaluated were: number of hospitalizations by health macro-region, and number of hospitalizations by age group (<1 year, 1 to 4 years, 5 to 9 years, 10 to 14 years, 15 to 19 years, 20 to 29 years, 30 to 49 years, 40 to 49 years, 50 to 59 years, 60 to 69 years, 70 to 79 years and ≥80 years), between 2008 and 2023 in the state of Ceará. The database used for data collection was Tabnet, which is supplied with data from the Unified Health System of the Brazilian. For graphic construction, the *GraphPad Prism* version 8.0 program was used, so that the *heatmap* model was applied to demonstrate the values. In addition, analyses of variance (*Two-way ANOVA*) were performed, followed by the Turkey test, to demonstrate significant differences between the groups. In the analysis of the number of hospitalizations per year and by age group, the evaluation showed that the peak in the number of cases occurred in 2012, with an average of 8.4 cases per macro-region, where the “Cariri” region stands out for the highest average of 5.3 cases per year, significantly higher only than that of the “East Coast/Jaguaribe”, of 1.25. Similarly, the average number of cases relative to the age group 1 to 4 was significantly higher than the others, with the exception of those related to patients aged ≥60 years. The study highlights the need for public policies aimed at improving sanitation conditions, health education and access to adequate treatment, with a focus on the most affected regions and vulnerable age groups.

Keywords: Amebiasis, Hospitalizations, Ceará.

TITLE: EPIDEMIOLOGICAL PROFILE OF SCHISTOSOMIASIS IN THE SANTA LOCATION ROSA IN THE MUNICIPALITY OF CONTENDAS DO SINCORÁ- BA

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Schistosomiasis mansoni is considered a serious public health problem and affects 240 million people in more than 50 countries. This study aimed to describe the profile of schistosomiasis cases in the locality of Santa Rosa, in the municipality of Contendas do Sincorá in 2019, according to demographic data, sex, age, education and parasite load.: This is a descriptive study with a quantitative approach, based on secondary data from the Schistosomiasis Control Program (PCE) and the Primary Care Information System (SIAB), referring to the population of the locality of Santa Rosa, Contendas do Sincorá-BA, in 2019.52 tests were performed, of which 21 (40.38%) were positive for schistosomiasis mansoni. Regarding the profile of confirmed cases of schistosomiasis in the locality, it was observed that 13 (61.9%) were female; showed that 9 (42.9%) arem from the age group between 20 and 59 years old and 10(47.6%) have little education. Regarding the parasite load, among those diagnosed with the disease, all presented a low parasite load. Conclusion: It is concluded that in the period studied in 2019, in the locality of Santa Rosa, the profile of infected individuals was mostly female, young adults, with little education and a light parasitic load. The findings suggest strategies for preventing and controlling the disease in the area, targeting the profile of individuals affected by the infection. Therefore, intersectoral actions within the scope of health promotion must be implemented to achieve the elimination of disease transmission foci in the locality.

Keywords: Neglected diseases; Schistosomiasis; Schistosoma mansoni

HEALTH EDUCATION FOR THE PREVENTION AND CONTROL OF TOXOPLASMOSIS IN VARIOUS ENVIRONMENTS

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Various parasitic zoonoses affect humans and can cause significant health issues. Among these diseases, toxoplasmosis, commonly referred to as "cat sickness," stands out. The objective of this project was to conduct a health education initiative aimed at disseminating accurate information regarding the prevention and control of toxoplasmosis. The project was implemented in the municipalities of Trindade and São Luís de Montes Belos, Goiás. A total of 22 primary schools, 5 secondary schools, and 7 institutions providing education for youth and adults were visited. In each setting, the language was adapted to ensure that the information could be more easily absorbed. In the case of children, a theatrical performance featuring actors dressed as cats and dogs was conducted to explain how to properly care for animals and improve personal and environmental hygiene conditions. For the presentations aimed at adolescents and adults, brochures and slides were the primary materials used, supplemented by social media posts that were shared with attendees. The initiative emphasized the use of simpler, more accessible language, always beginning with a discussion with students about their existing knowledge of toxoplasmosis. The topic consistently engaged the audience, as nearly everyone had some prior awareness of the disease and the associated risks of living with cats. However, many individuals still believed that the primary method of preventing the disease was to avoid contact with cats, remaining unaware of the key prophylactic measures that are more closely related to personal and environmental hygiene, as well as the proper consumption of animal-derived products. This project was significant for teaching ways to prevent toxoplasmosis, reinforcing appropriate measures, and dispelling unfounded information.

Keywords: animals, anthropozoonoses, prophylaxis.

KNOWLEDGE OF HORSE BREEDERS ABOUT TICKS IN THE MUNICIPALITY OF JEQUIÉ, BAHIA

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A major dilemma faced by horse breeders is tick control, as these parasites can cause economic losses and serve as vectors for disease agents such as babesiosis. The objective of this study was to investigate the level of knowledge horse breeders have about ticks. The study was conducted with horse breeders in the municipality of Jequié, Bahia, where horseback riding events frequently take place in both urban and rural areas. A questionnaire was administered to breeders to investigate the occurrence and areas of highest tick incidence in horses, as well as to inquire about their properties, animals, and level of knowledge about ticks. Nine horse breeders were interviewed, raising Mangalarga Marchador, Quarter Horse, and mixed-breed horses, all of which were saddle athletes participating in equestrian competitions and gait contests. Only one breeder kept their animal in a rural, forested area, while the others housed them in stables within the urban area of the municipality's headquarters, a caatinga region. Among the interviewees, 55.55% (5/9) reported no tick infestations, while the remaining breeders associated tick presence with rainy periods, reproductive seasons, and post-competition phases. All breeders were aware of the impact of ticks. Additionally, 66.7% (6/9) recognized the relationship between ticks, anemia, and the transmission of *Babesia*. Regarding tick control in the environment, four breeders did not use specific methods, while the others reported applying parasiticides or using methods such as lime. For tick control in animals, seven used pastes, two used powdered parasiticides, three used injectable parasiticides, and four used baths. Although breeders possess basic knowledge, their management methods are applied instinctively, highlighting the importance of disseminating information on tick prevention, control, and related health risks.

Keywords: *Babesia*, Horses, Prevention.

EDUCATIONAL ACTIONS ON ZOONOSES AND WATERBORNE DISEASES IN ACTIVITIES OF AN EXTENSION CURRICULAR COMPONENT

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Parasitology studies parasites that affect humans. The extensionist nature of the Human Parasitology curricular component enabled students to develop an intervention proposal in the Várzea Nova II location, in the municipality of Santo Estêvão-BA. The aim of this study was to inform the population about zoonoses and waterborne diseases. The presentation of the Parasitological Studies Extension Program (CONSEPE 07/99) was held, with an explanation of the main parasitic diseases, associated risks and the importance of good hygiene and environmental sanitation practices. Socioeconomic and health data were collected from families and samples were collected for parasitological examination of feces at the Clinical Analysis and Parasitology Laboratory of UEFS, after signing the free and informed consent forms. The exam results were delivered, and health education activities were implemented according to the epidemiological profile. The results of the exams were delivered, and health education activities were carried out in accordance with the epidemiological profile. The results indicated the presence of commensal and pathogenic amoebas. Epidemiological form recorded the presence of triatomines and the confirmation of cases of Chagas disease. The students developed the intervention proposal addressing waterborne diseases, with a dialogued presentation, a quiz on amoebiasis and the production of an educational video on Chagas disease. To encourage participation and complement the presentations, an exhibition of triatomine specimens was held to demonstrate the different species and differences between predatory, phyllophagous and hematophagous insects. The community actively participated in the presentations and discussions, which provided alignment between scientific knowledge and popular knowledge. These activities reinforce the role of extension in the academic training of students while enabling the reduction of vulnerabilities of populations exposed to different parasites.

Keywords: Human Parasitology; University Extension; Zoonoses.
Funding Source: PROEX/UEFS

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PROMOTING HEALTH EDUCATION FOR STRATEGIC LEADERS IN THE EDUCATIONAL AREA OF ITUIUTABA, MINAS GERAIS

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Abstract

Parasitic diseases create an organic deficit that often results in comorbidities that limit development and quality of life, especially among children. Recognizing the role of health education in disseminating preventive knowledge, in August 2024, we conducted an educational update for 43 managers of public elementary schools in Ituiutaba, Minas Gerais, Brazil. The event included theoretical explanations of intestinal, tissue, and blood parasites, followed by viewing some of them under a microscope or stereomicroscope. Post-intervention knowledge gain was assessed via pre- (43 respondents) and post-event questionnaires (36 respondents), focusing on parasitic disease definitions, enteroparasitosis (prevention, diagnosis, and symptoms), and the indiscriminate use of antiparasitic drugs. When asked about prior events on parasitic diseases, 34.9% of participants reported never having attended any, and 48.8% participated years ago. Participants demonstrated improved knowledge post-event, correctly identifying parasitic diseases as caused by protozoa and worms (76.7% pre-event, 94.4% post-event correct response rates), recognizing enteroparasitosis prevention (79.1% pre-event and 94.4% post-event), diagnosis (95.3% pre-event and 94.4% post-event), and symptoms (51.2% pre-event and 80.6% post-event). When asked how often antiparasitic drugs should be taken and stool tests performed, 16.3% and 18.6%, answered correctly pre-event; 94.4% and 63.9% answered correctly post-event, respectively. This reveals that although participants were familiar with some aspects of enteroparasitosis, they had questions about their treatment and diagnosis. However, doubts about the frequency of stool tests remained after the event. Since the region has good environmental sanitation services, stool tests are recommended only when symptoms are present. These findings underscore the importance of ongoing training for these professionals, especially because they act as strategic leaders in their communities.

Supported by: Institutional Program of Support Extension Activities at the State University of Minas Gerais.

Keywords: Parasitic diseases; Enteroparasitosis; Prevention.

THE ROLE OF DIGITAL MEDIA DURING THE COVID-19 PANDEMIC: AN ANALYSIS OF THE POPULARIZATION OF PARASITOLOGY

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The COVID-19 pandemic led to the implementation of measures such as physical distancing, while the use of technologies reshaped interpersonal relationships and work dynamics. In this context, social media (SM) emerged as the primary means of communication, although they also contributed to the spread of fake news, exposing the population to misleading information. Addressing this scenario, the extension projects Parasito Educac and the Parasitology League of the State University of Rio de Janeiro used social networks as a bridge between the university and society, promoting health education and disseminating general aspects of parasitology from May 2020 to January 2022. To achieve this, profiles were created on platforms like YouTube, Facebook, and Instagram, where content on topics such as basic concepts, medically significant diseases, news debates, ectoparasites, diagnostic methods, and health promotion was developed and shared. The material production involved a literature review using national and international databases, along with free tools like Canva and PowerPoint to create posts, videos, and polls. Over the period, 165 publications were made, with topics on diagnostic methods and parasite protection garnering the most attention from the audience. Analyzing the profile of the 32 participants involved in content creation revealed a mixed group of students, including those already experienced in similar activities. The main challenges reported were adapting scientific language to make it accessible to the target audience (43.7%), followed by ensuring clarity (18.7%) and conciseness (18.7%) of the content. It is concluded that the use of SM during the pandemic not only facilitated effective communication with diverse audiences but also contributed to the professional growth of undergraduate students, enhancing skills such as knowledge, creativity, and teamwork.

Keywords: Parasitology League. Health Education. Scientific Communication.

Comparative epidemiological data on visceral and tegumentary leishmania in Brazil between 2014 and 2025.

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Leishmaniasis was discovered by the pathologist William Boog Leishman. It is classified as a zoonotic disease of a tropical and subtropical nature, similar to other zoonoses, as the development of its vector is common in hot and humid regions. The phlebotomine, popularly known as the straw mosquito, is the main vector of leishmaniasis, and these protozoa affect various mammals, including humans. Leishmaniasis is a very heterogeneous pathology, in other words, it has various clinical forms, which differ depending on the species affected by the host. In Brazil there are two main subtypes of leishmaniasis, tegumentary (TL) and visceral (VL).

Therefore, the aim of this work is to carry out an epidemiological assessment of TL and VL in Brazil, as Leishmaniasis is seen as a neglected zoonosis, and several recent studies have sought to develop new therapeutic targets, such as drugs and even a vaccine. The methodology of this project consists of a systematic study using the DataSus database, between the years 2014 and 2025, evaluating all regions of Brazil, and as an exclusion criterion, all cases before 2014 were removed.

From the data obtained, 30,896 cases of VL were reported in Brazil, in which the main region affected was the Northeast (56.5%), followed by the Southeast (18.4%). In relation to TL, 181,075 cases were reported, in which the main region affected was the North (46.3%), followed by the Northeast (24.6%). In short, the results showed a strong relationship between the development of leishmaniasis in regions with less infrastructure and a more favorable climate for the development of the vector, among other factors that contribute to the increase in cases in these regions.

Keywords: Leishmaniasis; epidemiology; cases.

PARASITOLOGICAL EVALUATION OF LETTUCE (*Lactuca sativa*) AS A PRACTICAL TOOL FOR TEACHING BIOTECHNOLOGY

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Parasitology is the science that studies the relationship between parasites and hosts, modes of infection, diagnostic methods and health education strategies for prevention and control. Contamination of food consumed raw is a concern since one of the main sources of contamination is fecal-oral. The aim of this study was to assess the presence of parasitic evolutionary forms in lettuce (*Lactuca sativa*) sold in supermarkets, greengrocers and street markets in the municipality of Catalão - GO. One head of lettuce was purchased/collected from each establishment and placed in two separate thermal boxes for the supermarket and the market, which had previously been sterilized under ultraviolet light. The lettuce leaves were washed in the original plastic bag with one liter (1000ml) of sterile normal saline solution. After sedimentation and centrifugation, the material was observed under a 40x light microscope. *Giardia lamblia* cysts were found in all the samples. *Toxocara* sp eggs were seen in samples from the market and the sacolão. It was also possible to detect hookworm and *Taenia* sp eggs in the market samples. Hookworm rhabditoid larvae were present in the supermarket samples. The results found during this study made it possible to work with the students not only on parasitological diagnostic techniques, but also on health education and surveillance. It is hoped that the results will contribute to the development of local prevention programs.

Keywords: *Giardia lamblia*, *Taenia* sp., Lettuce

WHAT WORM IS THIS? AN ADVENTURE ABOUT *Ascaris lumbricoides*

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The Ascariasis is the most common intestinal parasitosis in Brazil, frequently affecting school-aged children. The lack of sanitation, combined with the socioeconomic conditions of the population, makes this parasitosis very prevalent in our environment. This study was part of a mandatory essay in the Parasitology course, conducted in groups within the Undergraduate Nursing Program, and involved developing a children's book to be used in communities served by extension projects. It also aimed to disseminate knowledge that could empower children in the prevention and promotion of their own health. The book was created with a playful approach to facilitate learning and understanding about ascariasis and its prevention methods. Its creation included a cognitive model designed to stimulate memory, attention, curiosity, and sensory perception. Materials such as EVA foam, fabric, cardstock, and poster boards with printed cutouts of the adult worm and eggs were used, as well as schematic models of organs involved in the life cycle. The language used was based on parasitology textbooks, adapted into children's story formats for better understanding by the target audience. The book's text tells the story of a girl who acquired ascariasis and noticed the presence of the worm in her faeces, prompting her to ask questions about the parasitosis, its transmission, prophylaxis, and ways to share the knowledge. The story unfolds through a dialogue of questions and answers between the girl and the narrator. It is acknowledged that using a children's book to prevent ascariasis among children is one of the most effective and far-reaching educational tools for ensuring understanding of this parasitosis and its prevention.The book was tested with about one hundred and twenty children attended by the “Teresa de Jesus Shelter” in a university extension event, called Parasitosis Prevention Fair. All children showed great interest and curiosity in the story of the book. In addition, great interest and attention was aroused in the topic addressed.

Keywords: parasitology, children's story, prevention.

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EVALUATION OF SERUM LEVELS OF INTESTINAL FATTY ACID BINDING PROTEIN (I-FABP) IN INDIVIDUALS WITH PARASITIC INFECTIONS

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Abstract

Metabolites secreted by parasites, associated with the production of cytokines and cytotoxic T cells, are related to epithelial damage and enterocyte apoptosis. Intestinal fatty acid binding protein (I-FABP) is expressed in epithelial cells and, when damage to the intestinal mucosa occurs, I-FABP is released into the circulation and its plasma concentration increases. Thus, this study aimed to evaluate the levels of I-FABP in polyparasitized and in monoparasitized individuals by different species of parasites. For this purpose, sera from individuals with negative parasitological examination using different techniques (HPJ, Baermann, Agar Plate, Kato-Katz and Faust), and from individuals diagnosed with parasitic infections, were obtained from participants of a study carried out in the rural area of Camumu, Bahia, and from users attended at the Clinical Analysis Laboratory of the School of Pharmacy, Federal University of Bahia. The following groups of sera were tested using a commercial immunoenzymatic assay for I-FABP, according to the results of their parasitological examinations: negative (n=8), polyparasitized (n=8), *Ascaris lumbricoides* (n=4), *Trichuris trichiura* (n=4), Hookworms (n=3), *Strongyloides stercoralis* (n=4), and *Giardia duodenalis* (n=4). The highest serum levels of I-FABP (expressed in ng/mL ± standard deviation) were observed in polyparasitized (3.077 ± 1.485 ng/mL), and in monoparasitized hosts with hookworms (4.007 ± 1.697 ng/mL) or *Trichuris trichiura* (4.075 ± 1.334 ng/mL), the latter being statistically significant (p<0.05), when compared with individuals without parasitic infections (0.546 ± 0.760 ng/mL). Preliminary data suggest an effect of helminth parasitism on the levels of an important marker of enterocyte damage. New experiments are underway to measure a larger number of sera, including sera from individuals with intestinal amoebas.

Supported by: CNPq/DECIT/MS - Grant term 442752/2024-5; CNPq and FAPESB scholarships for undergraduate students (PIBIC); and CAPES/MEC doctoral scholarship

Keywords: Parasitic infections; intestinal FABP; inflammation.

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FIOCRUZ BAHIA'S SCIENCE OUTREACH INITIATIVES IN PARASITOLOGY

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Abstract

The Oswaldo Cruz Foundation (Fiocruz) in Bahia, Brazil, has an institutional science outreach program that focuses on priority scientific areas, including neglected tropical diseases (NTDs). Fiocruz Bahia's science outreach initiatives aim to inspire curiosity and interest in scientific knowledge among children and youth, contributing to the popularization of science. Covering diverse topics such as health education, parasites, disease vectors, and insect biodiversity, these initiatives raise awareness and support efforts to prevent, control, and eliminate diseases like leishmaniasis, Chagas disease, and schistosomiasis. Through these efforts, Fiocruz Bahia demonstrates its commitment to science communication and its active role in combating NTDs, ultimately contributing to public health improvements.

Supported by: Fiocruz Bahia

Keywords: Science outreach, Neglected tropical diseases, Science communication, Popularization of science.

UFF LICE IN ACTION: DISSEMINATING INFORMATION TO RAISE AWARENESS AND CONTROL PEDICULOSIS

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Abstract

Pediculosis is a public health problem that affects individuals regardless of gender or age, being more prevalent among schoolchildren in Brazil. Health education actions and social media are effective strategies for disseminating information about infections/infestations and diseases, helping with prevention. This study aimed to promote awareness about pediculosis through activities carried out in schools and municipal early childhood education units (UMEI), events inside and outside the university and posts on Instagram. From June to December 2024, a face-to-face workshop was held which included theater with a "hot lice" fixation activity, a flipchart with observation of lice under a microscope and a lecture followed by an interactive quiz, using dynamic and participatory approaches adjusted according to the age of the participants. Instagram posts were produced on Canva. The workshop was presented to 500 elementary school students from the Niterói municipal education, which was positively evaluated by teachers and students. A meeting was held with 16 people responsible for children from a UMEI using the flipchart, identifying the circulation of correct information and myths. Information about lice was presented at two events open to the community, one within the University and the other at a fair on the sports court of a private school. Both at the workshop and at the events, after interacting with the public, folders and fine-tooth combs were distributed. On Instagram, 14 posts, 13 stories and one reels were published, reaching 1258 accounts and obtaining 3264 views. The actions developed contributed to the dissemination of information about pediculosis with a leading role among undergraduate students, encouraged the adoption of protective behavior, demystifying myths and strengthening health education as a complementary strategy for controlling pediculosis and improving health conditions for schoolchildren and the community in which they are included.

Supported By: PROEX-UFF

Keywords: louse, health education, social media

+Lugar Platform for Mapping Geohelminthiases and Arboviruses Risk: Data Integration for Epidemiological Surveillance

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Health as a right for all requires not only effective public policies but also active societal engagement. Collaborative mapping on platforms like OpenStreetMap enables the production and sharing of geospatial data. The new +Lugar platform, in addition to participatory zoonosis mapping, enables the identification of its predictors through gamification features and also ensures the inclusion of external projects aimed at social contribution. This study aimed to evaluate the use of +Lugar for participatory mapping of geohelminth infections and Aedes spp. infestation. The study areas were State University of Feira de Santana (UEFS) and the neighboring Campo Limpo district, with collection zones marked using Google Earth. Animal fecal samples were collected in public areas of both locations, while at UEFS, PneuTrap3D ovitraps were also distributed. Collection points and environmental information were recorded in +Lugar, and all samples were analyzed in the laboratory. The information in +Lugar was analyzed and associated with the laboratory data, building a database in Excel and performing statistical analyses using EpiInfo 7.2 and SaTScan 10.2.5. The platform facilitated the georeferencing of the samples, allowing for the precise location of collection sites and their association with environmental factors. Regarding fecal sample collection, the data obtained allowed for spatial analysis and the detection of clusters of positive samples within the Campus. For Aedes, the platform highlighted the presence of potential larval breeding sites, such as water and food bowls, water tanks, plant pots, tires, and bottles. It was noted that improvements to the platform's interface are needed to provide users with more autonomy in creating and editing projects. The integration of georeferenced, epidemiological, and environmental data into a user-friendly application makes epidemiological surveillance more efficient, supporting the planning of control and prevention actions.

Supported by Universidade Estadual de Feira de Santana

Keyword: Participatory Mapping, Geospatial Epidemiology, Zoonoses Surveillance

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SCIENCE AND THE SERTÃO: AN INTERACTIVE DIALOGUE IN THE PREVENTION OF CHAGAS DISEASE

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ABSTRACT

Chagas Disease, discovered by Carlos Chagas in 1909, remains a public health challenge, especially in endemic areas. Innovative health education strategies are essential to expand knowledge on the topic and promote prevention, particularly in an increasingly digitalized world. This study aimed to develop a comic book (HQ) that personifies *Trypanosoma cruzi* and engages the public in a playful and educational manner, bridging the scientific legacy of Carlos Chagas to modern Chagas disease prevention through interactive and accessible resources. The educational comic merges tradition and innovation to raise awareness about Chagas disease, incorporating regional elements from the Brazilian Northeast. Using accessible language and interactive visuals, it personifies the *T. cruzi* parasite to explain its biological cycle, the acute and chronic phases of the disease, and the pathogen-host relationship. Lighthearted dialogues between the parasite, the kissing bug (barbeiro), and humans blend entertainment with scientific information, emphasizing preventive measures. The digital and interactive format aims to broaden reach, promoting health in a playful yet informative way. The comic demonstrates high potential to democratize complex concepts in parasitology, facilitating identification and understanding among rural populations and fostering engagement. The inclusion of regional terms honored cultural memory, while the interactive approach enables better content assimilation by individuals from endemic communities, healthcare professionals, and students. This initiative shows that dialogue between scientific history and contemporary technologies can transform historical content into practical tools for prevention, early diagnosis, and stigma reduction. The comic transcends its original format, serving as a model for educational initiatives that unite scientific rigor, community engagement, and digital innovation, strengthening public health in a globalized context.

Supported by: CAPES, CNPq.
Keywords: *Trypanosoma cruzi.* Comic book. Neglected diseases. Information technology. Health education.

ANALYSIS OF CASES OF *SCHISTOSOMA MANSONI* IN THE NORTHEAST AND SOUTHEAST REGIONS OF BRASIL IN THE YEARS 2018-2021

PALLOMA DE OLIVEIRA MIRANDA VELOSO[1], MARIA EDUARDA DAMY DOS SANTOS PIMENTA E SILVA[1], FERNANDA NAZARÉ MORGADO[2], JÉSSICA LEITE DA SILVA[2], FÁTIMA CONCEIÇÃO SILVA[2], PATRICIA DE OLIVEIRA CAMERA[2]

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Schistosomiasis is caused by worms of the Trematoda class, with the *Schistosoma mansoni* species prevalent in South America. Initially asymptomatic, it can evolve into severe forms and lead to death. In Brazil, the most affected regions are the northeast and southeast, where it stands out as a major public health problem. We conducted a retrospective descriptive study, with a quantitative approach, based on data published in the Schistosomiasis Control Program, with the objective of evaluating the epidemiological data in these regions between 2018 and 2021. We were able to observe that the northeast region had a higher number of positive cases than the southeast region in the analyzed period, probably due to the socioeconomic characteristics of the region. A positive data observed in both regions was the high percentage of treated (above 70%) among the positive cases, which favors the control of transmission in these areas. Even during the pandemic, despite the decrease in the number of cases monitored, the percentage of cases treated remained high among the positive cases. Regarding the identification of the molluscs that are vectors of the disease, the predominance of the species *Biomphalaria straminea* was observed in the northeast region and *B. glabrata* in the southeast. However, the absence of *B. tenagophila* in the southeast during the period evaluated was noteworthy. Based on the data observed, we can conclude that cases are closely linked to the lack of basic sanitation, drinking water, and social vulnerability, and are classified above all as a public health problem. The availability of epidemiological data is of utmost importance so that strategies can be drawn up to raise awareness and improve the territory to reduce these numbers. The scarcity of updated data (the latest is from 2021) makes it difficult to make quick and accurate decisions, preventing the strategic allocation of resources for prevention and treatment of the endemic disease in the country.

Key words: Schistosomiasis; Prevention and Control; Public Health

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KNOWLEDGE AND PRACTICES OF THE RURAL POPULATION IN AN ENDEMIC AREA OF THE STATE OF PIAUÍ REGARDING CHAGAS DISEASE AND TRIATOMINE

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Abstract

Chagas disease (ChD) is a zoonotic disease caused by *Trypanosoma cruzi*, with triatomine insects as its primary vectors. Although vector-borne transmission has decreased in Brazil, the discontinuation of entomological surveillance activities has contributed to the infestation and colonization of domestic and peridomestic environments by infected triatomines, particularly in rural areas, including the state of Piauí. This study assessed the knowledge and practices of the rural population of Campinas do Piauí regarding triatomines and ChD. Knowledge was evaluated through the application of a questionnaire, supplemented by a display of hemipteran specimens for vector identification. Both adults and adolescents from rural communities participated in the study. A total of 256 households from 18 rural communities in Campinas do Piauí were visited, and 347 residents were interviewed. The data revealed that the rural population had good knowledge of the vectors and frequently reported triatomine invasions in their homes. Regarding ChD, 76.6% (266/347) of respondents were familiar with the disease and associated the heart as the main affected organ. Additionally, 76.5% correctly identified triatomines and reported that the most common practice upon encountering them in domestic or peridomestic environments was to kill them. Among control measures, environmental cleaning and the use of insecticides were the most frequently mentioned strategies. The results indicate that the rural population of Campinas do Piauí has a good understanding of triatomines and ChD in both groups studied, however, there are limitations regarding preventive strategies against household infestation. Therefore, continuous vector control actions, combined with educational campaigns, are essential to promote active community participation in disease prevention.

Supported by: CAPES, CNPq.

Keywords: Chagas disease; entomological surveillance; health education.

Genotypic characterization of *Giardia lamblia* and *Blastocystis* sp. in fecal samples from children in a Community, Florianópolis, SC

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Giardia lamblia and *Blastocystis* sp. are among the most frequently detected protozoa in human fecal samples and are major causes of diarrhea in children—especially those facing water and food insecurity. Recent laboratory and coproparasitological surveys have shown high positivity for *Blastocystis* sp., highlighting the need for molecular characterization to enhance epidemiological understanding. This study aimed to use *nested* PCR to identify and molecularly characterize *G. lamblia* and *Blastocystis* sp. in fecal samples from children in the Serrinha community of Florianópolis, SC. A total of 152 stool samples from children and adolescents aged 2 to 15 years, collected from institutions in the Serrinha community (Bairro Trindade), were preserved in SAF solution and examined by direct microscopy, spontaneous sedimentation, and Faust's method. Samples that were positive for *G. lamblia* and *Blastocystis* sp. by microscopy were then processed for DNA extraction, PCR optimization, sequencing, and classification of subtypes and assemblages. This study was approved by the Ethics Committee of the Federal University of Santa Catarina (CAAE 59641322.1.0000.0121). Among the 152 samples, 64 (42%) were positive for enteroparasites. Detected parasites included *Blastocystis* sp. (34.8%), *Endolimax nana* (21.2%), *Entamoeba coli* (16.7%), *Entamoeba histolytica/E. dispar* (9.1%), *Giardia lamblia* (7.6%), *Ascaris lumbricoides* (3%), *Iodamoeba butschlii* (3%), *Hymenolepis nana* (1.5%), *Enterobius vermicularis* (1.5%), and *Entamoeba hartmanni* (1.5%). Of the *Blastocystis* sp. positive samples, 84.8% were confirmed by nested PCR and classified as subtypes ST1, ST1b, ST2, ST3, and ST3a, which are often linked to symptomatic cases. *G. lamblia* samples were identified as assemblage B, which is common in human and mammalian infections and suggests potential zoonotic transmission. These findings contribute to improved epidemiological insights and help guide future monitoring and control measures.

Supported By CAPES and UFSC/PROEX
Keywords *Giardia lamblia*; *Blastocystis* sp.; Molecular characterization.

HEALTH EDUCATION: PLAYFUL STRATEGIES IN TEACHING PARASITIC DISEASES

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Health education is essential to promote disease prevention and the adoption of healthy habits. This paper describes an educational experience carried out with 9th grade elementary school students and 3rd grade high school students in public schools in Macaíba/RN, focusing on raising awareness about human and animal parasitic diseases. The highlight was the use of the game “Pass or Repass Parasitological” to make learning more dynamic and engaging. The activities took place at the Alfredo Mesquita Filho Rural Education Center and at the Ivonete Felipe de Souza Professora State School of Traíras, and were organized in three stages. Initially, there was a presentation of the project and the application of a questionnaire to collect information about socioeconomic profile, hygiene habits and previous knowledge about helminthiasis and protozoa. Then, the students participated in discussions about the importance of good personal and dietary hygiene habits, with an emphasis on parasitic diseases such as taeniasis, leishmaniasis and schistosomiasis. They were also able to observe macroscopic specimens and images of parasites, enriching their visual and practical understanding of the topics covered. In the second phase, the dynamics of the game “Pass or Repass Parasitological” were applied, which reviewed and reinforced the content in an interactive way, with questions and challenges that encouraged active participation from students. In the final phase, participants answered a questionnaire to evaluate the effectiveness of the activities. The results showed a significant increase in the students' knowledge, with advances in the identification of parasites, understanding of the forms of transmission and adoption of preventive measures. The game stood out for making learning more dynamic and participatory, facilitating the retention of content and proving to be an effective strategy in teaching complex health topics.

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Keywords: Health education; playfulness; parasitology.

Identification of parasites with zoonotic potential in domesticated dogs

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Parasites with zoonotic potential are common in domestic animals, especially companion dogs, and are a Public Health concern, especially due to the proximity of these animals to humans. Therefore, the investigation and identification of these agents in domesticated dogs is important. This study aimed to evaluate fecal samples from 103 domesticated dogs in Araçatuba, São Paulo state, Brazil. The occurrence and molecular diversity of *G. duodenalis* in dogs were analyzed using the TF test and a nested PCR of the 18s rRNA gene of *Giardia* spp., and positive samples were genotyped by amplification of the GDH gene. Of the total fecal samples investigated in the parasitological examination, six presented *Toxocara* spp. eggs and one presented *Ancylostoma* spp. eggs, but no cysts of *Giardia* spp. were observed. However, five samples (4.85%) were positive for *G. duodenalis* using nPCR of the 18S rRNA gene and four of these were successfully genotyped, identifying the BIV zoonotic assemblage in two, and the C assemblage in the others. The three parasites found in the samples are zoonotic agents responsible for visceral larva migrans, cutaneous larva migrans and giardiasis, respectively. Statistical analysis revealed a significant association between infection and sex of the animals, with a higher prevalence in males. Thus, it was possible to observe an occurrence of 5.83% of *Toxocara* spp.; 0.97% of *Ancylostoma* spp. and 4.85% of *G. duodenalis*, identifying the assemblages BIV and C, in dogs domiciled in Araçatuba, São Paulo, Brazil.

Funding: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)

Keywords: Public Health, Zoonosis, pets

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GUIDED TOURS FOR PROMOTING A SENSE OF BELONGING AND HEALTH AMONG WORKERS IN LABORATORY ENVIRONMENTS

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Abstract

Parasitic and infectious-contagious diseases are a significant public health challenge; their effective control requires coordinated actions in therapy, sanitation, and education. Thus, university extension programs serve as a valuable tool for supporting the learning process of the population. The aim of this study was to promote the visit and exchange of information for the cleaning staff of the Federal University of Uberlândia (UFU) to the practical Parasitology and Microbiology laboratory classrooms. Guided visits were conducted, and participants had access to the parasitology collection, microbiology materials, as well as information on various diseases and prevention methods. At the end of each visit, a discussion was held regarding the participants' perspectives on the topics presented. The visits were carried out in two sessions, with a total of 24 participants, who observed under the microscope slides of parasites (*A. lumbricoides*, *E. vermicularis*, *T. solium*, *T. saginata*, *S. mansoni*, *T. cruzi*, *G. duodenalis*, *P. humanus*, *P. pubis*, *R. sanguineus*, *T. penetrans*, and *A. aegypti*), as well as macroscopic specimens of *T. infestans* and flies; bacteria (Gram-positive: *S. aureus*; Gram-negative: *E. coli* and *Salmonella* spp.); and fungi (filamentous: *Aspergillus* spp., and yeasts: *C. albicans*). Parasites in formaldehyde and explanatory boards were also observed, and there was interaction with 3D models. Practical activities included hand hygiene techniques, disinfection and cleaning procedures, tooth brushing techniques, and the use of hand sanitizer and sodium hypochlorite. The responses from participants during the discussion revealed that many were curious about the activities in the labs and had numerous questions regarding prevention methods for the various agents presented. We concluded that working with a segment of the population often neglected in educational efforts enables the democratization of access to discussions on these topics, leading to societal changes.

Supported by: UFU
Keywords: Parasitology, university extension, health promotion.

SCHISTOSOMIASIS: HOSPITALIZATION COSTS DUE TO SCHISTOSOMA MANSONI INFECTION, BRAZIL, 2018 TO 2023

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Abstract

This study aimed to describe the epidemiological profile of hospitalization cases due to *Schistosoma mansoni* infection recorded in the Hospital Information System (SIH/SUS), Brazil, 2018 to 2023. For data selection, a filter was applied to the mortality tabulation list, CID-10 Morbidity List code B65 (Schistosomiasis), considering the place of residence of the cases. Descriptive statistics were used for data analysis. A total of 937 hospitalization cases due to schistosomiasis mansoni infection were recorded, with R$ 667.168,91 spent on hospitalizations, with highest number of records was among male individuals, aged between 50 and 59 years. The aggregated data from the studied period, the length of stay of patients with schistosomiasis in hospitalization beds totaled 7.481 days. Patients aged between 50 and 59 years had a longer hospitalization period, ranging from 130 to 329 days, aggregated data. The estimating the treatment costs for individuals in this study, aged ≥4, versus what was spent on hospitalization, it was observed that a total of R$ 641.623,40 could have been saved. The recommended treatment for *S. mansoni* infections is a single 600 mg dose of Praziquantel (price of one tablet is R$ 1,65), provided free of charge by the Ministry of Health. Hospitalization cases due to *S.mansoni* indicate the presence of severe forms of the disease, which can be prevented through early detection and immediate treatment of the infection, highlighting the need for improvements in schistosomiasis surveillance programs. This study observed that approximately R$650.000,00 could be saved if individuals had been properly treated. The WHO estimated that schistosomiasis causes the loss of healthy life or death of approximately 1.7 million people annually worldwide, the data this study may be underestimated. Additionally, the lack of hospital beds and units results in outpatient treatment of patients who need hospital care, and these cases are not recorded in the SIH.

Supported by: Secretariat of Health and Environment of Ministry of Health

Keywords: Schistosomiasis, hospitalization, Surveillance

PARAPREV - EDUCATION AND PREVENTION OF PARASITOSES: STRENGTHENING HEALTH IN BARREIRAS-BA

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Parasitic diseases are a serious public health problem, especially in areas with poor sanitation and education. This reality is experienced in Barreiras-Ba, since data published by the Bahia State Health Department (SESAB) reveal that in 2023, 19.7% of Chagas disease cases in Bahia were concentrated in the west of the state, and 13.5% in Barreiras alone. Furthermore, according to SESAB, from 2022 to 2023, there was an exponential increase in the incidence of leishmaniasis cases in the state, with the West macro-region taking fourth place. The project "ParaPrev - Education and Prevention of Parasitic Diseases: Strengthening Health in Barreiras-BA" aims to raise awareness among the population about the main parasitic diseases present in the region, their forms of transmission and prevention methods. With educational activities in schools and Basic Health Units (UBS), the initiative uses lectures, discussion groups, distribution of informative materials and the use of microscopes to demonstrate infectious forms of parasites. In addition, the dissemination of content through a digital platform on Instagram expands the reach of the information. The project impacts students, health professionals and the community, addressing diseases such as Chagas, schistosomiasis, leishmaniasis, giardiasis, amoebiasis, helminthiasis, dengue, cutaneous larva migrans, scabies and pediculosis. The methodology involves organized visits to schools and UBS, dividing the activities according to the main groups of parasitic diseases (waterborne, fecal-oral, vectorial and cutaneous transmission). The proposal seeks not only health education, but also critical training of the population on the impacts of parasitic diseases in the social and economic context. Thus, it is concluded that the initiative is of great importance in reducing the incidence of these infections, promoting a better quality of life for the population and minimizing treatment costs in the public health system.

Financial Support: Programa Institucional de Bolsas de Iniciação à Extensão (PIBIEX)

Keywords: Parasitosis. Education. Population

EVALUATION OF PARASITOLOGICAL DIAGNOSTIC METHODS IN PRESCHOOL CHILDREN IN NATAL-RN

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Intestinal parasites are the cause of numerous diseases, which affect a large portion of the world population, especially children. Several methods are used to diagnose these parasites, which makes it necessary to evaluate and compare them. Thus, the positivity of three parasitological diagnostic methods (Hoffman, Pons and Janer - HPJ, Rugai and Willis-Mollay), together with the perianal imprint by the Graham method, was analyzed in preschool children from Municipal Child Education Centers in Natal-RN, which was approved by the ethics committee (CAAE 59703022.7.0000.5537). A total of 66 stool samples were obtained from the children, which were tested for the HPJ, Rugai and Willis-Mollay methods, and 39 slides with the perianal imprint. From them 26 samples were positive (39.3%) for helminths and/or protozoa, regardless of the method used, with *Ascaris lumbricoides* being the most frequent, followed by *Entamoeba coli* and hookworms (25%, 22.72% and 20.45%, respectively). Among the 26 positive samples, monoparasitism (50%), biparasitism (30.8%) and polyparasitism (19.2%) were observed, with the most frequent association between *A. lumbricoides* and *E.coli* (30.8%). Regarding to the parasitological methods, Graham was positive for only three samples (7.7%), exclusively for *Enterobius vermicularis*, while HPJ, Rugai and Willis-Mollay obtained positivity of 25.8%, 21.2% and 10.6%, respectively. The HPJ method showed the highest positivity for helminths (52.9%) and Willis-Mollay showed the highest positivity for protozoa (57.1%). Thus, it could be concluded that these methods have limitations, and that the use of more than one method is essential to obtain a more accurate diagnosis.

Supported by: Coordination for the Improvement of Higher Education Personnel - CAPES,

UFRN.

Keywords: Helminths; Intestinal parasites; Protozoa.

ENTOMOLOGY

UNVEILING THE FEEDING PROFILE OF *Triatoma wygodzinskyi* LENT, 1951 (HEMIPTERA: REDUVIIDAE): GENETIC EVIDENCE OF LIZARD PREFERENCE

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Triatoma wygodzinkyi is a sylvatic triatomine, potentially a vector of *Trypanosoma cruzi*, that occurs in southeastern Brazilian states, where it is typically associated with rocky outcrops and occasionally found in human dwellings. It is closely related to species of the *T. pseudomaculata* complex and has been misidentified as *Triatoma arthurneivai* Lent & Martins, 1940 for over 40 years. There is a lack of information regarding host associations and trypanosomatid infections, highlighting this neglected species. Identifying blood meal sources (BMS) of vector insects enables the identification of species involved in maintaining enzootic parasite cycles, reveals the fauna supporting vector colonies, and provides insight into the faunal composition of the study areas. For this purpose, specimens identified as *T. wygodzinskyi* were collected from a rural area in *Vargem Grande do Sul* municipality, São Paulo state. Twenty-two triatomines collected from 5 colonies, including nymphs and adults, were selected for BMS identification and trypanosomatid diagnosis. The triatomines were dissected to obtain intestinal contents, followed by DNA extraction, PCR DNA Barcoding, and Sanger sequencing of the genetic markers *cyt*b, 12S rDNA, and 18S rDNA. Preliminary analyses showed negative for infection and BMS as *Tropidurus itambere*, a neotropical ground lizard, and *Hemidactylus mabouia*, the cosmopolitan house gecko. Lizards of the *Tropidurus* genus are well-documented as BMS for other *Triatoma* species, and *Hemidactylus* has been previously identified as BMS for *Triatoma petrocchiae*. In general, lizards are considered refractory to *T. cruzi*, infection, but recent studies highlight them as “silent hosts”. The present is the first genetic detection study of BMS and trypanosomatid diagnosis for *T. wygodzinskyi*, demonstrating an important feeding profile on lizards. These findings are essential for understanding the ecology of *T. wygodzinskyi* and its role in parasite transmission.

Supported by: CAPES, FAPERJ, CNPq, LPIP-IOC/FIOCRUZ

Keywords: Blood meal sources, DNA Barcoding, Vector ecology

BLOOD MEAL SOURCES, TRYPANOSOMATID INFECTION AND MICROBIOTA IN TRIATOMINES COLLECTED FROM A CHAGAS DISEASE OUTBREAK LOCALITY IN PERNAMBUCO, BRAZIL THROUGH HIGH-THROUGHPUT SEQUENCING

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The interactions of vectors, parasites, microbiota composition, and blood meal source (BMS) species constitute a dynamic process in the success of parasite transmission. In this study, we investigated these elements through High-Throughput sequencing (HTS) in triatomines from Ibimirim municipality, Pernambuco, a locality where a Chagas disease (CD) outbreak occurred in 2019. *Triatoma pseudomaculata* nymphs (n=28) were collected in bird nests (peridomestic ecotope) and tree barks (wild ecotope). DNA extracted from intestinal contents was submitted to the 12S rDNA, 18S rDNA, and 16S rDNA PCR to detect BMS, *Trypanosoma cruzi*/DTUs infection, and microbiota composition, respectively. A mix of the markers of 6/28 specimens was submitted to HTS on a MiSeq Platform (Illumina). The dataset obtained was processed using a genomic pipeline based on AdapterRemoval, SeqKit, and Blast for removing adapters and low-quality bp, following the comparison consensus to a custom-built reference database. BMS results revealed humans, *Felis catus*, *Mus musculus*, *Didelphis albiventris*, and *Carollia perspicillata*. The 5 BMS were detected in a single vector, and 2/6 vectors were *T. cruzi* positive. Nymphs from the peridomestic ecotope had the highest BMS and microbiota diversity. Vectors that fed on >1 BMS also had greater microbiota diversity. *Didelphis albiventris,* and *C. perspicillata*, ancient and significant reservoirs of *T. cruzi*, were identified in both wild and peridomestic environments. The recognition of opossums and bats suggests their role in sustaining the cycle of the parasite, which may have implications in the outbreak scenario. In the same way, the *M. musculus* and *F. catus*, proposed to actively link domestic and peridomestic environments, could had established the role of connector with humans. Microbiota specific profiles associated to BMS or *T. cruzi* infection were not observed. The study provided additional data for understanding the transmission scenario of the CD outbreak.

Supported by: FAPERJ, CNPq, IOC, LPIP-IOC/FIOCRUZ, CAPES.

Keywords: *Trypanosoma cruzi;* HTS, *Triatoma pseudomaculata*

FIRST GENETIC STUDY OF *Pediculus humanus capitis* De Geer, 1767 (ANOPLURA: PEDICULIDAE) FROM RIO DE JANEIRO, BRAZIL

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Pediculus humanus Linnaeus, 1758 (Anoplura: Pediculidae) is an obligate hematophagous and monoxenic human ectoparasite that was phylogenetically inherited from our human ancestors. Lice cause pediculosis with global distribution and high prevalence. In addition, they carry valuable information about their long coevolutionary history with *Homo* species. *Pediculus humanus* is divided into two ecotypes, commonly known as the head louse and body louse, *P. h. capitis* De Geer, 1767 and *P. h. humanus* Linnaeus, 1758, respectively. These ecotypes are genetically classified into six phylogenetic clades, designated A to F, with distinct geographic distributions. There is limited information about the lineages circulating in Brazil. This study investigated for the first time the genetic diversity of *P. h. humanus* from individuals in Rio de Janeiro municipality, RJ state, Brazil. During 2024, pools of adult lice and eggs from 76 individuals were donated by *Piolho Control*, a head lice removal center, which were collected in sterile Falcon tubes. DNA was extracted from one adult louse by individual and genetically analyzed by PCR and Sanger sequencing using the *cyt*b gene (360 bp). Preliminary results revealed PCR positivity in 15/76 individuals, with nucleotide sequences predominantly corresponding to haplotype A5, along with three putative novel haplotypes. Clade A is the *P. humanus* cosmopolitan *cyt*b lineage, and haplotype A5 is the most frequently detected haplotype in the current worldwide molecular epidemiology. In the country, haplotype A5 was only identified in Northern Brazil, and in America, it was found in French Guiana, Chile, Peru, Colombia, Ecuador, Panama, Honduras, Mexico, and the USA. Our findings of three putative novel haplotypes, in addition to the known A5 haplotype, in Rio de Janeiro, contribute novel data into the genetic diversity and distribution of *P. humanus* in Brazil, a continental dimensions country, and globally.

Support: Capes; Faperj; CNPq; Piolho Control; LPIP-IOC/FIOCRUZ.

Keywords: Phylogenetics, Pediculosis, Molecular Epidemiology

IMMUNE RESPONSE PROFILE OF PATIENTS INFECTED BY *LEISHMANIA* (*VIANNIA*) *GUYANENSIS* BEFORE TREATMENT WITH PENTAVALENT ANTIMONY (GLUCANTIME®).

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American cutaneous leishmaniasis (ACL) is a zoonosis caused by flagellates of the genus *Leishmania* and its pathogenesis is strongly influenced by factors inherent to the host, such as genetic factors and immune response, by factors inherent to the parasite, such as virulence of infecting species, and by factors related to the vector, such as vectorial capacity. In order to characterize the immune response of patients infected by *L. guyanensis* before treatment with Glucantime®, samples were collected from 17 individuals infected and 17 uninfected individuals (controls). Analysis of cellular profile Cell T and B lymphocytes, NK cells, monocytes, T regulatory cells and cytokines (IL-2, IL-5, IL-5, IL -6, IL-8, IL-10, IL-12, IFN-γ and TNF-α) were done. The results showed that a wide spectrum of clinical manifestations can develop in accordance to the host immune response. Proinflammatory cytokines such as IFN-γ and IL-12 showed significant results ($p<0,0001$) with higher concentration (561,99/6) in patients prior to initiation of treatment. There was an increase in the normal ratio of $CD4^+$ ($p=0,0050$) to $CD8^+$ ($p=0,0001$) before treatment in the infected group (12/5). The NKT ($CD3^+/CD16^+/CD56^+$) cells concentrations were markedly lower (76% / $p<0,0001$) in patients prior to initiation of treatment when compared to controls. The concentrations of various components of immune response in patients compared to the controls varied and there was no uniformity in the profile of patients with the same disease, suggesting the presence $T_H1/T_H2/T_H17$ in response to infection by *L. guyanensis*.

Supported by FAPEAM, CAPES and CNPq.

Keywords: American cutaneous leishmaniasis, immune response, *Leishmania* (*Viannia*) *guyanensis*.

House Dust Mites: Recombinant expression and IgE reactivity analysis of Blo t 23 and Der p 20

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Introduction: Allergies are a public health problem in several countries around the world. One of the main sources of allergic is house dust mites (APD), such as *Blomia tropicalis* and *Dermatophagoides pteronyssinus*. The molecular and immunological characterization of allergens from these two species is crucial for clinical applications. **Objectives:** Express and purify the allergens Blo t 23 and Der p 20 and compared the IgE reactivity of these two allergens with that of seven other allergens from APD. **Material and Methods:** Blo t 23 and Der p 20 coding sequences were cloned into pET21(a+) vectors. A colony of *E. coli* BL21(DE3) was transformed and proteins were expressed. After solubility testing, purification included anion exchange chromatography and size exclusion chromatography. The IgE reactivity was determined by indirect ELISA using rBlo t 23 and rDer p 20, along with seven additional allergens (rBlo t 1, 2, 5, 21 rDer p 1, 5, 21), were used as coating antigens. **Results:**The expression of rBlo t 23 and rDer p 20 was confirmed, with observed protein bands corresponding to their theoretical molecular weights of 13 kDa and 40 kDa, respectively. Purification steps were efficient, yielding pure proteins at final concentrations of 23.38 mg/L for rBlo t 23 and 4.47 mg/L for rDer p 20. The two proteins showed IgE binding capacity in sera from allergic patients, showing an IgE reactivity of 60% and 31% for rBlo t 23 and rDer p 20, respectively. However, rBlo t 23 displayed significantly lower IgE binding capacity compared to rBlo t 2, 5 and 21. Similar finding occurred for rDer p 20, but only in comparison to rDer p 21. **Conclusion:** *E. coli* BL21 (DE3) can be used as an effective expression vector to produce rBlo t 23 and rDer p 20, with high expression yields. Considering that they showed IgE reactivity in sera from patients allergic to HDMs, these recombinant allergens could, therefore, be used to explored as potential markers for disease severity.

Keywords: house dust mite; allergy; recombinant protein.

Support: Fapesb (5718/2023); Fundação Escola Politécnica da Bahia (FEP, Projeto 0156-A); CNPq (403336/2021-0).

PHENOTYPIC CHARACTERIZATION AND PHYLOGENETIC INFERENCE OF WING SHAPE IN SAND FLIES OF THE SUBTRIBE PSYCHODOPYGINA (DIPTERA: PSYCHODIDAE: PHLEBOTOMINAE)

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The sand fly subtribe Psychodopygina (Diptera: Psychodidae: Phlebotominae), stands out due to its involvement in the transmission of leishmaniasis agents. The use of supplementary tools, through integrative taxonomy approaches, such as Geometric Morphometrics (GM), can help to improve the taxonomy and systematics of this group, which remains advancing. Thus, we aimed to provide a phenotypic characterization and assess the phylogenetic signal of wing shape within Psychodopygina.

At least one species of each genus of Psychodopygina was selected, considering their availability on the collections - University of São Paulo Museum of Zoology, and Instituto Evandro Chagas. The coordinates of 13 landmarks from male wings were digitized using tpsDIG. MorphoJ software was used to estimate the shape variation, morphological diversity, calculate centroid size (CS), and perform principal component (PCA) and canonical variate (CVA) analyses. Phylogenetic inference was performed based on a matrix of Mahalanobis distance, subjected to analysis using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and Neighbor-Joining clustering algorithms.

The following *taxa*, *Bichromomyia* (1 sp., n = 47)*, Martinsmyia* (2 spp., n = 27), *Nyssomyia* (8 spp., n = 159), *Psathyromyia* (11 spp., n = 140*), Psychodopygus* (13 spp., n = 147)*, Trichophoromyia* (5 spp., n = 180) and *Viannamyia* (2spp., n = 25), were assessed; a representative of Brumptomyina, *Brumpromyia avellari* (n = 30)*,* was included as an outgroup, totaling 713 wings. All genera partially shared the same phylomorphospace on CVA and PCA. On CVA, however, *Trichophoromyia, Nyssomyia* and *Viannamyia* grouped further apart. *Viannamyia* comprised the most divergent genus based on CS, CVA, PCA and UPGMA, as well presented the greatest estimated morphological diversity. All CS were distinct on genera-comparisons, with exception of *Bichromomyia-Psychodopygus*. The topologies produced by the wing shape partially correspond with that of literature-based molecular data.

These results prove the importance and potential application of wing shapes in recovering the relationships among sand flies

Supported by: IEC/SVSA/MS; CNPq; CAPES
Keywords: wing; phylogeny; Psychodidae

LARVICIDAL EFFECT OF COFFEE (Coffea arabica) EXTRACTS ON Aedes aegypti: AN ECOLOGICAL ALTERNATIVE FOR VECTOR CONTROL

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Aedes aegypti is a vector of DENV, ZIKV, and CHIKV, which cause diseases with a significant impact on global health. Mosquito control is primarily conducted through insecticides; however, resistance has reached alarming levels, necessitating the discovery and/or development of new molecules. In this context, botanical products are gaining prominence, with this study focusing on coffee (Coffea arabica). A. aegypti larvae (Rockefeller strain, L3, n=15; quadruplicate) were subjected to gold-standard bioassays. The larvicidal effect of extracts obtained from green (ECV) and roasted (ECT) coffee beans diluted in Tween or DMSO with dechlorinated water (0.12 to 4 mg/mL) was evaluated. Control group larvae were exposed only to Tween/DMSO and dechlorinated water. Results were analyzed using PROBIT regression and ANOVA. The LC50 values in mg/mL were observed for ECV (DMSO 2.53±0.1; Tween 2.57±0.25) and ECT (DMSO 1.43±0.05; Tween 0.33±0.22), with the best effect observed for ECT in Tween (99.2%). Characterization of ECV and ECT by HPLC revealed caffeine (73.9 and 6.1 mg/g) and caffeic acid (43.8 and 4.5 mg/g) as the major compounds, demonstrating the impact of roasting on composition. The larvicidal effect of pure caffeic acid in Tween (0.26 mg/mL) was 25±0.5%. Despite being the major compound in ECT, the observed mortality suggests that caffeic acid is not the primary active component or acts synergistically with other compounds. Histological analyses suggest that the larvicidal effect is related to tissue damage in the mosquito's midgut. Based on these results, ongoing investigations include: i) the effect of the major compounds individually and in combination on populations susceptible and resistant to deltamethrin; ii) larvicidal effects related to midgut destruction and/or the involvement of adenosine and esterases; iii) the potential impact of coffee on vector physiology, with emphasis on reproductive capacity and vector competence.

FINANCIAL SUPPORT: UFLA, ICB/UFMG; CAPES; INCT-Entomologia Molecular; EMBRAPA-Café.
KEYWORDS: Aedes aegypti; Coffea arabica; Biopesticide.

IDENTIFICATION AND TESTING OF CANDIDATES FOR THE DEVELOPMENT OF LEISHMANIASIS TRANSMISSION BLOCKING VACCINES

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American visceral leishmaniasis (AVL) is considered a major neglected public health problem. There has been a significant increase in the number of human cases of visceral leishmaniasis in recent years, which in Brazil is caused by *Leishmania infantum chagasi*, transmitted by *Lutzomyia longipalpis.* There is a demand for new approaches in the control of leishmaniasis due to the lack of vaccines and limitations in the use of traditional vector control methods, such as insecticide resistance, costs, etc. An alternative strategy for controlling this disease is the use of transmission-blocking vaccines (TBVs), which consist of immunizing vertebrate hosts with vector or parasite antigens responsible for establishing the pathogen in the vector, thus stimulating the production of neutralizing antibodies that will interrupt the development of the parasite inside the vector, blocking transmission. We are working with several promising candidates in the parasite, both by testing insect infection with mutant parasites (either obtained through collaborations or by creating mutants through CRISPR) and performing artificial infections in the presence of antibodies against proteins of interest. Our group identified potential targets for TBVs in *Leishmania*, which may be essential for its development in the insect. Among them are LFR1 and LIT (related to iron metabolism), ATP11 and ABC (transporters of amino acids and other molecules, respectively) and a chitinase, which appears to play an important role in the transmission of Leishmania by the vector. We produced *Leishmania* chitinase mutants by CRISPR that were 100 times less efficient than controls in artificial transmission assays. From the identified targets, synthetic peptides were designed and inoculated into mice or rabbits, in the presence of different adjuvants. Finally, artificial infections of *L. longipalpis* with *L. i. chagasi* were performed in the presence of the sera. Initial results show promising results with the decrease of parasite load in insects infected in the presence of the immune sera.

Inova-Fiocruz, FAPERJ, INCT-Entomologia Molecular

Keywords: Leishmania, sandfly, transmission blocking vaccines.

CHARACTERIZATION OF THE REPELLENT ACTION OF INSECTICIDES ON Aedes aegypti (Diptera: Culicidae)

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Aedes aegypti is a vector of significant importance to public health, transmitting viruses responsible for diseases such as Dengue, Zika, and Chikungunya. Addressing these arboviruses primarily relies on the use of insecticides; however, prolonged usage has led to the selection of resistant populations. For this reason, many studies have been dedicated to understanding this phenomenon, yet little is known about the repellent effect of these compounds. Repellency is defined as the ability of a compound to induce behavioral avoidance in insects. In this study, we investigated the potential repellent effect on *A. aegypti* (Rockefeller strain, female adults, aged 1–9 days post-emergence, blood-deprived) of insecticides commonly used for field control (Fludora Fusion – deltamethrin 6.25% and clothianidin 50%, and Ficam VC80 – bendiocarb 80%) and their technical-grade equivalents (deltamethrin P.A. and bendiocarb P.A.). Control group assays were performed simultaneously with the test groups, using only the solvents (commercial products – water; technical grade – chloroform and vaseline). The assays were conducted with 30 insects in triplicate on different days, with test and control groups evaluated simultaneously. Contact repellency was assessed using an adapted HITSS methodology with WHO kit tubes, confirming repellency for commercial products (52% for Fludora, 42.4% for Ficam) and technical grades (63.2% for deltamethrin and 55.9% for bendiocarb). Spatial repellency was evaluated using "hand-in-cage" tests, showing effects for commercial products (72.4% for Fludora and 15.7% for Ficam) and only for technical-grade deltamethrin (34.6%). Repellency for technical-grade clothianidin will be evaluated. The information generated by this study could contribute to a better understanding of the interaction between A. aegypti and insecticides, potentially improving chemical control strategies for this vector.

Financial Support: CAPES, ICB-UFMG, INCT-Entomologia molecular

Key-words: Culicidae, *Aedes aegypti*, insecticide repellency, Hitss, hand-in-cage

IMPACT OF FEMALE AGE AND EGG QUIESCENCE OF Aedes aegypti ON INSECTICIDE SUSCEPTIBILITY

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The control of Aedes aegypti is primarily based on insecticides, which have selected resistant populations. While resistance mechanisms are extensively studied in this biological model, the impact of female age and egg quiescence on insecticide susceptibility remains unknown and was the focus of this study. To evaluate the influence of female age, three groups of 45 A. aegypti (Rockefeller strain, 1–3 days post-emergence, fed with 10% sucrose ad libitum) couples were assembled on day 0. On day 2, a blood meal was provided, followed by the insertion of an oviposition container. On day 5, the container was removed, and the paper strips with eggs were dried. On day 8, the eggs were hatched in dechlorinated water, and the L3/L4 larvae (n=30, triplicates) were subjected to deltamethrin GT (0.05 µg/mL in ethanol P.A.) following gold-standard bioassays. This procedure was repeated over three consecutive weeks with the groups in parallel, with egg strips originating from the 1st, 2nd, 3rd, and 4th gonotrophic cycles. Mortality data were analyzed using PROBIT regression and ANOVA. Lower susceptibility to deltamethrin was observed in larvae from the 2nd cycle (2.32%) compared to the 1st (17.80%) and 3rd (39.25%) cycles. Larvae from the 4th cycle could not be evaluated due to high parental mortality. To assess the impact of egg quiescence, the methodology above was applied using only eggs from the 1st gonotrophic cycle. The egg strips were divided into four parts and hatched 3, 30, 60, and 90 days post-oviposition. Larvae from eggs with 60 days of quiescence showed higher susceptibility (35.28%) compared to those hatched after 3 (24.44%), 30 (25.93%), and 90 (22.41%) days. These findings demonstrate that the age of A. aegypti females and egg quiescence significantly affect susceptibility to deltamethrin.

Financial support: ICB-UFMG, INCT-Entomologia Molecular

Keywords: *Aedes aegypti*, female age, egg quiescence

PARASITOSIS IN HIGH SCHOOL BIOLOGY TEXTBOOKS (PNLD 2018-2021): A QUALITATIVE ANALYSIS WITH AN EMPHASIS ON ENTOMOLOGY

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Textbooks are essential pedagogical tools that facilitate the comprehension of learning objectives. The Ministry of Education, through the National Textbook Plan (PNLD), provided ten educational collections for adoption by public schools across Brazil during the 2018-2021 triennium. Among the contents covered in 2nd-year high school biology textbooks, parasitology stands out. Despite variations in topic depth and approach, the material presented is expected to be accurate and appropriate for students. In this context, it is crucial to evaluate the textbooks provided by the PNLD and monitor the evolution and accuracy of their content with each triennium. This study aimed to analyze the representation of parasitosis, emphasizing entomology qualitatively, in PNLD 2018-2021 high school biology textbooks, aiming to later compare them with the PNLD 2022-2025 textbooks. Based on the "Guia de Bolso das Doenças Infecciosas Parasitárias" of the Ministry of Health, the analyzed content was classified as complete, sufficient, incomplete, or absent. None of the textbooks addressed all the topics outlined in the guide. On average, only 46% of the evaluated content was classified as complete. Among the neglected topics, "vector morphology" and the "symptoms of vector-borne diseases" were prominent. While all textbooks included figures, only two provided illustrations for all evaluated topics. In terms of exercises, two textbooks stood out in quality and quantity. The *Biologia* textbook (Silva Junior, 2016) was considered the most comprehensive, while *Biologia Unidade e Diversidade* (Favareto, 2016) presented the most in-depth treatment of the evaluated topics. The next phase of this study will involve analyzing the PNLD 2022-2025 textbooks to assess content quality and coverage improvements.

Keywords: textbook, biological sciences, review, entomology.

Funding: INTC-Entomologia Molecular, ICB-UFMG

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SILENCING OF LIPID METABOLISM PATHWAY GENES IN *RHODNIUS PROLIXUS* INFECTED BY *TRYPANOSOMA RANGELI*

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Triatomines are nocturnal hematophagous hemimetabolous insects that feed in large and spaced portions. Energy from food is stored in the form of triglycerides (TAG) in the fat body. On demand, these lipids are transported by the insect's hemolymph to different organs through a specific carrier, lipophorin. In addition to *Trypanosoma cruzi*, the etiological agent of Chagas disease, triatomines are capable of transmitting the protozoan *Trypanosoma rangeli*. Although it does not cause disease in humans, *T. rangeli* can cause different pathogenic effects in insects, in addition to physiological and behavioral changes. Studies conducted by our group have shown that infection by *T. rangeli* in *Rhodnius prolixus* promotes an increase in the amount of total lipids and diacylglycerides (DAG) in the hemolymph, as well as the accumulation of TAG in the fat body of fifth-instar nymphs. Thus, when evaluating the expression of the lipophorin (*lp*) and diacylglycerol acyltransferase (*dgat*) genes, which are involved in the transport and production of lipids, respectively, we found an increase in the expression of both genes. This suggests that *T. rangeli* is capable of manipulating the lipid metabolism of its insect vector. Based on these data, we performed the silencing of the *lp* and *dgat* genes, using the RNA interference (RNAi) technique, in 10 days old fifth-instar nymphs of *R. prolixus*. After one week of dsRNA injection, uninfected insects showed a silencing rate of 78.43% and 98.04% for *lp* and *dgat*, respectively. Eighteen days after silencing (28 days old insects), this rate increased to 96.94% (*lp*) and 99.49% (*dgat*), demonstrating the stability of silencing technique in this insect. Experiments evaluating the impact of gene silencing on *T. rangeli* infection showed a reduction in the number of parasites in the hemolymph of silenced insects in relation to control ones.

Supported by: VPPCB FIOCRUZ, INCT-EM, FAPEMIG, CNPq
Keywords: *Rhodnius prolixus*, *Trypanosoma rangeli*, Lipids

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ABSTRACT

GEOMETRIC MORPHOMETRY OF THE WINGS OF *Sciopemyia sordellii* (SHANNON & DEL PONTE, 1927) (DIPTERA: PHLEBOTOMINAE) POPULATIONS FROM BRAZILIAN BIOMES

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Abstract

Sciopemyia sordellii (Diptera: Psychodidae: Phlebotominae) is widely distributed across the Americas, with the most records occurring in Brazil. It is found in all five Brazilian geographical regions and across diverse biomes. This raises doubts about their identity as a single biological entity and points to the occurrence of species complex.

Here, we used geometric morphometric (GM) analyses to investigate differences in wing size and shape among *S. sordellii* populations from Brazilian biomes. After taxonomic studies, 262 specimens of *S. sordellii*, 133 males and 129 females, from four biomes, Amazon (n= 72), Caatinga (n= 31), Cerrado (n= 80) and Atlantic Forest (n= 39), were selected. These specimens come from biological collections in Brazil, and from fields collected from different research projects.

The right wing of each specimen was analyzed. To GM, Generalized Procrustes Analysis (GPA), Principal Component Analysis (PCA), Canonical Variable Analysis (CVA), Discriminant Functions (DF), and Mahalanobis distances (dM) were carried out using the MorphoJ program, and centroid size analyzed in GraphPad Prism.

Wing analyses revealed important differences in wing size and shape between *S. sordellii* specimens from the Caatinga biome and those from the Amazon, Cerrado and Atlantic biomes, in both males and females. Environmental factors, particularly the high temperatures and water scarcity characteristic of the Caatinga biome, may contribute to the observed differences in wing size and shape between this population and others.

Molecular investigations are currently being conducted on these *S. sordellii* populations to evaluate the concordance between genetic differentiation and the results obtained from geometric wing morphometry.

Supported by: CAPES

Keywords: Integrative Taxonomy, Phlebotominae, Species complex.

SOCIOECONOMIC PROFILE AND KNOWLEDGE ABOUT *AEDES AEGYPTI* AND ARBOVIRUSES OF THE RESIDENTS OF THE SANTOS DUMONT NEIGHBORHOOD IN THE CITY OF CÁCERES/ MATO GROSSO, BRAZIL.

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Abstract

Arboviruses such as dengue, chikungunya and Zika cause major outbreaks every year in many countries. The main control for these diseases is the fight against the *Aedes (Stegomyia) aegypti (L.)* mosquito. For this to happen, it is important that the population is informed and participative. The aim of this study was to survey the socioeconomic profile of residents of the Santos Dumont neighborhood in Cáceres/MT and their knowledge of *Aedes aegypti* and arboviruses. The study was approved by the Ethics Committee of the State University of Mato Grosso (opinion no. 6.717.917), in which the residents answered a structured questionnaire. Of all those interviewed, 52% were female and 48% male, and 76.2% had incomplete primary education and an income of up to one minimum wage. The majority (90%) were over 60 years old. With regard to knowledge of *Ae. aegypti,* 85.7% of the residents said they had heard of the mosquito, and around 52.7% had already contracted a disease transmitted by *Ae. aegypti.* However, 66.7% don't take part in community activities on arbovirus vectors and 75% think that the community, including the government and the population, are primarily responsible for combating the mosquito. Furthermore, 76.2% reported that awareness campaigns about *Aedes aegypti* could be more efficient and 31.6% said that endemic disease agents had never been to their neighborhood. Furthermore, 80% believe that *Aedes aegypti* has a significant impact on public health. The data reveals that, even with knowledge about the mosquito, there is a lack of preventive measures that contribute to the spread of arboviruses, showing that there are gaps in community participation and in the effectiveness of awareness campaigns. The data also reinforces the importance of integrated actions between the population and public authorities, prioritizing initiatives that increase community awareness.

Supported by: UNEMAT, FAPEMAT.

Keywords: *Aedes aegypti,* Arboviruses, Socioeconomic.

HUMAN-MITE INTERACTION: A CASE OF *ORNITHONYSSUS BURSA* (MESOSTIGMATA: MACRONYSSIDAE) AND ASSOCIATED PATHOGENS IN BRAZIL

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Abstract

Ornithonyssus bursa (Berlese), commonly known as the "tropical fowl mite," is a hematophagous mite found in domestic and wild birds, which occasionally bites humans. Human infestation primarily occurs when abandoned nests are near homes or when humans handle infested birds. In Brazil, the first report of human dermatitis caused by *O. bursa* was in the municipality of Viamão, State of Rio Grande do Sul. Since then, this mite has been reported in the states of Espírito Santo, Maranhão, Paraíba, Paraná, Rio de Janeiro, Rio Grande do Sul, and São Paulo, with most records concentrated in the Southeast and South regions of the country. In this study, we report a new case of *O. bursa* biting humans, accompanied by molecular characterization and the detection of pathogens and endosymbionts. One of the co-authors, residing in Campinas, São Paulo, observed the construction of a pale-breasted thrush (*Turdus leucomelas*) nest in her residence, which eventually also served as a breeding site for a chick of the parasitic shiny cowbird (*Molothrus bonariensis*). After the nest was completely abandoned, the co-author began experiencing mite bites that caused inflammation. The mites were collected and sent to the Laboratory of Zoological Collections, Butantan Institute, for morphological and molecular identification, including pathogen screening. After extracting DNA from 50 *O. bursa* specimens, we obtained partial *cox1* gene sequences, which revealed a single haplotype when compared. Furthermore, these specimens were screened for Anaplasmataceae pathogens, resulting in the detection of *Ehrlichia* sp. and *Wolbachia* sp. strains. This case represents a new locality and host record for *O. bursa*. Additionally, based on the analyzed material, we were able to provide, for the first time, partial *cox1* sequences, as well as the first records of the pathogenic bacterium *Ehrlichia* and the endosymbiont *Wolbachia* in *O. bursa* specimens from Brazil.

Supported by: FAPESP, CNPq.

Keywords: tropical fowl mite, parasitism, dermatitis

A NEW CASE REPORT OF CHIGGER MITES (TROMBIDIFORMES: TROMBICULIDAE) PARASITIZING HUMANS IN BRAZIL

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Abstract

Chigger mites, known as “mucuim” or “trombiculídeo” in Brazil, includes three active stages, and only the larvae are parasites, while the other stages are free-living predators. Once attached to a host, the larvae feed on the host’s epithelial tissue for several days, gradually forming the stylostome. An inflammatory reaction can be formed during this feeding process, known as trombiculiasis or trombiculosis. In Brazil, two forms of trombiculiasis can occur in humans: the nodular form, caused by the parasitism of *Apolonia tigipioensis* Torres & Braga, and the superficial form, caused by the parasitism of species from the genus *Eutrombicula* Ewing. One of these species, *Eutrombicula batatas* Linnaeus, has been recorded in the states of Amazonas, Maranhão, Mato Grosso, Pernambuco, Pará, Paraná, Rio de Janeiro, Rio Grande do Sul, and São Paulo, associated with different species of birds and mammals. Parasitic mites with low host specificity, such as *E. batatas*, can exhibit anthropophilic behavior. This has been observed in other parts of the Neotropical region, where this species has been recorded biting humans, including in Brazil (Maranhão State). The material analyzed in the present study was obtained from case reports of individuals who had been bitten in a region of the Amazon Forest in the Monte Negro municipality, Rondônia State. The specimens were cleared, slide-mounted, and identified as *E. batatas*. The slides were deposited at the Acarological Collection of the Instituto Butantan (IBSP) under the number IBSP 18658. Here, we provide the first record of *E. batatas* in Rondônia state and the second record of superficial trombiculiasis by *E. batatas* in humans in Brazil. Identifying this species in a new region highlights the widespread distribution of *E. batatas* and its potential to cause human parasitism in the Neotropical region, further emphasizing the importance of monitoring and studying parasitic mite species to understand their impact on human health better.

Supported by: FAPESP, CNPq, EpiAmo

Keywords: Chiggers, trombiculiasis, Humans

INVESTIGATION OF THE EFFECTS CAUSED BY THE INTRODUCTION OF THE ENDOSYMBIONT *WOLBACHIA PIPIENTIS* INTO *LUTZOMYIA LONGIPALPIS* AND THE INFECTION OF THIS VECTOR BY *LEISHMANIA*

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Leishmaniasis, caused by *Leishmania* spp. and transmitted by sand flies, remains a major public health challenge. *Lutzomyia longipalpis* is the primary vector of visceral leishmaniasis in the Americas, and insecticide-based control strategies have shown limitations. *Wolbachia pipientis*, an endosymbiont successfully used to control arboviruses in *Aedes aegypti*. This study evaluated the establishment of *Wolbachia* in *L. longipalpis* and its effects on *Leishmania infantum* infection. Our purpose in this work is to investigate the use of this endosymbiont in the control of leishmaniasis transmitted by sandflies. We tested the co-infection of sandflies with the bacteria through artificial blood feeding from Lulo embryonic cells previously stably infected with the wMelPop-CLA strain together with *L. infantum*, or only infected Lulo embryonic cells as control. A pool of 10 insects was collected for RNA extraction and cDNA synthesis, using RT-qPCR. We evaluated the permanence of the bacteria in the insect for up to 144 hours after infection, targeting the wsp gene of Wolbachia. We also verified that the presence of the bacteria showed a reduction in the parasite load in relation to the group without the endosymbiont, through the quantification of the Leishmania Actin gene. We also studied the expression of insect immune genes, and verified that the presence of the endosymbiont activated the Toll pathway, demonstrating that *Wolbachia* can modulate this immune pathway. To study the potencial establishment of of the endosymbiont in the sandfly, females were fed with cells transfected with Wolbachia and detection of the bacteria occurred for up to 10 days after feeding. We also evaluated the possibility of vertical transfer of *Wolbachia* and detected the bacteria in first generation male and female insects. Our findings suggest that *Wolbachia pipientis* could be a promising tool for the biological control of *L. longipalpis*, contributing to new strategies for leishmaniasis prevention.

Supported by: PGBCM/IOC/Fiocruz.

Keywords: Vector control; *Lutzomyia longipalpis*; *Wolbachia pipientis*.

ANALYSIS OF THE ALLERGENIC POTENTIAL EXTRACT OF THE HOUSE DUST MITE *Glycycometus malaysiensis*.

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Introduction: Dust mites are one of the primary sources of aeroallergens and are closely associated with allergic diseases such as asthma and rhinitis. Among these, *Glycycometus malaysiensis* is a mite species found in house dust, which is morphologically similar to *Blomia tropicalis*, making its identification challenging. Studies have shown that patients with asthma and rhinitis often test positive for *G. malaysiensis* through skin prick tests and specific IgE reactivity. Despite its potential clinical significance, the specific allergens and allergenic potential of *G. malaysiensis* remain poorly characterized. Investigating these allergens is crucial for understanding the species' contribution to allergic diseases and for developing more effective diagnostic and therapeutic tools. **Objectives** To cultivate *G. malaysiensis* in a laboratory setting, extract its antigens to produce an allergenic extract, and evaluate its reactivity using serum from atopic and non-atopic individuals through ELISA. Additionally, to analyze the mite's proteins using one-dimensional electrophoresis and immunoblotting and compare the IgE reactivity of *G. malaysiensis* with other clinically important mites. **Material and Methods:** Dust mite cultures were selected and characterized using PCR-based molecular identification. Protein extracts were prepared from pure cultures containing *B. tropicalis* and *G. malaysiensis*, while the *Dermatophagoides pteronyssinus* protein extract was obtained from mites purchased from a specialized supplier. These protein extracts were utilized in indirect ELISA assays and immunoblotting to evaluate IgE reactivity, using sera from atopic and non-atopic individuals from Salvador, Brazil. **Results and conclusions:** The analysis revealed that atopic patients exhibited similar IgE reactivity to the extracts containing *B. tropicalis* and *G. malaysiensis*. Interestingly, IgE reactivity to the *Dermatophagoides pteronyssinus* extract was lower compared to the extracts from *B. tropicalis* and *G. malaysiensis*. This study underscores the immunogenic potential of *G. malaysiensis* and its significance within the tested population from Salvador, Brazil.

Support: Foundation for Research Support of the State of Bahia (FAPESB).

Keywords: House dust mites**,** allergies**,** *G.malaysiensis*

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MAINTENANCE TECHNIQUES FOR *LUTZOMYIA LONGIPALPIS* REARING IN AN INSECTARY AT GONÇALO MONIZ INSTITUTE, FIOCRUZ-BAHIA

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Abstract

Lutzomyia longipalpis (Diptera: Phlebotominae) is the primary vector of *Leishmania* parasites, the causative agents of visceral leishmaniasis. These insects are holometabolous, as their larval stages occur in soil rich in organic matter. Females are obligate hematophages, requiring blood meals from vertebrate hosts to support oogenesis, a critical factor in disease transmission. Given their medical and epidemiological importance, maintaining laboratory colonies is essential for research on parasite-vector interactions and developing innovative control strategies. This study details rearing techniques applied at the only *L. longipalpis* insectary in Northeast Brazil, located at Fiocruz-Bahia, during the year of 2024. Colony maintenance follows the species' natural life cycle. Eggs are surface-sterilized using 1% hypochlorite and 70% alcohol, then transferred to dental plaster-lined pots and incubated under controlled humidity, temperature, and photoperiod conditions. Upon hatching, larvae receive a specialized diet formulated by the insectary team, with feeding protocols adjusted according to density and developmental stage. Following pupation, adults are released in entomological cages and provided continuous access to a 70% sucrose solution. After an acclimation period of 4–5 days, females undergo blood-feeding on an anesthetized hamster. Engorged females are subsequently separated, quantified, and placed in oviposition pots lined with plaster, initiating a new reproductive cycle. These optimized rearing methods enable the production of over 20,000 female sand flies per month, sustaining a colony exceeding 100,000 individuals annually. This robust system supports experimental studies involving more than 1,000 sand flies weekly without compromising production capacity. By ensuring a continuous and reliable supply of vector specimens, these methodologies significantly contribute to research on visceral leishmaniasis pathogenesis and the advancement of vector control strategies.

Supported by:
Keywords: insect rearing, Sandfly, *Lutzomyia longipalpis*

TITLE: EVALUATION OF *Spirulina maxima* IN STIMULATING OVIPOSITION AND ATTRACTIVENESS OF *Aedes aegypti*

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Abstract:

Aedes aegypti is a vector of medical importance responsible for the transmission of arboviruses with a high impact on public health. Ovitrap traps use water and bait to attract females to lay eggs, but they compete with natural and artificial breeding sites found in the environment. Studies show that microorganisms associated with water solutions seem to favor the attraction of mosquitoes. Scientific evidence has indicated that organic compounds present in microalgae of the Spirulina genus could influence the oviposition behavior of *Ae. aegypti*. In this context, the present study sought to evaluate the bioactivity of the biomass of the microalga *Spirulina maxima* as an oviposition and attraction stimulant for *Ae. aegypti*. The activity index for *Ae. aegypti* oviposition was compared using pure *Sp. maxima* biomass at concentrations of 0.01 g/L, 0.02 g/L, 0.04 g/L, 0.08 g/L and 0.16 g/L. The control group was restricted to distilled water. The attractiveness of *Sp. máxima* was also compared to brewer's yeast (*Sccharomyces cerevisiae* - SC), recommended by the Ministry of Health, in three different concentrations: 0.4 g/L (suggested by the Ministry of Health), 0.02 g/L and 0.08 g/L. In all the tests, 30 females and 10 males age between 7 and 10 days were used, placed in cages measuring 30x30x30 cm. Five replicates were carried out for each experimental group. The headspace technique coupled with gas chromatography-mass spectrometry (GC-MS) was then used to identify the volatile and semi-volatile compounds released by the experimental solutions of *Sp. máxima*. The Two Way ANOVA test was used for statistical analysis.

All the *Spirulina maxima* solutions tested at different concentrations showed a significant effect on the oviposition of *Ae. aegypti* when compared to the control groups (distilled water). With regard to the comparison with yeast (SC), the results showed that *Sp. maxima* (0.8 g/L) proved to be the most effective option, outperforming yeast (0.4 g/L), while at the concentration (0.4 g/L), both showed similar performance. Yeast (SC) was superior to *Sp. maxima* when its concentration was 20 times higher than that of Spirulina (0.02 g/L). Chemical analysis revealed the presence of 11 compounds, with D-alanine standing out, representing more than 40% of the total area detected in the chromatographic analysis. These findings suggest that the pure biomass of *Sp. maxima* has the potential to be used as an attractant in ovitrap traps, contributing to vector control strategies. The results show that *Sp. maxima* can be used as an alternative in traps for monitoring and controlling *Ae. aegypti* in the field.

Supported by Coordination for the Improvement of Higher Education Personnel – CAPES

Keywords: *Aedes aegypti*, *Spirulina máxima*, oviposition stimulation, vector control

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EVALUATION OF THE IMPACTS OF A SECOND BLOOD FEEDING ON *LUTZOMYIA LONGIPALPIS* INFECTED WITH *LEISHMANIA INFANTUM*

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Abstract

Visceral leishmaniasis is a neglected disease in the Americas, caused by *Leishmania infantum*. Its transmission cycle involves the sand fly vector *Lutzomyia longipalpis*, and vertebrate hosts (humans and dogs), contributing to the persistent spread of the disease in urban areas of Brazil. During blood feeding, infected sand flies inoculate vertebrate hosts with the metacyclic promastigote forms of *Leishmania*. It is known that sand flies, like other hematophagous insects, take multiple blood meals throughout their life cycle. However, the impact of a second blood meal with uninfected blood on the parasite-vector interaction, and its implications for protozoan transmission, remains poorly understood. This study aims to assess the effects of a second blood meal on *Leishmania infantum*-infected sand flies by comparing parasite load and metacyclic promastigote forms between single and second-blood-fed groups, as well as monitoring post-infection mortality. Female sand flies reared at LAIPHE were artificially infected using parasite-laden dog blood. After six days, half of the surviving insects were offered a second blood meal with uninfected hamster blood. On Day 12 post-infection, midguts were dissected to quantify parasite load and metacyclic promastigote forms. The study hypothesizes that a second uninfected blood meal may enhance metacyclic promastigote differentiation in sand flies, thereby influencing the epidemiology of the disease and providing new avenues for research. Mortality was monitored daily, with a significant challenge being the maintenance of sand fly survival until Day 12. In the initial trials, 1,000 insects were used, with an average survival of 500 by Day 6. Of these, 250 sand flies were offered a second blood meal. Preliminary findings from Day 12 dissections indicate that the second-blood-fed group exhibited higher parasite loads and a greater number of metacyclic promastigotes compared to the single-blood-fed group.

Supported by: FAPESB, FIOCRUZ, CAPES, CNPq

Keywords: *Leishmania infantum*, *Lutzomyia longipalpis*, second blood meal

INSULIN AND ITS ROLE IN INTESTINAL ALKALINIZATION OF *LUTZOMYIA LONGIPALPIS*

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Lutzomyia longipalpis is an insect belonging to the family Psychodidae. This insect is also the primary vector of *Leishmania infantum* in the Americas, the etiological agent of American visceral leishmaniasis. When this insect feeds on blood, large changes occur in the intestinal pH value. This pH change influences the production and activity of digestive enzymes, as well as the developmental process of *L. infantum*. In *L. longipalpis* females fed on sucrose, the luminal pH is observed to be around 6.0. However, when females feed on blood, the intestinal pH increases, reaching values close to 8.1. Three main mechanisms have been identified as responsible for this pH elevation: the first is related to the release of CO_2 from the ingested blood; the second involves hormonal signaling triggered during blood feeding; and the third consists of a transport system in which certain amino acids are co-transported with H^+ ions from the intestinal lumen to the enterocyte cytoplasm. The present study aims to identify and describe intra- and extracellular steps of the alkalinization mechanism associated with hormonal signaling after blood feeding. It has been demonstrated that the influx of Ca^{2+} into enteroendocrine cells of *L. longipalpis* females leads to the release of a molecule into the insect's hemolymph, which induces intestinal alkalinization in a 100% of tested midguts. Furthermore, the ex vivo application of bovine insulin to the intestines of *L. longipalpis* females reproduces the same effects as hormones released by enteroendocrine cells, with all midguts showing alkalization response. Gene expression results for insulin and its receptor (LLONM1_008043 and LLONM1_009708, respectively) confirm the presence of these genes in the midgut of *L. longipalpis* females. Although not significant, blood-fed females showed a 4.6-fold increase in insulin receptor expression. These findings indicate that insulin is the hormone responsible for intestinal alkalinization.

Supported by: CAPES; FAPEMIG; CNPq; INCT-EM
Keywords: *Lutzomyia longipalpis*; intestinal pH; insulin

How does *Plasmodium vivax* infection modulate gene expression in *Anopheles aquasalis*?

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Malaria remains a major global health issue, with 263 million cases and 597,000 deaths reported worldwide. In the Americas, Brazil, Venezuela, and Colombia account for most cases, with the Amazon region representing 99.6% of infections. *Anopheles aquasalis*, a primary *Plasmodium vivax* vector in coastal regions, is a key laboratory model for malaria research, plays a critical role in the study of vector-parasite interaction. This study aimed to analyze changes in gene expression of *An. aquasalis* during *P. vivax* infection to identify potential molecular targets related to vector-parasite interactions. The female of *An. aquasalis* were fed via membrane with blood from adult volunteers (≥18 years) infected with *P. vivax* (CAAE: 13653019.2.0000.0005) and inactivated blood was used for the control group. Fifteen days post-infection, a sub-sample of mosquitoes were dissected, to establish the infection rate, and afterwards, the RNA was extracted from pools of 10 females using TRIzol™ (Thermo Fisher Scientific, MA, USA). RNA-seq libraries were prepared and sequenced on the Illumina HiSeq2000 platform. Quality-checked was done by using FastQC v0.11.8 and downstream analyzes was performed using CLC Genomics Workbench v23 (Qiagen, Germany). RNA-seq libraries were prepared and sequenced on the Illumina HiSeq2000 platform. The *An. aquasalis* genome, available through VectorBase, was used to map the sequences, and the determination of the differentially expressed genes was selected by the following criteria; log2 fold change ≥ 1.5 and FDR $p<0.05$. Comparative analyses revealed 279 differentially expressed genes, with 244 showing a fold change ≥1.5. Of these, 209 (85.66%) were upregulated, and 35 (14.34%) were downregulated. Functional analysis identified genes involved in proteolysis ($p = 0.006$), carbohydrate metabolism ($p = 0.0008$), and cation binding ($p = 0.012$). Genes associated with peptidase activity ($p = 0.003$) and extracellular region localization ($p = 0.006$) were also identified. In conclusion, *P. vivax* infection induces gene expression changes in *An. aquasalis*, activating genes related to biological processes, molecular functions, and cellular components, providing new insights into vector-parasite interactions and vector control.

Supported By: Capes, CNPq and INCTEM

Keywords: *Plasmodium vivax*; gene expression; vector control

IMPORTANCE OF RABBIT FARMING IN THE MAINTENANCE OF WILD TRIATOMINAE

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The domestic rabbit (*Oryctolagus cuniculus*), typically raised in floor systems or cages, may facilitate the maintenance of triatomine bugs, which are vectors of *Trypanosoma cruzi*, due to their confinement in restricted areas. This study aims to report the occurrence of kissing bugs in rabbit farming at the Instituto Federal Baiano - Campus Santa Inês. The municipality of Santa Inês is situated in the Jiquiriçá Valley in the southwestern region of Bahia. This Institute hosts resident students and various livestock species, including goats, sheep, horses, cattle, pigs, birds, and rabbits. Rabbit farming is conducted in a shed adjacent to native vegetation, consisting of Gigante de Flandes, Nova Zelândia, and Borboleta breeds, which are distributed on the ground and in cages. Since the first specimen of the kissing bug was identified in the rabbit farming facility in 2023, six other T. cruzi vectors have been captured on the premises. All captured insects belong to the species *Triatoma melanocephala*, a triatomine considered to be wild. Due to a lack of knowledge among the employees in the rabbit farming sector regarding the identification of kissing bugs, they reported having observed other insects resembling *T. melanocephala* without appropriate diligence. The species identification and subsequent reporting were conducted at the Pathology and Molecular Biology Laboratory of the Instituto Gonçalo Moniz/FIOCRUZ, to which the insects from the Parasitology Laboratory of the Instituto Federal Baiano were sent for initial identification and serving as a point of reference for receiving suspected insects at the Institution. Entomological surveillance is also crucial due to the associated risks of infections in both animals and individuals who reside on campus.

Keywords: Rabbit farming, Chagas disease, *Triatoma melanocephala*

CHAGAS DISEASE: FROM UNKNOWLEDGE TO THE RISK OF VECTOR TRANSMISSION

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Chagas disease is a parasitic infection caused by *Trypanosoma cruzi* and has historically been transmitted by insect vectors of the Triatominae subfamily. The objective of this study was to report the risk of vector transmission of Chagas disease in the municipality of Santa Inês, Bahia. Located in the southwestern region of Bahia, within the Jiquiriçá Valley, Santa Inês has no recorded autochthonous cases of Chagas disease and is therefore classified as a silent area for this condition. However, in 2022, following an incident in which a child was bitten by a Triatominae bug, the Parasitology Laboratory of the Instituto Federal Baiano – Campus Santa Inês initiated investigations about the presence of these vectors in the region. After an initial screening, the collected insects were sent to the Pathology and Molecular Biology Laboratory (LPBM) at the Gonçalo Moniz Institute (FIOCRUZ), where, by 2024, 34 specimens of barber bugs—identified as *Panstrongylus geniculatus* and *Triatoma melanocephala* were confirmed. These specimens were captured on a private farm, at a rural educational institution, in the municipal headquarters, and in three other rural locations. Reports generated by the LPBM were subsequently sent by IF Baiano to the Municipal Health Department, alerting authorities to the need for enhanced surveillance. The spatial distribution of the barber bugs underscores a potential risk for vector transmission of Chagas disease in Santa Inês, Bahia. These findings highlight the importance of continuous monitoring and the implementation of preventive measures to mitigate the risk of Chagas disease in the region.

Keywords: Triatominae, Parasitology, *Trypanosoma cruzi*

INVERTEBRATE-DERIVED DNA (iDNA) TO IDENTIFY SANDFLIES' BLOODMEAL.

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Metabarcoding data obtained by Next Generation Sequencing (NGS) has been used to determine the species in mixed biological samples, like DNA obtained through the gut content of invertebrates that feed on vertebrates (invertebrate-derived DNA, iDNA).
In the present study, we identified the vertebrate species from iDNA samples of female sand flies using the metabarcoding approach. We also compared the results among a mammal-specific (16SrRNA), broader vertebrate-specific (12SrRNA) mini-barcodes, and a barcode of the *CytB* mitochondrial gene.
Phlebotomine sand flies were collected in an endemic area of Cutaneous Leishmaniasis (CL) located in the Cacao Region, southeastern of Bahia, Brazil. We analyzed forty female sand flies distributed in thirteen samples of seven different sand fly species including CL vectors in Brazil.
The Operational Taxonomic Units (OTUs), obtained after metabarcoding sequencing, were compared with the sequences available in the GenBank NCBI® for species identification. The criteria of high percentage of matches (98%-100%) was used for OTU assignment at species level, combined to reference databases of the expected species occurrence in the study area.
Metabarcoding results revealed twenty-one OTUs of vertebrate's species, distributed in forty GenBank® sequences, including primates (four OTUs), rodents (four) and ungulates (six). Surprisingly, non-mammals' species, such as reptiles (one OTU) and amphibians (three) were also detected. The mini-barcode 16SrRNA identified twelve OTUs in 69 detections, 12SrRNA and CytB eleven OTUs in 30 and 38 detections, respectively.
Canis lupus, *Equus asinus*, *Equus caballus* and *Homo sapiens* were the sandflies' bloodmeal identified by the three targets used, presenting the highest ratio of detection.

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Keywords: Phlebotominae, Metabarcoding, *Leishmania*.

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INFECTION BY *Trypanosoma cruzi* AND FOOD SOURCE OF TRIATOMINES FROM RURAL AREAS OF MUNICIPALITIES IN THE SOUTHEAST OF THE STATE OF PIAUÍ, BRAZIL

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Abstract
High rates of peri- and intradomiciliary infestation by triatomines infected with *Trypanosoma cruzi* and an estimated seroprevalence of 5.8% for human infection have been reported in the southeastern region of the state of Piauí. Thus, this study aims to evaluate the natural infection of triatomines by *T. cruzi* through fresh examination of intestinal contents and xenoculture. The identification of the food source of these vectors was performed using sequencing of the mitochondrial 12S rRNA gene to detect the blood in the triatomine's intestine. The consensus DNA sequences from the intestinal contents were compared with sequences deposited in GenBank using the BLASTn tool to identify the blood in the triatomine's intestine. A total of 520 triatomines were captured, belonging to the species *Triatoma brasiliensis* (n=468), *Triatoma pseudomaculata* (n=48), and *Panstrongylus lutzi* (n=4). Nymph and adult stages of *T. brasiliensis* were found in both peri- and intradomiciliary environments, while *T. pseudomaculata* was captured in peridomiciliary areas. *P. lutzi* was found exclusively in the peridomiciliary environment in the adult stage. The infection rate in *T. brasiliensis* was 10% (10/100) in the intradomicile and 5.9% (22/368) in the peridomicile, while for *P. lutzi*, it was 25% (1/4) in the peridomicile. No infected specimens of *T. pseudomaculata* were identified. The food source was identified in 19 infected specimens of *T. brasiliensis*. *Homo sapiens* DNA was observed in 42.1% (8/19), *Felis catus* in 26.3% (5/19), and *Canis familiaris* in 21% (4/19). The presence of infected *T. brasiliensis* specimens and the detection of human blood in their intestinal contents indicate a risk of *T. cruzi* transmission to the rural population. These findings reinforce the need for continuous actions to control these vectors and combat the infection in the region.

Supported by: CAPES, CNPq

Keywords: vector transmission; PCR; domestic environment.

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ECOLOGICAL DYNAMICS AND LEISHMANIASIS TRANSMISSION IN URBAN SAND FLIES: FIRST EVIDENCE FROM PORTO VELHO, RONDÔNIA

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[1]LABORATÓRIO DE ENTOMOLOGIA - FUNDAÇÃO OSWALDO CRUZ- FIOCRUZ, RONDÔNIA, BRASIL [2]PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA EXPERIMENTAL -FIOCRUZ, RONDÔNIA/UNIR, BRASIL. [3]GRUPO DE ESTUDOS EM LEISHMANIOSE, INSTITUTO RENÉ RACHOU, FUNDAÇÃO OSWALDO CRUZ, BELO HORIZONTE, MINAS GERAIS, BRASIL. [4]INCT-EpI-AmO – INSTITUTO NACIONAL DE EPIDEMIOLOGIA DA AMAZÔNIA OCIDENTAL.

Abstract

This study was conducted for the first time in remaining urban forests of Porto Velho, Rondônia, Brazil, and aimed to characterize the ecological aspects of the phlebotomine phlebotomine transmission cycle of leishmaniasis and other agents found in insects. Ten locations in urban forest fragments were selected for collection in 2021 (October, November, December), followed by quarterly collections in 2022 and 2023. HP light traps were installed in two forest strata: canopy and ground. Males were mounted and identified, while females were dissected, with the head and the last three segments used for species identification. The thorax and abdomen were grouped into pools of 2 to 10-20 specimens from the same location, species, and stratum or analyzed individually for Trypanosomatidae DNA research using PCR targeting the V7V8 fragment (18S gene). Engorged females were analyzed using PCR targeting the 358 bp fragment of the conserved region of the mitochondrial DNA (cytb) of vertebrates. A total of 2,371 phlebotomines, representing 52 species in 12 genera, were collected. The analysis revealed DNA of *Leishmania infantum* in females of *Bichromomyia flaviscutellata* (1), *Nyssomyia* complex *Antunesi* (6), *Nyssomyia umbratilis* (1), *Nyssomyia sp.* (1), *Psychodopygus ayrozai* (1), *Psychodopygus davisi* (3), *Psychodopygus paraensis* (1), and *Sciopemyia sordellii* (1). DNA of *Trypanosoma minasense* was found in two samples of females from *Ny.* complex *Antunesi* and *Psychodopygus* series *Guyanensis*. DNA of *Trypanosoma sp.* was detected in *Sc.* sordellii (4), *Sciopemyia spp.* (1), and *Sciopemyia vattierae* (1). Sequencing of the cytb confirmed *Homo sapiens*, *Dasypus novemcinctus*, and *Tamandua tetradactyla* as blood sources for different phlebotomines. The identification of *Le. infantum* in phlebotomines collected in urban forest areas highlights the need to develop further studies to identify hosts/reservoirs in these regions.

Supported by: CNPq, CAPES, FAPERO, INCT- EpI-AmO.
Keywords: Plebotominae, Amazônia, Trypanosomatidae.

PHLEBOTOMINE SAND FLIES (DIPTERA, PSYCHODIDAE) IN AN ENDEMIC AREA FOR VISCERAL LEISHMANIASIS IN THE STATE OF PIAUÍ, BRAZIL

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In Brazil, visceral leishmaniasis (VL) is a life-threatening zoonosis caused by *Leishmania infantum*, transmited mainly by *Lutzomyia longipalpis* sand flies (Diptera, Psychodidae) and predominant in the Northeast region, including Piauí state. The knowledge of the sand fly fauna and behaviour is essential for taking better prevention and control measures against VL in endemic locations. Thus, the objective of the present study was to identify the sand fly fauna from Picos, an endemic municipality for visceral leishmaniasis in the southeast of Piauí state. Sand flies were collected using light traps set in five points in neighbourhoods with reported cases of VL in the urban area of the city. Traps were installed for two consecutive nights from 5 PM to 8 AM. The collections occurred from november 2024 to february 2025 and the sand flies were identified to species level using light microscopy following the classification proposed by Galati. A total of 31 collections were performed and 762 sand flies were captured. The mean of collected sand flies per trap was 24,6, ranging from 0 to 248. Sand flies were catched in all points of collection. November was the month with the lowest number of collected flies (58) and the highest number of captured flies was registered in february (392). Males represented 71,5% of the sampled flies and females, 28,5%, in a proportion of 1:2,5 (F:M). All (100%) of the sand flies were identified as *Lutzomyia longipalpis.* Male *L. longipalpis* populations 1S (one spot) and 2S (two spots) were detected occuring in sympatry in the studied area. Our findings indicate that sand flies are present throughout the urban area of Picos, with a particular prevalence of *Lutzomyia longipalpis*, the primary vector of *L. infantum*. This underscores the potential risk of leishmaniasis transmission in urban environments. Additionally, there is a noticeable trend of increasing vector populations at the beginning of the year, coinciding with the region's rainy season.

Supported by: Universidade Federal do Piauí

Keywords: *Lutzomyia*, Psychodidae, leishmaniasis.

ANALYSIS OF THE EFFICACY OF DOUBLE BR-OVT COMBINED WITH *AEDES AEGYPTI* LARVAL EXTRACT AND BTI BIOLARVICIDE AS AN APPROACH TO ATTRACTIVE AND LETHAL TRAPS.

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Aedes aegypti and *Culex quinquefasciatus* are important representatives in the transmission of several arboviruses, such as Dengue, Zika, Chikungunya and parasites. Traps associated with oviposition baits are integrated control methods that function as stimulants. The application of attract-and-kill baits leads us to studies that explore methods such as the use of *Aedes aegypti* larval extract as a stimulant and the biolarvicide Bti for larval elimination. The experiments were initially conducted under insectarium conditions. The larvae were desiccated in an oven at 65°C for 15 hours and then aqueous extracts were produced at 0.33 larvae/mL. To evaluate the bait, paired tests were performed with different solutions: one containing 100 mL of aqueous extract (test) and another containing 100 mL of distilled water (control). Twenty pregnant females per species were introduced into separate cages and evaluated for 7 days in 12 replicates. Tests were also performed using both species together per cage. The simulated field experiment was conducted in an outdoor screened area (26 m²) where baiting potential was observed in paired Double BR-OVTs traps: Test (1 L of larval extract +2g Bti) and control (tap water only + 2g Bti). Fifty pregnant females per species were used in each of the 12 replicates over a 3-day period, with the traps collected and the eggs or rafts counted. The results of the laboratory investigation demonstrated that the preferred oviposition site was the extract for both species, with an OAI = +0.51 ($p < 0.0001$) for *A. aegypti*, while *C. quinquefasciatus* had an OAI of +0.67 and +0.43, respectively. Preliminary laboratory tests resulted in an OAI of +0.50 for *Aedes* and +0.93 for *Culex*. In a simulated field, the test and control traps were similar but indicated an attraction level for the test trap of OAI +0.09 for Culex and an OAI +0.14 for *Aedes*. Larval extracts are effective baits for stimulating mosquito oviposition.

Supported By: IAM_FIOCRUZ-PE.

Keywords: Semiochemicals, vector surveillance, vector control.

Evaluation of natural infection by *Trypanosoma cruzi* in the Truká Indigenous land, in Pernambuco, Brazil

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The state of Pernambuco, located in the Northeast region of Brazil, is considered endemic for Chagas disease and the presence of *Trypanosoma cruzi* vectors is frequently reported in households. In 2018 alone, the presence of infected triatomines was recorded in 40 municipalities. Recently, the epidemiological bulletin of Chagas disease showed the occurrence of acute cases in the brazilian indigenous population, corresponding to 2.7% and 0.8% of reported chronic cases. Therefore, for better control and planning of actions in vulnerable regions, such as indigenous lands, the entomological investigation process must be associated with sustained environmental surveillance actions. Therefore, the objective of this study was to evaluate the natural infection by *T. cruzi* in triatomines captured in the Truká indigenous land, in the municipality of Cabrobó-PE. The collections were carried out in the peridomiciliary environment, between the years 2023-2024, and the captured insects were identified and examined for the detection of *T. cruzi* through direct examination and xenoculture. In addition, the data regarding the spontaneous demand for collection of triatomines provided by the local endemic agent between the years 2017-2019 were also analyzed. During this period, 97 triatomines were captured and identified as *Triatoma brasiliensis*, with a percentage of infection by *T. cruzi* corresponding to 6.2%. Among the active search, a total of 37 species were captured between the years 2023-2024, all of the subspecies *Triatoma brasiliensis macromelasoma*, belonging to the *Triatoma brasiliensis* species complex. Regarding infection, all specimens were negative for *T. cruzi*. These data demonstrate the need for constant epidemiological surveillance to prevent contact between infected triatomines and humans and the consequent transmission of *T. cruzi*, as well as the implementation of chemical control.

Supported by: Capes and CNPq.

Keywords: Truka indigenous land, Kissing bug, Chagas disease.

EXPANSION OF ENTOMOLOGICAL SURVEILLANCE OF *Aedes sp*. USING OVITRAPS IN THE STATE OF MINAS GERAIS

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The entomological surveillance of urban arboviruses has been conducted for years through the Rapid Index Survey for *Aedes aegypti* (LIRAa/LIA in portuguese). To complement this methodology, the Ministry of Health of Brazil recommended the use of ovitraps throughout the national territory. The ovitrap consist of a black container filled with water and equipped with a wooden paddle inside. Simulating a breeding site for *Aedes aegypti*, eggs laid on the paddle can be counted and used to generate entomological indicators. These indicators are useful to evaluate pre- and post-control interventions, as well as to assess the implementation of new vector control technologies. Therefore, the State Health Department of Minas Gerais (SES/MG) monitored the implementation of ovitraps in 14 municipalities over an 18-month period. In light of this observation, a methodology was developed for the effective implementation of ovitraps across all 853 municipalities. It involves the active engagement of municipal health managers, comprehensive theoretical and practical training on ovitraps, the provision of a detailed manual for operational teams, and the implementation of a systematic methodology for continuous monitoring of these municipalities. Additionally, criteria were established to indicate a higher likelihood of the permanent implementation of ovitraps, as well as indicators for evaluating the execution of this policy. A strategy has been formulated to decentralize the methodology, enabling its replication across all 28 regional health units of the state of Minas Gerais.

Supported by: Not applicable

Keywords: entomological surveillance, *Aedes aegypti*, ovitrap

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ABSTRACT

FIRST REPORT OF *Sciopemyia vattierae* (LE PONT & DESJEUX, 1992) (DIPTERA: PSYCHODIDAE) FROM STATES OF AMAZONAS AND PARÁ, NORTHERN BRAZIL

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Abstract

Sand flies (Diptera, Psychodidae, Phlebotominae) are a diverse group and important for public health because they are vectors of pathogens such as protozoa of the genus Leishmania. This subfamily comprises about 1060 species distributed worldwide, where more than half of the species, 555, occur in the Americas. *Sciopemyia vattierae* was described by Le Pont & Desjeux (1992) from the collection of nine females and six males in the Amazon rainforest of Yucumo, Bolivia. There are also records of the species in Colombia and Peru. In Brazil, *S. vattierae* was first found in 2014, in the municipality of Rio Branco, state of Acre. Since then, there has been only one more record of S. *vattierae* in Brazil, in 2022, in the municipality of Porto Velho, state of Rondônia. Here, we present the first record of *S. vattierae* for two other states in the northern region of Brazil, Amazonas and Pará. In the Amazonas, the specimens were collected in the Hi Merimã and Paumari do Lago indigenous lands, located in the municipality of Lábrea, and in the Urucu River region, in the municipality of Coari. In the Pará, the specimens were collected in the Amazon National Park, in the city of Itaituba. The specimens were collected between 2011 and 2023, using different traps. In total, four males and six females of *S. vattierae* were identified in the municipalities of Amazonas, while in the state of Pará only female specimens were found (n=3). The finding of this species in Brazilian states east of its first record in the country reinforces the suggestion that *S. vattierae* is a cis-Andean species.

Supported by: CNPq, CAPES, FAPs
Keywords: Geographical distribution, Phlebotominae, Taxonomy.

FOOD SOURCES AND *Trypanosoma cruzi* INFECTION IN *Panstrongylus megistus*: EVIDENCE OF THE RISK OF DOMICILIARY VECTOR TRANSMISSION

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Chagas disease remains a public health concern in Brazil due to the persistent risk of vector-borne transmission by autochthonous triatomines with a high colonization capacity. *Panstrongylus megistus* is the primary vector in the municipalities covered by the Regional Health Superintendence of Divinópolis, Minas Gerais-Brazil. This study aimed to identify the feeding sources and *Trypanosoma cruzi* infection rate in *P. megistus* specimens collected between 2021 and 2024 in this region.The intestinal contents of 167 *P. megistus* specimens (125–74.8% intradomiciliary; 42–25.1% peridomiciliary) were extracted, and genomic DNA was isolated and amplified for molecular diagnosis of infection (kDNA) and food source identification (12S rRNA locus). Food source identification was performed by comparing the obtained sequences with those deposited in GenBank using BLASTN. Eight vertebrate orders were identified, corresponding to 18 species. In the intradomicile, human blood was detected in 91 (55%) specimens (42 nymphs and 49 adults), of which 20 (22%) were infected with *T. cruzi*. Dog blood was found in 10 adult triatomines, with 3 (30%) being infected. Additionally, infected triatomines feeding on opossums, rodents, chickens, cats, and bats were identified, among others that showed no association with infection. The highest prevalence of human blood was found in the bedroom, with 35 nymphs (58.3%) and 25 adults (41.7%). In the living room, human blood was detected in 19 (95%) adults and 1 (5%) nymph. In the peridomicile, the analysis of 42 insects revealed that most had fed on birds (23–54.8%), followed by rodents (7–16.7%) and dogs (3–7.14%), among other sources. Of the 5 (11.9%) infected specimens, 2 had fed on *Rattus rattus*, and 1 each on *Mus musculus*, *Gallus gallus*, and *Bos taurus*. These results highlight the infection risk to which the population is exposed, especially due to the presence of infected insects in the intradomicile, including nymphs capable of feeding on humans.

Supported by: Fapemig, Fiocruz Minas

Keywords: Chagas disease; Triatominae; Vector

LARVAL THERAPY: POSSIBILITY OF ADHERENCE TO THE TECHNIQUE BY PATIENTS WITH CRONICAL WOUNDS

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The application of sterile fly larvae to hard-to-heal wounds, with the aim of promoting biological debridement and disinfection, is known as Larval Therapy (LT). Although this technique has been known for centuries and is regularly used in the United States and European countries, in Brazil, the first application of LT was only performed in 2012 at the University Hospital of the Federal University of Rio Grande do Norte.

The aim of this study was to analyze the knowledge and possibility of adherence to the LT technique by patients with chronic wounds in the city of Maceió, Alagoas.

This is a descriptive qualitative and quantitative study conducted at the Salgadinho Medical Care Center, a reference center for the treatment of chronic wounds located in Maceió, Alagoas. The study was submitted to and approved by the Research Ethics Committee of the State University of Health Sciences of Alagoas, under CAAE 71110423.0.0000.5011. For data collection, questionnaires were applied, and medical records of patients undergoing treatment for chronic wounds were analyzed. After administering the questionnaire, patients received clarification about how LT works and its benefits. Doubts, as well as whether they accepted the use of LT were recorded.

A total of 24 patients were interviewed. Most patients with chronic wounds (91.27%) were unaware of LT and its mechanism of action. However, if the procedure were available through the Brazilian Unified Health System, 70.83% of patients would be willing to undergo the treatment. The main reasons cited included "health improvement", "better treatment", and "faster wound healing".

These findings highlight the need to promote and implement innovative therapies, such as LT, through official health agencies, so that patients with chronic wounds have an additional treatment option.

Supported by: Fundação de Amparo à Pesquisa do Estado de Alagoas – FAPEAL

Keywords: wounds; flies; facultative myiasis; maggot therapy.

ENTOMOLOGICAL SURVEY OF *Culicoides* spp. IN AN AREA WITH OROPOUCHE FEVER TRANSMISSION IN PIAU, MINAS GERAIS, BRAZIL

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Entomological monitoring of vectors in areas with arbovirus transmission is crucial for understanding transmission dynamics and implementing control measures. This study presents the results of an entomological survey of *Culicoides* spp. conducted in Piau, Minas Gerais, in January 2025, in an area with reported cases of Oropouche fever. The municipality is characterized by a tropical climate, diverse vegetation including Atlantic Forest fragments, and has banana cultivation as its main economic activity. The presence of banana trees throughout both rural and urban areas creates favorable environmental conditions for *Culicoides* proliferation across the entire municipality. The study employed multiple collection methodologies at eight strategic points, three in rural areas and five in urban areas, with outdoor traps consistently placed near banana trees in both settings. CDC light traps baited with CO_2 from yeast-molasses fermentation were installed in duplicate (indoor and outdoor) and operated during early morning and late afternoon periods. Additionally, active searches were performed using electric aspirators in indoor and outdoor environments and protected human landing catches in outdoor areas during late afternoon. Collected specimens were preserved in liquid nitrogen for viral diagnosis, with a subsample maintained in 70% alcohol for morphological identification. A total of 1,035 specimens were captured, with 96.56% collected through CDC traps. Spatial distribution indicated higher abundance in rural areas (63%) compared to urban areas (37%), despite the presence of banana trees in both areas. Samples are currently undergoing molecular analysis for viral detection and species identification. Preliminary results provide important indicators about the distribution and density of these vectors in the study area, contributing to the understanding of the local epidemiological scenario and its relationship with widespread banana cultivation.

Supported by: Not applicable.

Keywords: Oropouche Fever; Entomological Surveillance; Arbovirus.

GENOMIC AND ULTRASTRUCTURAL CHARACTERIZATION AND CYTOPATHOLOGICAL EFFECTS OF NOVEL *BACILLUS* SPP. FROM RECIFE, BRAZIL: AN ALTERNATIVE FOR THE POPULATION CONTROL OF VECTOR MOSQUITO VECTORS OF PARASITIC DISEASES.

MARCELO SOARES DE BARROS; TÚLIO DE LIMA CAMPOS; BRENA BELFORT; MARIA EDUARDA DE OLIVEIRA PEREIRA ROCHA; JOÃO LUIZ DE LEMOS PADILHA PITTA; KARINA LIDIANE ALCÂNTARA SARAIVA; OSVALDO POMPILIO DE MELO NETO, CLÁUDIA MARIA FONTES OLIVEIRA; TATIANY PATRÍCIA ROMÃO.

INSTITUTO AGGEU MAGALHÃES – IAM/FIOCRUZ-PE, RECIFE, PERNAMBUCO, BRASIL.

The control of mosquito populations is a critical strategy in attempts to interrupt the transmission cycle of several vector-borne pathogens, with bacteria of the *Lysinibacillus* and *Bacillus* genera currently being widely used as mosquito control agents. The selection of resistant mosquitoes constitutes a concerning challenge, however, with the search for new entomopathogenic bacteria offering a promising alternative for managing vector resistance. Here, we aimed to evaluate the genomic, ultrastructural, and toxic characteristics of two novel bacterial isolates exhibiting excellent and specific larvicidal activity. To achieve this, we first performed whole genome sequencing and annotation, revealing them to be phylogenetically related to *Lysinibacillus sphaericus* (Ls) and *Bacillus thuringiensis* HD-789. Genes encoding several novel and known Cry toxins were identified in the genomes. Ultrastructural analyses of sporulated inoculums were then performed using transmission electron microscopy (TEM), revealing, respectively, a triple crystal formation and parasporal bodies with toxic crystals, for the two isolates. Larvicide toxicity was also evaluated through bioassays, showing 100% larvicidal activity against *Culex quinquefasciatus* colonies resistant to the reference Ls2362 strain for the first isolate, with 100% larvicidal activity against *Culex* and *Aedes* species observed for the second isolate. Cytopathic effects in mosquito larval intestines were also visualized via TEM, revealing complete destruction of microvilli and mitochondrial damage. These analyses indicate that these isolates possess biotechnological potential to produce novel biolarvicides.

Supported by: PROEP IAM/FIOCRUZ-PE, FACEPE, CAPES, NPT-FIOCRUZ.

Keywords: Biolarvicides, Mosquitocidal Toxins, Culicidae.

FIRST RECORD OF *Panstrongylus megistus* (BURMEISTER, 1835) (HEMIPTERA, REDUVIIDAE, TRIATOMINAE) ON ILHA GRANDE, RIO DE JANEIRO, BRAZIL

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Chagas disease is a parasitic infection caused by the parasite *Trypanosoma cruzi*, transmitted by insects such as *Panstrongylus megistus*, which was the first triatomine recognized as a vector of the disease. This study investigated the presence of *P. megistus* in Ilha Grande, Rio de Janeiro state. The insect was collected, identified, and analyzed to determine the *T. cruzi* infection and their blood meal source. The presence of *P. megistus* was morphologically confirmed. The genetic analysis showed *Gallus gallus* as the blood meal source, but it was negative for *T. cruzi* infection. The findings expand the known distribution of *P. megistus* and highlight the need for the continued monitoring of this Chagas disease vector. Herein, we present the first record of *P. megistus* in the region, and due to the potential of *T. cruzi* transmission in the area, the installation of physical barriers to reduce this risk is recommended.

Keywords: Chagas disease. kissing bugs. *Trypanosoma cruzi*.

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THE INFLUENCE OF MIDGUT BACTERIAL MICROBIOTA OF *ANOPHELES AQUASALIS* ON SUSCEPTIBILITY TO *PLASMODIUM VIVAX* – PRELIMINARY DATA

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The intestinal microbiota of mosquitoes is an important factor in vector competence, with microorganisms capable of blocking or enhancing susceptibility to pathogens. The *Anopheles aquasalis* was colonized under laboratory conditions for studies of interaction with the *Plasmodium vivax*, as it has high infection rates, ranging from 45 to 90%. Recent work at the Insectary of the Leônidas and Maria Deane Institute showed a drastic reduction in the infection rate of this mosquito with *P. vivax* (5%), leading the research group to hypothesize that the composition of the bacterial microbiota could be influencing susceptibility. To investigate this influence, 4 groups of adult female mosquitoes were formed, with 3 groups fed for 3 days with a 5% sugar solution + antibiotic (Group 1: Kanamycin + Carbenicillin; Group 2: Carbenicillin; Group 3: Kanamycin), and the fourth group was fed only 10% sucrose (Control). On day 4, all groups were fed with a 10% sugar solution. On the fifth day, the females were fed with blood infected with *P. vivax*, using the artificial membrane feeding system. After seven days of feeding, the midguts of the females were dissected and stained for oocyst counting. Five intestines from each group were placed in 3 ml of TSB broth and cultivated in an incubator at 37°C for 24 h. The grown colonies were plated on different culture for isolation. Preliminary results showed that the females that were fed with Kanamycin and Carbenicillin separately had infection rates of 75% and 80%, respectively; the group treated with the two antibiotics had 10%, and the control group had 5%. These results indicate that bacteria that are part of the colony microbiota and are susceptible to Kanamycin and Carbenicillin may be inhibiting the development of the parasite. Further experiments will allow us to identify the bacteria that influence the infection and that could be targeted for controlling/blocking malaria transmission.

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Keywords: Malaria, *Anopheles aquasalis*, Intestinal microbiota.

Enhancing *Aedes aegypti* Surveillance: A Combined Approach Using PneuTrap3D® Ovitraps and +Lugar Platform

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Aedes aegypti, the primary vector of arboviruses, is a synanthropic mosquito of great importance for Public Health. PneuTrap3D® is a trap developed for egg capture, allowing monitoring and quantification of mosquitoes' presence. The aim of this experiment was to monitor the infestation of *Aedes* on the campus of State University of Feira de Santana - UEFS, as well as the implementation of +Lugar Platform to assess risk areas. For this, PneuTrap3D® ovitraps were distributed at pre-established points, during the periods of January to May (I) and December (II), totaling 137 and 30 traps, respectively. After one week, the traps were collected, and the eggs were counted under a stereomicroscope at 20x magnification. In period II, the collection points were marked on the +Lugar platform, which allowed for georeferencing and environmental data collection. The results were transcribed into Excel, and the Ovitrap Positivity Index (IPO = number of positive ovitraps/total number of ovitraps) and the Egg Density Index (IDO = total number of eggs counted/number of positive ovitraps) were calculated. In period I, the IPO was 80 (58.39%; 95% CI = 50.00-66.32) and the IDO was 34.01. In period II, 30 traps were used, of which two yielded positive results, with an IPO of 6.37% and an IDO of 1.50. At the end of November, the UEFS campus underwent a pest control process, which may have contributed to the low IPO and IDO in the second period. Additionally, the second period was dry and without rain, which may have also contributed to the results. The use of +Lugar revealed 11 points with the presence of potential breeding sites for larvae, such as water pots, pet food, water tanks, flowerpots, tires, and bottles. Thus, +Lugar enabled the georeferencing of potential risk areas, information that can be used to implement preventive measures, which, combined with egg count-based monitoring, could impact the control of *Aedes* populations and the incidence of arboviruses.

Supported by Universidade Estadual de Feira de Santana.

Keyword: Aedes aegypti monitoring, Georeferenced risk assessment, Vector surveillance.

MOSQUITO SURVEILLANCE IN THE UNIVERSITY OF BRASÍLIA USING LIGHT-WEIGHT ASPIRATORS

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In 2024, Brazil faced its most severe dengue epidemic to date. Additionally, data from the Brazilian Ministry of Health revealed that the Federal District reported the highest dengue incidence across the country. Given the importance of understanding transmission dynamics in areas with frequent human movement, this study aimed to analyze the spatial distribution of adult *Aedes aegypti* (Linnaeus, 1762) and *Culex quinquefasciatus* (Say, 1823) across different locations of the University of Brasília. Mosquitoes were captured over two days each month from August 2024 to January 2025 by teams of researchers using handheld battery-operated aspirators across three areas on campus I and campus II as a whole. Each day, eight predetermined sites were sampled within each area, with two rounds of aspiration at each site, lasting up to five minutes per round, and a one-hour interval between rounds. The captured insects were then transported to the laboratory for identification and counting. A total of 1,451 mosquitoes were captured, comprising 581 *Ae. aegypti* and 870 *Cx. quinquefasciatus*. Area 2 of campus I had the highest total number of mosquitoes captured, with 881 mosquitoes (360 *Ae. aegypti* and 521 *Cx. quinquefasciatus*), while campus II had the lowest, with only 46 mosquitoes (14 *Ae. aegypti* and 32 *Cx. quinquefasciatus*). The month with the lowest total mosquito capture was August 2024, during the dry season, while the highest number of captures occurred in November 2024, during the rainy season. In area 2 of campus I, the peak of *Cx. quinquefasciatus* captures was observed in September, while the peak of *Ae. aegypti* was recorded in November. These results show variation in mosquito abundance between areas, with *Cx. quinquefasciatus* being more frequent overall. Moreover, these findings indicate that the aspiration technique could detect seasonal fluctuations in adult mosquito populations, with both species exhibiting varying peak times depending on the area.

Supported By: UNB, CNPq, CAPES.

Keywords: Dengue, vector capture, spatial distribution.

SAND FLY FAUNA (DIPTERA, PSYCHODIDAE) IN THE AMAZON, *CERRADO* AND *CAATINGA* BIOMES IN AREAS OF LEISHMANIASIS TRANSMISSION IN NORTH-EASTERN BRAZIL

RONAYCE CONCEIÇÃO DE JESUS SERRÃO PIMENTA, JUDSON CHAVES RODRIGUES, ROSA CRISTINA RIBEIRO DA SILVA, ANTONIA SUELY GUIMARÃES E SILVA, MAXCILENE DA SILVA DE OLIVEIRA, KAREN BRAYNER ANDRADE PIMENTEL, LUCAS SANTOS RIBEIRO, FABIANO CORRÊA, VALÉRIA CRISTINA SOARES PINHEIRO

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Phlebotomine Sand flies (Diptera, Psychodidae, Phlebotominae) are insects of medical and veterinary importance that transmit leishmaniasis causative agents. Entomological surveys to identify biotypes help to update our knowledge of the composition of species distributed in different habitats. The main objective was to assess the diversity, abundance and spatial distribution of sand flies species between the Amazon, *Cerrado* and Caatinga biomes in the states of Maranhão e Piauí. The study was conducted in six districts in the states of Maranhão (Amazon and *Cerrado*) and Piauí (*Caatinga*). The specimens were captured between 2022 and 2023 using CDC-type light traps. In each area, the specimens were collected on two consecutive nights during the dry season. A total of 680 specimens were collected, spread over four subtribes, 11 genera and 30 species. The *Cerrado* had the highest relative abundance (51.76%) and richness (19 species), followed by the Amazon (32.65%; 15 species) and the *Caatinga* (15.59%; eight species). However, the Amazon region had the highest diversity (H′=1.88). The most abundant species in the Amazon was *Bichromomyia flaviscutellata* (25.6%), in the *Cerrado Evandromyia lenti* (42.6%) and in the *Caatinga Nyssomyia whitmani* (42.5%). The latter species is the main vector of cutaneous leishmaniasis in Brazil and was detected in all three biomes, demonstrating its good adaptation to different habitats. In addition, a total of eight proven or putative *Leishmania* vectors were identified. The differences observed between biomes in terms of richness, abundance and diversity emphasize the need for more studies about interactions between species and their role in the transmission of leishmaniasis. Therefore, this work shows that more studies in these areas are essential to support conservation measures for the wild habitats of sand flies, which could be a measure to control the spread of the disease in urban areas.

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Keywords: Entomological survey. Biota. Phlebotominae

SANDFLIES DIVERSITY AND NATURAL INFECTION BY TRIPANOSSOMATIDS IN WILD AND SYNANTROPIC ENVIRONMENT, FROM TWO LOCALITIES IN THE BRAZILIAN AMAZON REGION.

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Sandflies are known as important vectors of pathogens that cause human and animal disease around the world. In the Amazon Region, this insects present a high diversity, with many species known as vectors of *Leishmania* parasites, that cause leishmaniasis. Futhermore, some studies carried out over the past decades, have recorded natural infection by other members from the Tripanossomatidae family in this insects, but their role in the emergence of infectious disease still poorly understood. The alarming climatic and environmental crisis in the Amazon region has been shown that some sandflies species have become adaptaded to anthropic lands, which increase the risk of contact with new pathogens and emergence of new transmission cycles. Therefore, the aim of this study is evaluate the composition and diversity of sandflies fauna in differents landscapes, and the natural infection by tripanossomatids, from two areas in the Brazilian Amazon region. Sandflies samples were carried out between May/2023 to March/2024, in the municipalities of Presidente Figueiredo - PF (North from Amazonas river) and Urucurituba - UR (South from Amazonas river), using CDC light traps, in the forest and crop environments. After the sampling, sandflies were transported and sorted in the field laboratory. The females were dissecated on PBS 1x, and the midgut were analysed under microscopy for search of flagellate forms. If positive, the midgut were isolated in LIT culture médium, suplemented with 10% of FBS, and stored at 27ºC, for parasite grown. Negative midguts were stored in 99% etanol for molecular detection of tripanossomatids. Females and males were identified to species or genera level using the cladistic keys from Galati (2022). For calculate the minimum infection rate, we used the following formula: number of sandflies / number of sandflies analised x100.The data were tabulated on Microsoft Excel, the ecological analyses were performed on the the programs Past, R Studio and Dives. A total of 2275 sandflies were collected, belonging to 15 genera and 80 species. The genera most abundant were *Evandromyia* (14 species) and the most abundant species was *Micropygomyia rototaensis* (613 individuals). We recorded 8 new ocurrences of sandflies species for Amazon state: *Evandromyia aldafalcaoae, Evandromyia andersoni, Lutzomyia carvalhoi, Brumptomyia mesai* and *Pressatia calcarata*. The abundance and richness in the forest was higher than in the crop environment, on both locations, however, the diversity was higher in crop environment in the PF municipality, and no statistical difference in the diversity between both municipalities was observed. Natural infection were recorded, under microscopy, in 9 sanflies species: *Lutzomyia* sp. (1 individual/ forest environment), *Sciopemyia nematoducta* (3 individuals / 2 forest, 1 crop) *Trichophoromyia ubiquitalis* (1 / forest) *Micropygomyia rorotaensis* (1/ forest) *Evandromyia inpai* (1/forest) *Evandromyia walkeri* (1 / forest) *Nyssomyia antunesi* (1 / crop), all collected at PF municipality. The minimum infection rate was 1,85%. This results suggest a high diversity of sanflies, on both localites, even in environments under human action, and the record of tripanossomatids in different sandflies species in both environments indicate the ocurrence of different transmission cycles in this landscapes.

Supported by: CAPES, FAPEAM, FIOCRUZ, CNPQ

KEYWORDS: tripanossomatidae, sandflies, Amazon

DISSECTION OF SAND FLIES (DIPTERA, PSYCHODIDAE) FOR THE DETECTION OF *Leishmania* PROMASTIGOTES (KINETOPLASTIDA, TRYPANOSOMATIDAE) IN URBAN LEISHMANIASIS TRANSMISSION FOCI IN CAXIAS, MARANHÃO, BRAZIL

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In endemic regions, the investigation of *Leishmania* in the gut of sand flies, the detection of DNA and the quantitative infection rate are of fundamental importance to explain the role of the species as a vector. The aim of this study was to detect *Leishmania* promastigotes in sand flies and to analyze the monthly distribution of vectors in urban areas of Caxias, Maranhão. The collections were carried out in two districts (Bacuri and Pirajá) over eight months during the dry and rainy seasons. The insects were collected with CDC light traps and the live females were dissected to expose the gut. A total of 5,997 sand flies were collected, of which 70% were males and 30% females belonging to the species *Lutzomyia longipalpis*, *Nyssomyia whitmani, Evandromyia lenti* and *Ev. evandroi*. The vector *Lu. longipalpis* was the most abundant species (98 %), which confirms its adaptation to urban areas and its resistance to climatic variations, as this species was captured in both the dry and rainy seasons. Of the total number of captured females (N= 1,782), the gut was dissected from 389 (84.6 % *Lu. longipalpis*, 13.1% *Ev. evandroi* and 2.3% *Ny. whitmani*), and no *Leishmania* promastigotes were detected. It is possible that the negative result is due to the low number of females examined. Although the number of specimens collected was relatively high, not all were dissected as several females died over time, reducing the number of samples examined. However, the fact that no infected females were found cannot be considered a definitive result, as the technique of dissecting female sand flies is considered to be not very sensitive. We conclude that there is a large number of sand flies vectors in the urban area of Caxias and that the non-detection of promastigotes in females does not indicate that the parasites are not present in the region. Furthermore, the main vector of L. infantum, the species Lu. longipalpis, is well adapted to the urban areas of Caxias.

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Keywords: Promastigote. Dissection technique. Vectors

VALIDATION OF AN ANTI-*TRYPANOSOMA CRUZI* IMMUNOCHROMATOGRAPHIC TEST IN TRIATOMINE FECES SAMPLES COMPARED TO TWO DIFFERENT METHODOLOGIES (PCR AND ELISA).

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Immunochromatographic tests, also called rapid tests, are tools for execution, reading, and interpretation of results widely used in medicine. Immunochromatographic tests were developed to detect antibodies in a variety of tropical diseases, including Malaria, Leishmaniasis, Schistosomiasis, and Chagas disease. In the literature, immunochromatographic tests are also found for the detection of a variety of antigens, such as BHCG, viral antigens, and more recently, *Entamoeba histolytica* antigens in fecal samples. Antigens from other intestinal protozoa such as *Giardia lamblia* and *Cryptosporidium parvum* have also been identified in fecal samples. In this context, this study aims to develop and optimize an immunochromatographic test to be used on triatomine feces for the initial screening of the infection rate of these insects in endemic areas. In this study, two sensitive and specific techniques were performed to validate the tested chromatographic strips: ELISA and conventional PCR. The obtained results demonstrate that the strips showed a positive test band in 10 fecal samples of *Triatoma infestans* experimentally infected, while 10 negative fecal samples of the same species of triatomines did not mark the test band. The ELISA tests were able to detect 10^4 parasites per well of the plate, suggesting that this is the detection limit of this technique using the chosen pairs of antibodies for the assay. The PCR assays also demonstrated 100% positivity in positive samples and 100% negativity in negative samples. It is concluded that the chromatographic test is promising in the tested model, requiring field testing using other species of triatomines, as well as a more significant number of specimens to confirm its effectiveness in epidemiological screenings.

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Keywords: Rapid test. *Trypanosoma cruzi*. Chagas disease.

DUST MITES IN BRAZIL: CULTIVATION, MOLECULAR IDENTIFICATION, AND CHARACTERIZATION OF *B. TROPICALIS* AND *G. MALAYSIENSIS*

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Introduction: Asthma and rhinitis are among the most prevalent respiratory allergies, primarily triggered by exposure to house dust mites. The most common mite species associated with these allergies include *Blomia tropicalis*, *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, and *Glycycometus malaysiensis*, all recognized for their significant allergenic potential. Objectives:To identify and cultivate mites from the genera *Dermatophagoides* and *Glycycometus*. House dust mite extracts are essential for allergy diagnosis, treatment, and scientific research. Therefore, ensuring the quality of these extracts is crucial, as variability in their composition can compromise the diagnosis and treatment of allergic patients. Materials and Methods: House dust mites were collected from household dust samples in Salvador, Brazil. The dust was enriched with spirulina and yeast as nutrient sources and then cultured under controlled temperature and humidity conditions. After the cultivation period, DNA was extracted using the NucleoSpin® DNA Insect kit (MN), and molecular identification was performed using a PCR assay based on ribosomal DNA (rDNA) sequences ITS1 and ITS2. These sequences are located in conserved genes, allowing species identification. The resulting PCR products were analyzed by electrophoresis on a 1% agarose gel. Additionally, protein extracts were prepared and analyzed using SDS-PAGE gel electrophoresis. Results: Molecular analysis of the dust samples confirmed the presence of mites from the species *B. tropicalis* and *G. malaysiensis*. These mites were isolated and cultivated in pure cultures. After culture expansion, protein extracts of *B. tropicalis* and *G. malaysiensis* were produced and subsequently evaluated alongside *D. pteronyssinus* extracts (Stallergenes Greer) using a 12% SDS-PAGE gel. Differences in protein profiles among the three mite species were observed. The species *B. tropicalis* and *G. malaysiensis* exhibited a high degree of similarity in their protein profiles, whereas *D. pteronyssinus* showed significant differences compared to the other studied species. Conclusion: It was possible to cultivate and identify the mites *B. tropicalis* and *G. malaysiensis*. Additionally, protein extracts from these species were successfully produced and characterized.

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KEYWORDS: HOUSE DUST MITES**,** ALLERGIES**,** IDENTIFICATION

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Exploring natural breeding sites of Phlebotominae Sandflies in xerophytic areas of the Dry Chaco Bioregion, Argentina.

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Abstract

In Argentina, eco-epidemiological studies of leishmaniasis are mainly focused on the abundance and diversity of adult vectors. However, little is known about the immature stages of Phlebotominae and their preferred breeding sites. This study aims to determine the potential breeding sites of Phlebotominae in the Dry Chaco, a xerophytic area where most cutaneous leishmaniasis (CL) cases in the province are reported. The study was conducted from April 2023 to March 2024 in four households located in rural settlements near Juan José Castelli, Chaco. Monthly surveys were carried out to identify natural breeding sites of Phlebotominae through organic substrate (OS) collection and the installation of emergence traps (ETs) in different ecotopes: household, peridomestic area, forest, and *vizcacha* burrows (*Lagostomus maximus*). OS samples were observed in the laboratory for 60 days and then subjected to flotation with a supersaturated NaCl solution to detect immature stages and/or emerged adults. ETs were rotated every 60 days within the same ecotope. Due to the impossibility of installing ETs inside burrows, sticky traps (STs) were placed at burrow entrances to indirectly study adult emergence. Out of 360 OS samples, a single *Migonemyia migonei* female was found in a peridomestic area in April 2023. A total of 200 ETs were installed, capturing 40 Phlebotominae: *Mg. migonei* (75%), *Cortelezzii complex* species (22.5%), and *Nyssomyia neivai* (2.5%). The peridomestic area had the highest emergence rate (80%), followed by the household (17.5%) and the forest (2.5%). STs placed at burrow entrances captured 104 Phlebotominae, with *Evandromyia chacuensis* (26.9%) being the most common, followed by *Ev. cristacapita* (20.2%), *Ev. sallesi* (16.3%), *Mg. migonei* (11.5%), *Cortelezzii complex*, *Ev. termitophila*, *Ev. cortelezzii*, and *Pintomyia torresi* (<10%). The findings suggest that peridomestic areas and burrows could be the most suitable sites for vector development in these xerophytic areas.

Supported by: CONICET

Keywords: sand fly, vector, immature stages

DEVELOPMENT OF A PARATRANSGENIC LUTZOMYIA *LONGIPALPIS* BY ENGINEERED BACTERIA DRIVING REFRACTORINESS TO *LEISHMANIA INFANTUM* INFECTION.

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The parasite *Leishmania infantum* is the etiologic agent of American visceral leishmaniasis (AVL), a neglected disease transmitted by the sandfly *Lutzomyia longipalpis*. The current scenario of territorial expansion with increasing incidence and lethality of AVL highlights the need for new strategies to control the spread of this disease. Paratransgenesis is an approach, in which symbiotic bacteria of insect vectors are engineered to drive antiparasitic molecules and reduce their vectorial capacity. The aim of the present work is to select molecules with anti-leishmanial activity to produce a paratransgenic sandfly to block *L. infantum* transmission. To select an effector molecule, we used the MTT assay to test the cytotoxic effect of 6 antimicrobial peptides (AMPs). Artificial infection experiments were performed to assess the AMP effect on parasite load and sandfly fitness. We determined that 16μM of the AMP-3 was toxic to *L. infantum* in axenic cultures and artificially infected *L. longipalpis* females, but had no deleterious effects on the vector. To establish a delivery system for the AMP in the vector, bacteria from the gut of female *L. longipalpis* were isolated after blood feeding, identified by sequencing the 16S rRNA, and tested for hemolytic activity and resistance to AMP. We selected an *Escherichia* sp. non-sensitive to this AMP and genetically modified with a plasmid for constitutive expression and signal peptide-mediated secretion of AMP. This plasmid was electroporated into bacteria and AMP secretion and their activity was tested through Dot Blot and MTT assay. We observed that the conditioned media from the transgenic bacteria was able to reduce the *L. infantum* axenic culture viability. Our next steps include testing these transgenic bacteria effects on parasite viability during their development in *L. longipalpis*. In this way, we propose the development of a paratransgenic sandfly with reduced vectorial capacity to transmit the *L. infantum*.

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Lutzomyia longipalpis; Leishmania infantum; Paratransgenesis.

LARVAE PRESENCE IN LARVICIDE DISSEMINATION STATIONS TREATED WITH DIFFERENT PRODUCTS AT THE UNIVERSITY OF BRASÍLIA

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Dengue represents a significant public health challenge in Brazil. The 2024 epidemic registered the highest incidence rates ever documented in the country. Innovative technologies, such as larvicide dissemination stations (DS), are being integrated into vector control campaigns. This technique commonly utilizes Pyriproxyfen (PPF), an insect growth regulator (IGR), to hinder mosquito development. However, concerns regarding the potential emergence of mosquito resistance to PPF highlight the need for alternative insecticides. Diflubenzuron (DFB), another IGR, is a potential substitute for PPF. In this study we evaluated the larvae presence in DS treated with PPF or DFB. By the end of October 2024, 600 DS were deployed across 12 areas at UnB. Each area was assigned 50 DS. Four areas were randomly chosen to receive either PPF, DFB or a product with no larvicide effect as a control. Over three months, researchers conducted regular maintenance of the DS, assessing their integrity and recording the presence of *Aedes aegypti* (Linnaeus, 1762) larvae. Results showed that 20.73% of DS with PPF contained larvae, closely matching the 22.15% observed in control areas. In contrast, DS treated with DFB exhibited a lower presence of larvae (4.56%). These findings suggest that PPF has a subtle mode of action when compared to DFB, which apparently disrupts larval development more immediately.

Supported By: UNB, CNPq, CAPES.

Keywords: Dengue, innovative control, insecticides.

ENTOMOLOGICAL SURVEILLANCE OF MOSQUITOES IN AREAS OF RIPARIAN VEGETATION AND URBAN PERIDOMICILES OF PORTO VELHO, RONDÔNIA

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Mosquitoes are the most important arthropod vectors in the transmission of pathogens that cause viral and parasitic diseases in humans and animals. This study aimed to conduct entomological surveillance of mosquitoes in areas of urban riparian vegetation and peridomiciles. Collections were carried out in 10 locations distributed in the urban area of Porto Velho, Rondônia, with the presence of riparian vegetation and peridomicile areas of human dwellings close to these areas. For each locality with riparian vegetation (10 locations), three areas were selected, totaling 30 peridomiciles. Sampling occurred quarterly from 2021 to 2023, using the electric aspiration technique (adapted Nasci aspirator) for 15 minutes in each location. A total of 3,914 mosquitoes were collected, 2,545 males and 1,369 females, identified in 11 genera (*Aedes*, *Aedeomyia*, *Anopheles*, *Coquillettidia*, *Culex*, *Limatus*, *Mansonia*, *Psorophora*, *Trichoprosopon*, *Uranotaenia* and *Wyeomyia*) and 21 species. The species with the greatest abundance were *Culex quinquefasciatus* (73.93%), *Aedes albopictus* (8.91%), and *Aedes aegypti* (7.05%). Regarding the collection habitat, there was a greater abundance of had a higher abundance of mosquitoes peridomicile, with 2,285 specimens (58.38%), while in the riparian vegetation areas, 1,629 mosquitoes were captured (41.62%). In the riparian vegetation, greater species richness was found (20 species) compared to the peridomicile (12 species). The species *Cx. quinquefasciatus*, *Li. durhamii* and *Ps. ferox* were found in all locations. Surveillance of urban culicid fauna should be continuous, as it allows the identification of circulating species that may be participating in the dissemination of pathogens, as well as directing local vector control and prevention methods.

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Keywords: Amazon region, Culicidae, Vectors.

POTENTIAL OF Coffea sp. AS A BIOINSECTICIDE: A SYSTEMATIC REVIEW ON THE CONTROL OF AGRICULTURAL, URBAN PESTS, AND VECTORS

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Insects are the most diverse class in the animal kingdom, playing crucial roles in ecosystems. From an environmental health perspective, agricultural and urban pest insects, as well as vectors of pathogens, stand out. The control of these organisms typically involves insecticides, which over time have driven the selection of resistant populations. This phenomenon highlights the urgent need for new active compounds, with plant biodiversity being a promising field of research. In this context, the present systematic review aimed to answer the question: “What is known about the insecticidal effects of botanical derivatives from Coffea sp. on agricultural and/or urban pests?” The databases consulted included BVS, Google Scholar, PubMed, ScienceDirect, Scopus, and Web of Science. Only original articles published between 1920 and 2024 in English, Spanish, and Portuguese were considered. Search terms such as "Coffea, coffee, insecticide, bioinsecticide, bioassay, and insect," combined with Boolean operators AND/OR, were used. Of 3,273 studies initially selected, only 24 were included in this review. Of these, 7 (29.2%) investigated insecticidal effects on agricultural pests, 1 (4.2%) on urban pests, and 16 (66.6%) on vectors. Four Coffea species were used in various forms, including coffee grounds, fruit husks, roasted and green beans, and leaves. Tests on agricultural pests were conducted via ingestion (4 studies, 57.1%) and contact (3 studies, 42.9%). For urban pests, tests were ingestion-based (100%), while vector studies used World Health Organization bioassays (100%). Among the 24 evaluated studies, 13 (40.6%) demonstrated insecticidal effects of coffee derivatives, with 3 (23.1%) on agricultural pests and 10 (76.9%) on vectors. Based on a scoring evaluation, 14 studies (58.3%) were considered of moderate quality, mainly due to insufficient detailing of key points, warranting caution in interpreting the results.

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KEYWORDS: Insecticide; Coffea; Review.

ANALYSIS OF THE REPELLENT ACTION OF INSECTICIDES ON Aedes aegypti (DIPTERA: CULICIDAE): A SYSTEMATIC REVIEW

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Aedes aegypti is a vector of significant arboviruses in global health, such as Dengue, Zika, and Chikungunya viruses. It is a well-studied biological model, with special attention to its biology and control, particularly due to its widespread resistance to the most known and available insecticides worldwide. However, little is known about its repellency to these insecticides. In this context, we conducted a systematic review guided by the following question: "What is known about the repellent action of insecticides on A. aegypti?" The databases consulted were MEDLINE (PubMed) and the "Biblioteca Virtual em Saúde" (BVS). Only original articles published between 1990 and 2023 in English, Spanish, and Portuguese were considered. The descriptors used were repellency, insect repellents, *A. aegypti*, organochlorates, pyrethroids, organophosphates, carbamates, neonicotinoids, and growth inhibitors. Of the 543 studies initially selected, 47 were included in this review, with 46 presenting positive results for repellency. In total, 185 insecticide repellency bioassays were performed in the 47 studies, of which 75.5% suggested some degree of repellency, regardless of the resistance/susceptibility level of the population, type of essay, and the insecticide used. The repellency effect in resistant populations was observed in 67.6% of the essays, while the same effect was observed in 87.6% of essays with susceptible insects. Nearly 80% of the essays tested repellency for pyrethroids. Of the most used insecticides, DDT exhibited the highest frequency of repellency effects in the bioassays (93.8%), while 78.7% of the assays with deltamethrin and 71.7% of the assays with permethrin showed repellency effects. Only four insecticides (including Temephos) showed no repellency effects in any study. These findings underscore the importance of studying insecticide repellency to understand vector behavior and improve integrated vector management strategies.

Financial Support: CAPES, ICB-UFMG, INCT-Entomologia molecular

Key-words: Culicidae, *Aedes aegypti*, insecticide repellency, Hitss, hand-in-cage

PARASITO2025
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RHODNIUS PROLIXUS IMPAIRS TRYPANOSOMA CRUZI GROWTH THROUGH COLD-SEEKING BEHAVIORAL THERMOREGULATION

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Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, is the main neglected vector borne illness in terms of public health burden in Latin America, affecting ca. 8 million people. It is transmitted by kissing bugs of the Triatominae sub-family, and mainly by *Rhodnius prolixus* and *Triatoma infestans* (Hemiptera: Reduviidae) in South America. Temperature is the main abiotic factor impacting the life and ecology of insects as they are ectotherms. However, *R. prolixus* plays an active role in mediating the effects of temperature through its thermopreference and its ability to select shelters based on thermal cues, behaviorally thermoregulating. Crucially, the interplay between *T. cruzi* infection and thermopreference in *R.prolixus* remains unexplored. In our project we investigated the effects of infection by *T. cruzi* on the thermopreference of *R. prolixus*. To do so we recorded the position of infected and uninfected individuals in a temperature gradient at 6 different times of day over the period from blood feeding up to two weeks after molting. Additionally, we compared the evolution of parasite load between insects allowed to select their environmental temperature and individuals maintained at a fixed temperature, providing insights into the interaction between behavioral thermoregulation and infection dynamics. We demonstrated that *R. prolixus* seeks colder temperatures (22.39 ± 0.05 ºC) when infected by *T. cruzi* compared to their uninfected counterparts (which preferred 23.40 ± 0.07 ºC), a behavior called behavioral anapyrexia. This reaction led to a diminution of parasite load when compared to individuals maintained at 26ºC, and to a higher proportion of insects which cleared the infection. These results highlight the importance of considering the behavior of the insect when investigating the interaction between triatomines and *T. cruzi*.

Supported by: Institut Pasteur, CNPq, CAPES, FAPEMIG, INCT-EM

Keywords: thermobiology, vector biology, trypanosomiasis

OCCURRENCE OF MOSQUITOES IN GALLERY FORESTS IN PRIMEIRA CRUZ, MARANHÃO, BRAZIL.

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The distribution of mosquito species in the state of Maranhão has been little studied, except for anopheline species. Northern Maranhão is one of the least studied areas of Cerrado in terms of mosquito diversity in Brazil. Therefore, systematic surveys could provide fundamental elements in terms of faunal diversity and public health. We analyzed the occurrence of diurnal and nocturnal mosquitoes at the beginning of the rainy season in Cerrado gallery forests in the municipality of Primeira Cruz, Maranhão. We collected in three gallery forests (G1, near Lagoa do Cassó, G2, next to the road 10 km from the lagoon and G3, Brejo) for 5 days in December 2024. We used 3 collection methods: CDCs, manual capture and Shannon traps, at different times between 8am and 10pm. In forests G1 and G2, we installed 10 CDCs, carried out manual collection with a Castro aspirator (1 man/hour) and collection with a Shannon trap (pair of traps, 4 hours), over 4 days/nights. In G3 we only used Shannon traps for 2 nights. Mosquitoes were identified using keys. We analysed the richness and number of mosquitoes collected using different methods. We collected 862 specimens belonging to 7 genera and 13 species, mainly with Shannon (n=429, 49.8%), followed by CDC (n=374, 43.4%) and manual capture (n=59, 6.8%). We collected more mosquitoes at night in forest G1 (n=556, 64.5%) and G2 (n=204, 23.7%). The diurnal species were *Aedes serratus*, *Sabethes glaucodaemon*, *Sa. chloropterus*. The nocturnal species were *Anopheles* sp, *Coquillettidia albicosta*, *Cq. arribalzagae*, *Cq. lynchi*, *Uranotaenia nataliae* and *Uranotaenia* sp. We found potential vector species of Yellow Fever - YF *(Sa. glaucodaemon* and *Sa. chloropterus*). Most of the mosquitoes were found at night in the gallery forest near Lagoa do Cassó. The presence of YF vector species in the forest near the lagoon indicates a potential risk of transmission to locals and tourists.

Supported By: CNPq, CAPES

Keywords: mosquitoes, traps, cerrado.

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A SYSTEMATIC REVIEW: ANALYSIS OF THE NATIVE MICROBIOTA OF SANDFLIES AND ITS INTERACTION WITH *LEISHMANIA*

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Abstract

Sandflies are highly adaptable to various environments and climatic conditions. They are responsible for the transmission of Leishmania, and their biology, including different feedings, could influence their microbiota and consequently their ability to allow the proliferation of the parasite and their transmissibility. Research on the diverse microbial communities in the midgut of phlebotomines and their interactions with *Leishmania*—including the potential benefits or drawbacks for protozoan transmission—remains limited. To address this gap, a systematic review was designed to compile and assess existing data on sandfly microbiota and its relationship with *Leishmania*, potentially suggesting effective control strategies. The review protocol was published on the Open Science Framework platform, and database searches were conducted shortly thereafter. The search strategy employed three key themes: sandflies, microbiota, and *Leishmania*, resulting in 1,315 studies—404 from Web of Science, 429 from PubMed, and 482 from Scopus. After the initial screening, which involved removing duplicates, non-English articles, and non-research papers, 576 articles remained for further evaluation. Titles and abstracts were then assessed according to predefined inclusion and exclusion criteria. The first reviewer shortlisted 31 studies for full-text analysis, while the second selected 29. A third reviewer resolved discrepancies, finalizing 30 studies for full-text review, data extraction, and bias assessment using an adapted SYRCLE Risk of Bias tool. After the full-text review of the 30 papers, data will be collected and recorded in REDCap for risk of bias analysis. Studies with a lower risk of bias will undergo detailed data extraction on sandflies, microbiota, and *Leishmania*, followed by a meta-analysis using multivariate statistical methods.

Supported by: FAPESB, FIOCRUZ, CAPES, CNPq

Keywords: *Leishmania*, sandfly, microbiota

ECOLOGY OF *Corethrella* (DIPTERA: CORETHRELLIDAE), VECTORS OF TRYPANOSOMATIDS IN ANURANS FROM FORESTED AND ANTHROPIZED ENVIRONMENTS IN AN AMAZONIAN SETTLEMENT

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Coretrelids are popularly known as frog-biting midges due to the habit of their females to exclusively blood feeders of anurans. Because of this habit, these insects are also vectors of trypanosomatids on anurans. Thereare little informations about the diversity and ecology of these insects, especially in the Amazon. This study aimed to compare the effect on the abundance of these insects in forested and anthropized landscapes in the rural settlement of Rio Pardo, Presidente Figueiredo, Amazonas. Collections were carried out in the municipality of Presidente Figueiredo in the Rural Settlement of Rio Pardo, located 160 km from Manaus. Corethrelids were collected by simple HP model light traps and HP model light traps with loudspeakers ("frog-song traps"). The coretrelids were screened and are grouped according to morphological similarity, gender, females with eggs and engorged females according to morphological similarity in microtubes containing alcohol for later identification and molecular techniques. Subsequently, the specimens will be dissected and mounted on slides in phenol balsam. To date, 3,442 specimens of Corethrella have been screened. Of these, 2,417 specimens are from the forest environment and 1,025 specimens are from the Peridomicile, showing a significant difference (Mann Whitney t-test $p<0.01$). The abundance of coretrelids is higher in forested areas compared to anthropized areas, demonstrating a probable effect on the population in impacted areas.

Key words: Frog Biting midges, bioindicators, Amazon basin

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SURVEY OF PHLEBOTOMINE SANDFLY (DIPTERA: PSYCHODIDAE) FAUNA AND *Leishmania* spp. INFECTION IN THE DESTERRO ENVIRONMENTAL CONSERVATION UNIT, FLORIANÓPOLIS, SANTA CATARINA, BRAZIL

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Abstract

Santa Catarina (SC) was the last state to report autochthonous cases of Visceral Leishmaniasis (VL) in Brazil. The first case of Canine Visceral Leishmaniasis (CVL) was reported in Florianópolis (SC, Brazil) in 2010, and it remains the only municipality in the state with autochthonous cases in dogs and humans. The main vector, *Lutzomyia longipalpis*, has not been detected in SC but has been reported in the neighboring states of Rio Grande do Sul and Paraná. This study aimed to survey the sandfly fauna in the Desterro Environmental Conservation Unit (UCAD) in Florianópolis, and investigate natural *Leishmania* spp. infection in sandflies, and analyze the influence of climatic factors on their monthly distribution. Twelve monthly sandfly collections were carried out using two CDC light traps, one set in the tree canopy and the other at ground level. The presence of *Leishmania* spp. in female sandflies was assessed using conventional PCR, targeting the ITS1 region of rDNA, with DNA sequencing performed to determine the species involved. In total, 458 sandfly specimens were captured, belonging to the following species: *Brumptomyia cunhai*, *Brumptomyia nitzulescui*, *Expapillata firmatoi*, *Migonemyia migonei*, *Nyssomyia neivai*, *Pintomyia fischeri*, *Psathyromyia lanei*, *Psathyromyia limai*, *Psathyromyia pascalei* and *Psychodopygus ayrozai*. This study identified the first record of the species *Psathyromyia limai* in Southern Brazil. Among the 347 females analyzed for natural *Leishmania* spp. infection, two tested positive by PCR. However, DNA sequencing results were inconclusive regarding the specific trypanosomatid species involved. Analysis of climatic factors showed a positive correlation between sandfly abundance and mean temperature and precipitation, though without statistical significance. These findings contribute to increasing the knowledge of sandfly fauna in SC and reinforce the need for continued surveillance of leishmaniasis vectors.

Supported by: IOC/Fiocruz, CNPq, INCT-EM

Keywords: Phlebotominae, PCR, visceral leishmaniasis

Climate and *Aedes* Infestation: Evaluating Statistical Models for Ovitrap Positivity and Egg Density

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Entomological surveillance using ovitraps is essential for controlling arboviruses. This study evaluated the influence of climatic variables on Aedes spp. oviposition in Buritizeiro (MG) using PneuTrap3D® traps. From April to December 2024, 65 to 75 traps were placed in households and regularly inspected for Aedes eggs and larvae. After one to two weeks, the traps were collected, and eggs were counted under a stereomicroscope (20x). The Ovitrap Positivity Index (IPO = positive ovitraps/total ovitraps) and Egg Density Index (IDO = counted eggs/positive ovitraps) were calculated. Climatic data (temperature, humidity, and rainfall) were obtained from INMET. Generalized Linear Models (GLM) with Gamma and Quasi-Poisson distributions, Generalized Additive Models (GAM) for nonlinear patterns, and Random Forest models were applied to assess variable importance. All data analysis and statistical modeling were performed using R software (version 4.4.1). The mean IPO and IDO were 32.87±13.16 and 38.59±14.74. GLM models for IDO did not identify significant climatic predictors. For IPO, the Quasi-Poisson model provided the best fit, indicating that rainfall had a negative effect, close to statistical significance (P Value = 0.0547), suggesting that rainy periods reduce ovitrap positivity. The reduced model with only rainfall lost significance, suggesting that other variables contributed to IPO variation. GAM did not detect significant nonlinear relationships, and Random Forest confirmed the low influence of climatic factors on IPO and IDO. These findings suggest that rainfall may affect IPO but does not explain IDO variation. Additional factors, such as land use and local environmental characteristics, may be key determinants in Aedes spp. infestation.

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Key-words: Entomological surveillance, Ovitrap Positivity Index, Climatic variables.

IT MIGHT LOOK LIKE A TRASH BIN, BUT IT IS NOT: MAINTENANCE OF LARVICIDE DISSEMINATION STATIONS IN THE UNIVERSITY OF BRASÍLIA

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The dengue vector control methods implemented by Brazilian health authorities in 2024 were insufficient to manage mosquito populations. Innovative technologies such as larvicide dissemination stations (DS) have emerged as alternatives to strengthen control efforts. We focus on the deployment of DS at the University of Brasília (UnB). Unlike its common use in residential settings, in this research we used DS in unrestricted human circulation areas. This leads to challenges such as people mistakenly identifying the DS as trash bins or removing them from their designated locations, compromising the intervention's effectiveness. On October 2024, we installed and mapped a total of 600 DS across 12 areas at UnB, nine of which were located on campus I, and three on campuses II, III and IV. Each area was assigned 50 DS. Following installation, we performed regular maintenance on the DS, recording observations about their integrity and trash presence over a period of three months. Considering all study areas and months combined, 8.79% of the DS contained trash, while 4% had been moved from their original installation sites. After three months, area 2 of campus I (UnB hospital) exhibited the highest percentage of DS with trash (23.20%), followed by campus IV (20%). In terms of DS removal, area 3 of campus I had the highest percentage (16%), followed by campus III (9.25%). Trash presence decreased monthly. In November, the percentage was 14%; 8.76% in December and 6% in January. We emphasize the critical role of regular maintenance and proper management of DS, highlighting the need for health education initiatives, as the method relies on public understanding and cooperation. The evaluation of the implementation of DS in areas with free public circulation is noteworthy, as such environments are less common for this type of control measure. Addressing these challenges is essential to ensure the optimal functioning and long-term sustainability of the technique.

Supported by: UnB, CNPq, CAPES.

Keywords: Dengue, vector control, health education.

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Distribution patterns of Phlebotominae fauna in the major biogeographical regions of Argentina.

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Abstract

In Argentina, the most common form of leishmaniasis is American Cutaneous Leishmaniasis (ACL), which has been recorded since 1930, with sporadic cases and outbreaks in 12 provinces. American Visceral Leishmaniasis (AVL) was initially observed only in scattered rural human cases until 1970. However, the first autochthonous case was reported in Misiones in 2006, and the disease has since spread to 8 provinces. Knowledge of Phlebotominae fauna in Argentina is still evolving. Before the 1960s, only 12 species were documented, but systematic surveys since the 1990s have increased this number to 46 species. Epidemiological transmission risks are influenced by Phlebotominae species assemblages. To date, Phlebotominae have been documented in 14 provinces across Argentina, spanning several biogeographic regions. Most leishmaniasis cases are concentrated in three main bioregions: Paranaense, Chaco, and Yungas, each with its own characteristics and transmission dynamics. In the Paranaense region, with *Ny. whitmani* as the primary vector, ACL outbreaks occur in Misiones, particularly in areas near deforestation. In the Chaco region, *Mg. migonei* dominates dry areas, while *Ny. neivai* is more common in humid zones, with variations in species dominance due to anthropogenic changes. The Yungas region, characterized by low Phlebotominae diversity, exhibits peri-domestic and peri-urban transmission cycles, with *Ny. neivai* as the dominant species, often associated with *Mg. migonei*. All three regions also host *Lu. longipalpis*, primarily in urban or peri-urban areas, which is considered the main vector of *Le. infantum*, though other species may contribute to sporadic transmission. Given the shifting transmission limits due to environmental changes, a better understanding of the factors shaping population assemblages could enhance efforts to prevent or control leishmaniasis transmission in Argentina.

Supported by: CONICET
Keywords: sand fly, sandflies, leishmaniasis

HELMINTOLOGY AND MALACOLOGY

Neuroinflammatory and Cognitive Impacts of Toxocara canis Infection in a Murine Model

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Toxocara canis causes toxocariasis, a disease that primarily infects dogs. In paratenic host, such as humans and other animals the parasite does not complete its life cycle but instead undergoes larval migration, a condition known as “larva migrans”. The larvae migrate through various organs, including the brain. Here we sought to investigate how T. canis lodges in the central nervous system (CNS) of C57BL/6 mice infected with 1,000 larval eggs after 14 days post infection. We aimed to assess larval migration patterns, characterize neuroinflammatory responses using spectral flow cytometry, evaluate neurotrophic factor alterations by ELISA, and analyze behavioral changes. Larval recovery via the Baermann-Moraes method confirmed preferential brain lodging over the cerebellum. Neuroimmunological profiling using flow cytometry revealed significant systemic leukocytosis without peripheral cell activation but pronounced inflammatory infiltration in the CNS, predominantly by activated neutrophils and eosinophils. Microglial activation indicated a local inflammatory response to larval presence. Additionally, decreased CX3CL1 levels both systemically and in the brain, along with reduced GDNF in brain tissue, suggested impaired inflammatory regulation and neuroprotection. Infected mice also exhibited signs of memory deficit. Our findings suggest that infection with T. canis induced a disruption in neuroimmune homeostasis and impaired neuroprotection, which may contribute to the observed memory deficits. These results highlight the potential for lasting neurological consequences of T. canis infection and reinforce the need for further studies to elucidate its impact on brain function and behavior.

Supported by CAPES, CNPq, Fapemig.

Key-Words: *Toxocara canis*, Neuroinflammation, Neurotrophic factors

GENETIC TRANSFORMATION OF TOMATOES WITH *ASCARIS* SPP. IMMUNOGENIC GENE

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Ascariasis, a neglected parasitic disease, represents a global public health problem due to its high morbidity and frequent reinfection. Vaccines represent a promising strategy for control, and several immunogenic proteins from *Ascaris* sp. have been studied for this purpose. The aim of this study was to genetically transform tomato (*Solanum lycopersicum* cv. Micro-Tom) with an immunogenic gene from *Ascaris* sp. The coding sequence of the BOT protein was optimized for expression in the plant using JCAT and synthesized in pUC57 (Genscript). The gene was cloned into pCR8 (Gateway system), transformed into *Escherichia coli* and selected in LB/spectinomycin. After recombination with pMDC32, a new transformation and selection in LB/kanamycin was performed. Colonies were confirmed by PCR using M13 primers. The pMDC32/DNA plasmid was digested with SpeI and HindIII enzymes for gene confirmation, and then introduced into *Agrobacterium tumefaciens*. The cotyledons of tomato seedlings were transformed with the recombinant bacterial suspension and selected with hygromycin. DNA from young leaves (T2 generation) was analyzed by PCR using primers from the vector and the target sequence, confirming gene integration in 10 out of 13 transformants (77%). Tomatoes offer a promising platform for antigen expression due to their ease of cultivation and high biomass yield. Successful transformation with the *Ascaris* gene paves the way for protein analysis and potential application as an edible vaccine against ascariasis.

Supported by: CAPES, CNPq

Keywords: Genetically Modified Plants. Ascariasis. Biotechnology.

OCCURRENCE OF PARASITISM BY *Schistosoma mansoni* IN LIMNIC MOLLUSKS IN AN URBAN AREA IN THE INLAND OF SERGIPE

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Schistosomiasis mansoni is a neglected tropical disease affecting more than 200 million people in 78 countries. It is endemic in Brazil, mainly in coastal regions and the Northeast. Environmental factors, such as water bodies containing intermediate host mollusks and favorable climatic conditions, influence its prevalence. This research aimed to investigate the occurrence of parasitism by *Schistosoma mansoni* in mollusks of the genus *Biomphalaria* in an urban area in the municipality of Riachuelo/SE. The collections took place at a single point, distributed in three distinct periods: November 2023 (Collection 1), April 2024 (Collection 2) and July 2024 (Collection 3). The researchers collected the gastropods using tweezers and metal shells, then placed them in plastic bags and sent them to the Laboratory of Entomology and Tropical Parasitology (LEPaT) of the Federal University of Sergipe (UFS), where they counted, measured, and kept them in containers with non-chlorinated water. Evaluated the positivity of *S. manson*i by exposing the mollusks to artificial light for approximately one hour. We reexposed the mollusks that did not release cercariae to light weekly for forty days. The authors identified the released cercariae using dichotomous keys for classification. 260 mollusks were obtained, all belonging to the species *B. glabrata*. In Collection 1, 136 mollusks were found, of which eighteen (13%) were positive for *S. mansoni*. In Collection 2, 62 mollusks were captured, ten (16%) positive. In the last collection, 62 mollusks were obtained, of which five (8%) were positive. We concluded that the studied area presents a potential risk to the health of the local population due to the environmental conditions and the direct interaction of people with the foci of schistosomiasis transmission, which confirms the need to provide subsidies for the implementation of more targeted and effective public health measures in the battle against schistosomiasis in the region.

Supported by PIBIC-COPES.

Keywords: Mollusks; *Schistosoma mansoni*; *Biomphalaria sp*.

ARTIFICIAL INTELLIGENCE/MACHINE LEARNING APPROACH FOR TAXONOMIC SPECIES DISCRIMINATION USING HELMINTH COLLECTIONS DATA: APPLICATION ON ARCHAEOLOGICAL MATERIAL FROM THE NEW AND OLD WORLDS

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Modern parasitological records show that around 300 species of helminth capillariids have been described in all vertebrate taxa, making it a very diversified group. Capillariid eggs have been reported in archaeological material in both the New and Old Worlds, mainly in Europe and South America. They have been found in coprolites, sediments from latrines, pits, or burials. The main proposal of this study was to characterize and identify capillariid eggs found in Brazilian and European archaeological sites, using an artificial intelligence/machine learning approach for taxonomic species discrimination based on helminth collection data.

A total of 39 archeological samples from Europe, deposited in the Paleoparasitological Collection of the University Marie et Lois Pasteur, Besançon, France was analyzed. In addition, 80 coprolites from the pre-Columbian archaeological site *Gruta do Gentio II*, Brazil, deposited in the Paleogenetic Laboratory at IOC, FIOCRUZ, Rio de Janeiro, were evaluated. Capillariid eggs were classified according to morphometric, morphological, and ecological parameters and processed using artificial intelligence/machine learning technology and reference dataset of specimens by both Institutional Helminthological Collections.

Ten samples from Europe and 4 from Brazil were positive for capillariid eggs with 13 different morphotypes. As European samples were mainly collected from latrines and pits, parasite-host information was absent, and consequently, species identification was impaired, in contrast to the Brazilian coprolites. In the Brazilian sites, the new methodology indicates *Capillaria exigua* in feline coprolite, *Baruscapillaria resecta* in opossum, *Aonchotheca bovis* in bovid. In European sites, *C. venusta*, *A. myoxinitelae*, *Eucoleus madjerdae*, *B. spiculata* were found.

The study provides new results by applying an innovative methodology for parasite species identification and gaining insights into past human or animal host-parasite relationships.

Supported by: CNPq, FAPERJ, LPIP-IOC/FIOCRUZ

Keywords: Taxonomy, Helminths, Paleoparasitology.

GASTROINTESTINAL PARASITES FOUND IN *Conepatus amazonicus* (CARNIVORA: MEPHITIDAE) IN AN AGROECOSYSTEM IN SOUTHEASTERN GOIÁS

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The Cerrado is notable for its rich diversity and endemism, but faces degradation due to hunting, fire management and deforestation, making it a "hot spot" of threatened biodiversity. Members of the Mephitidae family, such as *Conepatus amazonicus*, Hog-nosed Skunks, are nocturnal and omnivorous animals that have a perianal gland that produces a strong odor for defense; they have a wide distribution including Atlantic Forest and Cerrado in Brazil, being adaptable to disturbed environments, but are still poorly studied. Parasitic infections, while generally non-lethal, deteriorate the health and behavior of animals, increasing their vulnerability to predation and other environmental pressures. The scarcity of data on the prevalence of parasites in wild animals poses a risk to the health of populations and humans. Thus, the objective of this work is to identify the evolutionary parasitic forms found in carcasses of *C. amazonicus* collected after being run over on roads in the Cerrado of Goiás. For this, the adult worms were dissected and their structures analyzed, comparing with works found in the literature. Eggs were also visualized in order to confirm the identification. Three genera of parasites were identified, *Spirometra* sp., *Prosthenorchis* sp., *Physaloptera* sp., and the biological cycles of the parasites were compared to the feeding habits of *C. amazonicus*, showing the zoonotic potential of the helminths. The data obtained encourage further work on the epidemiology of wild animal parasites, as well as studies of these parasites with molecular biology, for specific identification.

Key-words: *Conepatus amazonicus, Spirometra*, *Prosthenorchis*

COMBINED ANTHELMINTIC AND ANTIBIOTIC TREATMENT REDUCES BACTERIAL TRASNLOCATION IMPACTS IN EXPERIMENTAL TRICHURISIS

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Soil-transmitted helminths (STH) remain a major public health issue, particularly in impoverished regions, despite advancements in healthcare. Approximately 1.5 billion people are infected worldwide, with Brazil reporting a 5.41% prevalence of human trichuriasis. Albendazole has been used for over 40 years in mass drug administration programs to treat STH. However, research on the interaction between intestinal helminths and bacteria remains limited. The recent discoveries about bacterial translocation in association to the growing issue of drug resistance further underscore the need for new treatment strategies. Trichuriasis induces tissue damage, bacterial translocation, and intestinal inflammation. This study aimed to evaluate the combined effects of albendazole and piperacillin-tazobactam during chronic experimental trichuriasis. Swiss Webster mice were infected with 150 embryonated *T. muris* eggs. Thirty-five days post-infection, the mice were treated for 8 days with antibiotics, a single dose of anthelmintics, or both. Four groups were established: Group 1 (antibiotic treatment), Group 2 (anthelmintic treatment), Group 3 (combined treatment), and Group 4 (control, no treatment). Kato-Katz exams were performed on days 0, and 7 of treatment. We analyzed parasitological, cell inflammatory and histopathological parameters of the cecum, as well as the viability of the worms. Egg analysis revealed morphological and morphometric changes in the anthelmintic-only and antibiotic-only groups. Antibiotic treatment significantly reduced epithelial submucosa thickening and inflammatory infiltrate. The combination of anthelmintics and antibiotics led to distinct changes in the interaction between the nematode, bacteria, and host tissue during chronic infection. These results suggest that combined treatment could be an effective approach for treating trichuriasis in humans and animals, although further studies are needed to confirm its potential.

Supported by: FAPERJ; CNPq; CAPES; FINEP.
Keywords: Trichuriasis; treatment; bacteria.

PEPTIDE-BASED APPROACH FOR DIFFERENTIAL IMMUNODIAGNOSIS OF *ASCARIS SUUM* INFECTIONS AND HELMINTH CROSS-REACTIVITY IN MICE

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Ascariasis, a disease of significant medical and veterinary importance, is caused by parasitic nematodes that primarily infect humans and animals (*Ascaris lumbricoides* and *Ascaris suum).* The World Health Organization classifies ascariasis as one of the most prevalent Neglected Tropical Diseases, disproportionately affecting populations in endemic regions with poor sanitation and limited healthcare access. Helminth infections caused by species such as *Toxocara canis* and *Schistosoma mansoni* share similarities with *Ascaris* spp., leading to cross-reactivity in diagnostic tests and complicating the development of specific diagnostic tools. This study adopted an unconventional approach, using peptides derived from the Tc-CTL-1 protein of *T. canis*, to evaluate the reactivity of sera from infections with different helminths, focusing on *A. suum*. Immunoblotting assays were performed with sera from mice infected and uninfected with *T. canis*, *A. suum*, and *S. mansoni*. IgG1 and IgG2a antibodies were used for detection, followed by densitometric analysis with ImageJ software and statistical evaluation. Results showed strong recognition of specific peptides by IgG2a antibodies against *A. suum*, while IgG1 antibodies exhibited lower reactivity. Peptides with optical density (OD) ratios ≥ 2.5 for IgG2a (n = 14) and ≥ 1.5 for IgG1 (n = 3) were identified as promising candidates for differential diagnosis. BLASTp analysis revealed alignment between selected peptides and proteins of *T. canis* and *S. mansoni*, indicating conserved regions among these parasite proteins. These findings suggest that this unconventional peptide selection approach may effectively identify superior targets for the differential diagnosis of *A. suum* infections. Further investigation and validation using immunoenzymatic methods are planned to confirm the diagnostic potential of these peptides, contributing to improved diagnostic tools for helminth infections in endemic regions.

Supported by CNPq and FAPEMIG

Keywords: Ascariasis Diagnosis, Helminth Cross-Reactivity, Peptide-Based Immunodiagnostics

Schistosoma mansoni infection associated with dyslipidemia in an experimental model: implications for visceral adipose tissue after praziquantel treatment

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Schistosomiasis is a neglected disease that affects millions of people in endemic areas and it's heavily influenced by changes in the host's metabolic profile. To investigate the effects of praziquantel (PZQ) treatment on acute schistosomiasis infection in animals subjected to a high-fat diet (HFD), female Swiss webster mice were fed either an HFD or a standard diet (SD) for 34 weeks. The animals were subcutaneously infected with 100 *Schistosoma mansoni* cercariae (LE strain) in the 24th week, and received by gavage 60 mg/kg of PZQ in the 32nd week. Body mass (BM) and food intake (FI) were monitored weekly. At week 34 of the experiment mice were euthanized, blood samples were collected for biochemical analyses (lipids levels), and macrophages (MAC) were cultured for indirect evaluation of nitric oxide (NO) production. Visceral adipose tissue (VAT) was removed, fixed, histologically processed, and stained with Hematoxylin and Eosin for stereological and histopathological analyses. The results showed that HFD promoted a progressive and sustained increase in BM, high lipids levels compared to animals fed an SD, even with a reduction in FI, inducing dyslipidemia. However, the treatment appeared to attenuate the protective effect of the infection against excessive lipid accumulation in the host. When analyzed individually, both HFD and infection enhanced the inflammatory response in stimulated macrophages. However, the combination of these conditions revealed an inhibitory effect on NO production, suggesting a remodeling of the inflammatory response. Stereological and histopathological analyses indicated that HFD promoted adipocyte hypertrophy, while infection induced hyperplasia. Treatment, on the other hand, resulted in a mixed profile of these cellular changes. In conclusion, the interaction between HFD, acute schistosomiasis infection, and PZQ treatment impacts the course of the infection, modulating both metabolic and inflammatory parameters.

Funding: CAPES, FAPERJ.
Keywords: Schistosomiasis, Hypercholesterolemia, Treatment.

Helium Ion Microscopy (HIM) and Transmission Electron Microscopy (TEM) combined with High-Pressure Freezing and Freeze Substitution (HPF/FS) for detailed characterization of the *Schistosoma mansoni* adult worm tegument

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Electron microscopy (EM) is crucial for studying *Schistosoma mansoni* morphology, particularly the tegument, a key target for drug and vaccine development. EM reveals the plasma membrane, spines, and papillae. The ventral sucker contains sensory papillae and large spines that aid in host attachment, with structures as small as 1 µm. Helium ion microscopy (HIM) provides high resolution and depth of focus without the need for metal coatings, while transmission electron microscopy (TEM) enables nanoscale analysis. This study investigates the ultrastructure of the tegument using advanced fixation and microscopy techniques. For cryofixation, samples were placed in aluminum holders with 1-hexadecene, mounted in high-pressure freezing holders, and frozen using a Bal-Tec 010 HPF system. After freezing, samples were stored in liquid nitrogen, immersed in a substitution medium, and processed for TEM analysis using an HT7800 RuliTEM microscope. For HIM, cryofixed samples were dehydrated with ethanol, dried in CO_2, and mounted on metal stubs. Specimens were analyzed using an ORION microscope. HIM revealed sub-500 nm details, including dorsal tubercles and spines in males, while females displayed a smooth tegument. Both sexes had spines on the suckers, primarily on the dorsal face. TEM of the male body wall showed well-organized single structures, large spines, vacuoles, vesicles, cytoplasmic inclusions, a nucleus, and muscle layers. Cryofixation minimized distortion, preserving ultrastructural integrity better than chemical fixation. These advancements in HIM and TEM enhance the understanding of *Schistosoma mansoni* ultrastructure, supporting future research on parasite biology and therapeutic targets.

Keywords: *Schistosoma mansoni*, freezing and microscopy.

IN VITRO TOXICOLOGICAL ASSESSMENT AND IN VIVO IMMUNOTHERAPEUTIC INVESTIGATION OF RECOMBINANT PROTEINS FROM *Trichuris trichiura*

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Introduction: Allergic diseases affect millions of people worldwide. Helminths have shown potential in modulating allergic responses in the host. Omics studies conducted by our group identified TtMIF and Ttc4299 proteins as potential modulators of inflammatory responses. **Objective:** To evaluate the cytotoxicity of the recombinant proteins rTtMIF and rTtc4299 from *Trichuris trichiura* in different cell types and rTtc4299 immunomodulatory potential in a murine model of *Blomia tropicalis*-induced allergy. **Methods:** In this study, rTtc4299 was heterologously expressed in E. coli BL21 (DE3), purified using an ÄKTA Pure 25® system with a HisTrap FF Ni Sepharose column, and confirmed by SDS-PAGE and Western blot analysis. The cytotoxic effects of recombinant proteins were evaluated on the human macrophage cell line (THP-1) using the MTT assay. The immunomodulatory capacity of rTtc4299 was assessed through cytokine quantification in cell supernatants using ELISA. A/J mice were sensitized and challenged with *Blomia tropicalis* extract, followed by treatment with dexamethasone or rTtc4299 protein via subcutaneous administration. Regulatory T cell production in splenocytes' culture was verified by flow cytometry. **Results:** Stimulation with the proteins did not induce toxicity in THP-1 cells. The rTtc4299 stimuli induced the increased secretion of IL-10, IFN-γ, and IL-1β levels, while reducing TNF levels, indicating its immunomodulatory properties. Total and differential leukocyte counts in the bronchoalveolar lavage fluid of mice were modulated by rTtc4299 treatment. Antibodies (IgG, IgG2a and IgE) and cytokine responses (IL-10, IL-4, IL-5 and IFN-) were also modified in mice treated with rTtc4299. Flow cytometry analyses revealed that rTtc4299 treatment influenced T cell regulatory responses. **Conclusion:** *T. trichiura* recombinant proteins seem to be non-toxic and capable of modulating the Th2 immune response *in vitro* and *in vivo* promoting a mixed immunological profile with regulatory and Th1 cytokine production. However, additional assays are still required to confirm these findings.

Keywords: Allergy, immunomodulation, whipworm
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NEMATODES OF PUBLIC HEALTH CONCERN IN SYNANTHROPIC TERRESTRIAL MOLLUSKS IN BRASILIA, FEDERAL DISTRICT, BRAZIL

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Terrestrial gastropods (slugs and snails) are important components of Brazilian biodiversity due to their richness of species. However, some species are considered agricultural pests or carriers of nematodes of interest to Public Health, such as *Angiostrongylus cantonensis*, etiological agent of the zoonosis eosinophilic meningitis (EoM). After the report of a suspected case of EoM in a child in Ceilândia, Brasília, an epidemiological study was carried out involving the survey of transmitting terrestrial mollusks in the patient's home, as well as in 03 neighbourhoods with previous reports of mollusk infestation. Four mollusk collections were carried out during the dry and rainy periods (2021 and 2022), performing 38 collecting sites. Nematode larvae recovered by the technique of artificial digestion of mollusks. Obtained larvae were identified based on the analysis of mitochondrial cytochrome *c* oxidase I (MT-CO1). We collected 806 mollusks from nine species of seven families: Achatinidae (Achatininae and Subulininae), Agriolimacidae, Ariophantidae, Bradybaenidae, Bulimulidae, Limacidae, and Veronicellidae. Part of the collected mollusks (n=527) was parasitologically analyzed, and the others (n=279) were fixed for morphological identification and deposit in the Mollusk Collection of the Oswaldo Cruz Institute. A total of 134 specimens (25%) were naturally infected by nematodes: *A. cantonensis* (at 03 sites), *Aelurostrongylus abstrusus* (05), and *Cruzia tentaculata* (08). The African snail was the most infected species (53,7%), including the neighbourhood where the child with suspected EoM resides. The results also indicated 07 new reports of mollusk species for Brasília. They reinforce the importance of surveying the mollusks to better understand the epidemiology of transmission of parasitic diseases such as EoM, which is essential for directing more effective prevention and control measures, aiming at promoting the health of the population.

Supported by: Instituto Oswaldo Cruz – IOC/Fiocruz

Keywords: *Achatina* (*Lissachatina*) *fulica, Angiostrongylus cantonensis*, Eosinophilic Meningitis.

Expression and Purification of Recombinant Protein of the *Toxocara canis*

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Introduction: *T. canis* and *T. cati* are gastrointestinal nematodes of the genus *Toxocara spp.* which complete their life cycle in the digestive tract of dogs and cats, and in paratenic hosts, including man, this cycle cannot be completed and the larvae remain in the host, passing between organs and systems. Toxocara spp., infection triggers immune responses from the innate immune system, activating the adaptive system and consequently producing Th2 cytokines that cause eosinophilia and an increase in IgE. Currently, toxocariasis is diagnosed using the larval excretory-secretory antigens of this nematode (TES). Still, the cross-reactivity of antibodies (IgG or IgG4) with antigens from other helminths can lead to a false positive diagnosis. **Objective:** To evaluate the reactivity of the rFULL protein, a *T. canis* specific fusion protein (TES-26 and CTL-4), in the immunodiagnostic Toxocariasis. **Methods:** A synthetic plasmid containing the FULL sequence was transformed into different strains of E. coli, and expression of the heterologous protein was induced with IPTG for 4 hours. The bacterial extracts were solubilized in a pH 9 buffer solution and purified using affinity chromatography. Western Blot confirmed the expression and purification of the protein. Finally, the reactivity of the protein was evaluated with serum from patients using indirect ELISA. **Results and Conclusions**: The rFULL protein was constructed with all the probable B-cell epitopes found through the genetic sequences of two molecules (rTES-26 and rCTL4), resulting in a 38 kDa protein. The expression of the protein was observed and confirmed by western blotting after 4 hours of expression in the bacterial culture, and affinity purification was also carried out using the histag inserted at the C-terminal end of the protein. Through indirect ELISA, it was possible to confirm the immunogenic potential of the rFULL protein, with sensitivity of 71% and specificity of 87% to IgG, and respectively 62% and 91% para IgG4 when compared to TES.

Keywords: Toxocariasis, Immunodiagnosis, recombinant proteins, Toxocara spp.
Support by: fapesb.

IMMUNE CHARACTERIZATION OF CHIMERIC ELASTASE WITH T-CELL EPITOPES (Q1) DERIVED FROM SCHISTOSOMA MANSONI

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Hygiene hypothesis supports the prevalence of allergies in developing countries. Such chronic immune disorders lack definitive cures, highlighting the need for effective treatments. Dust mites, such as *Blomia tropicalis*, are major aeroallergen sources, especially in Brazil. In this context, allergen-specific immunotherapy represents a viable alternative to pharmacotherapy due to its ability to modulate the immune response. Since helminth infections activate immune mechanisms similar to allergic responses, promoting Th2 polarization through immune evasion, the use of recombinant molecules from *Schistosoma mansoni,* such as cercarial elastase (SmCE) are a promising treatment option. This study evaluated the immunoregulatory and immunomodulatory effects of Q1, a chimeric protein designed with T-cell epitopes from SmCE, using *in vitro* stimulation of peripheral blood mononuclear cells (PBMCs) from allergic and non-allergic individuals sensitized to *B. tropicalis*. Q1 was designed using T-cell epitope predictions from SmCE isoforms, with peptide ligands incorporated into the sequence. For protein expression, the cDNA encoding Q1 was synthesized into plasmids, transformed into *E. coli* strains (Star and Rosetta). The protein was purified, dialyzed, and quantified. Mite extract was also prepared for comparison. Blood samples from *B. tropicalis*-sensitized individuals were analyzed for IgE reactivity via indirect ELISA. PBMCs were isolated and stimulated with Q1 for 48 and 72 hours. Cell viability and cytokine production (IL-1β, IL-4, IL-5, IFN-γ, IL-10, IL-13, TNF-α) were measured. Results demonstrate Q1 significantly increased IL-1β and reduced IL-13 in both allergic and non-allergic individuals, while other cytokines remained at basal levels. Cell viability was unaffected, indicating no adverse effects. Although more studies are needed, these results point Q1 as a viable candidate for allergen-specific immunotherapy, offering an alternative to corticosteroid-based treatments.

Supported by: CNPQ, FAPESB

Keywords: Allergy, Immunotherapy, Biotechnology

A promising new chimeric protein for the recognition of *Schistosoma mansoni* infection

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The trematode *Schistosoma mansoni* is the etiological agent of mansonic schistosomiasis, one of the most globally relevant neglected tropical diseases due to the high prevalence of its severe forms and the significant number of associated deaths. The diagnostic method currently recommended by the World Health Organization is the Kato-Katz parasitological technique, which performs well in areas with high infection intensity. However, in regions with low parasitic load, its sensitivity decreases significantly, making case detection more challenging, underestimating the true disease prevalence, and compromising epidemiological control strategies. In this context, the present study aimed to evaluate the efficacy of a chimeric protein named QSmP, derived from 17 peptides of the *S. mansoni* proteome, for infection detection using the ELISA method. Serum samples from 88 infected individuals from the endemic area of Januária/MG and the metropolitan region of Recife/PE were analyzed (67 with low intensity, 13 with moderate intensity, and 8 with high intensity), along with 12 serum samples from uninfected individuals from a non-endemic area. Among the 88 analyzed serum samples, 45% were reactive to the QSmP protein (29 with low intensity, 8 with moderate intensity, and 3 with high intensity). Notably, none of the negative serum samples showed reactivity. These findings suggest that the use of chimeric proteins derived from the *S. mansoni* genome represents a promising strategy to improve case identification, particularly in areas with low infection intensity, contributing to enhanced diagnosis and epidemiological monitoring of schistosomiasis.

Key words: *Schistosoma mansoni*; Serological diagnostics; Chimeric protein.

Support and financing: FAPEMIG, CAPES e CNPq.

METAGENOMIC ANALYSIS OF INTESTINAL MICROBIOTA CHANGES IN MICE INFECTED WITH *TRICHURIS MURIS* DURING ACUTE AND CHRONIC PHASES

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The nematode experimental model *Trichuris muris* is frequently used to study infection processes in soil-transmitted helminths (STH) and host-parasite interactions. However, the associations between helminths and bacteria remains poorly known, particularly regarding the impact of this interaction on the pathophysiology of intestinal infections. In this study, we highlight bacterial virulence in the context of an altered host microbiota. During chronic trichuriasis, a inflammatory process occurs due to lesions caused by adult worms onthe intestinal epithelium. Our group has previously demonstrated that *T. muris* alters gut microbiota composition, and the resulting tissue damage facilitates bacterial invasion into the submucosal layer, leading to dysbiosis and bacterial translocation from the acute to the chronic phase of infection. The aim of the present study is to identify the main bacterial groups present in the microbiota of C57BL/6 mice infected with low doses of *T. muris* at different stages of infection and compare them with a control group. Fecal samples were collected from infected and control mice at 90 minutes and at 10, 17, 22, and 35 days post-infection. Microbiome analysis was performed using 16S rRNA sequencing on the Illumina MiSeq platform. Results showed that the control group exhibited a higher abundance of *Lactobacillales* compared to infected mice, where this abundance was markedly reduced. The *Clostridiales* order increased during the acute phase but declined in the chronic phase, although it remained more abundant in the chronic phase compared to the control group. The *Bacteroidales* order showed an initial increase during the acute phase, followed by a significant rise in the chronic phase compared to both the controls and the acute phase. In conclusion, trichuriasis induces significant alterations in gut microbiota composition, reducing beneficial bacteria while promoting the proliferation of pathogenic ones, which directly impacts host health.

Supported by: CAPES, FAPERJ-JCNE, FINEP, CNPq

Keywords: trichuriasis, microbiota, Metagenomic

FUNCTIONAL ROLES AND ECOLOGICAL PERSPECTIVE IN NEMATODES PARASITE OF SMALL MARSUPIALS

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Host-parasite interactions shape ecosystems, affecting host physiology, behavior, and ecology. This study analyzed helminth community structure and function using ecological networks and the core-satellite concept. Data were collected in 2014, 2022, and 2023 in Pantanal, Mato Grosso do Sul. Helminths were identified morphologically and molecularly. Interactions were assessed via network analysis, with species classified by Zi (within-module degree) and Pi (participation coefficient) (Guimerà & Amaral, 2005). Abundance categories followed Holmes & Price (1986): core (>2), secondary (0.6–2), satellite (0.2–0.6), and rare (<0.2). Ultra-peripheral species (Pi=0) included *Pterygodermatites* sp., *Spirura guianensis*, *Spirura mexicana*, and hosts *Marmosops ocellatus* and *Marmosa (Micoureus) budini*. Peripheral species, such as *Spirura* sp., *Gracilioxyuris agilisis* and *Pterygodermatites jagerskioldi* interacted mainly within modules (0.05<Pi≤0.62). Non-hub connectors including *Didelphoxyuris thylamisis* and the host *Gracilinanus agilis* linked multiple modules (0.62<Pi≤0.80). Hosts *Thylamys macrurus* and *Gracilinanus agilis* had high Zi values (15.0), indicating potential hubs. Core species included *Didelphoxyuris thylamisis* and *Gracilioxyuris agilisis* (>2), secondary *Physaloptera herthameyrae* (0.6–2), satellite *Pterygodermatites hymanae* (0.2–0.6), and rare *Subulura eliseae*, *Spirura guianensis*, *Spirura* sp., *Pterygodermatites jagerskioldi*, *Pterygodermatites* sp., *Spirura mexicana* (<0.2). This study integrates network analysis with core-satellite theory, highlighting key species in marsupial-helminth communities. *Didelphoxyuris thylamisis* and *Gracilioxyuris agilisis* were monoxenous; others were heteroxenous. Further analyses revealed that monoxenous and heteroxenous helminths differ in prevalence, which influences functional connectivity in the network (Pi). These suggest that helminths with distinct life cycles contribute differently to the ecological structure of the parasitic network.

Supported By: FAPERJ-JCNE, CNPq-Universal, UERJ, FINEP.
Keywords: MARSUPIALS, NEMATODE, NETWORK

Histopathological, Morphometric and Stereological Analysis of the Effects of Alcoholism on the Intestine of Schistosomiasis-Infected Mice

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Although the impact of ethanol and *Schistosoma mansoni* infection on small intestine damage has been extensively studied individually, the intestinal histopathological effects of their comorbidity remain uncertain. To investigate this gap, male Swiss Webster mice infected with 80 cercariae received 200 µL of 18% ethanol orally for 28 consecutive days. The animals were divided into the following groups: C (Control), E (Uninfected and ethanol intake), I (infected without ethanol intake), and IE (infected and ethanol intake). After nine weeks of infection, the mice were euthanized, and the small intestine (jejunum) was removed and fixed in 10% formalin. A fragment of the intestine underwent routine histological processing and Hematoxylin-Eosin staining for histopathological, morphometric and stereological analysis. The group C presented the four intestinal layers preserved and with characteristic morphological features. In group E, altered Paneth cells, protein denaturation in intestinal glands extending to the mucosal layer, submucosa, and muscularis due to ethanol-influenced pH changes, and reduced vascularization of enterocytes were observed. In group I, schistosomiasis-induced inflammation in the mucosa and submucosa, with Paneth cells, goblet cells, and leukocyte infiltration in all layers except the serosa, led to villus destruction. In group IE, there was an increase in the muscular layer thickness compared to both group E (129%) and group I (61%). Additionally, an increase in the number of Paneth cells was observed compared to both group E (200%) and group I (125%), and a reduction in goblet cells counts (-67%) compared to both groups. Leukocyte infiltration increased by 133% in quantity compared to the ethanol group (E). However, the inflammatory response was comparable to that of the infected group (I). The association of schistosomiasis infection with ethanol intake exacerbated the inflammatory process, leading to greater tissue damage in the intestine.

Supported By: CAPES

Keywords: *Schistosoma mansoni*, Intestinal histopathology, Ethanol-induced intestinal damage

EOSINOPHILIA INDUCED BY *A. SUUM* INFECTION MAY BE ASSOCIATED WITH REGRESSION OF METASTASES IN 4T1 MURINE BREAST CARCINOMA

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Some studies suggest that eosinophils play a beneficial role in tumor regression. Our group has shown that *A. suum* infection triggers a persistent immune response, leading to eosinophil recruitment to tissues. However, their role in cancer progression remains unknown. Thus, we used female BALB/c mice infected with 250 or 2500 *A. suum* eggs to investigate parasite-induced inflammation in breast cancer. After 14 days, 1×10^6 4T1 cells were subcutaneously inoculated into the right flank. Tumor growth and survival were assessed. After 21 days, animals were euthanized, and lung tissue was collected for histopathological analysis and cytokine quantification, while tumor and BAL samples were used in flow cytometry. To improve metastasis detection, mice received an intravenous injection of 100µCi of radiolabeled peptide, and scintigraphic images were obtained four hours later and lung radioactivity was measured using a gamma counter. Mice infected with 250 eggs had reduced survival. Histopathology analysis showed increased inflammatory infiltrate in the 2500-infected group, while the 250-infected group had a weaker response, associated with less metastasis control.Higher-dose infection led to increased inflammatory infiltration and cytokine expression but reduced metastatic foci. Conversely, lower-dose infection resulted in a weaker immune response, lower cytokine levels, and greater metastatic burden, confirmed by radioisotope imaging. Flow cytometry showed increased BAL cellularity, with eosinophil predominance in the high-dose group. Given *A. suum*-induced eosinophilia, we conducted a pilot study comparing survival between wild-type (WT) and GATA1-/- mice with cancer. The absence of GATA1 led to decreased survival with a Hazard Ratio of 3.429. In summary, high-dose *A. suum* infection reduced metastatic foci in the lung without worsening the clinical condition. Our findings suggest that eosinophils play a role in controlling metastases, warranting further investigation.

Supported by: FAPEMIG, CNPq and CAPES.

Keywords: Eosinophils; breast cancer; helminth infection

ABSTRACT

Unveiling the Role of SmFES Kinase in Schistosoma mansoni: Functional Insights and Novel Compounds for Schistosomiasis Control

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Abstract
The SmFES protein kinase is implicated in signal transduction pathways in *Schistosoma mansoni*, particularly during larval transformation after host penetration. Previous studies from our group identified SmFES as essential for parasite reproduction, hepatic granuloma formation in murine models, and parasite attraction to host mucus molecules. This study further investigates SmFES functionality and screens compounds that may disrupt the parasite's lifecycle. Mice infected with Smfes-knocked-down schistosomula exhibited a 23% higher mortality rate compared to the nonspecific control group exposed to GFP-dsRNA. Liver cytokine analyses showed reductions of 72.3% in CCL2 and 46.3% in IL-6. Histological examination of liver tissues revealed increased collagen deposition and reduced cellularity. Fluorescence in situ hybridization detected Smfes transcription in reproductive regions (uterus, ootype, vitellaria) and the encephaloesophageal region in adult female worms. To explore control strategies, in silico-predicted SmFES-binding compounds were tested on egg and intermediate life stages of the parasite. SmFES17 reduced miracidia hatching rates by 8-12.9%. Compounds SmFES9, SmFES15, SmFES18, and SmFES22 decreased miracidia attraction to *Biomphalaria glabrata* mucus. Snails exposed to miracidia treated with these compounds showed a 30.7% to 58% reduction in cercariae release after 40 days. These findings suggest Smfes influences immune modulation, cellular recruitment, and fibrosis in the liver, contributing to increased mortality in Smfes-knocked-down infections. Its transcription in reproductive and neurological regions highlights potential roles in egg-laying and neural processes. Identified compounds demonstrated efficacy in reducing cercariae release and miracidia hatching, offering promising avenues for schistosomiasis control.
Supported by: CNPq, CAPES, FAPEMIG, FIOCRUZ
Keywords: SmFES, Host-parasite interaction, Schistosoma mansoni, kinase inhibitors

Neuromodulatory peptides of *Biomphalaria glabrata* and their relationship with snail locomotor behavior and susceptibility to *Schistosoma mansoni* infection

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In Brazil, *Biomphalaria glabrata* is the most important snail host species of *Schistosoma mansoni*, the etiological agent of schistosomiasis. The geographical distribution of *B. glabrata* is often associated with schistosomiasis, a disease that requires efficient efforts for eradication. In this context, studies aiming to control the snail population and reduce/block the *S. mansoni* replication/transmission are extremely relevant. One approach to achieving these goals is to deepen our understanding of the snail's nervous system. The FMRFamide belongs to a class of neuromodulatory peptides (FaRPs) that affect the locomotion, circadian cycle, and physiology of many invertebrates. Therefore, these peptides have a potential for the development of molluscicides of low or no toxicity to non-target organisms. Our study investigated the relationship among FaRPs, the circadian cycle, and the locomotory behavior of infected and uninfected *B. glabrata*. Snails were studied using gene expression analysis, behavioral activity, and gene silencing. Behavioral activity was evaluated by filming snails, and quantifying movement patterns. For gene silencing mediated by RNAi, dsRNA was injected into snails followed by phenotypic, behavioral, and gene expression evaluation. Our results suggest that the FaRP gene expression is perturbed by *S. mansoni* infection; and may indicate a possible link between the gene function and the snail locomotor behavior; the infected snails displayed reduced movement compared to controls. Behavioral analysis also provided insights into the circadian cycle, suggesting that snails may be more active at night. Preliminary gene silencing experiments induced a gene expression knock-down, an important achievement given the challenges of gene expression perturbation approaches in snails. These findings highlight the significance of combined molecular and behavioral studies in producing insight that may lead to the development of future control strategies.

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Keywords: FMRFamide, invertebrate nervous system, behavioral studies

Does *Strongyloides stercoralis* modulate liver damage in alcoholic patients?

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Alcoholic patients are vulnerable to several comorbidities of multifactorial etiology, resulting from the combination of malnutrition, imbalance of both the immune response and the endocrine system, as well as liver dysfunction. Previous studies demonstrate an elevated *Strongyloides stercoralis* infection frequence in alcoholic individuals, as well as a higher risk of severe strongyloidiasis.
This is a case-controlled study with 240 alcoholic patients, 60 infected and 180 non-infected with *S. stercoralis*. All patients were admitted for alcoholic detoxification at an Alcoholic Care and Treatment Center. The blood biomarkers evaluation was performed by automated methods (hematological biomarkers), commercial kits (biochemical biomarkers) and nephelometry (IgE dosage).
A high frequency of alcoholics with a low hemoglobin content was observed, but there was no difference between the infected and non-infected groups. The percentage of individuals with eosinophilia, 58.3 (35/60) and 26.1% (47/180), and the concentration of total IgE, 2,882 and 1,400 IU/mL, were significantly higher ($p<0.05$) in alcoholics infected with *S. stercoralis* than in non-infected individuals, respectively. ALT and AST levels were high in both groups compared to the reference values. However, in the infected group, lower AST levels and a smaller frequence of individuals with high levels of ALT were found. Also, AST levels were higher in individuals with a parasite load greater than 100 larvae/g of feces compared to those with a parasite load less than 10 larvae/g of feces, 90.80 ± 39.9 and 56.42 ± 31.9 U/L, respectively ($p<0.05$). A similar result was found for ALT levels.
This study indicates possible protection from alcohol-induced liver damage in *S. stercoralis*-infected individuals, which could be associated with a parasitic immunomodulation of the inflammatory process and may depend on the parasite load.

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Keywords: *Strongyloides stercoralis*; Alcoholics; Liver alteration

SILENT INVASION: HOW TRICHURIS ALTERS THE GUT FROM EARLY INFECTION TO CHRONIC DISEASE

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The dissemination of neglected diseases and their impacts make the continuous study of pathogens essential to ensuring public health. Trichuriasis is a neglected intestinal parasitosis caused by the soil-transmitted helminth (STH) *Trichuris* spp. In the chronic phase, it leads to systemic changes and severe damage to intestinal tissue due to the presence of the parasite and the process of bacterial translocation. However, little is known about when these changes begin and which larval stage they are associated with. Seeking to elucidate this issue, this study aims to track alterations in the cecum during the development of trichuriasis and determine the period in which bacterial translocation occurs in association with the larval stage in a low-parasitic-load infection. Additionally, we will analyze whether the parasite remained inserted in the intestinal mucosa throughout the infection's development. To investigate this, we infected C57BL/6 mice (CEUA 059/2018) with a low dose (50 eggs) and *Swiss* mice infected with high dose (150 eggs). We performed histopathological analysis, SEM, and FISH of the cecum, from larval hatching and mucosal penetration to the presence of the adult worm, based on the following time points: 90 min, 10, 17, 22, and 35 days post-infection (d.p.i.). Our results show that at 10 d.p.i., L2 already causes lesions in the intestinal mucosa. At 17 d.p.i., we found bacteria adhered to the parasite's cuticle, inside the tunnel formed by L3 in the mucosa, and invading the intestinal submucosa. Additionally, we observed that L3 emerges from the mucosa, exposing its anterior region and stylet, suggesting a sewing-like movement as it migrates through the intestine. Histopathological changes in the cecum intensify at 22 and 35 d.p.i., even in a low-dose infection. These findings associated with changes in immunology responses in different tissues may redefine the life cycle of *Trichuris* spp. and pave the way for new therapeutic approaches.

Supported By: CNPq; FAPERJ; CAPES; FINEP.

Keywords: Trichuriasis; Bacterial translocation; Acute phase.

NEAR INFRARED SPECTROSCOPY APPLIED TO THE DIAGNOSIS OF NATURALLY INFECTED *Biomphalaria glabrata* IN THE MUNICIPALITY OF SUMIDOURO/ RJ.

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The near infrared spectroscopy (NIRS) has been applied to species differentiation and parasitological diagnosis of *Biomphalaria glabrata* infected with *Schistosoma mansoni* under experimental conditions. However, the use of NIRS to diagnose infected molluscs collected in the field had not been carried out. The aim of this work was to develop spectral models for the parasitological diagnosis of live specimens of *Biomphalaria* spp collected in the field in areas of schistosomiasis transmission. The municipality chosen was Sumidouro, in the mountainous region of the state of Rio de Janeiro. The molluscs were collected at two collection points over a twelve-month period. A Spectral solutions MyNIR-R portable spectrophotometer (900 to 1700 nm) was used to collect the NIR spectrum. The specimens were collected, surface-dried and placed in the window of the NIR equipment, individually, with the left side facing downwards to obtain the spectrum. The molluscs from the field were kept individually in the laboratory for weekly observation of cercariae elimination. The spectra of the positive molluscs were collected again with the NIR. For chemometric analysis of the spectra and construction of the models, pre-treatment and principal component analysis (PCA) and Linear Discriminant Analysis (LDA) were used. A total of 756 specimens of *B. glabrata* were collected in the municipality of Sumidouro, 409 of which survived for evaluation of cercariae elimination in the laboratory and 89 of which eliminated cercariae of trematodes of veterinary interest. It was not possible to identify *S. mansoni* cercariae. The spectrophotometric model built with infected and uninfected *B.glabrata* in the field showed a differentiation between the two groups in PC-1 of 80% with a hit rate in LDA of 65.8%. These results demonstrate the efficiency of portable NIRS as a parasitological diagnostic tool for naturally infected animals.

Support by CAPES 0001 and PAEF IOC-008-FIOCRUZ-22-2-6

Key words: schistosomiasis, diagnostic and *Biomphalaria* spp

Texto em POortuguês – precisou ser reduzido

The near infrared spectroscopy (NIRS) have been apllied na diferenciação de espécies e no diagnóstico parasitológico de *Biomphalaria glabrata* infectada por *Schistosoma mansoni* em condições experimentais. Foram construídos modelos espectrais de diferenciação de moluscos infectados nos períodos patente e pré patente da infecção. No entanto, o uso do NIRS para diagnóstico de moluscos infectados coletados no campo não tinha sido realizado. O objetivo desse trabalho foi desenvolver modelos espectrais para o diagnóstico parasitológico de espécimes vivos de *B. glabrata* coletadas no campo em áreas de transmissão da esquistossomose. O município escolhido foi Sumidouro, na região serrana do estado do Rio de Janeiro, antigo foco ativo de transmissão da esquistossomose. Os moluscos foram coletados em dois pontos de coletada por um período de doze meses. Para coleta do espectro NIR foi utilizado o espectrofotômetro portátil Spectral solutions MyNIR-R com faixa espectral NIR de 900 a 1700 nm. Os espécimes foram coletados, secos superficialmente e posicionados na janela do equipamento NIR, individualmente, com o lado sinistro voltado para baixo para a obtenção do espectro. Para a construção de um modelo espectrofotométrico de diagnóstico parasitológico com o NIR portátil, os moluscos oriundos do campo foram mantidos individualmente em laboratório para observação semanal de eliminação de cercárias. Os espectros dos moluscos positivos foram novamente coletados com o NIR. Para análise quimiométrica dos espectros e construção dos modelos foram utilizados pré-tratamentos e as análises de componentes principais (PCA) e Análise Discriminante Linear (LDA) como método de classificação. Foram coletados 756 espécimes de *B. glabrata* no município de Sumidouro, 409 sobreviveram para realização da avaliação de eliminação de cercárias em laboratório e 89 eliminaram cercárias de trematódeos de interesse veterinário. Não foi possível identificar cercárias de *S.mansoni*. O modelo espectrofotométrico construído com moluscos infectados e não infectados em campo demonstrou diferenciação dos dois grupos no PC-1 com 80% com taxa de acerto (classificação dos grupos) no LDA de 65,8%. Esses resultados demonstram a eficiência do NIRS portátil como ferramenta de diagnóstico parasitológico dos animais infectados naturalmente.

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Key words: schistosomiasis, diagnostic and *Biomphalaria* spp

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OPTIMIZATION OF THE HELMINTEX METHOD FOR THE DIAGNOSIS OF SCHISTOSOMIASIS MANSONI

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Schistosomiasis mansoni, caused by *Schistosoma mansoni*, is a parasitic infection affecting millions of individuals, particularly in tropical and subtropical regions. Early diagnosis is crucial for appropriate treatment and disease control. The Helmintex method is widely employed due to its ability to concentrate helminth eggs in fecal samples. However, its efficiency can be compromised by prolonged processing times and the presence of large sediment particles. To optimize this process, we aimed to introduce a three-dimensional (3D) filtering device, replacing the sieve-based step, to reduce filtration time and enhance egg recovery.

The prototype test employed a 3D device attached to a 60 mL syringe, in which pre-counted *S. mansoni* eggs were inoculated at the top of the filter, followed by the addition of 70% ethanol up to a total volume of 60 mL. With the plunger attached, the liquid was released into a 50 mL tube and centrifuged at 300 × g for 10 minutes. The filtered sediment was analyzed microscopically, demonstrating improved egg concentration and detection.

With this adaptation, the average filtration time was reduced, and the retention of larger particles enabled more efficient egg concentration. The variation in recovery after five repetitions was only 4%, indicating the method's reliability. This innovation has the potential to benefit large-scale diagnostic laboratories, particularly in endemic areas, contributing to more effective disease control. Further studies and cost-benefit analyses are necessary to validate this approach and ensure its applicability in diverse settings.

Supported by: : FAPES, PPSUS, CNPq
Keywords: Schistosomiasis diagnosis; Three-dimensional (3D) filtration; Helmintex optimization

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ELIMINATION OF LYMPHATIC FILARIASIS AS PUBLIC HEALTH PROBLEM IN BAHIA STATE, NORTHEAST BRAZIL

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Abstract
Objetive: Salvador and Castro Alves were considered endemic foci of lymphatic filariasis (LF) in Bahia State, in the Northeast Region of Brazil, until the end of the 1960s decade. Since then, the cities have not been reassessed to attest the elimination of parasite transmission. To confirm the absence LF autochthonous cases and *Wuchereria bancrofti* transmission in those municipalities, blood and serological surveys, as well as xenomonitoring, were carried up. **Methods:** In the city of Salvador, thick blood smears from 11,324 night school students and 663 school employees were collected and examined to detect *Wuchereria bancrofti* microfilariae. In addition, blood samples were obtained from 510 children aged from six to ten years old to test for circulating *W. bancrofti* antigens using immunochromatographic test. Simultaneously, vector infection was evaluated by xenomonitoring, using the Polymerase Chain Reaction, with the collection of 23,580 female *Culex quinquefasciatus* mosquitoes that were found inside houses in former LF endemic area in Salvador. In the municipality of Castro Alves, 3,105 children and adolescents were examined for *W. bancrofti* antigenemia. **Results:** No cases of microfilaremia or antigenemia were detected in the samples collected in the cities of Salvador and Castro Alves, and no mosquitoes were found to be positive for *W. bancrofti* DNA. **Conclusions:** The results confirm that LF transmission has been interrupted in the former endemic areas of Salvador and Castro Alves in the Northeast region of Brazil. These data were important to certificate the elimination of LF transmission in Brazil by the World Health Organization.

Supported by: Ministry of Health of Brazil and Health Department of Bahia State.
Keywords: *Wuchereria bancrofti*; lymphatic filariasis; elimination.

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FECAL SEDIMENT SPREADING, ADHESION AND AURAMINE STAINING OF *Schistosoma mansoni* EGGS ON MICROSCOPE SLIDES PRODUCED BY HELMINTEX METHOD

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It is crucial to develop more sensitive methods for the detection of *Schistosoma mansoni* eggs in feces, considering the global impact of schistosomiasis. Thus, the elimination of this disease as a public health problem depends on advancements in diagnosis. In Helmintex, a highly sensitive diagnostic method for schistosomiasis, three different methodologies were implemented for improvement, aiming to evaluate the impact of sample distribution on glass slides (1), the persistence of egg adhesion and Auramine(Au-O) staining over sample aging (2), and to characterize fecal sediments through granulometry (3). In methodology (1), slides prepared with 30 µL of feces, 90 µL of 0.9% saline solution, and 90 µL of 5% Tween 20 were categorized as "good" or "poor" spreading based on the level of particle aggregation on the slide. Methodology (2), using the same solutions with the addition of eggs, had its material analyzed under light and fluorescence microscopy at different time intervals. Methodology (3) involved slides with 30 µL of feces, 90 µL of 0.9% saline solution, and 70 µL of 5% Tween 20, which were subsequently analyzed using ImageJ and categorized by the quantity and size of granules. It was concluded that slides with good spreading exhibited larger areas, indicating a more effective sample distribution, while poor spreading showed smaller areas, reflecting greater sediment aggregation, emphasizing the importance of homogeneous distribution for better visualization. Additionally, it was observed that approximately 68.7% of eggs remained in their positions after storage, with stable fluorescent staining for up to 90 days. Finally, a higher quantity of granules was found in smaller size categories, with significant differences between the mean values of the categories, indicating variation in the distribution of particles retained by sieves. The sum of these efforts ensures better microscopic visualization of the eggs, consequently aiding in a faster and safer diagnosis.

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Keywords: Helmintex, Eggs, *Schistosoma mansoni*

PARASITE-HOST RELATIONSHIP IN MICE ENVIRONMENTALLY CONTAMINATED WITH SYPHACIA SPP IN ANIMAL KEPT IN RESEARCH FACILITIES

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Syphacia sp is a cosmopolitan nematode that parasitizes small rodents. Its eggs are easily dispersed, potentially interfering with experimental studies conducted in environments with weakened sanitary conditions. To evaluate the parasitic interaction of Syphacia sp with isogenic (C57BL/6) and non-isogenic (Swiss Webster) mice, the animals were divided into four groups after carrying out Graham's parasitological examination: BLN (C57BL/6 negative); BLP (C57BL/6 positive); SWN (Swiss Webster negative); and SWP (Swiss Webster positive). After collecting peripheral blood for cell profile evaluation (leukogram), the rodents were euthanized, and the cecum and peri-anal region were removed for histopathological and morphometric analysis. In the BLN and SWN groups, the cecum region showed standard morphology of the mucosal, submucosal, and muscular layers, along with goblet cells. However, the BLP and SWP groups presented hyperplasia of goblet cells with increased mucus production, leukocyte infiltration, and submucosal expansion, while the muscular layer remained preserved. In the peri-anal region, only the BLP group showed alteration with the transformation of epithelial tissue into glands with goblet cells and the loss of structures such as sebaceous glands and hair follicles, associated with rectal prolapse. The infected BLP and SWP groups exhibited an inflammatory cell profile, but the isogenic animals demonstrated greater susceptibility. The morphometry of the cecum showed that both infected groups had significant morphological alterations, but the non-isogenic group showed less damage compared to the infection. The results indicate that *Syphacia sp* significantly interferes with the health of rodents, especially isogenic mice, potentially impacting and compromising the validity of experimental studies conducted in contaminated environments.

SUPPORT: FCM, FAPERJ, CAPES

KEYWORDS: *Syphacia spp*, Morphometry, Peri-anal

DIAGNOSIS OF ENTEROBIASIS: WHY USE THE GRAHAM TECHNIQUE?

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Abstract

Enterobiosis is caused by *Enterobius vermicularis*, a nematode that inhabits the large intestine, being common in children. Its most common symptomatology is intense anal itching, generally nocturnal, due to oviposition by the female, which determines the recommendation to carry out parasitological techniques that enable the identification of these eggs directly from the perianal region. Despite of this knowledge, few studies use the Graham Technique, carrying out the parasitological examination with techniques that enable the detection of parasitic structures in fecal samples, determining the underestimation of positivity for *E. vermicularis*. The aim of this study was to diagnose enterobiosis using parasitological techniques in fecal samples and at perianal material. The study was carried out with children aged two to six years old from three community daycare centers in Niterói. It was requested to collect three slides of perianal material from different days for the Graham Technique and two pots of total feces to perform the Ritchie technique modified by Young et al., Faust et al. and fine mesh screening. Samples were received from 95 children, of which 88 submitted at least one fecal sample, totaling 151 samples, and 92 children submitted at least one slide of perianal material, totaling 255 slides. *E. vermicularis* was diagnosed only using the Graham Technique in 12% (11/92) of the children, reinforcing the importance of its use in diagnosing this parasitosis, as this was the most common parasite. 74/92 children submitted all three slides, of which 8 (10.8%) were positive. The use of only one slide showed positivity in 4/5 children. The insertion of a second slide increased the diagnostic efficiency, with results statistically close to the use of three slides. The use of the Graham technique was mandatory to diagnose entetobiaisis, and the use of two slides increased the effectiveness of the diagnosis, minimizing cost and discomfort/work in obtaining samples.

Keywords: parasitological technique. *E. vermicularis*. Nematode

New Occurrence of *Braga patagonica* (Isopoda: Cymothoidae) in the Eastern Amazon: Morphology, Parasitism, and Distribution

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Ectoparasitic isopods, despite their small size, are intriguing due to their remarkable adaptation to parasitic life and the diverse effects they exert on their host organisms. These organisms can be susceptible to ectoparasites, such as isopod crustaceans. This study reports the presence of the isopod *Braga patagonica* parasitizing *Hoplias malabaricus* in the state of Maranhão, located in the Eastern Amazon, Brazil. Isopod specimens were collected from the fish body in two sampling periods conducted between 2021 and 2022 and identified using dichotomous keys based on morphological analyses and parasite measurements. Additionally, parasitological indices were calculated, including prevalence rate (P%), mean intensity (MI), and parasite abundance (PA). A total of 25 parasites, including both females and males, were found attached to the gill cavity, tegument, and near the pectoral and pelvic fins. During the first sampling period, the hosts exhibited a prevalence rate of 100%, with MI and PA values of 1.0 ± 0.00, whereas in the second sampling period, the prevalence rate decreased to 66.67%, with MI of 0.06 ± 0.04 and PA of 0.04 ± 0.29. Females were larger, measuring approximately 2.09 cm in length and 1.09 cm in width, while males reached 1.96 cm in length and 0.86 cm in width, and exhibited a lighter coloration compared to females in both sampling periods. *B. patagonica* has been previously reported in the major rivers of the Amazon basin, particularly in northern Brazil, with low prevalence in fish from natural environments, found in various native fish species such as *Serrasalmus* spp., *Colossoma macropomum*, *Pygocentrus nattereri*, and *Hoplias malabaricus*. This study establishes a new geographical record of *Braga patagonica* in the state of Maranhão, in the northeastern region of Brazil. The findings provide valuable insights into the distribution of the Cymothoidae family in the Eastern Amazon.

Keywords: Fish parasites, Isoposa, Crustacea

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Dog stool collected in public areas as indicators of environmental contamination by zoonotic geohelminths: a collaborative mapping investigation in Feira de Santana, Bahia, Brazil

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Zoonotic geohelminth infections, particularly in urban areas with high animal traffic, where fecal deposition in shared spaces can lead to environmental contamination, pose a potential risk for human infection. This cross-sectional observational study aimed to determine the prevalence and spatial distribution of these parasites in dog stool collected from public areas in Feira de Santana, Bahia. Sample collection followed predefined trails, and collection sites were georeferenced using the +Lugar platform, which also recorded environmental variables. In the laboratory, samples were analyzed using the Gordon & Whitlock method with hypersaturated sodium chloride and zinc sulfate solutions. Statistical analysis included absolute and relative frequency calculations, Pearson's chi-square test, McNemar's test, and logistic regression in EpiInfo, as well as spatial analysis using a space-time scan model in SaTScan. Of the 272 samples analyzed, 19.49% tested positive for parasites, with Ancylostomidae (17.28%) being the most prevalent, followed by *Cystoisospora* spp. (5.51%) and *Toxocara* spp. (2.94%), with no significant difference between the solutions used in the tests. Six spatial clusters were identified, one of which was significant within the university campus (rr=3.00; p=0.021), warranting further investigation to determine the contributing factors. No significant associations were found between environmental variables and parasite positivity rates. Collaborative mapping conducted through the +Lugar platform contributed to identifying these conditions, facilitating result interpretation for future control measures. The detection of geohelminths in public areas highlights the need for continuous monitoring, educational initiatives, and animal management strategies aimed at preventing and controlling zoonoses within the One Health framework.

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Keyword: Zonotic geohelminths, Spatial analysis, One Health

Metamicrocotyla macracantha (ALEXANDER, 1954) (POLYOPISTHOCOTYLA: MICROCOTYLIDAE) OF *Mugil liza* VALENCIENNES, 1836 (OSTEICHTHYES: MUGILIDAE) IN TWO LAGOONS ENVIRONMENTS FROM RIO DE JANEIRO STATE, BRAZIL

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Abstract

The state of Rio de Janeiro has artisanal fishing as a very important subsistence activity. *Mugil liza* is one of the most common fish, popularly known as mullet. The Maricá lagoon is the largest in the Maricá-Guarapina lagoon system. The Jacarepaguá lagoon system is formed by rivers that originate on the slopes of the Maciço da Tijuca and Pedra Branca, and which flow to the sea along the coastal plain until they flow into the region's lagoons. The present work aimed to compare the occurrence of *Metamicrocotyla macracantha* in these two environments. A total of 44 specimens of *M. liza* were collected from Maricá lagoon and 11 from Jacarepaguá lagoon. The fish were purchased from artisanal fishermen, transported to the Laboratório de Biologia e Ecologia de Parasitos at ICBS/UFRRJ, where they were necropsied. Of the 44 mullets analyzed in Maricá lagoon, eight (24%) were infested by *M. macracantha* with mean intensity of 1.8 and mean abundance of 0.34. Four out of 11 mullets from Jacarepaguá lagoon were infested (36%), with mean intensity of 3.7 and mean abundance of 1.3. Although only 11 fish were collected in Jacarepaguá lagoon, the parasitic parameters of *M. macracantha* were higher from that location. Exposure to contaminants can directly or indirectly impact infestation intensities. The indirect impact would be linked to the effect of pollution on the host's immune system, which could increase infestation and the production of mucus, which serves as food for polyopisthocotylans. In March 2024, the Jacarepaguá lagoon presented a poor to very poor IQA (Water Quality Index), while the Maricá lagoon presented a medium IQA, which may have favoured the greater infestation of mullets from the Jacarepaguá lagoon. The study of the parasitic fauna of economically important fish, such as mullet, is pivotal because ectoparasites can directly affect the development of these animals and polyopisthocotylans ones can be used as bioindicators of environmental quality.

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Keywords: Lagoon systems, Mullets, Platyhelminthes

MACROPHAGE MIGRATION INHIBITORY FACTOR FROM *Trichuris Trichiura* IMPROVES SOME THERAPEUTIC PARAMETERS OF AN ALLERGY VACCINE

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Introduction: *Blomia tropicalis* is major source of mite allergens, triggering symptoms of allergic rhinitis and allergic asthma. Allergen-specific immunotherapy, while a promising therapeutic approach to induce allergen tolerance, needs improvement on aspects of safety and efficacy. Helminth-derived immunoregulatory biomolecules offer potential as allergy vaccine adjuvants. Through omics studies, our group identified the macrophage migration inhibitory factor (TtMIF) from *Trichuris trichiura* as one of this potential immunoregulatory adjuvants.
Objective: To test the recombinant TtMIF as an adjuvant for a recombinant hypoallergenic vaccine (BTH2) in a murine model of asthma induced by *B. tropicalis*.
Methods: Mice were sensitized and challenged with *B. tropicalis* extract and treated with BTH2 + Alum®, or BTH2 + Alum® + rTtMIF, in a protocol lasting a total of 4 months. After euthanizing the animals, several immunological efficacy parameters were evaluated by various in vitro assays.
Results: Presence of rTtMIF during treatment reduced allergenic markers (eosinophils, IgE, Th2 cytokines) compared to placebo, while increasing specific IgG and IgA, that may have acted as blocking antibodies, since there was inhibition of IgE-binding capacity of human sera reactive to *B. tropicalis* allergens. Compared to BTH2 alone, rTtMIF+BTH2 displayed: (i) high stimulation indexes of splenocytes; (ii) up-regulation of IL-10 and TGF-β in lungs and secreted by splenocytes; (iii) higher production of IgA in sera and lungs; and (iv) stronger induction of serological blocking antibodies. Interestingly, it was observed strong correlation between IgA and TGF-β in the mice' lungs.
Conclusions: rTtMIF acted as an adjuvant of BTH2, improving its efficacy by up-regulating regulatory cytokines and blocking antibodies. Although these results are appealing, additional chronic mice models are still warranted to confirm the findings, the exact T cell responses and rTtMIF safety without Alum®.

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Keywords: Immunoregulation, Adjuvant, Whipworm.

INTESTINAL BACTERIAL TRANSLOCATION IN SCHISTOSOMIASIS MANSONI-INFECTED C57BL/6 MICE FED ON A HIGH-FAT DIET: INSIGHTS FROM FLUORESCENCE *IN SITU* HYBRIDIZATION (FISH)

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There is increasing evidence that the coexistence of schistosomiasis mansoni and obesity exacerbates liver, spleen, and intestinal damage by altering the architecture and physiology of these organs. Studies highlight the role of the intestinal microbiota in hepato-intestinal schistosomiasis, shedding light on the complex interactions between adult worms, intestinal microbiota, host immunity and the pathophysiology of infection in the acute and chronic phases. Therefore, we sought to understand this relationship using a model of diet-induced obesity. C57BL/6 male mice fed either a high-fat diet (HFD, 60% fat) or a standard diet (10% fat) for 13 weeks were infected with 100 *Schistosoma m*ansoni cercariae (BH strain). Mice were divided into four groups: USC (uninfected, standard diet), UHFC (uninfected, high fat diet), ISC (infected, standard diet) and IHFC (infected, high fat diet). Obesity development was assessed by lipid profiles, glucose levels, oral glucose tolerance, body mass and adiposity. For fluorescence in situ hybridization (FISH), mouse jejunum sections were collected and fixed in 10% buffered formalin for 48 hours. FISH was performed on each slide using conditions and buffers with 30% formamide in the hybridization buffer and probes for eubacteria. IHFC showed a reduction in lipid profile, an increase in HDL-c and better oral glucose tolerance and reduction in body mass. The results obtained by FISH indicate the occurrence of bacterial translocation in the infected groups, especially in those subjected to the high fat diet. This process is known to occur due to increased permeability of the intestinal mucosa stimulated by HFD, increased bacterial growth, a weakened immune system and lesions caused by *S. mansoni* eggs during their passage into the intestinal lumen. Altogether findings suggest that concurrent obesity and acute schistosomiasis have a significant impact on the architecture of the jejunum in C57BL/6 mice.

Keywords: *Schistosoma mansoni*. Obesity. Gut microbiota.

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EVALUATION OF THE OVICIDAL POTENTIAL OF QUERCETIN IN MANSONI SCHISTOSOMIASIS IN A MURINE MODEL

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Schistosomiasis is a neglected and endemic disease in Brazil. The only drug used for treatment is praziquantel, and the lack of alternative therapeutic options has raised concerns within the scientific community due to the potential emergence of worm resistance, as this drug acts only against the adult worm. Quercetin is a natural polyphenolic flavonoid with low toxicity, possessing antioxidant, anti-inflammatory, and immunomodulatory activities, as well as in vitro antiparasitic action. The objective of this study was to evaluate the ovicidal potential of quercetin administered during the juvenile stage of Schistosoma mansoni in a murine model, analyzing the effects on eggs after 55 days of infection. This was an in vivo experimental study conducted with 39 male albino Swiss Webster mice, experimentally infected with Schistosoma mansoni. Quercetin was administered via gavage at the following doses: 800 mg/kg, 1600 mg/kg, and 2400 mg/kg, with treatments performed according to the infection days: 5th and 15th days post-infection or 10th to 14th days post-infection. Feces collection was performed after 55 days of infection, and egg counting was conducted using the Kato-Katz method. Results were expressed as median and 25th and 75th percentiles of the egg count in the feces across the different groups. In statistical model 1, which compared the infected control group to the treated groups, no statistically significant difference was observed. Similarly, statistical model 2, which evaluated the different quercetin concentrations and treatment durations, also did not indicate any statistical difference. The results obtained in this study suggest that quercetin, at the doses used, does not possess ovicidal activity against S. mansoni when administered during the juvenile stage of the parasite.

Keywords: Schistosomiasis; Schistosoma mansoni; Quercetin.

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HEPATIC AND RENAL EFFECTS OF *Rosmarinus officinalis* ESSENTIAL OIL IN MICE INFECTED WITH *Strongyloides venezuelensis*

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Strongyloidiasis is a parasitic disease prevalent in developing countries, where climatic conditions favor its spread. Treatment relies on albendazole, thiabendazole, mebendazole, or ivermectin, the latter being the drug of choice. However, in addition to potential hepatic and renal side effects, reports of parasitic resistance have emerged. Thus, the search for alternative therapies is necessary, and natural products have been investigated as potential low-cost and accessible antiparasitic agents. *Rosmarinus officinalis* is traditionally used in medicine for its antifungal, antibacterial, anti-inflammatory, and antioxidant properties. However, its potential against *Strongyloides* sp. and its effects on the host's hepatic and renal function remain underexplored. This study evaluated the hepatotoxic and nephrotoxic effects of *R. officinalis* qt. Cineol essential oil in mice infected with *Strongyloides venezuelensis*. Male Swiss mice, aged between 8 and 10 weeks and weighing approximately 20 g, were infected with 1500 filariform larvae (L3). After 24 hours, the animals were divided into four groups: GI (infected and treated with water), GII (infected and treated with ivermectin 2 mg/kg), GIII (infected and treated with essential oil 200 mg/kg), and GIV (non-infected control). Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, and creatinine were measured. Mice treated with ivermectin showed increased ALT and AST levels compared to other treatment groups. Urea levels were higher in mice treated with essential oil than in those treated with ivermectin. A slight increase in creatinine was observed in ivermectin-treated animals. The results indicate the need for further studies to evaluate the safety of rosemary essential oil as a therapeutic alternative for strongyloidiasis.

Funding support: CAPES

Keywords: Strongyloidiasis, medicinal plants, treatment

THERAPEUTIC POTENTIAL OF *Rosmarinus officinalis* AGAINST *Strongyloides venezuelensis:* AN IN VIVO APPROACH

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Strongyloidiasis is a parasitic disease prevalent in developing countries, often associated with environmental conditions that facilitate its transmission. In immunocompetent individuals, the infection is typically confined to the gastrointestinal tract; however, in immunosuppressed individuals, it can progress to severe and potentially fatal forms. Ivermectin is the drug of choice for treatment, but parasitic resistance and therapeutic failures highlight the need for new therapeutic approaches. In this context, *Rosmarinus officinalis* has been investigated for its pharmacological properties, including its potential anthelmintic effect. This study aimed to evaluate the anthelmintic activity of *R. officinalis* ethanolic extract in mice infected with *Strongyloides venezuelensis*. Male Swiss mice, aged between 8 and 10 weeks and weighing approximately 20 g, were infected with 1500 filariform larvae (L3) of *S. venezuelensis*. After 24 hours, the animals were divided into five groups and treated for six consecutive days: Group I – treated with *R. officinalis* ethanolic extract (600 mg/kg); Group II – treated with *R. officinalis* ethanolic extract (200 mg/kg); Group III – treated with ivermectin (2 mg/kg); Group IV – treated with water, and Group V – non-infected control. On the seventh day post treatment, the animals were euthanized. The 600 mg/kg concentration showed 88.4% efficacy, while ivermectin achieved 92.5%, directly reducing parasite fecundity. No significant changes were observed in leukocyte counts. However, alterations in hepatic and renal markers were detected in groups treated with 600 mg/kg of *R. officinalis* and ivermectin. In conclusion, *R. officinalis* ethanolic extract demonstrated potential as a therapeutic alternative for strongyloidiasis, but further studies are needed to assess its effects on the host.

Supported By: CAPES

Keywords: Phytotherapy, antiparasitic treatment, medicinal plants.

PRESENCE OF HELMINTHS IN FECAL SAMPLES OF *Lycalopex vetulus* (CARNIVORA: CANIDAE) IN CORUMBAÍBA, GOIÁS

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Although the Cerrado is famous for its diversity and abundant endemism, it is being damaged by hunting, extensive grain monoculture, industrial pollution and deforestation, making it a “hotspot” of endangered biodiversity. Members of the Canidae family, such as the *Lycalopex vetulus*, hoary fox, are nocturnal and crepuscular, omnivorous animals with short snouts and large ears; they have a wide distribution in the Cerrado biome, in the central-southern region of Brazil, such as in the states of Minas Gerais, Mato Grosso do Sul, Mato Grosso, São Paulo, Goiás, Tocantins, southwest Bahia and western Piauí, in the Serra da Capivara National Park. The animals' health and behavior are impaired by parasitic infections, which are generally not fatal, but increase their susceptibility to predation and other environmental disturbances. A threat to the health of wildlife populations such as hoary foxes and humans is the lack of data on the prevalence of parasites in wild animals. In view of this, the aim of this study was to carry out laboratory tests on fecal samples of hoary foxes (*Lycalopex vetulus*) collected at Fazenda Pontal in Corumbaíba (GO), for parasitological identification. For this purpose, eggs were visualized in the samples and then identified by comparing works found in the literature. Three genera of parasites were identified, *Ancylostoma* sp., *Prosthenorchis* sp. and *Taenia* sp., demonstrating zoonotic potential given their biological cycles. The information gathered encourages new epidemiological research into wild animal parasites, as well as the identification of species using molecular biology techniques.

Key-words: *Lycalopex vetulus*, Cerrado, Helminths.

BIODIVERSITY OF HELMINTHS IN THE *Thylamys macrurus* (OLFERS, 1818) (DIDELPHIDAE: THYLAMYINI) FROM BRAZILIAN PANTANAL, MATO GROSSO DO SUL, BRAZIL

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The biome Pantanal, located in central South America, a seasonally fire-prone region, drew international attention after the unprecedented 2020 wildfires. The Pantanal serves as a vital biodiversity sanctuary, harboring numerous endemic species of Brazilian flora and fauna. Among didelphid marsupials, *Thylamys macrurus* (Olfers, 1818) is endemic to parts of Mato Grosso do Sul, where it plays an important role in the local ecosystem. However, increasing environmental threats, such as habitat loss and climate-driven wildfires, endanger the region's unique biodiversity, underscoring the need for conservation efforts. In this way, the aim of this study was to characterize the helminth community of *T. macrurus* in the Pantanal, specifically in the municipality of Corumbá, state of Mato Grosso do Sul, Brazil. Four small marsupials *T. macrurus* were collected from the Pantanal (SISBIO 13373-5; 73761) using Sherman live traps placed on the soil and in the trees. The helminths were recovered from the gastrointestinal tracts, washed in saline (NaCl) and fixed in 70% ethanol for light microscopy. All captured hosts were parasitized and 228 helminth specimens were recovered from them. The helminths were first identified based on morphological and morphometrical characterization. We identified four different genera of nematode and one Cestode. We recovered two *Spirura* sp. in stomach, one *Pterygodermatites jagerskioldi*, 110 *Didelphoxyuris thylamisis*, 110 *Gracilioxyuris agilisis*, and two cestodes. The helminth species found in *T. macrurus* have also been reported in other species of the tribe Thylamyini, corroborating previous studies on helminth communities within the genus belonging to the tribe. This study provides the first analysis of the helminth community in *T. macrurus*, offering valuable insights and highlighting the importance of research on helminth biodiversity in small marsupials, especially those living in habitats increasingly affected by environmental degradation.

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Keywords: Marsupials, Pantanal, Nematode, Cestode

PARASITOLOGICAL RESEARCH IN A BIOFERTILIZER LAGOON FROM THE ANAEROBIC BIODIGESTION OF BOVINE FAECES

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Brazil has the largest cattle commercial herd of in the world, with a system for raising dairy cattle in confinement, where there is greater concentration of animals and, consequently, a high production of cattle waste generated from the production. These wastes contain pollutants, which, when handled incorrectly, can cause serious environmental problems, in addition to enabling the spread of parasitic diseases in the herd. From this impasse, anaerobic bio-digestion in biodigesters emerges as a sustainable alternative for waste treatment arising from cattle raising, enabling the generation of high-energy (biogas) and nutritive (biofertilizer) by-products. The use of biofertilizers in agriculture promotes plant growth and protects the crop, but it may contain pathogens that have the capacity to cause infections in animals, and when this is irrigated in pastures, it ends up compromising the health of cattle. Thus, the present work had as general objective to perform the parasitological control of biofertilizer from the anaerobic decomposition of cattle manure using the direct microscopic method and the modified Bailenger method, which was standardized in the laboratory where the study was conducted. The biofertilizer samples analyzes performed by the direct method revealed the presence of *Toxocara vitulorum* eggs, while those performed by the modified Bailenger method did not identify evolutionary parasitic forms of bovine parasites. As for the preparation of the cattle manure sample processed by the modified Bailenger method, it was possible to identify *Oesophagostomum radiatum.* The standardization of the methodology modified by Bailenger had good sensitivity, reduced cost and easy execution. Both methods of analysis proved to be useful for carrying out parasitological monitoring of the biofertilizer studied in this work, but the direct microscopic method proved to be more efficient than the modified Bailenger method, providing faster results with greater precision.

Key-words: Biodigester; Renewable Energy; Parasitological Control; Bovine Helminths.

COMPARISON BETWEEN IMMUNOBLOT AND ELISA ASSAYS FOR IMMUNODIAGNOSIS TO NEOTROPICAL ECHINOCOCCOSIS IN VULNERABLE POPULATIONS IN THE NORTHERN REGION OF BRAZIL

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Neotropical echinococcosis (NE), endemic to the northern region of Brazil, is a disease caused by *Echinococcus vogeli*. Immunodiagnosis for NE can minimize health complications and mortality by identifying potentially infected individuals while they are still asymptomatic. This study aims to compare the serological assays ELISA (EL) and Immunoblot (IB) (gold standard) in their ability to detect IgG antibodies against *Echinococcus* spp. in human serum samples from vulnerable populations in northern Brazil. A total of 960 serum samples collected between 2022 and 2024 were analyzed using the two assays. In 2022, 106 serum samples were collected in Porto Velho, Rondônia (RO), with no reactive samples in IB and 14 (13.21%) reactive in EL. In 2023, 530 samples were collected in Alto Juruá municipalities (AJ), Acre (AC), with 87 (16.41%) testing reactive in IB and 136 (25.66%) in EL. Also in 2023, 63 samples were collected in Porto Velho (RO), with 2 (3.17%) testing reactive in IB and 8 (12.7%) in EL. In 2024, 261 serum samples were collected in AJ (AC), with 109 (41.76%) testing reactive in IB and 112 (42.91%) in EL. The higher percentage of reactive samples in EL compared to IB may be due to the lower specificity of ELISA in comparison to IB. Both assays still need to be evaluated for cross-reactivity using an internal serum panel of other helminthic infections. Due to its lower specificity, ELISA serves as a good preliminary test to screen potentially reactive samples, considering its automation and ability to process a higher number of samples simultaneously. IB, being the gold standard, offers higher specificity; however, as an in-house assay, it requires more time and manual labor. Therefore, the combination of both assays could optimize time and labor requirements for serological screening of NE-vulnerable populations.

Supported by: CAPES; CNPq; LPIP-IOC/FIOCRUZ.
Key-words: Neotropical hidatidosis/echinococcosis; ELISA; Immunoblot.

GENETIC INSIGHTS INTO NEOTROPICAL *Echinococcus* SPECIES: AN OVERVIEW OF AVAILABLE DATA

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This study describes the genetic data available for neotropical echinococcosis (NE) species in sequence databases. The terms "*Echinococcus vogeli*" and "*Echinococcus oligarthrus*" were searched in GenBank (GB) and WormBase ParaSite (WBPS) databases, and the retrieved DNA sequence information (length, region, coding status, genome, and publication) was compiled into a spreadsheet and analyzed using Excel 365 (Microsoft, USA). *Echinococcus oligarthrus* has a complete genome (PRJEB31222) from Argentina in WBPS and 45 sequences in GB, including 1 mitogenome (NC_009461) from Panama, 5 complete CDS (18S rRNA*, ef1a,* 5.8S rRNA and ITS1: Panama; and *atp*6: Brazil), and 39 partial CDS (genomic: *pold, pepck, rpb2, ef1a, elp, AgB1*; mitochondrion: *nad*1*, atp*6*, cox*1). *Echinococcus vogeli* lacks a complete genome, but GB contains 86 sequences: 1 mitogenome (NC_009462) from Colombia, 2 complete CDS for *atp*6 from South America (unknown country), and 83 partial CDS (genomic: 18S rRNA*, pold, pepck, rpb2, ef1a, eg95, elp,* 5.8S rRNA and ITS1*, AgB1, hbx2, AgB, act II, mdh*; mitochondrion: *cox1, atp*6*, nad*1*,* 12S rRNA). In addition, we compared *E. vogeli* and *E. oligarthrus* mitogenomes using BLAST/NCBI, revealing 86.18% genetic identity with 100% coverage. The data is consistent with previous interspecific analyses of *Echinococcus* spp. based on mtDNA (~85%). Intraspecific analysis based on mitogenomes from cystic echinococcosis species has already shown significant genetic diversity, even among isolates from the same country. So, expanding genetic studies, including additional whole-mitogenome sequencing, is crucial to improve species identification, clarify epidemiological roles, and support control strategies for NE. In this context, the study supports the necessity of projects focused on enhancing genetic information of these species in Brazil, even though there are difficulties of obtaining samples, due to the parasitic nature of *E. vogeli* and *E. oligarthrus*.

Supported by: CAPES; CNPq; LPIP-IOC/FIOCRUZ.

Keywords: Genetic data, *Echinococcus oligarthrus*; *Echinococcus vogeli*.

CHIMERIC PROTEINS FROM SCHISTOSOMA MANSONI IN MURINE MODEL FOR ALLERGY

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Helminths derived molecules, particularly *Schistosoma mansoni*, show potential in modulating allergy, one of the most prevalent chronic diseases worldwide. *S. mansoni* infection activates immune mechanisms similar to allergic responses, including IgE production and eosinophil recruitment, while promoting Th1 polarization and IL-10 production, which may control atopy. Our work evaluates the immunomodulatory potential of chimeric proteins from *S. mansoni* elastase (Q1, Q2) in a murine allergy model induced by *Blomia tropicalis*. AJ mice were divided into groups: non-allergic group, Negative Control, and four allergic groups: Positive Control (untreated), Q1-treated, Q2-treated, and dexamethasone-treated. Allergy was induced by intraperitoneal injections of *B. tropicalis* extract (BtE), followed by intranasal challenges over one week. Mice were then treated. Serum IgE, IgA, and IgG2a levels were analyzed, along with bronchoalveolar lavage (BAL) and lungs. Splenocytes were cultured with BtE and either Q1 or Q2. Cytokine production was assessed by ELISA, splenocyte proliferation by MTT, and cell populations by flow cytometry. BAL cells underwent total and differential counting, and peroxidase levels were measured in lungs and BAL. No significant differences in antibody levels were observed. In Q1-treated mice lung, eosinophils and neutrophils were increased, while macrophages and lymphocytes were decreased, suggesting worse allergic conditions. IL-5 was reduced in BAL but increased in lung homogenates. Q2-treated mice had more macrophages and less IFN-γ in BAL than positive control, along with lower IL-5 levels, indicating inflammation control. Lung IL-4 and IL-13 levels were lower in control groups. In splenocyte cultures, Q2 increased CD4+ cells, with higher IFN-γ production, suggesting Th1 stimulation, crucial for Th1/Th2 balance and immune tolerance. Finally, peroxidase levels indicated improved allergic conditions. These findings point Q2 as a promising allergy treatment.

Supported by: FAPESB, CAPES, FEP

Keywords: *S. mansoni* , allergy, chimeric proteins.

FACTORS ASSOCIATED WITH HELMINTH INFECTIONS IN SMALL RUMINANTS FROM THE SEMI-ARID REGION OF BAHIA

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Gastrointestinal helminth infections are an important cause of losses in goat farming, especially in semi-arid regions. But are specific animal characteristics associated with a higher positivity for these infections? This study aimed to answer this question by identifying factors associated with helminth positivity in goats and sheep through multivariate analysis using a robust Poisson regression model. Data from 1,915 animals from 95 farms in the semi-arid region of Bahia, Brazil, were analyzed. The variables studied included species (goat or sheep), age (young, young adult, and adult), and body condition score (BCS). Fecal samples were analyzed at the Laboratório de Saúde Única of UFOB. The results indicate an association between animal characteristics and vulnerability to helminth infections. Goats showed a 16% higher prevalence compared to sheep (RP = 1.160; 95% CI: 1.094 – 1.231; $p < 0.001$), suggesting a possible difference in susceptibility between species, potentially related to management practices and greater exposure to contaminated areas. Young animals showed a higher prevalence compared to adults (RP = 1.093; 95% CI: 1.021 – 1.171; $p = 0.011$), which may be attributed to an immature immune system, emphasizing the need for more intensive control strategies for this group. Animals with a lower BCS showed a 35.5% higher prevalence (RP = 1.355; 95% CI: 1.062 – 1.760; $p = 0.018$) compared to those with a BCS between 3 and 4, increasing to 57% higher (RP = 1.570; 95% CI: 1.213 – 2.064; $p < 0.001$) in the most debilitated animals. These data reinforce that poor body condition reduces the animal's ability to combat infections, exacerbating the effects of parasitism. These findings highlight the need for preventive strategies for goats, young animals, and those with poor body condition, as well as studies integrating management and environmental factors to better understand their influence on helminth positivity.

Supported by: FAPESB, INCITE Call (2022)
Keywords: Veterinary Parasitology, Multivariate Analysis, Goat Farming

TARGETING GPCRS IN *SCHISTOSOMA MANSONI*: ENHANCING RNAi KNOCKDOWN FOR EFFECTIVE CONTROL OF SCHISTOSOMIASIS

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Abstract

Schistosoma mansoni is a parasitic flatworm responsible for schistosomiasis, a neglected tropical disease affecting millions worldwide. Control of schistosomiasis is limited by the reliance on Praziquantel (PZQ) as the sole treatment, highlighting the urgent need for alternative drug targets. The reproductive biology of schistosomes is unique, as the female requires continuous pairing with a male to achieve sexual maturation. This study investigates G protein-coupled receptors (GPCRs) as potential drug targets, given their crucial roles in biological processes and druggability. However, despite extensive research on GPCRs in vertebrates, their roles in invertebrates like schistosomes remain poorly understood.We focused on the functional characterization of *S. mansoni* GPCRs differentially expressed in pairing-experienced males, pairing-inexperienced males, and females, hypothesizing their involvement in male-female interactions and reproduction. To overcome low RNA interference (RNAi) efficiencies, we developed a novel two-dsRNAs-one-target RNAi approach, achieving knockdown (KD) efficiencies of 92–99% for candidate GPCRs. Physiological effects were monitored over 21 days *in vitro*, evaluating pairing stability, attachment capacity, stem-cell activity, and egg production. Our findings revealed significant phenotypes following RNAi-mediated GPCR KD, including curling, tegumental damage, reduced motility, decreased stem-cell proliferation, and a decline in mature oocytes. These results strongly suggest that the selected GPCRs play critical roles in the vitality and reproductive biology of *S. mansoni*. In conclusion, this study optimized RNAi techniques for GPCR characterization and identified key candidates essential for schistosome survival and reproduction. Targeting these GPCRs could disrupt crucial processes in the schistosome lifecycle, providing promising alternatives to PZQ for combating schistosomiasis.

Supported by: Deutsche Forschungsgemeinschaft (DFG)

Keywords: *Schistosoma mansoni*, GPCRs, RNAi

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SEROEPIDEMIOLOGY OF STRONGYLOIDIASIS IN SAMPLES FROM RECIFE BLOOD BANK: A NEGLECTED DISEASE

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Abstract

The World Health Organization (WHO) recently defined the strongyloidiasis as one of the Neglected Tropical Diseases (NTDs). More detailed epidemiological data on the global distribution of strongyloidiasis are needed. In this context, the aim of this study was to perform a seroepidemiological evaluation of *Strongyloides stercoralis* infection, to detect anti-*Strongyloides* IgG antibodies present in serum samples from blood bank donors in the city of Recife, Pernambuco, Brazil. The study analyzed 766 serum samples from blood donors from the Hematology and Hemotherapy Foundation of Pernambuco (HEMOPE). A heterologous extract of the cuticle of infective larvae of *Strongyloides venezuelensis* was prepared to be used in the enzyme-linked immunosorbent assay (ELISA). To determine the cut-off of the reaction, control serum samples with positive parasitological results for *S. stercoralis* (n=28), negative (n=53) and positive for other parasitoses (n=20) were used. Samples with absorbance values above the cut-off were considered reactive. The ROC curve generated with the control samples showed an area of 0.996 (95% CI = 0.989-1.003; p< 0.0001). The cut-off was 0.420, with a sensitivity of 96.1% (95% CI = 80.4% to 99.9%) and specificity of 96.2% (95% CI = 87% to 99.5%). The optical density values ranged from 0.117 to 2.720 (median 0.396). Of the 766 serum samples analyzed, 343 were classified as reactive, corresponding to 44.8% positivity (mean 0.815±0.451). These results may suggest a significant rate of exposure or response to the nematode *S. stercoralis* in the studied population. Furthermore, it may be important for the implementation of public policies to control this neglected helminthiasis.

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Keywords: human strongyloidiasis, soroepidemiology, Brazil

IMMUNOMODULATION POTENTIAL OF CHIMERIC ELASTASE FROM *SCHISTOSOMA MANSONI* ON HUMAN PERIPHERAL BLOOD CELLS FROM ALLERGIC AND NON-ALLERGIC INDIVIDUALS

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The hygiene hypothesis suggests that reduced exposure to infectious agents in early childhood may impair immune development, increasing allergy risks, particularly in high-income countries. Helminth infections, like allergies, induce Th2 responses, with CD4+ T cells producing IL-4 and IL-5. However, helminths also promote IL-10 production and activate regulatory T cells (Tregs), helping to control inflammation. Thus, allergen-specific immunotherapy based on parasite molecules offers promising alternatives to corticosteroids, which are palliative and often cause side effects. Among *Schistosoma mansoni* proteins, elastase (SmCE) plays a key role in immune evasion during cercarial invasion, inducing both Th1 and Treg responses, which may help rebalance Th2-dominant allergic responses. This study aimed to evaluate the immunoregulatory potential of a chimeric protein (Q2) based on *S. mansoni* elastase. Q2 was expressed in *Escherichia coli* strains through IPTG induction. Protein extracts were solubilized in Tris-HCl buffer and purified by affinity chromatography. Identity and purity were confirmed by Western blot using a 6×His tag. Peripheral blood mononuclear cells (PBMCs) from healthy and allergic donors were isolated by Ficoll-Hypaque separation and stimulated with Q2 for 48 and 72 hours. Unstimulated cells served as controls. Polymyxin B was used to neutralize residual endotoxins. After incubation, cell viability was assessed, and cytokines (IL-1β, IL-4, IL-5, IFN-γ, IL-10, IL-13, TNF-α) were measured in the culture supernatant. We observed that Q2 stimulation increased TNF-α production without altering baseline IL-10 levels, suggesting a Th1 response without suppressing Treg activity. These findings highlight the potential of Q2 as an immunotherapeutic agent, but further studies are needed to confirm these preliminary results and ensure its safety and efficacy.

Supported by: CNPQ, FAPESB

Keywords: Cercariae, allergies, immunomodulation.

The Impact of antibiotic treatment on *Trichuris muris* Excretory/Secretory Products and their role in host-bacteria interactions

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Trichuriasis is a disease caused by soil-transmitted helminths of the genus *Trichuris*. They infect the cecum of mammals, invade epithelial mucosa, and cause bacterial translocation and dysbiosis. These parasites produce excretory/secretory products (ESPs), which play a role in modulating the host's immune response. This study aimed to extract ESPs from *T. muris* recovered from antibiotic-treated and untreated mice to determine whether antibiotic treatment alters the protein composition of ESPs. Additionally, we assessed the antimicrobial activity of these ESPs and their potential influence on bacterial interactions with T84 and Caco-2 cells *in vitro*. Mice were infected with *T. muris* and either received antibiotics or remained untreated. Parasites were maintained in RPMI medium at 37°C with CO_2 for 24 h. Some samples underwent MS/MS analysis for protein identification, followed by data extraction and quantification, including concentration measurements, KEGG pathway analysis, gene ontology, signal peptide identification, and network analysis. We also evaluated the ESPs' ability to inhibit bacterial growth and their interactions with *Escherichia coli* 042, *E. coli* DH5α, and *Salmonella* c20 in T84 and Caco-2 cells for 6 h. We observed differences in the protein composition of ESPs between treated and untreated groups. The ESPs from antibiotic-treated mice exhibited a more complex protein profile, with a broader range of functional proteins. Some proteins identified in both groups displayed antimicrobial and immunomodulatory activities. However, antibiogram analyses revealed that ESPs did not inhibit bacterial growth, although *Staphylococcus aureus* exhibited altered aggregation behavior. Additionally, we detected changes in bacterial aggregation and invasive potential, particularly in *E. coli* 042 and *E. coli* DH5α. Our findings suggest that the composition of ESPs varies depending on the host's microbiota, potentially influencing bacterial interactions within the host.

Suported by CNPQ, CAPES, FAPERJ

Keywords: *Trichuris,* excretory/secretory products, parasite-microbiota-host interactions

EVALUATION OF A MULTIPLEX BEAD ASSAY FOR *Strongyloides stercoralis* DIAGNOSIS USING THE RECOMBINANT ANTIGEN rSs-NIE-1

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Strongyloides stercoralis diagnosis is still a challenge to public health once the parasitological methods are ineffective and the serological tests are limited by several factors, such as the difficulty in the antigen production and the false-positive results. Multiplex assays offer the advantage of testing multiple parameters simultaneously, which could enable to detect several parasitic infections at once. This study aimed to evaluate the sensitivity and specificity of a Multiplex Bead Assay (MBA) in MagPix® platform for *S. stercoralis* diagnosis using a recombinant antigen to detect different specific antibodies isotypes. This study enrolled 330 alcoholic male patients. Parasitological examination was performed on three fecal samples by spontaneous sedimentation, Baermann-Moraes and Agar Plate Culture methods. Sera samples were tested for IgG, IgG1, IgG4, IgA1 and IgE antibodies against *S. stercoralis* recombinant antigen rSs-NIE-1 and IgG antibody against recombinant antigen of *Toxocara canis* excretory–secretory antigen (Tc-CTL-1) and microsomal fraction of *Schistosoma mansoni* adult worms (MAMA) by MBA. In parasitological methods, *S. stercoralis* was the parasite with the highest frequency, 21.2% (70/330). The MBA did not presented reactions for IgA1 or IgE detection. The highest sensitivity was observed in NIE-IgG (95.7%), while the highest specificity was demonstrated in NIE-IgG1(92.3%). However, based on the likelihood ratio (LR), the best diagnostic test was NIE-IgG4 (LR 9.96). Possible cross-reactions were observed in all isotypes, mainly in NIE-IgG, where there was a positivity of 14.5% (10/69) and 58.8% (10/17) in patients with high levels of antibodies for *S. mansoni* (MAMA) and *Toxocara* species (Tc-CTL-1) without *S. stercoralis* larvae in the feces, respectively. This study demonstrates that the MBA can be used as an alternative in *S. stercoralis* diagnosis, particularly for detection of IgG4-specific antibodies.

Supported by: Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)

Keywords: *Strongyloides stercoralis*, Multiplex Assays, Recombinant Antigens.

Identification of Protective Epitopes for a Multiepitope Schistosomiasis Vaccine: Insights from Human and Animal Models

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Schistosomiasis is endemic in 78 countries and has the highest morbidity among helminth infections. A vaccine would be a valuable tool for control and elimination. Most vaccines tested so far have targeted single antigens, none reaching final development. We propose using multiple immunogenic epitopes at the host-parasite interface, associated with protective immune responses. Our group mapped protective epitopes in murine models immunized with attenuated cercariae and in auto-cured rhesus macaques. This study aims to identify epitopes uniquely recognized in resistant to reinfection (RR) individuals versus susceptible (SR) ones in a schistosomiasis-endemic area and compares them with those previously identified in animal models. To achieve this, sera collected 18 months after Praziquantel treatment were used to screen peptide microarrays containing 55 secreted or exposed proteins from the alimentary tract or tegument. The most reactive epitopes were found in Sm25 and ADP-ribosyl cyclase (tegument), MEG-12, MEG-4.1, and LAMP (alimentary tract). Notably, two tegument targets (Sm25 and ADP-ribosyl cyclase) displayed three epitopes differentially recognized by RR sera. In general, RR individuals recognized more tegument epitopes, while SR individuals recognized more alimentary tract epitopes. Immunohistochemistry confirmed that RR individuals showed a stronger response against native tegument proteins than SR individuals. We then compared these epitopes with those previously mapped in protected animal models. Mice recognized 126 epitopes, human 56, and rhesus macaques 38. Our findings highlight the specificity and diversity of the humoral response in each model. Despite the high number of epitopes identified, only 11 were shared across all three models - two from the tegument and nine from the alimentary tract. In conclusion, these epitopes warrant further evaluation as vaccine candidates.

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Keywords: Schistosoma mansoni, vaccine, epitopes

PREVALENCE OF INTESTINAL ENTEROPARASITOSIS IN A RURAL BRAZIL-BOLIVIA BORDER COMMUNITY

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In areas with poor basic sanitation, intestinal parasites continue to be a major public health problem. The aim of this study was to assess the prevalence of intestinal enteroparasitosis in the Vila Picada community, located in the district of Porto Esperidião – Mato Grosso, on the border with Bolivia. The quantitative descriptive study was carried out as part of a research project by the Parasitology Laboratory (LaBPar) of the State University of Mato Grosso (UNEMAT), approved by the Research Ethics Committee (CEP – 6.447.376). Thirty-six fecal samples from individuals of different age groups were collected and analyzed using the Hoffman method. Of the total number of samples analyzed (36), 18 showed parasites, resulting in na overall prevalence of 50% (18 samples positive for parasites/36 total samples × 100). The positive samples identified the following parasites: *Blastocystis sp.* (22.22%), *Endolimax nana* (13.89%), *Entamoeba coli* (5.56%), *Entamoeba dispar* (2.78%), *Giardia lamblia* (2.78%) and *Entamoeba histolytica* (2.78%). Half of the positive samples were dominated by *Blastocystos sp*. And *Endolimax nana*, suggesting that these two species may be the most prevalent in the community and possibly the main culprits of the intestinal infections observed in the region. On the other hand, 24 samples showed no parasites, probably due to a low parasite load or adequate hygiene practices. It can be concluded that the absence of parasites can be attributed to factors such as effective sanitation measures and a reduction in the parasite load in the population. The presence of parasites in half of the positive samples reveals that parasitic infections continue to be a public health problem. Thus, preventive actions and improvements in basic sanitation are essential for controlling parasites, and continued surveillance is fundamental to guaranteeing the health of the local population.

Supoported by: FAPEMAT.

Keywords: Intestinal enteroparasitosis, prevalence, public health

THE WIDESPREAD OF *ANGIOSTRONGYLUS CANTONENSIS* IN SABARÁ, MG, WARNS OF THE RISK OF OCCURRENCE OF THE ZOONOSIS EOSINOPHILIC MENINGITIS

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The current wide distribution of the giant African snail *Achatina fulica* has contributed to the dispersal of the nematode *Angiostrongylus cantonensis*, leading to an increasing number of human cases of eosinophilic meningoencephalitis (EoM), an emerging disease in Brazil. In Minas Gerais state, this parasite was recently reported to Belo Horizonte, the capital of the state. Given this, the present study aims to identify infectious larvae of the *Angiostrongylus* spp. recovered from *A. fulica* collected in the municipality of Sabará, located in the metropolitan region of Belo Horizonte. Municipal health department agents and researchers involved in the study have been monitoring infected snails in neighborhoods previously known to be infested with African snails since April 2024. So far, six neighborhoods were sampled: Vila Rica (VR) (n=63 snails); General Carneiro Parte Alta (GCA) (n=11); General Carneiro Parte Baixa (GCB) (n=33); Nações Unidas (NU) (n=24); Alvorada (AL) (n=40) and Fátima (FA) (n=37), totaling 208 snails obtained and analyzed. The parasitological analysis followed the technique of artificial digestion of mollusks in HCl 0.7% and nematode larvae recovered were identified based on the analysis of mitochondrial cytochrome *c* oxidase I (MT-CO1). Larvae of *A. cantonensis* were found in five (7.9%) *A. fulica* specimens from VR, three (*27%*) from GCA, eight (24.2%) from GCB, five (20.8%) from NU, 19 (47.5%) from AL, and one (2.7%) from FA, totaling 41 (19%) *A. fulica* specimens testing positive. Given the presence of *A. cantonensis* in the region and the high infestation rate of *A. fulica*, in addition to continuing the collections and parasitological examinations, health agents will also receive training for recognition of the species, control measures, and public education efforts. Furthermore, a health education project will be implemented to raise awareness among residents in the affected areas aiming to prevent infection and spread of EoM.

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Keywords: eosinophilic meningitis, *Achatina fulica*, emerging disease.

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DETERMINANTS OF DOG DEWORMING PRACTICES: INSIGHTS FROM A BRAZILIAN CROSS-SECTIONAL STUDY

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Abstract

Zoonotic diseases can be caused by helminths that use dogs as definitive hosts, including parasites from the genera *Ancylostoma* and *Toxocara*. In this context, canine deworming is essential to safeguard both animal and human health. This study investigated factors associated with adherence to or non-adherence to deworming practices in a Brazilian municipality. A total of 501 dog guardians in Divinópolis, Minas Gerais, were randomly selected and interviewed in person. The questionnaire collected data on guardian's socioeconomic characteristics and their dogs' individual attributes. Bivariate chi-square tests were conducted to assess associations between these factors and deworming practices. The results revealed a significant positive association ($P < 0.05$) between deworming practices and higher education levels, female ownership, households with fewer than four inhabitants, residences with more than one bathroom, and ownership of a laptop. Conversely, non-adherence to deworming was significantly associated with the absence of a washing machine or car, failure to vaccinate dogs against rabies, and never taking dogs to a veterinarian. No significant associations were found for individual dog characteristics such as sex, breed, size, or age at acquisition (puppy or adult). Additionally, the presence of children in the household showed no correlation with deworming practices. These findings highlight the need for health education initiatives emphasizing basic dog care, particularly among socioeconomically vulnerable populations. Strengthening veterinary access and public awareness may also contribute to improved adherence to deworming protocols.

Supported by: CNPq, CAPES.

Keywords: One Health; Zoonotic parasitosis; responsible pet guardianship.

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ELEVATED IGG ANTIBODIES TO *Ascaris lumbricoides* IN RHEUMATOID ARTHRITIS PATIENTS: EXPOSURE OR SUSCEPTIBILITY?

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Rheumatoid arthritis (RA) is a chronic autoimmune disease affecting the joints, leading to inflammation and pain. The use of immunosuppressive therapy in RA patients increases their susceptibility to infections. *Ascaris lumbricoides* is one of the most prevalent helminths in humans, known for its immunomodulatory immune responses on the host. However, their impact on autoimmune diseases patients remains unclear. This study evaluated IgG reactivity against *A. lumbricoides* in RA patients and its potential association with disability. Serum samples from 89 RA patients receiving treatment at the Hospital de Clinicas da Universidade Federal de Uberlândia (HC-UFU) were collected (RA group), only six patients submitted fecal samples for parasitological exams, all of them had negative results. The control group (CT group) consisted of individuals residing in the same geographical area, sex, age, and income, with no chronic diseases. IgG antibodies against *A. lumbricoides* were detected using an enzyme-linked immunosorbent assay (ELISA) with plates coated with a synthetic peptide predicted as B-cell epitope to this helminth. A significantly higher IgG reactivity index (RI) was observed in the RA group compared to the control group ($p < 0.0001$). This could indicate an increased susceptibility to infection *to A. lumbricoides* in RA patients due to immune dysregulation or immunosuppressive therapy. However, no significant difference was found in HAQ-DI disability scores between seroreactive and non-seroreactive individuals. Demographic factors such as age, gender, and residence area did not significantly influence seropositivity. These findings suggest that RA patients exhibit higher IgG seroreactivity to *A. lumbricoides*, but this exposure does not appear to impact disease-related disability. Further studies are needed to determine if the exposure to *A. lumbricoides* in RA patients has meaningful immunomodulation on these patients.
Supported by: FAPEMIG and CAPES.

Keywords: Autoimmune disease, Helminth infections, Immunosuppression.

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DIFFERENTIAL IGG SEROREACTIVITY TO *Toxocara canis* IN IMMUNOCOMPETENT AND AUTOIMMUNE RHEUMATIC PATIENTS: FREQUENCY, ASSOCIATIONS, AND FUNCTIONAL CORRELATIONS

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Toxocara canis is a zoonotic helminth transmitted through ingestion of embryonated eggs from contaminated soil, water, or food, and by consuming raw or undercooked meat from paratenic hosts such as poultry, swine, and small mammals. Immunocompromised individuals may be more vulnerable to chronic infection.

This study evaluated IgG seroreactivity to *T. canis* in immunocompetent (children and adults) and autoimmune rheumatic patients, comparing antibody frequency within and between these groups and their controls.

A total of 424 serum samples were analyzed: 168 from immunocompetent individuals (51 children, 117 adults) and 256 from immunosuppressed patients (88 rheumatoid arthritis [RA], 57 spondyloarthritis [SpA], 76 systemic lupus erythematosus [SLE], 18 other lupus [LU], and 17 other autoimmune rheumatic diseases). ELISA was performed using *Toxocara canis* excretory-secretory (Tc-TES) proteins, and positives were confirmed with a highly specific recombinant chimeric protein (99.3% sensitivity, 97.8% specificity).

Seroprevalence was 8.93% in immunocompetent individuals, 32.42% in immunosuppressed patients, and 23.11% overall. RA and SLE patients had higher IgG anti-*T. canis* seroreactivity ($p = 0.0071$ and $p = 0.0007$) and antibody frequency ($p = 0.0130$ and $p < 0.0001$) than controls. In RA, the IgG reactivity index showed a significant inverse correlation with physical disability (HAQ-DI, $r = -0.2508$, CI = -0.4423 to -0.0373, $p = 0.0092$).

RA patients had significantly higher seroreactivity than adults ($p = 0.0430$) and children ($p < 0.0001$), SLE patients showed higher values than adults ($p = 0.0445$) and children ($p < 0.0001$), and SpA patients had higher levels than children ($p = 0.0081$). Among immunocompetent individuals, adults showed greater seroreactivity ($p = 0.0009$) and antibody frequency ($p = 0.0217$) than children, suggesting increased exposure with age.

These findings highlight distinct serological profiles in immune-compromised and healthy individuals, reinforcing the need for further research on *T. canis* exposure, immune responses, and clinical impact.

Supported by: FAPEMIG, CAPES.

Keywords: Rheumatic diseases, Human toxocariasis, Serological analysis.

MEDICINAL PLANTS IN THE ANTHELMINTIC CONTROL OF SMALL RUMINANTS IN THE SEMI-ARID REGION OF BAHIA: THE VISION OF SMALL PRODUCERS

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Abstract:

Medicinal plants have been used empirically for years by different traditions due to their bioactive and chemical nature. Herbal medicines are widely used in veterinary medicine because of their anthelmintic properties, which can replace industrialized drugs and delay parasite resistance, a current challenge in the fight against gastrointestinal parasites in small ruminants.

This research aims to understand the use of medicinal plants by small farmers in the treatment of worms in goats and sheep in the semi-arid region of Bahia. To this end, a survey was carried out of the main plants used by small ruminant producers in the rural area of the municipality of Buritirama, Bahia, using a questionnaire containing 19 questions about the use of plants in animal health, forms of administration, processing and collection. The study was approved by the CEP-UFOB. Fifteen farmers were interviewed, 93.3% of whom confirmed that they use plants as anthelmintic alternatives, the most commonly cited being: Mastruz (66.6%), Neem (33.3%) and Batata de Pulga (26.6%).

Other plants mentioned to a lesser extent were: Babosa, Papaconha and Caatinga de Porco. The popular names were kept, as no plant has yet been collected to identify the species. Around 76.9% of the participants reported using these plants to control infections in general and 50% used them mainly for worms, especially in sheep and goats. All the interviewees learned about the use of the plants through family tradition and administer them orally, mixed with feed or water.

Previous studies have evaluated the anthelmintic efficacy of most of the plants mentioned, but with divergent results depending on the part of the plant evaluated, processing and form of use. Most of the farmers interviewed have used medicinal plants orally to control anthelmintic infections in small ruminants, and their use is based on popular knowledge, with plants that are easily accessible in the region and based on family tradition.

Supported By: INCITE/FAPESB

Key words: Phytotherapy, verminosis, ethnopharmacology.

INFLUENCE OF REPRODUCTIVE STATUS ON THE PARASITE LOAD OF SHEEP RAISED IN THE SEMI-ARID REGION OF BAHIA

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Abstract:

Gastrointestinal parasites are one of the main health challenges in sheep farming, compromising the productive performance of the flock. However, few studies have investigated the influence of reproductive status on the intensity of infection.

This study compared the parasite load and clinical parameters between two groups of sheep in different reproductive periods. In January 2025, samples were collected from 16 ewes (9 in puerperium and 7 in the early third of pregnancy) of the Santa Ines breed, aged between 1 and 2 years and raised under similar conditions of grazing and sanitary management at the UFOB School Farm. Parasitic (OPG) and physiological (FAMACHA test and hematocrit) data were analyzed. Among the ewes in puerperium, 66.6% had OPG over 700, while only 14.28% of the ewes in early pregnancy had high OPG. As for the hematocrit assessment, 77.7%of the ewes in puerperium had a hematocrit of less than 25%, in contrast to those in early pregnancy,where none had such a reduction. When assessing the degree of FAMACHA, it was found that 55.5% of puerperal ewes had FAMACHA higher than 3, in contrast to only 14.28% of ewes in the early stages of pregnancy.

These findings suggest that lactation directly influences susceptibility to parasitic infection, potentially due to an increase in the production of cortisol, oxytocin and prolactin,which reduce the animal's immune capacity.There is also a reduction in cytokine production and a detour of nutrients to milk production, increasing the risk of anemia and infection.

Therefore, proper knowledge of this impact is fundamental for adopting strategies such as feed supplementation, since,in addition to hormonal effects, nutrition also influences the sheep's immune response.Furthermore,the division of flocks can also help with sanitary control, minimizing negative impacts on production.

Supported By: INCITE/FAPESB

Key words: Gastrointestinal parasitosis, sheep farming, reproductive development.

Anthelmintic Activity of 1,10-Phenanthroline-5,6-Dione-Derived Metallodrugs Against *Schistosoma mansoni* and *Angiostrongylus cantonensis*

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Parasitic worm infections represent a serious global health problem, affecting more than two billion people, especially in low-income regions. The limited availability of effective treatments highlights the need for new therapeutic strategies and approaches. In this context, metallodrugs stand out as a promising alternative due to their structural versatility and ability to modulate biological targets. This study evaluates the anthelmintic activity of 1,10-phenanthroline-5,6-dione (phendione) and its metal complexes, $Cu(phendione)_3(ClO_4)_2.8H_2O$ and $Ag(phendione)_2(ClO_4)$, against *Schistosoma mansoni* and *Angiostrongylus cantonensis*. Cu-phendione exhibited the highest potency (EC_{50} = 2.3 µM for *S. mansoni* and 6.4 µM for *A. cantonensis*), followed by Ag-phendione (EC_{50} = 6.5 µM and 12.7 µM, respectively). Cytotoxicity assays revealed a favorable safety profile, with selectivity indices exceeding 86.9 for *S. mansoni* and 31.2 for *A. cantonensis*. Selective toxicity was further supported by high lethal dose values in *Caenorhabditis elegans*, a free-living nematode used as a toxicity model. These findings highlight the potential of phendione-based metallodrugs as effective and safe therapeutic candidates for parasitic diseases. .

Supported by: CAPES and FAPESP.

Keywords: Neglected disease; Antiparasitic; Metallodrugs and Phendione.

EVALUATING THE IMPACT OF ALBENDAZOLE ON *Trichuris trichiura* PREVALENCE IN AN ENDEMIC AREA OF BAHIA, BRAZIL

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ABSTRACT

Introduction: In Brazil, soil-transmitted helminths (STH) predominantly affect the North and Northeast regions, where access to water, sanitation, and hygiene is limited. Albendazole (ALB) is the primary treatment for STH. However, it is crucial to assess its efficacy to determine if ALB can be recommended. This study aims to evaluate the effectiveness of ALB in treating *Trichuris trichiura* (Tt) infections in an endemic area of Brazil. **Methodology:** This temporal analysis study was conducted in the rural area of Conde, Brazil. Residents were followed before and after ALB treatment (ALBt). Participants were evaluated using the Kato-Katz method to quantify the presence of Tt and *Ascaris lumbricoides* (Al) eggs. In 2004, 777 individuals were assessed, and follow-up evaluations were conducted after two rounds of ALBt in May and October 2005. Post-treatment collections occurred in December 2006. In 2018, a new study of 340 individuals was included, with evaluations conducted before and 30 days after ALB treatment. The parasite load was measured in eggs per gram (EPG) of feces. **Results**: In 2004, 70.8% of individuals tested positive for Tt and 56.6% for Al. Post-treatment analysis showed that 87.5% of individuals still had Tt eggs and 67.9% still had Al eggs. In 2018, 40.3% and 53.8% of individuals tested positive for Al and Tt, respectively. Post-treatment evaluations revealed that the positivity for Al reduced to 7.3% while 52.6% still had Tt eggs. At that timepoint the median parasite load for Al decreased from 7542 (1452-24810) to 6912 (2412–17778) EPG. For Tt, the median parasite load was reduced from 420 (120–1236) to 288 (96–927) EPG. **Conclusion**: Over time, the prevalence of Al appears to decrease, indicating a better response to ALBt. In contrast, Tt infections persist, suggesting a possible therapeutic failure of ALB. These findings highlight the need for alternative treatment strategies and continual monitoring in endemic areas to effectively control Tt infections.

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Keywords: *Trichuris trichiura*, Albendazole, Treatment.

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29º CONGRESSO BRASILEIRO DE PARASITOLOGIA

HELMINTH SEROREACTIVITY IN SYSTEMIC LUPUS ERYTHEMATOSUS: INFLUENCE OF IMMUNOSUPPRESSION ON IGG ANTIBODY LEVELS

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The relationship between helminth infections and autoimmune diseases, such as systemic lupus erythematosus (SLE), has gained interest due to their immunomodulatory potential. However, patients with autoimmune diseases frequently undergo immunosuppressive therapy, increasing their susceptibility to infections. This study aimed to evaluate IgG seroreactivity to *Strongyloides stercoralis* and hookworm synthetic B-cell epitopes in SLE patients and a control group, investigating both immune alterations and the relevance of pathogen control in immunocompromised individuals.
Serum samples from 76 SLE patients and matched controls were analyzed using an enzyme-linked immunosorbent assay (ELISA). The significantly higher IgG seroreactivity observed in SLE patients may indicate a history of greater exposure or an increased susceptibility to these pathogens. However, the association between corticosteroid therapy and reduced seroreactivity to *S. stercoralis* reinforces the hypothesis that these antibodies reflect past exposure rather than active infection, as immunosuppressive therapy may influence antibody production. No correlation was found between antibody levels and physical disability index (HAQ-DI), nor with demographic and environmental factors such as age, gender, income, place of residence, or pet contact.
Importantly, the immunosuppressed state of SLE patients increases their risk of opportunistic infections, reinforcing the necessity of monitoring and controlling parasitic infections, particularly in endemic areas, to prevent severe complications. These findings enhance our understanding of the complex interactions between helminth infections and autoimmune diseases, highlighting the importance of preventive measures and the need for further studies on the immunological impact of helminths in immunocompromised individuals.

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Keywords: Helminth infections, Autoimmune disease, Immunosuppression.

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DETECTION OF ANTI-STRONGYLOIDES IGG ANTIBODIES: A SEROEPIDEMIOLOGICAL STUDY IN BLOOD DONORS FROM CURITIBA

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Abstract

Strongyloidiasis is an intestinal parasitic disease caused by the nematode *Strongyloides stercoralis*, with a worldwide distribution and an estimated 600 million people infected. This study aims to detect anti-*Strongyloides* IgG antibodies in blood donors from the Paraná Center for Hematology and Hemotherapy (HEMEPAR) using the Enzyme-Linked Immunosorbent Assay (ELISA) technique. For this purpose, a heterologous extract of the cuticle from infective larvae of *Strongyloides venezuelensis* was prepared using the CTAB detergent. A total of 823 serum samples from donors were analyzed. To determine the reaction cut-off, control samples were used: parasitological positive for *S. stercoralis* (n=28), negative (n=53), and positive for other parasitic infections (n=20). The cut-off value was 0.488, with a sensitivity of 100.0% (95% CI = 86.77% to 100.0%) and a specificity of 96.55% (95% CI = 88.09% to 99.58%). Absorbance values (450 nm) ranged from 0.079 to 2.339 (median of 0.344). Of the 823 samples analyzed, 230 were classified as reactive, corresponding to a positivity rate of 28% (mean 0.414 ± 0.260). Given that strongyloidiasis is a neglected helminthiasis, the present results can be used as a basis for future investigations and contribute to public health strategies.

Supported by: FAPESP (2022/02401-1 and 2024/10786-6)

Keywords: human strongyloidiasis, soroepidemiology, Brazil

HUMAN ASCARIASIS AS A RISK FACTOR FOR BREAST CANCER PROGRESSION: THE LINK BETWEEN INFECTION, INFLAMMATION AND LUNG METASTASIS IN A MURINE MODEL

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Human ascariasis is a neglected disease with significant socioeconomic impact. Studies suggest that helminths can influence the development of cancers, including breast cancer. We believe that this relationship has some risk, due to *Ascaris* spp. constant contact with society and the environment, thus modulating the immune response and the possible tumor microenvironment. We investigated the relationship between *Ascaris suum* infection, breast cancer progression, and its lung metastases. For this purpose, female BALB/c mice were infected with 250 larval eggs of *A. suum*. After 14 days, they received $1x10^6$ 4T1 cells subcutaneously in the right flank to induce breast cancer. Tumor growth and survival were evaluated. After 21 days, some of the animals were euthanized for analysis of bronchoalveolar lavage by flow cytometry, lung histopathology, and cytokine quantification. For better accuracy in detecting lung metastases, a solution containing 100 µCi of the 99mTc-HYNIC-βAla-Bombesin(7-14) complex was administered intravenously to a portion of the animals, and scintigraphic images were obtained four hours after administration. The findings indicated that the infection decreased survival of animals with cancer and contributed to tumor growth and the appearance of ulcerated areas. Radioisotope experiments revealed greater lung metastasis area, confirmed by histopathology that showed increased metastatic foci count, which resulted in an increase in tissue lesion score, metastatic foci, and total diameter area. Cytokine analysis indicated a significant reduction in IL-10, IL-5, and IL-13 and a significant increase in IL-17, TNF-α, and TGF-β. Bronchoalveolar lavage revealed changes compatible with clinical worsening and we observed that there was no increase in the intensity of inflammatory cells in animals previously infected. We conclude that prior infection with *A. suum* at low doses can aggravate breast cancer and its prognosis in mice due to chronic inflammation.

Supported by: FAPEMIG, CNPq and CAPES.

Keywords: Nematodes, tumor microenvironment, neglected diseases.

COPROPARASITOLOGICAL EXAMINATION IN ARMADILLOS FROM THE PANTANAL AND CERRADO OF MATO GROSSO DO SUL.

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Abstract

Little is known about the prevalence, diversity, and distribution of endoparasites in armadillos in Brazil, as well as the risk and impact on public and animal health. The role of wild animals as sources and amplifiers of emerging pathogens in humans and domestic animals has received considerable attention in recent years. The increase in anthropogenic activities and urban expansion in areas of habitat for wild animals contributes to the spread of parasites. Therefore, investigating the parasitic fauna of zoonotic importance in wild animals is essential for health management and surveillance. This study, conducted in partnership with the Institute for the Conservation of Wild Animals (ICAS), analyzed fecal samples from armadillos in the Pantanal and Cerrado regions of Mato Grosso do Sul. A total of 59 fecal samples were analyzed: 51 from the six-banded armadillo (Euphractus sexcinctus), 2 from the giant armadillo (Priodontes maximus), 3 from the southern naked-tailed armadillo (Cabassous squamicaudis), and 3 from the nine-banded armadillo (Dasypus novemcinctus). The samples were collected after spontaneous defecation of the animals, induced by the stress of manual capture, and from animals killed in vehicle collisions on highways. The feces were placed in collection jars, preserved in sodium dichromate, and sent to the Veterinary Parasitology Laboratory of the Institute of Health Sciences-UFBA in September 2023. The coproparasitological techniques used were Willis (qualitative flotation) and Hoffman, Pons, and Janer (spontaneous sedimentation). The results obtained from the two techniques, respectively, were eggs of the Order Strongylida (65.52%; 77.19%), eggs of Aspidodera (24.14%; 24.56%), eggs of Trichuridae (3.45%; 3.50%), and oocysts of Eimeria spp (74.14%; 77.19%); suggesting that coexistence with domestic animals could influence such parasitism and reinforcing the hypothesis that armadillos may act as potential reservoirs of endoparasites.

Supported by: ICAS

Keywords:helminth;protozoan;armadillo

Antischistosomal Activity of Sesquiterpene Polygodial from *Drimys brasiliensis*: *In Silico*, *In Vitro* and *In Vivo* Investigations

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The World Health Organization highlights the urgent need for innovative therapeutic strategies to enhance helminthiasis control. In this context, Brazilian biodiversity represents a valuable source of bioactive molecules. This study employed bioactivity-guided fractionation of the hexane extract from *Drimys brasiliensis* (Winteraceae) branches, leading to the isolation and characterization of the sesquiterpene polygodial. The compound was then evaluated for its activity against *Schistosoma mansoni*, the causative agent of schistosomiasis. *In vitro*, polygodial exhibited significant potency against adult worms (EC_{50} = 10 µM) while showing no cytotoxicity in Vero cells (CC_{50} > 200 µM). *In silico* analyses revealed favorable pharmacokinetic properties (ADME) with no PAINS violations. *In vivo*, *S. mansoni*-infected mice treated with polygodial (400 mg/kg) showed a 44.0% reduction in worm burden, along with reductions of 71.8% in fecal egg excretion and 69.5% in intestinal egg load. These findings highlight polygodial as a promising lead compound for schistosomiasis treatment.

Supported by: CAPES and FAPESP.

Keywords: Anti-*Schistosoma*; Natural product; *Drimys brasiliensis; Schistosoma*; Anthelmintic.

ENDOPARASITES FOUND IN GUIANA DOLPHINS (*Sotalia guianensis*) IN NORTHEASTERN BRAZIL

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Cetaceans are considered environmental sentinels in the ecosystems they inhabit. Parasitic infections are common, being one of the main causes of strandings, although they are not always associated with the cause of death. Public data from the Aquatic Biota Monitoring Information System (SIMBA) were used to identify parasites found in stranded Guiana dolphins (*Sotalia guianensis*, Van Beneden, 1864) along the PMP-SE/AL stretch, which is monitored from the municipality of Conde (BA) to Pontal do Peba (AL). SIMBA allows the standardization and systematization of data generated during the activities of the Beach Monitoring Project (PMP). The taxonomy filter "*Sotalia guianensis*" was applied to the sections "Target Fauna Occurrences" and "Anatomopathological Examinations" to find the total number of stranded and necropsied individuals, respectively. Data obtained from anatomopathological examinations were analyzed to determine the proportion of stranded animals with the presence of any endoparasite. Finally, in the "Laboratory Tests/Images" section, the filters "*Sotalia guianensis*" and "Coprological/Parasitological" were applied to access the identification of the parasites. Between 10/01/2020 and 27/08/2024, 196 Guiana dolphin strandings were recorded, 162 of which underwent anatomopathological examination. Of these, 29 (17.9%) had the presence of some endoparasite, and 12 specimens were collected for identification. *Halocercus brasiliensis* was identified in 6 of the 12 animals (50%) in the lungs; *Anisakis spp.* in 1 animal (8.3%) in the stomach; *Halocercus brasiliensis* and *Anisakis spp.* in 2 animals (16.7%) in the lungs and stomach, respectively; and *Contracaecum spp.* in 3 animals (25%) in the stomach. These parasites, in addition to being associated with pathologies that hinder the animal's survival in nature, are also poorly studied and increase the risks to human health due to the zoonosis resulting from their parasitism in humans.

Keywords: Cetacean, Stranding, Parasitism.

EVALUATION OF RECOMBINANT AND CRUDE ANTIGENS USING IMMUNOBLOTTING FOR *Strongyloides stercoralis* INFECTION DIAGNOSIS

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Strongyloides stercoralis infection is usually asymptomatic but can develop into a severe condition in immunocompromised patients, such as alcoholics, making its diagnosis and monitoring essential. Parasitological methods have low sensitivity and are limited by the larval output. Immunoblotting has demonstrated elevated sensitivity and specificity in several infections' diseases, but there is still no defined standard of immunoreactive bands in the strongyloidiasis diagnosis. The aim of this study is to evaluate the sensitivity and specificity of *S. stercoralis* immunoblotting in the detection of specific IgG and IgA1 antibodies using *S. stercoralis* (SsS) and *S. venezuelensis* (SvS) soluble antigens, *S. venezuelensis* membrane antigen (SvM) and the recombinants r-Ss-NIE-1 and rSsIR. There were selected 50 male chronic alcoholic patients from a previous published study. Among them, 15 tested negative for any parasitic disease, while 35 were monoparasitized with *S. stercoralis* (n = 20), *S. mansoni* (n = 8) or Hookworm (n = 7). For diagnosis, there were selected bands with sensitivity and specificity greater than 50 and 80%**,** respectively. Immunoreactivity varied according to the tested antigens and isotypes. The membrane antigen of *S. venezuelensis* showed the best diagnostic performance, with 100% sensitivity for both IgG and IgA1 and specificities of 90.0 and 93.3%, respectively. The 26 and 75 kDa bands demonstrated high diagnostic potential. In contrast, the recombinant antigens r-Ss-NIE-1 and rSsIR exhibited high cross-reactivity rates, especially SsIR (87%). The findings in this study indicate that IgA1 detection with immunoblotting can be used in *S. stercoralis* diagnosis. Also, the 17, 26, 75 and 90 kDa bands of *S. venezuelensis* membrane antigen can be used as references. Additionally, the results highlight the need to develop new and more specific recombinant antigens for use in endemic areas.

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Keywords: *Strongyloides stercoralis*, Antigens, Immunoblotting.

RECORDS OF HELMINTHS ISOLATED FROM GREEN TURTLES (*Chelonia mydas*) IN NORTHEAST BRAZIL

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Sea turtles play a fundamental role in the conservation of marine ecosystems, serving as bioindicators of ocean health. Of the seven known species, five occur in Brazil, including the green turtle (*Chelonia mydas*, Linnaeus, 1758). The study of endoparasites in these animals is essential for assessing population and environmental health, indicating the presence of emerging diseases with potential zoonotic risks, and contributing to species conservation. This study utilized publicly available data from the Aquatic Biota Monitoring Information System (SIMBA). SIMBA is a platform containing data on marine tetrapod strandings systematically recorded by the Beach Monitoring Project (PMP), which was developed to meet the requirements of federal environmental licensing conducted by IBAMA for offshore oil and natural gas exploration and production activities by PETROBRAS. Between 22/10/2019 and 18/09/2024, 1470 strandings of green turtles were recorded between Conde (BA) and Pontal do Peba (AL). Of these, 187 individuals underwent pathological examinations, with 39 (20,85%) showing endoparasite infections, primarily affecting the intestines (41,02%) and kidneys (30,7%). Thirty samples from 23 animals were subjected to identification tests, resulting in the isolation of 10 different parasites. Among the 23 animals, 13 (56,52%) were infected with *Neoctangium travassosi*; 4 (17,39%) with *Learedius learedi*; 3 (12,04%) with *Cricocephalus albus*; 3 (12,04%) with *Pyelosomum cochlear*; 2 (8,69%) with *Metacetabulum invaginatum*; 2 (8,69%) with parasites belonging to the family *Hapalotrematidae*; 1 (4,34%) with *Deuterobaris intestinalis*; 1 (4,34%) with *Pleurogonius longiusculus*; 1 (4,34%) with *Schizamphistomum scleroporum*; and 1 (4,34%) with a parasite belonging to the order *Trypanorhyncha*. Sea turtles exhibit a diverse helminth fauna, and the observed results enhance the understanding of parasitism in these animals and their geographical distribution.

Keywords: Testudines, Parasitism, Stranding.

EPIDEMIOLOGICAL PROFILE OF CHILDREN AGED 3 MONTHS TO 6 YEARS IN A SCHISTOSOMIASIS-ENDEMIC RURAL AREA OF BAHIA, BRAZIL

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ABSTRACT

Introduction: Helminthiases, including infections by *Ascaris lumbricoides* (Al), *Trichuris trichiura* (Tt), and *Schistosoma mansoni* (Sm), represent a public health challenge, especially in rural areas with poor socioeconomic and sanitation conditions. This study aimed to describe the epidemiological profile of children aged 3 months to 6 years from a schistosomiasis endemic rural area in Conde, Bahia, Brazil. **Methodology:** A cross-sectional study, part of an ongoing clinical trial, was conducted between December 2024 and February 2025, involving 161 children, of whom 125 provided stool samples for parasitological analysis. The Kato-Katz result for Al and Tt was evaluated qualitatively, while for Sm, it was evaluated quantitatively and qualitatively. **Results:** The median age of children was 3 years old, with 82 girls (50.9%) and 79 boys (49.1%). Among the 125 tests, 4% were positive for Sm, 38.4% for Al and 31.2% for Tt, with frequent co-infections. The overall parasite load had a median of 12 (IQR 12-48) EPG. All children who tested positive for Sm belonged to families with an income below one minimum wage and consumed treated water, but without additional treatment. Most reported contact with river water and the disposal of waste in inappropriate places. In the biochemical analysis of the five children positive for Sm, only one (20%) had AST, PCR, and CK-MB levels above the reference value. **Conclusion:** The data revealed a high rate of infections by *Ascaris lumbricoides* and *Trichuris trichiura* among children, whereas schistosomiasis presented a lower rate. However, there is a strong indication of an association between socioeconomic conditions and the incidence of infections. The relationship between inadequate sanitation and contact with contaminated water sources highlights the urgent need for public health interventions and improvements in the living conditions of the local population.

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Keywords: Neglected Tropical Diseases, Childhood Helminthiasis, Epidemiological Profile.

Production of Recombinant Proteins Containing Immunogenic Epitopes of Schistosoma mansoni

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Schistosomiasis is a neglected tropical disease endemic in 78 countries, primarily affecting low- and middle-income regions. Although Praziquantel (PZQ) is available, concerns about drug resistance and limited access to treatment in endemic areas persist. A vaccine would be a valuable addition to the toolbox for control and elimination. Most vaccines tested so far have targeted single antigens, none of which have reached final development. We believe that multi-epitopes vaccines present a promising strategy. Our group previously conducted peptide microarray assays to screen 54 proteins from the tegument, esophageal gland, and gastrodermis, using sera from mice protected by the *Schistosoma mansoni* attenuated cercariae vaccine. This study aims to produce three chimeric proteins: (P1) 20 epitopes from esophageal gland, (P2) 23 epitopes from the tegument, and (P3) 30 epitopes from the gastrodermis. The three synthetic, *E. coli* codon-optimised constructs, which include an AAY spacer between epitopes, were cloned into pET21a vector and expressed in *E. coli* BL21(DE3). The proteins were expressed as inclusions bodies and solubilized using varying concentrations of urea or guanidine. Protein expression was confirmed via SDS-PAGE and Western blot using an anti-His antibody; and purified using nickel affinity chromatography with a linear imidazole gradient. The fractions containing the main protein peak were pooled and stepwise dialyzed/refolded in PBS 1x with decreasing concentrations of chaotropic agents. P1 showed high expression levels, with a yield of 106 mg/L of soluble protein after purification. The tegument protein (P2) had intermediate expression, yielding 29.3 mg/L, but with significant precipitation. The gastrodermis protein (P3) displayed low expression, yielding 1,1 mg/L of soluble protein. The next step is to use these proteins, either individually or in combination, in an immunization and challenge assay using C57BL/6 mice.

Keywords: Epitope, Recombinant protein, *Schistosoma*

Funding: FAPESB

COPROPARASITOLOGICAL FINDINGS IN RACCOON (Procyon cancrivorus) – CASE REPORT

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The Brazilian raccoon (*Procyon cancrivorus*) is widely distributed across Brazil and the Americas. Wild animals could be hosts for various parasites, including zoonotic agents, making stool parasitological analysis highly relevant to public health. An individual of *P. cancrivorus* was treated at the UENP Veterinary School Hospital with bilateral pelvic limb paralysis and underwent complementary diagnostic tests, including coproparasitological analysis. Fecal samples collected from the rectal ampulla were examined using the Willis-Molay and Hoffman methods. The Willis-Molay technique identified four oval, smooth-surfaced eggs belonging to *Ancylostoma* spp., a parasite transmitted via oral and percutaneous routes, posing zoonotic risks such as enteroparasitosis and cutaneous larva migrans in humans. Spontaneous sedimentation revealed four bioperculated eggs consistent with *Trichuris* spp., a genus rarely reported in procyonids but previously detected in *Procyon lotor* in Germany. Additionally, 18 non-sporulated coccidia of *Cystoisospora* spp. and *Eimeria* spp. were identified. While *Eimeria* spp. has not been previously reported in *P. cancrivorus*, *E. procyonis* is known to infect *P. lotor*, occasionally causing clinical coccidiosis in captive and free-living raccoons. In conclusion, coproparasitological analysis of *P. cancrivorus* revealed infections by *Ancylostoma* spp., *Trichuris* spp., and coccidia, emphasizing the potential zoonotic risk and the importance of continued parasitological surveillance in wildlife to better understand parasite-host interactions and their implications for public and animal health.

Supported by: Fundação Araucária.

Keywords: coproparasitology; zoonotic parasites, wildlife health.

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POTENTIAL OF SURFACE ANTIGENS FROM THE INFECTIVE LARVAE OF *STRONGYLOIDES VENEZUELENSIS* IN IMMUNODIAGNOSIS OF HUMAN STRONGYLOIDIASIS

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Abstract

Human strongyloidiasis affects approximately 613.9 million people worldwide. *Strongyloides venezuelensis* is a rodent parasitic nematode that is frequently used to obtain heterologous antigens for immunological diagnosis of human strongyloidiasis. The present study aimed to identify the components of the cuticle extract of the infective larvae of *S. venezuelensis* (iL3), obtained with the detergent CTAB, that may be relevant for immunodiagnosis. For this purpose, western blotting was performed, followed by mass spectrometry. Different antigenic components were recognized by IgG antibodies from the sera of patients with strongyloidiasis. The greatest recognition was observed for a mass range of 28–40 kDa. The immunoreactive proteins identified with greater abundance were galectin, proteins with CAP domain, metalloproteinase and arginine kinase. The present results reinforce the use of iL3 cuticle extract from *S. venezulensis* in the immunodiagnosis of human strongyloidiasis, in addition to pointing to future studies on biology and diagnostic markers.

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Keywords: human strongyloidiasis, Brazil, immunodiagnosis

HOW CAN BIOINFORMATICS TOOLS HELP TARGET NEW IMMUNOGENS? AN *IN SILICO* ANALYSIS OF TWO KNOWN IMMUNOGENIC CHIMERA PROTEINS FROM *ASCARIS* SP.

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Ascariasis, a neglected disease caused by parasites of the genus *Ascaris*, affects millions of people and has high morbidity rates. Due to the recurrence of reinfections, even after drugs treatment, a vaccine is essential for the control of the disease. This study aims to evaluate the immunogenic potential of two *Ascaris* proteins previously studied as vaccine candidates. Using bioinformatics tools, the proteins were evaluated for their physicochemical properties, ability to induce immune response and immunogenic potential. The physicochemical properties were analyzed by ProtParam and Protein-Sol, the immunogenicity was predicted by VaxiJen and the immune response was simulated by C-IMMSIM. Tertiary structure prediction was carried out using I-TASSER. The BOT sequence (217 amino acids, 22 kDa) showed a pI of 4.48, stability (instability index: 35.92) and solubility (predicted solubility: 0.924). Immunogenicity was classified as high (1.0778), with simulation indicating an increase in IFN-γ, active memory Th cells and memory B cells. The tertiary structure showed low similarity (C-score of -4), with a predominance of α-helix regions. The ASC-VAC sequence (382 amino acids, 39 kDa) had a pI of 10.04, high instability (74.30) and solubility (0.719). It was classified as immunogenic (0.5057), with a robust simulated response, an increase in IFN-γ, TGF-β, memory Th cells and persistent B cells. The tertiary structure prediction obtained a C-score of -0.30 and a Tm-score of 0.67±0.12, with several α-helix, β-sheet and active site regions characteristic of proteins rich in molecular recognition domains. *In silico* analyses confirm the physicochemical properties and immunogenicity of the proteins, reinforcing the viability of the sequences as vaccine candidates, with the potential to induce a long-lasting immune response. The C-IMMSIM simulations indicate the best time for cellular evaluation, allowing further analysis to ensure the safety and efficacy of the immunogens.

Supported by: CAPES, CNPq

Keywords: Ascariasis. Vaccine. Computacional Biology.

TEMPORARY BREEDING SITES OF *Biomphalaria glabrata* IN AN AREA OF HIGH ENDEMICITY FOR SCHISTOSOMIASIS MANSONI IN THE MUNICIPALITY OF RIACHUELO/SE, BRAZIL

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Schistosomiasis mansoni, a chronic or acute infectious parasitic disease caused by *Schistosoma mansoni*, is a neglected tropical disease endemic in various regions of Brazil. This study conducted a malacological survey in temporary breeding sites in the municipality of Riachuelo/SE. The primary objective was to understand the local malacological fauna and evaluate the potential for schistosomiasis transmission during the dry season in the Brazilian Northeast. The collections were carried out at three different points, in the post-rainy and pre-rainy periods, using tweezers and metal shells. The group sent the collected mollusks to the Laboratory of Entomology and Tropical Parasitology (LEPaT) of the Federal University of Sergipe (UFS) for counting, measuring, and preserving containers with non-chlorinated water. The survey revealed the presence of *Biomphalaria glabrata*, *Drepanotrema cimex*, *Physa sp.*, and *Pomacea sp*. In the post-rainy period, 208 mollusks of the species *Biomphalaria glabrata* were collected, of which 43 were dead, with a positivity rate of 11% for *S. mansoni* amid the living mollusks. In contrast, the researchers collected 152 mollusks in the pre-rainy period, of which 52 were dead, presenting a positivity rate of 10% for *S. mansoni* among the live mollusks. The biometric evaluation of live *B. glabrata* mollusks indicated shell diameters ranging from 18 mm to 6 mm. Under laboratory conditions, the authors observed ovogenial capsules 10 days after malacological collections, underlining the mollusk's reproductive potential. The study highlights the significant role of the malacological survey in understanding the different forms of interaction with water collections, including temporary ones that can determine various epidemiological patterns of infection and transmission of schistosomiasis mansoni, especially in places conducive to the proliferation of these mollusks.

Supported by PIBIC-COPES.

Keywords: Schistosomiasis mansoni; *Biomphalaria*; Sergipe.

Hepatic tissue remodeling in swiss webster mice with acute schistosomiasis and subjected to a hyperlipidic diet

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Dyslipidemia and schistosomiasis are significant public health concerns, particularly in endemic regions. This study investigated liver tissue remodeling in acute experimental schistosomiasis and diet-induced dyslipidemia in female Swiss Webster mice. The mice were fed either a high-fat diet (HFD) or a standard diet (SD) for 29 weeks. At week 20, the animals were subcutaneously infected with 100 *Schistosoma mansoni* cercariae (BH strain). After 9 weeks of infection, the mice were euthanized, and blood samples were collected for biochemical analysis. Additionally, peritoneal lavage was performed to evaluate cytokine profiles. Liver tissues were collected, fixed, processed, and stained with Hematoxylin and Eosin, Giemsa, Gomori's Reticulin, and Picrosirius Red for histopathological, stereological, morphometric, and collagen granuloma analyses. Biochemical tests confirmed dyslipidemia in the HFD-fed mice, although lipid and glucose levels were notably lower in the infected animals. Peritoneal cells exhibited a proinflammatory immune response, and liver tissues displayed a highly inflamed architectural morphology. Morphometric analysis of centrilobular and hepatic veins revealed that both HFD and schistosomiasis altered vessel morphology. The findings suggest that diet-induced dyslipidemia interferes with certain aspects of acute experimental schistosomiasis, contributing to immune cytokine profile modulated by macrophages, liver tissue disorganization, and remodeling of the morphometric parameters of centrilobular and hepatic veins.

Funding: CAPES

Keywords: Schistosomiasis, Hypercholesterolemia, Hepatic Tissue.

IN VIVO IMMUNOTHERAPEUTIC EVALUATION OF MACROPHAGE MIGRATION INHIBITORY FACTOR FROM *Trichuris trichiura* IN A CUTANEOUS LEISHMANIASIS MODEL

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Introduction: Leishmaniasis is a parasitic disease with approximately 2 million new cases reported annually. In Brazil, the primary etiological agent of cutaneous leishmaniasis (CL) is *Leishmania braziliensis*, which predominantly induces a Th1 immune response with increased production of IFNγ and TNF. Studies conducted by our group identified the macrophage migration inhibitory factor from *Trichuris trichiura* (TtMIF) as a potential regulator of inflammation, capable of downregulating TNF and IFN-γ production while promoting TGF-β and IL-10 secretion. **Objective:** To evaluate the immunomodulatory potential of subcutaneous administration of the recombinant protein TtMIF in the treatment of cutaneous leishmaniasis caused by *L. brasiliensis*. **Methods:** In this study, rTtMIF was heterologously expressed in *E. coli* BL21 (DE3), purified using an ÄKTA Pure 25® system with a HisTrap FF Ni Sepharose column, and validated by SDS-PAGE and Western blot analysis**.** The *L. braziliensis* promastigotes (strain MHOM/BR/01/BA788) used in experimentation were cultured in Schneider's medium at 23°C for approximately 7 days to enable parasites to reach stationary phase. BALB/c mice were infected with *Leishmania braziliensis*. One group received subcutaneous prophylactic administration of rTtMIF, while another group received both prophylaxis and treatment. A positive control group was infected but received no intervention. Ear lesion progression was monitored weekly. The draining lymph node cell culture was performed, and subsequently, the supernatants were collected, and the levels of IL-10 e IFN-γ, were determined by ELISA. **Results:** The treatment with the rTtMIF changed the parasite load in Balb/c mice in both the prophylactic and treatment groups. Cytokines immunomodulation has also occurred in these groups. **Conclusion:** The rTtMIF protein seems to modify the inflammation caused by *L. braziliensis*, indicating that further investigation into these effects is needed to confirm rTtMIF as a therapeutic strategy.

Keywords: recombinant protein, *L. braziliensis*, parasite
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CHEMICAL CHARACTERIZATION AND 3D RECONSTRUCTION OF *Trichuris muris* EGGS: EXPLORING THEIR ULTRASTRUCTURE WITH EDS-SEM AND FIB-SEM

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Trichuris trichiura – the etiological agent of the Neglected Tropical Disease trichuriasis – affects millions of people worldwide from underserved communities. Infection occurs through the ingestion of embryonated eggs present in contaminated soil and food, therefore, the first structures to establish contact with the host. *Trichuris* eggs possess a rigid eggshell, which provides resistance to environmental variations, and two polar plugs, that interact with the host's microbiota and play a crucial role in promoting the hatching of the first-stage larva (L1). Given their fundamental role in the parasite's life cycle, studying *Trichuris* eggs may provide insights into novel strategies for controlling and mitigating trichuriasis. Based on this, the present study aims to perform a chemical and morphological analysis of *Trichuris muris* eggs – an experimental model for trichuriasis – employing state-of-the-art techniques such as Energy-Dispersive X-ray Spectroscopy (EDS-SEM) and Focused Ion Beam Scanning Electron Microscopy (FIB-SEM). The samples were cryofixed by high-pressure freezing (HPF) and chemical fixed through a freeze-substitution process using Bal-Tec 010 HPF machine and a Leica EMP apparatus, respectively. Subsequently, the eggs were embedded in Polybed 812 epoxy resin and the solid blocks were trimmed. The analyses were performed by ThermoFisher Helios NanoLab 660 dual-beam microscope (LNNano/CNPEM). FIB-SEM serial images provided a 3D view of the whole egg and its main structures, including: the three major eggshell's sublayers – *Pellicula ovi*, Chitinous layer, and Electron-dense parietal coating –, and the L1 larva and its granules, while the EDS-SEM technique mapped chemical elements present on those egg's structures, including Cr, S, Cl, P, and Ca elements. Both microscopy methods provided new insights into the morphology and chemistry of trichurid eggs.

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Keywords: *Trichuris*, FIB-SEM, 3D reconstruction.

IMPACT OF A HIGH-FAT DIET ON RENAL HISTOPATHOLOGY IN MURINE SCHISTOSOMIASIS

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Schistosomiasis is a neglected tropical disease commonly associated with renal complications, including glomerular damage. To investigate the effects of a high-fat diet (HFD) on renal glomerular histopathology and cytokine production, twenty female Swiss Webster mice were fed their respective diets for 29 weeks, with infection introduced after 21 weeks (infected with 100 cercariae from BH stain) and divided into four groups: CDP (control), CDH (HFD, uninfected), IDP (SD and infected), and IDH (HFD and infected). Cytokine production (IL-6, IL-12, TNF-α, IL-10, MCP-1/CCL2, and IFN-γ) were also evaluated. To evaluate glomerular areas, tissue damage, and capillary vascularization, histological sections were stained in Hematoxylin-Eosin and Picrosirius Red to assess collagen deposition. The HFD induced glomerular hypertrophy, increasing total glomerular area (63%) (CDH vs. CDP), along with increased collagen deposition and vascular alterations. These changes were exacerbated in infected mice. Schistosomiasis infection led to glomerular disorganization and a reduction in capillary areas in CDH and IDP groups (42% and 58% respectively). Histopathological changes included Bowman's space expansion, mesangial hypertrophy, and vascular remodeling in IDP, CDH and IDH groups. TNF-α levels were elevated (533%) in the HFD-infected group (IDH vs. CDP), indicating a synergistic pro-inflammatory effect of diet and infection. In contrast, IL-10 levels increased (346%) in IDH and IDP groups, suggesting an anti-inflammatory response, while MCP-1/CCL2 levels rose sharply (680%) (IDH vs. IDP), indicating increased monocyte recruitment. These findings highlight the interaction between high-fat diets and schistosomiasis in exacerbating renal damage, emphasizing the dual role of pro- and anti-inflammatory cytokines.

Funding: CAPES

Keywords: schistosomiasis; glomerulopathy; renal schistosomiasis.

NEW DATA ON DACTYLOGYRIDAE PARASITES OF THE "TAMBATINGA" (*Colossoma macropomum* X *Piaractus brachypomus*) FROM A FISH FARM LOCATED IN RIO BRANCO, STATE OF ACRE, BRAZIL

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Abstract

The "tambatinga" (♀ *Colossoma macropomum* x ♂ *Piaractus brachypomus*) stands out as an important fishery resource in Brazil. This species is a hybrid that exhibits great resistance to low water temperatures when compared to the parental species (*C. macropomum*), and its organoleptic characteristics make it well accepted for cultivation and consumption. For this reason, it is the third most cultivated species in Brazilian fish farms. Cultivated species are frequently infected by helminths, particularly by monopisthocotylans belonging to the family Dactylogyridae. This survey aimed at identifying species of Dactylogyridae parasites of the "tambatinga" (*Colossoma macropomum* x *Piaractus brachypomus*) from a fish farm in the state of Acre, Brazil. Five specimens of the "tambatinga" were previously necropsied, the gills were placed in vials with ethanol 70% and sent to the "Laboratório de Helmintos Parasitos de Peixes, Instituto Oswaldo Cruz, FIOCRUZ", Rio de Janeiro. Gills were placed in Petri's dishes and examined under a dissecting microscope for dactylogyrids. The specimens recovered were mounted in Hoyer's medium and analysed in a light microscope with phase contrast. Four species of Dactylogyridae have been identified so far: *Anacanthorus spatulatus* Kritsky, Thatcher & Kayton, 1979, *Anacanthorus penilabiatus* Boeger, Husak & Martins, 1995, *Mymarothecium boegeri* Cohen & Kohn, 2005, and *Mymarothecium viatorum* Boeger, Piasecki & Sobecka, 1992. The four dactylogyrid species found in the present study are reported for the first time in the Acre State. In addition, *A. penilabiatus* and *M. viatorum* are reported for the first time parasitising the "tambatinga", contributing to the knowledge of the host-parasite relationships. These preliminary results indicate that further studies on the diversity of dactylogyrid species associated with commercially important fishes are necessary to investigate the impact of these parasites on the vital conditions of the cultivated hosts.

Supported by: VPPCB/Fundação Oswaldo Cruz, PIBIC

Keywords: Gill parasites, Hybrid species, Monopisthocotyla

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OPTIMIZATION OF THE HELMINTEX METHOD: EVALUATION OF MAGNETITE POWDER AS AN ALTERNATIVE TO PARAMAGNETIC BEADS FOR *SCHISTOSOMA MANSONI* EGG RECOVERY

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The Helmintex (HTX) method employs paramagnetic beads to capture *Schistosoma mansoni* eggs in a magnetic field. It is considered a standard technique for evaluating diagnostic methods, particularly for cost-effective screening in low-endemic areas. However, the high cost of paramagnetic beads limits its broader application. This study aimed to assess the efficiency of magnetite powder (Fe_3O_4) as a cost-effective alternative for *S. mansoni* egg recovery after fecal sample fixation and filtration. The procedure followed Teixeira et al. (2007), with modifications by Favero et al. (2017). After debris reduction, 50 *S. mansoni* eggs were seeded in samples and exposed to a magnetic field using the Multi-6 Microcentrifuge Tube Separator (BioMag®). Recovery efficiency was compared between paramagnetic beads (50 mg/ml Fe_3O_4) and Fe_3O_4 powder at 50 mg/ml (A), 25 mg/ml (B), and 12.5 mg/ml (C), with three replicates per concentration. Eggs were stained using auramine-O (Au-O) and analyzed by fluorescence microscopy. At concentration A, excessive Fe_3O_4 deposition impaired fluorescence and hindered microscopic analysis, while concentration C yielded only 22.2% recovery. Concentration B achieved 80.5% recovery, surpassing the 55% obtained with paramagnetic beads. Although preliminary, these findings suggest that Fe_3O_4 powder is an efficient and lower-cost alternative to paramagnetic beads in the HTX method..

Supported by: CNPq, FAPES
Keywords: Helmintex, Magnetite, *Schistosoma mansoni*

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CHARACTERIZATION OF OPTIMAL SPREADING OF FECAL SEDIMENTS PRODUCED BY HELMINTEX METHOD TO OPTIMIZE AUTOMATED IMAGE ANALYSIS DETECTION OF *Schistosoma mansoni* EGGS

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This study aimed to evaluate the impact of sample distribution on glass slides for microscopic analysis of sediment in the Helmintex (HTX) method used to diagnose *Schistosoma mansoni* eggs. The images were obtained from slides containing fecal samples, considered of low quality under the microscope. Prior to microscopic analysis, the slides were prepared to ensure a homogeneous distribution of the sediment for adequate visualization. The photographs were cropped to focus only on the slides, ensuring that the model could concentrate on areas of interest. To standardize the model input, all images were converted to RGB format and resized to a fixed size of 512x1536 pixels, in accordance with the slide dimensions. Padding was applied to maintain the correct aspect ratio without information loss. A total of 361 images were used in the study. Of these, 85% (306 images) were used for model training, with 15% allocated for validation. The final test set consisted of 55 images, all obtained from distinct slides and samples. Various convolutional neural network architectures were tested for classifying sediment distribution adequacy on the slides, including ResNext101, InceptionV4, EfficientNetB2, ResNet50, MobileNetV3, ViT (Vision Transformers), and Swin Transformers. These architectures are renowned for their performance in image classification, employing a range of feature extraction techniques from deep convolutional blocks to attention mechanisms used in transformer models. The model's performance was evaluated based on accuracy, sensitivity, and specificity with respect to the test dataset. InceptionV4 yielded impressive results in validation with an accuracy of 1.0 and a loss (BCE) of 0.033. It also achieved perfect sensitivity of 1.0, confirming its capacity to efficiently classify slide images. For the test dataset, ResNet50, MobileNetV3, and Swin Transformers demonstrated superior classification abilities, with accuracies also reaching 1.0 and sensitivity levels of 1.0. The results enabled the identification of the most efficient architecture for classifying slide images, contributing to the automation of the process for assessing sample suitability for microscopic analysis.

Supported by: CNPq, CAPES, FAPES
Keywords: Helmintex, spread, IA

FIRST REPORT OF THE ANISAKID *Contracaecum jorgei* SARDELLA, MANCINI, SALINAS, SIMÕES & LUQUE, 2020 (NEMATODA: ANISAKIDAE) IN COMMON SNOOK *Centropomus undecimalis* (BLOCH, 1792) (OSTEICHTHYES: CENTROPOMIDAE) FROM BRAZIL

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Abstract

Centropomus undecimalis, common snook, occurs in marine and estuarine waters, and to a lesser extent, in freshwater from North Carolina, down through Florida in the United States, to Rio de Janeiro, Brazil, including Central America. This species is economically important throughout the Brazilian coast, as well as for sport fishing and potential for fish farming. Belonging to the Anisakidae family, *Contracaecum jorgei* was originally described in *Hoplias argentinensis* from Argentina. The records of anisakids, especially in commercial fish species, should not be neglected, as anisakiasis in humans is a gastrointestinal parasitic infection that results from the accidental ingestion of infective larvae. The present study aimed at identifying and recording this anisakid in *C. undecimalis* from Brazil, based on morphology and molecular analysis. A total of 15 specimens of *C. undecimalis* were collected by artisanal fishermen in Rodrigo de Freitas lagoon, state of Rio de Janeiro, Brazil. These specimens were transported, in ice-cold styrofoam boxes and individually packaged inside plastic bags, to the Laboratório de Biologia e Ecologia de Parasitos of the Universidade Federal Rural do Rio de Janeiro, for examination. Genomic DNA was extracted using DNeasy® Blood and Tissue Kit (Qiagen) and the amplification was carried out using the cytochrome c oxidase subunit 2 (*cox*2) as molecular marker. Among the common snooks collected, 10 were parasitized by at least one third-stage larval specimen (L3) of *C. jorgei*, encysted in the abdominal cavity, with prevalence 66.7%. The measurements and morphology were compatible with the recent description of *C. jorgei* from *H. argentinensis*. The nucleotide sequences obtained from the *cox*2 had 100% of query cover and 99.4% similarity with sequences of *C. jorgei* deposited in GenBank. Therefore, the molecular analysis corresponded with the morphological identification confirming that the L3 larvae of *C. jorgei* collected from *C. undecimalis*.

Supported by: CAPES, FAPERJ, VPPCB – Fundação Oswaldo Cruz
Keywords: fish parasites, integrative taxonomy, Rodrigo de Freitas lagoon

ADIPOSE TISSUE REMODELING IN SCHISTOSOMIASIS MANSONI-INFECTED C57BL/6 MICE WITH METABOLIC DISORDER

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Schistosomiasis mansoni is a neglected disease affecting several countries, with approximately 700 million people currently living in areas at risk of infection. Changes in the nutritional profile of the population are reflected in an increase in metabolic disorders that may influence the course of human schistosomiasis. In this study, we sought to evaluate adipose tissue remodeling in a diet-induced obesity model with concomitant schistosomiasis mansoni. C57BL/6 male mice fed either a high-fat diet (60% fat) or a standard diet (10% fat) for 13 weeks were infected with 100 *Schistosoma m*ansoni cercariae (BH strain). The mice were then divided into four groups: USC (uninfected fed standard diet), UHFC (uninfected fed high fat diet), ISC (infected fed standard diet) and IHFC (infected fed high fat diet). The development of obesity was assessed by blood lipid profile, including TC (total cholesterol), TG (triglyceride levels), LDL-C (low-density lipoprotein cholesterol), HDL-C (high-density lipoprotein cholesterol) and VLDL-C (very low-density lipoprotein cholesterol) and glucose concentrations, OGTT (oral glucose tolerance test), body mass and adiposity index. Samples of visceral adipose tissue were fixed in 10% formalin, then processed and embedded in paraffin, and sectioned at 5 µm, deparaffinized and stained with hematoxylin and eosin (H&E). Adipose tissue showed structural changes due to the inflammatory process of infection. The mean cross-sectional area and diameter of adipocytes were greater in animals fed a high-fat diet, whether infected or not. We conclude that the relationship between schistosomiasis mansoni and the high-fat diet promotes significant changes in several physiological parameters and in adipose tissue remodeling.

Keywords: Schistosomiasis. Metabolic disorder. Obesity.

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ANTI-HELMINTHIC EFFECT OF ETHANOLIC EXTRACT AND ESSENTIAL OIL OF *Rosmarinus officinalis* IN MICE INFECTED WITH *Strongyloides venezuelensis*

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Strongyloidiasis, caused by *Strongyloides stercoralis*, affects populations in developing countries, with manifestations ranging from asymptomatic to potentially fatal. Conventional treatment, primarily based on ivermectin, faces challenges such as parasitic resistance and limited accessibility. Thus, natural compounds have been explored as alternative therapeutic options. *Rosmarinus officinalis* (rosemary) has recognized pharmacological properties, but its anti-helminthic effect remains underexplored. This study evaluated the anti-helminthic activity of ethanolic extract and essential oil (qt. cineol) from *R. officinalis* in male Swiss mice aged 8 to 10 weeks, weighing approximately 20g, infected with 1,500 filariform larvae (L3) of *Strongyloides venezuelensis*. Treatment began 24 hours post-infection, and animals were divided into four groups: GI (infected and treated with water), GII (infected and treated with ivermectin – 2 mg/kg), GIII (infected and treated with ethanolic extract – 200 mg/kg), and GIV (infected and treated with essential oil – 200 mg/kg). The treatment was administered orally for six consecutive days, and on the seventh day post-treatment, animals were euthanized. Parasitic load was assessed by counting eggs in feces, the number of parthenogenetic females, and fecundity rate. A significant reduction in egg count in feces was observed in treated groups compared to the control (p=0.0284). The lowest parasite burden was found in the ivermectintreated group, which showed a significant reduction in eggs per gram of feces and intestinal females compared to other treatment groups (p=0.0154). Although the ivermectin treated group had the lowest fecundity rate, there was no statistically significant difference among treatment groups. These findings indicate that *R. officinalis* bioactive compounds reduced parasitic load and fecundity, suggesting therapeutic potential against strongyloidiasis.

Supported By: CAPES

Keywords: Strongyloidiasis, Treatment, Medicinal Plants.

IN VITRO EVALUATION OF THE ANTHELMINTIC POTENTIAL OF *Rosmarinus officinalis* qt. CINEOL ESSENTIAL OIL AGAINST *Strongyloides venezuelensis*

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Strongyloidiasis is a neglected parasitic disease, with severe cases predominantly affecting immunocompromised individuals, potentially leading to fatal outcomes. The search for alternative treatments is essential due to therapeutic failures and the increasing resistance of parasites to conventional treatments. The therapeutic use of medicinal plants, such as phytotherapy, is an ancient practice that has gained recognition among complementary integrative practices. Brazil, with its rich biodiversity, harbors numerous plants with bioactive compounds that remain underexplored, including *Rosmarinus officinalis*. The essential oils of this plant contain pharmacologically active compounds, making them strong candidates as anthelmintic agents. In this context, the present study aimed to evaluate the in vitro anthelmintic potential of *R. officinalis* qt. Cineol essential oil. The larval motility inhibition test was conducted using *S. venezuelensis* L3 larvae incubated with different concentrations of the essential oil (200, 160, 140, 120, 80, 40, 20, and 10 mg/mL). Ivermectin (0.00036 mg/mL) served as the positive control, while water and PBS-DMSO (1%) were used as negative controls. Samples were analyzed after 24, 48, and 72 hours in triplicate. At higher concentrations, the oil achieved 100% inhibition of larval motility, similar to the positive control. The IC50 values obtained were 17.77 mg/mL at 24 hours, 14.88 mg/mL at 48 hours, and 22.26 mg/mL at 72 hours of exposure. Inhibition increased over time at concentrations of 40, 20, and 10 mg/mL, but without full effectiveness. The effectiveness of *R. officinalis* L. qt. Cineol essential oil was more dependent on concentration than on exposure time. In conclusion, *R. officinalis* essential oil, particularly its cineol fraction, exhibited anthelmintic activity against *S. venezuelensis* larvae, and further studies are needed to assess its in vivo efficacy.

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Keywords: Phytotherapy, antiparasitic treatment, medicinal plants.

Ultrastructural characterization of *Gracilioxyuris agilisis* and *Didelphoxyuris thylamisis* parasites of small marsupials in the Brazilian Pantanal

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Nematodes are a group which is highly taxonomically challenging due to their diversity, and lack of specialists. In this study, we report the occurrence of oxyurids: *Gracilioxyuris agilisis* (Feijó et al, 2008) and *Didelphoxyuris thylamisis* (Gardner & Hugot, 1995) in marsupials from Corumbá, state of Mato Grosso do Sul, Brazil. Using integrative taxonomy, employing light microscopy and scanning electron microscopy, we were able to characterize and distinguish these genera. The differentiation is based on the male morphology, in *G. agilisis*, the tip of the spicule is straight and pointed, and the mamelons of the rough area extend from the posterior region to the middle of the body. In *D. thylamisis*, the tip of the spicule is rounded, and the mamelons of the rough area extend from the posterior region almost reaching the anterior region. In both, the anterior part is very similar, with three distinct lips, four labial papillae around the oral opening, two amphids, a lateral wing composed of two longitudinal ridges and three esophageal teeth. In females, the differences were observed in the anterior part and are almost imperceptible. In *D. thylamisis*, the cephalic structures are arranged in a “hollow-headed”, while in *G. agilisis*, the anterior end has a “dome-shaped”, where all the cephalic structures are grouped. In addition, we identified other imperceptible differences in the females: in *D. thylamisis*, there is a defined cephalic wing, which extends to the beginning or middle of the bulb. In *G. agilisis*, the females do not have this cephalic wing, although their cuticle can, in some situations, be confused with this structure. The characterization and morphological identification of females of *G. agilisis* and *D. thylamisis* expands the knowledge about parasite diversity in tropical environments. Their high similarity highlights the taxonomic complexity of oxyurids, reinforcing the need for integrative approaches for accurate identification.

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Keywords: Nematoda, Marsupials, Morphology

NEMATODES OF VETERINARY MEDICAL INTEREST IN AN URBAN RESERVOIR IN THE MUNICIPALITY OF CATALÃO, SOUTHEASTERN GOIÁS STATE, BRAZIL

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Recreational areas near bodies of water can be an ideal place for leisure time in a natural environment, even within the city, but they can also be favorable places for the presence of parasites because they combine ideal climatic conditions, water and a large circulation of people and animals. Periodic monitoring is necessary in order to control infections of medical and veterinary interest. The aim of this study was to carry out parasitological analyses on soil samples from the Bica reservoir in the municipality of Catalão, in the south-east of the state of Goiás. The area around the dam was divided into five plots, and samples were collected from two points in each plot, upper and lower. Four samples were collected on alternate days, totaling 40 samples of approximately 50g of topsoil, with a maximum depth of five centimeters. The samples were processed in the Parasitological Diagnostic Sector of the Molecular Biology Laboratory of the Federal University of Catalão (UFCAT). For the parasitological analyses, we used five methods found in the literature, Rugai, Willis, spontaneous sedimentation, Ritchie, and modified Ritchie. By the Rugai method, it was possible to detect rhabditoid larvae of the genus *Ancylostoma* and *Strongyloides*. Using the modified Ritchie method, eggs of Ascaris lumbricoides and *Ancylostoma* sp were found. The presence of evolutive forms in the soil of the reservoir indicates contamination by animal and human waste, favoring the establishment of biological cycles of the species found and of other different zoonoses. The data obtained demonstrated the importance of raising the population's awareness of health promotion measures besides preventive actions and health education programs.

Keywords: Geo Helminths, Ancylostoma sp., *Strongyloides stercoralis*.

A NEW DIGITAL DROPLET PCR ASSAY FOR THE INTESTINAL SCHISTOSOMIAS DIAGNOSIS

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In Brazil, intestinal schistosomiasis is caused by the *Schistosoma mansoni* and its laboratory diagnosis is performed by the Kato-Katz technique, as recommended by WHO. However, this technique presents limitations on sensitivity when applied in low parasite burden individuals. Droplet digital PCR (ddPCR) has been presenting high accuracy for diagnosis of other diseases and appears as a choice for intestinal schistosomiasis. The aim of this study was to standardize and validate a ddPCR assay for the intestinal schistosomiasis diagnosis. For that, the total DNA was extracted from adult worms and 248 samples of individuals living in the municipality of Conde in Bahia, Brazil, an area of moderate endemicity for the disease, using QIAamp® Power Fecal Pro DNA® Kit, as recommended by the manufacturer. The ddPCR assay was performed using primers and probes targeting a 90 bp from Sm1-7 region of the *S. mansoni* genome. To define the best laboratory conditions for the assay, reactions were performed at different annealing temperatures, primers and probes concentrations and total DNA amount, using ddPCR consumables and reagents from Bio-Rad Laboratories. Repeatability was performed using 5 samples repeated 6 times, reproducibility using 7 samples repeated 5 times and analytical sensitivity using DNA extracted from adult worms. The conditions were defined as an annealing temperature of 56,5° C, primer concentration of 700 nM, probe concentration of 150 nM and total DNA amount of 40 ng. The ROC curve indicated a cut-off value of 10 copies/µL and the analytical sensitivity demonstrated a limit of detection (LOD) of 0.1 pg of DNA. Repeatability showed a coefficient of variation (CV) ranging from 6.8% to 11.1%, while reproducibility exhibited a CV ranging from 5.4% to 20.34%. The positive rate detection was 51.61%, compared to 51.21% for Kato-Katz combined with Helmintex. Thereafter, the results from ddPCR will be compared to those from qPCR and LAMP using the same DNA samples.

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Keywords: intestinal schistosomiasis, laboratorial diagnosis, ddPCR

SURVEY OF THE OCCURRENCE OF ENTERIC PARASITES IN FREE-LIVING ROBUST CAPUCHIN MONKEYS (*Sapajus nigritus*)

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Parasitic diseases in wild animals are of great relevance in the One Health context, especially in non-human primates, which can act as reservoirs for several parasites with zoonotic potential. However, investigations of endoparasites in these animals are scarce. Thus, this study aimed to investigate the occurrence of enteric parasites in free-living robust capuchin monkeys (*Sapajus nigritus*) that inhabit the university campus of the UNESP, School of Dentistry, Araçatuba, São Paulo, Brazil. A total of 44 animals were investigated in this research. Fecal samples were collected directly from the soil after spontaneous defecation, bottled in universal collection pots and sent for processing. Enteric parasites were detected by coproparasitological techniques (Willis–Mollay, Faust and Hoffman Pons and Janer). Of the animals investigated, 43 were positive by at least one technique, identifying Ancylostomatidae eggs (41/44 [93.18%]), *Strongyloides stercoralis* eggs and larvae (34/44 [77.27%]), Spiruridae eggs (8/44 [18.18%]) and *Hymenolepsis diminuta* eggs (10/44 [22.72%]). Regarding the positive animals, 86.04% presented polyparasitism, 48.83% (21/43) were infected with Ancylostomatidae and *S. stercoralis*, 16.27% (7/43) with Ancylostomatidae, *S. stercoralis* and *H. diminuta*, 11.62% (5/43) with Ancylostomatidae, *S. stercoralis* and Spiruridae, 4.65% (2/43) with Ancylostomatidae and *H. diminuta*, 2.32% (1/43) with Ancylostomatidae and Spiruridae and 2.32% (1/43) with Ancylostomatidae, Spiruridae and *H. diminuta*. Thus, we can identify the occurrence of enteric parasites in 97.72% of the capuchin monkeys investigated in this study, with the identification mainly of Ancylostomatidae eggs and *S. stercoralis* larvae.

Keywords: helminths, primates, zoonosis.

Occurrence of schistosomiasis in the Northeast Region of Brazil in 2023.

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Schistosomiasis is a parasitic disease caused by *Schistosoma mansoni* that can evolve into severe clinical forms that can lead to the death of those affected, making it a public health problem. This is a retrospective descriptive study using data from SINAN-NET-MS, with the aim of finding out about the occurrence of the disease in the northeast and providing information for prevention. A total of 3059 cases of the disease were reported in Brazil in 2023, of which 1008 (1008- 32.95%) in the Northeast Region in the states: Bahia (544 -53.97%) Pernambuco (143 - 14.18%), Sergipe (100- 9.92%) Paraíba (79- 7,), Maranhão (24-2.38%), Ceará (17 -1.69%), Rio grande do Norte (22- 2.18%) Piauí (04 -0.40%), As for gender: Male (570- 56.55%), female (438- 43.45%), it was possible to confirm cases by qualitative analysis in positive in 405, and quantitative analysis in 454 (more than one egg of the parasite found). The most affected race was brown (735) and white (136), and indigenous people were reported (05). The most affected age group was 40-59 years (343-34.02%), and those aged 60-80 (254-25.20%). The chronic clinical forms were: intestinal (397- 39.38%), hepato-intestinal (39- 3.87%), hepato-splenic ((67- 6.64%). and 41 died. Considering that schistosomiasis is a neglected disease and that it is related to access to basic sanitation (sewage disposal) and that we still have a population in the Northeast of Brazil with limited access to sanitation measures, the disease is present in the region with severe forms and deaths, making total access to basic sanitation essential for the elimination and control of the disease.

Keywords: Schistosomiasis, *Schistosoma mansoni*, Northeast

HELMINTH DIVERSITY OF WILD AND SYNANTHROPIC SMALL MAMMALS IN THE MUNICIPALITY OF MONTES CLAROS, NORTHERN MINAS GERAIS REGION

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Small mammals can serve as definitive or intermediate hosts for numerous species of parasitic helminths, some of which may have zoonotic potential. This study aims to identify helminth species morphologically and molecularly from small mammals captured in the municipality of Montes Claros, Minas Gerais. Sampling was conducted in Sapucaia Municipal Park, Milton Prates Municipal Park, and the neighborhoods of Carmelo, Vila Greice, and Alto São João. For captures, Sherman and Tomahawk traps were used, remaining open for five consecutive nights at each site and checked every morning. The captured animals were weighed, sedated, and euthanized with an intravenous overdose of general anesthetic. The carcasses of the collected small mammals were deposited and cataloged in the Coleção Integrada de Mamíferos Silvestres Reservatórios do Instituto Oswaldo Cruz (FIOCRUZ-RIO) after species identification. A total of 34 animals were captured: 11 in Sapucaia (three *Didelphis albiventris*, six *Thrichomys apereoides*, and two *Kerodon* sp.), two in Milton Prates, five in the Carmelo neighborhood, 12 in Vila Greice, and four in Alto São João, all belonging to the *Rattus* genus. Out of the 34 necropsied animals, 44% (15/34) tested positive for helminths from the phyla *Nematoda* and *Acanthocephala*, as well as the class *Cestoda*, including the species *Calodium hepaticum*; the genera *Physaloptera* sp., *Trichuris* sp., *Cruzia* sp., *Heligmostrongylus* sp.; the families Oxyuridae and Trichostrongylidae and strobilocercus parasitizing the liver of rodents. The identification of small mammal species and helminths is still in progress. While numerous helminthological surveys have already been conducted on wild and synanthropic small mammals, further exploration of the parasitic fauna in different environments is essential, especially for detecting potential reservoirs of helminths with zoonotic potential and preventing the potential transmission of these parasites to humans.

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Keywords: Zoonoses, Rodents, Helminths

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HIGHER *Strongyloides stercoralis* IGG SEROPREVALENCE IN IMMUNOSUPPRESSED RHEUMATIC PATIENTS COMPARED TO IMMUNOCOMPETENT INDIVIDUALS

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Strongyloides stercoralis is a soil-transmitted helminth capable of causing chronic infections, particularly in immunosuppressed individuals. This study aimed to evaluate IgG seroreactivity and antibody frequency in immunosuppressed rheumatic patients and immunocompetent individuals, using a synthetic peptide predicted to be species-specific and recognized by B cells.
A total of 425 serum samples were analyzed, including 256 from immunosuppressed patients (88 rheumatoid arthritis [RA], 57 spondyloarthritis [SpA], 76 systemic lupus erythematosus [SLE], 18 other lupus [LU], and 17 other autoimmune rheumatic diseases [ORD]) and 169 from immunocompetent individuals (51 children, 118 adults). The overall seroprevalence was 38.11%, with significantly higher prevalence in immunosuppressed (50.19%) than in immunocompetent individuals (19.64%). By group, IgG prevalence was 31.46% in RA, 68.42% in SpA, 59.21% in SLE, 50% in LU, 47.06% in ORD, 24.79% in immunocompetent adults, and 7.84% in children.
Comparing seroreactivity between rheumatic diseases and their respective controls, higher IgG seroreactivity was observed in SLE ($p < 0.0001$) and in immunocompetent adults compared to immunocompetent children ($p = 0.0048$). In contrast, RA patients showed reduced seroreactivity compared to their controls ($p = 0.0189$). Regarding antibody frequency, higher IgG levels were found in SLE ($p < 0.0001$), SpA ($p = 0.0227$), and immunocompetent adults compared to children ($p = 0.0110$). These findings suggest that *S. stercoralis* exposure and immune response differ across immune conditions, with higher prevalence in immunosuppressed patients, particularly in SLE and SpA, and in immunocompetent adults compared to children. This highlights the importance of screening for *S. stercoralis* in immunosuppressed individuals to prevent potential complications.

Supported by: FAPEMIG, CAPES.

Keywords: *Strongyloides stercoralis*, Autoimmune diseases, Seroprevalence

DEVELOPMENT OF A NANOTECHNOLOGY TOOL FOR THE DIAGNOSIS OF SCHISTOSOMIASIS

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Schistosomiasis is a public health problem that infects over 1.5 million people in Brazil. The gold standard test for diagnosing this disease is Kato-Katz, based on the detection of eggs in stool samples. However, it often yields false negative results for patients with a low parasitic load. Therefore, it is essential to develop more sensitive tests for the diagnosis of schistosomiasis. Currently, the use of biosensors has been used as promising tools for the diagnosis of other pathogens, such as SARS-CoV. Therefore, the objective of this study was to use EPEL technology - Portable Light Scattering Spectrometer (patent BR1020160097657) - to standardize a new form of diagnosis for schistosomiasis. EPEL performs analytical detection measurements based on the principle of dynamic light scattering in depolarized mode (DLS) emitted by the optical signal produced by gold nanorods bio-conjugated with recognition elements of interest. In this study, the tips of the nanorods were conjugated with a high molecular weight protein fraction extracted from the adult worm of *Schistosoma mansoni* (SmJ1). For standardization, the soluble extract of the adult worm was initially produced and fractionated in an exclusion column to obtain the SmJ1 fraction; then, the production of gold nanorods (150pM) and their functionalization with SmJ1 was carried out through chemical bonding mediated by linker 11-mercaptoundecanoic acid. After confirmation of the successful functionalization, serum from patients in the endemic region of Januária (Minas Gerais, Brazil) were tested at different concentrations. Preliminary tests indicate that the biosensors with SmJ1 were able to recognize and differentiate the association profile of the sensor with serum proteins from an individual infected with *S. mansoni* and an uninfected individual, indicating that this is a promising tool for the diagnosis of schistosomiasis.

Supported by: FAPEMIG, CAPES

Keywords: *Schistosoma mansoni*, nanorods, EPEL

PARASITOLOGICAL SURVEY BASED ON DATA OBTAINED FROM A CLINICAL ANALYSIS LABORATORY IN SALVADOR-BA

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Intestinal parasitoses are diseases caused by etiological agents such as helminths and protozoa, which, at some stage of their biological cycle, inhabit the human digestive system and can cause pathological conditions. They represent a public health issue as they result from a lack of basic sanitation, inadequate education, misinformation regarding hygienic-sanitary practices, high population density, degrading environmental conditions, and the virulence capacity of parasites. Around 3.5 billion people worldwide are affected by enteroparasitoses, with children being the most affected group. In Brazil, it is estimated that 55.3% of all children are infected with enteroparasitoses. Therefore, it is evident that the study of parasitology aids in the prevention and treatment of parasitic diseases that can affect both humans and animals. Knowledge about different species, the number of existing infectious forms, the age, and the condition of the host provides essential information not only for combating the parasite or disease but also for prevention and understanding human health conditions. In this context, the present study determined the occurrence of gastrointestinal parasites based on the results of stool parasitological examinations of patients from a SUS-affiliated clinical analysis laboratory, Labimuno, located at the Institute of Health Sciences – ICS/UFBA, in Salvador-BA. The study correlated the findings with the variables of patient age and sex. The methodology consisted of analyzing spreadsheets using Microsoft Office Excel®, version 2019, constituting an analytical retrospective cross-sectional study of stool parasitological test results from human patients between May 2018 and October 2020. The study identified the occurrence of helminths and protozoa, as provided by Labimuno. The study was approved by the ICS/UFBA Research Ethics Committee, under protocol number 4268282. A total of 34,906 stool parasitological test results were analyzed, showing an enteroparasite occurrence of 13.3%, with protozoa predominating in 90% of cases. The most prevalent species were *Endolimax nana* (46%) and *Entamoeba coli* (35%), with lower occurrences of *Ascaris lumbricoides*, *Trichuris trichiura*, *Schistosoma mansoni*, *Strongyloides stercoralis*, hookworms, *Enterobius vermicularis*, and *Taenia* spp. The average patient age was 40.19 years. Women comprised 70.4% of the sample, with an average age of 41.14 years, while men had an average age of 34.94 years. The median age was higher for women (43 years) than for men (41 years). The male population had a higher proportion of normal results (60%) compared to the female population (57.2%). Among positive cases, women had a lower prevalence (12.3%), while men had a prevalence of 15.7%. Understanding the percentage of the parasitized population helps implement preventive and curative measures to reduce or mitigate the damage caused by endoparasitoses. Additionally, it ensures a valuable database that will contribute to the literature, which remains scarce on this subject. Finally, this study highlights the need for further research to expand the epidemiological and parasitological knowledge of enteroparasitoses in Salvador-BA, analyzing the various scenarios and specific characteristics of this municipality.

Keywords: helminths, protozoa, humans

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SERPIGINOUS AND PRURITIC DERMATITIS: A CASE REPORT OF CUTANEOUS LARVA MIGRANS

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Abstract

Cutaneous larva migrans, also referred to as serpiginous and/or pruritic dermatitis, is a condition commonly known as "creeping eruption" or "geographic worm." It is an inflammatory skin reaction that occurs in accidental human hosts following the penetration of helminth larvae, primarily Ancylostoma caninum and Ancylostoma brasiliense, whose definitive hosts are dogs and cats. This study presents a typical case initially misdiagnosed as contact dermatitis “without parasitic involvement.” A 23-year-old male student from Recôncavo da Bahia developed intense itching on the buttocks and part of the right thigh after spending a week in Morro de São Paulo (Cairu, Bahia). During a private consultation, a general practitioner diagnosed the condition as a cutaneous allergy and prescribed a topical antihistamine. However, after three days without symptom relief, the patient sought a dermatologist, who identified serpiginous, pruritic lesions with irregular lines. Given the patient's recent exposure to sand, the dermatologist diagnosed migratory cutaneous edema, a presentation consistent with cutaneous larva migrans caused by hookworm larvae migration, without the need for additional tests. The patient was prescribed topical thiabendazole ointment and a single dose of oral ivermectin (two tablets). The lesions were resolved within two weeks. The location of the rash, its characteristic serpentine pattern, and the patient's exposure history were pathognomonic for this parasitic infection. Considering that the initial misdiagnosis delayed effective treatment and that this parasitosis is relevant to One Health, studies on the differential diagnosis of dermatitis—integrating clinical presentation with epidemiological data—are essential for improving patient outcomes.

Supported by: CNPq.
Keywords: host-parasite interactions, environment , public health.

CHANGES IN THE PREVALENCE OF INTESTINAL PARASITES IN AN ENDEMIC AREA OF BAHIA OVER THE PAST 20 YEARS

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ABSTRACT

Introduction: Despite the availability of treatments for various parasitic diseases, endemic areas require continuous monitoring to assess the prevalence of these infections and to understand the dynamics of these parasitosis. **Methodology:** This is a time-series study using data from two cross-sectional surveys conducted between 2001 and 2019. **Results:** In 2001, the spontaneous sedimentation method (HPJ) was performed on 1025 samples revealing high prevalences of Hookworms (71.2%), followed by *A. lumbricoides* (65.3%), *T. trichiura* (63.5%), and *S. mansoni* (42.4%). Protozoa detected included *E. nana* (27.3%), E. coli (45.3%), and *Giardia lamblia* (4.8%). In 2003, using the same HPJ method, the main parasites found were Hookworms (27.5%), *A. lumbricoides* (47.5%) and *T. trichiura* (52.5%), followed by *S. mansoni* (20.0%). Protozoa such as *E. nana* (2.5%), *E. coli* (10%) and *Giardia lamblia* (2.5%) were less frequently detected. In 2014, *S. mansoni* had the highest prevalence (66.6%). However, a progressive reduction was observed in subsequent years, with prevalences of 55.6% in 2018 and 34.9% in 2019. The prevalence of *A. lumbricoides* showed a continuous decrease after 2014, reducing to 8.6% between 2018 and 2019. During the same period, *S. mansoni* and *T. trichiura* decreased to 25.1% and 19.9%, respectively, along with a reduction in parasite loads. Hookworm analysis revealed low positivity (2.5%), possibly due to the slide reading time. **Conclusion:** Despite the interventions implemented over the past 20 years, the reduction in prevalence was limited for certain species. S. mansoni showed a 20.6% reduction between 2001 and 2018, while T. trichiura decreased by 36.4%. A. lumbricoides experienced an approximate 50% decline in the same period. These findings highlight the need for the continued implementation of parasite control strategies in endemic regions to further reduce infections and improve public health conditions.

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Keywords: Helminths, Protozoa, Neglected Diseases.

EVALUATION OF THE OCCURRENCE OF ENDOPARASITES IN CATTLE USED IN OX CARTS DURING THE TRINDADE PILGRIMAGE, GOIÁS

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The ox cart pilgrimage in Trindade is a nationally recognized event, as it is not only the largest ox cart pilgrimage in the world but also acknowledged as a heritage site by the National Institute of Historical and Artistic Heritage. During the event, over 400 ox carts traverse Trindade, Goiás, making it a national event that attracts animals from various parts of Brazil. Each ox cart typically carries an average of eight animals. Given the large concentration of livestock, there are sanitary risks, such as the dissemination of diseases and parasites. The transportation of cattle requires an animal transit guide, which ensures basic compliance measures, such as proof of vaccination. However, there is no regulation concerning parasitic diseases, such as helminthiasis. The objective of this project was to analyze the occurrence of endoparasites in the cattle present during the pilgrimage and to explore potential prophylactic measures. A total of 164 cart drivers were interviewed using a standardized questionnaire containing questions about endoparasite prophylaxis. Additionally, representative samples were collected from 97 ox carts, with at least four samples per cart. Regarding the use of anthelmintics, 75.61% reported using some chemical compound, 20.73% did not use any, and 3.66% were unable to respond. Among the interviewed cart drivers, 31.71% indicated that their animals consume water from streams, 9.14% from ponds, 5.49% from artesian wells, 3.66% directly from rivers, and 2.44% from cisterns and tanks. The primary parasites identified were Eimeria sp. (17.52%), strongyles (6.18%), and Moniezia sp. (1.03%). The parasitic load identified for helminth species was low, while that for Eimeria was relatively higher, which may indicate risks of environmental contamination at sites with high animal concentration if appropriate prophylactic measures are not implemented.

Keywords: cestodes, helminths, parasitology.

PROTOZOOLOGY

THERAPEUTIC POTENTIAL OF *MELALEUCA LEUCADENDRA* (L.) L. FLOWERS IN THE TREATMENT OF VISCERAL LEISHMANIASIS

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Visceral Leishmaniasis (VL) is a significant public health issue with high prevalence in Brazil and worldwide. The limited availability of effective and safe treatments, combined with the side effects of current therapies, underscores the need for alternative therapeutic options. The plant *Melaleuca leucadendra* has gained attention due to its promising biological properties. The study aimed to evaluate the *in vitro* leishmanicidal activity of the crude extract (CE) and its fractions: hexane (HE), ethyl acetate (AE), dichloromethane (DM), and butanol (BUT), from the flowers of *M. leucadendra* against *Leishmania infantum*. The experiment was conducted at various treatment times (24, 48, and 72h), with Amphotericin B as a positive control (PC). The CE was extracted using percolation with ethanol and then fractionated. Cytotoxicity was assessed in RAW 264.7 murine macrophages via the Sulforhodamine B (SRB) method, revealing low cytotoxicity with CC_{50} values above 150 µg/mL at all tested time points. The leishmanicidal activity against *L. infantum* promastigote forms (OP46 strain) was determined by the MTT assay, with the DM fraction showing the greatest inhibition, exhibiting an IC_{50} of 20.27 µg/mL and a selectivity index of 23.5 after 48 hours of treatment. Activity against amastigote forms was assessed using flow cytometry (FACS Calibur) with RAW 264.7 macrophages infected with *L. infantum* transfected with GFP. The DM and BUT fractions significantly reduced the percentage of infected macrophages (85.86% and 80.6%, respectively, at the highest concentrations after 24 and 48h), compared to the control. Additionally, the HE, DM, and BUT fractions reduced the parasite load by 82.28%, 82.89%, and 78.24%, respectively, after 48 hours. The DM fraction showed significant leishmanicidal activity in both promastigote and amastigote forms with low cytotoxicity, suggesting its potential as a candidate for new therapeutic agents against VL. However, further investigations are needed.

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Keywords: Visceral Leishmaniasis, *Leishmania infantum*, *Melaleuca leucadendra.*

NEUROINFLAMMATION AND BEHAVIORAL ALTERATIONS INDUCED BY VISCERAL LEISHMANIASIS IN A MURINE MODEL

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Visceral leishmaniasis (VL) is a severe zoonosis that, if untreated, can be fatal. While its general aspects are well-studied, its effects on the central nervous system (CNS) remain unclear. This study investigated neuroinflammation and behavioral changes in a murine model of *Leishmania* infantum infection. Female BALB/c mice (5–7 weeks old) were divided into two groups: non-infected (NI) and infected (LI-35). The LI-35 group received 5×10^7 *L. infantum* ITMAP-263 promastigotes and remained infected for 35 days. From days 27 to 35, all animals underwent behavioral tests (open field, elevated plus maze, Y-maze, and splash test) to assess anxiety, depression, and memory. After euthanasia, brain and serum samples were analyzed for parasite load, cytokine expression, enzymatic activity (NAG, MPO, EPO), histopathology, and fractalkine quantification by ELISA. Behavioral tests revealed significant differences between groups. The LI-35 group showed reduced distance traveled in the open field and elevated plus maze tests, indicating decreased exploratory behavior. In the Y-maze test, infected mice exhibited lower distance traveled and arm alternations, suggesting memory impairment. The splash test revealed reduced grooming behavior, consistent with depressive-like behavior. CNS analyses confirmed *L. infantum* presence in the brain and increased expression of pro-inflammatory cytokines, notably IL-33, a damage-associated alarmin, and TNF-α, a key pro-inflammatory cytokine. These findings suggest that *L. infantum* infection induces CNS inflammation, contributing to behavioral and cognitive alterations in infected individuals.

Supported by: FAPEMIG; CNPQ; CAPES.

Keywords: neuroinflammation; visceral leishmaniasis; behavior.

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IN VITRO CO-CULTURE MODEL OF *Trichomonas* vaginalis, *Candida* albicans AND *Lactobacillus crispatus*

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Trichomoniasis, caused by the protozoan *Trichomonas vaginalis,* is the most common non-viral sexually transmitted infection globally and increases the risk of HIV/AIDS transmission and acquisition. It often coexists with a dysbiotic vaginal environment characterized by reduced *Lactobacillus spp.,* particularly *L. crispatus. S*imilarly, C*andida albicans*, the most predominant vaginal yeast, disrupts the microbiota, causing vulvovaginal candidiasis, which frequently recurs. Both infections are increasingly challenged by treatment-resistant isolates, highlighting the urgent need for novel therapies. In this study, we aimed to standardize the co-culture of *T. vaginalis*, *C. albicans*, and *L. crispatus* to simulate the vaginal microenvironment, providing a platform for testing active compounds and developing new drugs. MRS liquid medium was used, with initial densities of ATCC or fresh clinical isolates of *T. vaginalis* (1.00 x 10^6 trophozoites/mL), *C. albicans* (3.33 x 10^4 CFU/mL), and *L. crispatus* (5.53 x 10^6 CFU/mL, LC2, co-culture with ATCC isolate; or 5.53 x 10^7 CFU/mL, LC1, co-culture with fresh isolate). After 24 hours, densities reached 2.00 x 10^6 trophozoites/mL (*T. vaginalis*), 2.6 x 10^6 CFU/mL (*C. albicans*), and 7.9 x 10^6 CFU/mL (LC2), and 8.3x10^6 CFU/mL (LC1) for *L. crispatus*. Higher *L. crispatus* densities reduced *T. vaginalis* and *C. albicans* viability. MIC for metronidazole against *T. vaginalis* isolates and MFC for fluconazole against *C. albicans* decreased in co-culture (twofold for ATCC isolate and fourfold for clinical isolate). Co-culture increased cytotoxicity of ATCC isolate, inhibited *C. albicans* biofilm formation (up to 92%), reduced its metabolic viability (up to 90%), and suppressed the yeast-to-hyphal transition (up to 70%). These findings suggest that this *in vitro* system is an effective tool for evaluating antimicrobial efficacy against pathogens causing vaginitis and for studying microorganism interactions.

Keywords: Antimicrobial activity; Co-culture; Vaginal microenvironment.

NUTRIENT STARVATION IMPACTS PURINERGIC SIGNALING AND PATHOGENICITY OF THE HUMAN PARASITE *Trichomonas vaginalis*

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Trichomonas vaginalis (Tvag) is an extracellular parasite and the causative agent of trichomoniasis, the most common non-viral sexually transmitted infection worldwide. The parasite's primary energy source derives from glucose metabolism, and Tvag is auxotrophic for essential metabolites such as purines and pyrimidines, relying on salvage pathways for nucleotide synthesis. Tvag is routinely cultivated in Diamond's medium, which contains high levels of carbohydrates and is supplemented with serum as a source of purines. The parasite encounters variable levels of glucose and purines in the infection site due to its virulence that leads to cellular lysis. Here, we aimed to investigate the effects of glucose and serum limitation on purinergic signaling and pathogenicity traits (cytotoxicity and extracellular vesicles) of Tvag. Both starvation conditions increased NTPDase and E-5′-N enzymatic activities in all tested Tvag isolates. Interestingly, this effect was reversed by adenosine but not by glucose. When evaluating the final step of the purinergic cascade, we observed that starvation reduced adenosine deamination in Tvag isolates. Serum limitation enhanced Tvag-induced cytotoxicity against vaginal epithelial cells, an effect that was reversed by adenosine alone or in combination with erythro-9-(2-hydroxy-3-nonyl)adenine, an adenosine deaminase inhibitor. Considering the importance of adenosine, we investigated the expression levels of nucleoside transporters (ENTs). Interestingly, serum limitation and glucose restriction caused downregulation and upregulation of ENT expression in Tvag, respectively. Finally, we demonstrated that starvation modulates extracellular vesicles release from trophozoites, suggesting a potential role for nutrient sensing in mediating cellular responses. Our findings enhance the understanding of critical survival pathways in Tvag and highlight nucleoside transporters as potential targets for therapeutic interventions.

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Keywords: *Trichomonas vaginalis*, Adenosine, Nucleoside Transporters

Evaluation of the trypanocidal activity of marine microalgae extracts

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Chagas disease, transmitted by *Trypanosoma cruzi*, is one of the Neglected Tropical Diseases (NTDs) classified as endemic in Latin America. Its treatment is limited to drugs that cause adverse effects and are not very effective in treating the chronic phase, which is associated with heart disease, gastrointestinal disorders, and other complications. In this regard, microalgae stand out as potential sources of new trypanocidal substances, as these organisms produce bioactive compounds with various pharmaceutical applications.

Given the evidence of leishmanicidal and trypanocidal activity in some microalgae species such as *Chlorella vulgaris*, this study aims to evaluate the trypanocidal activity of extracts from marine microalgae that have not been previously tested. For this research, the microalgae were obtained from the Microalgae Bank of the Bioprospecting and Biotechnology Laboratory (LABBIOTEC-UFBA) and cultivated in a properly sterile and illuminated environment to ensure their full development. Upon reaching the stationary phase, the wet biomass was collected by centrifugation, freeze-dried, and the extract was prepared by sonication with absolute ethanol, followed by separation of the supernatant and evaporation of its solvent.

To evaluate the trypanocidal activity of the extracts, the tested concentrations ranged from 250 µg/mL to 600 µg/mL, and a colorimetric cell viability assay of the epimastigote forms of *T. cruzi* (strain Y) was performed using MTT. The absorbance results were detected using a spectrophotometer and interpreted by plotting graphs that allowed visualization of the median inhibitory concentration (IC50) and the correlation coefficient. The extracts tested so far were from the following microalgae: Cm, Pt, Ig, Cc, Dsp, No, Pv, and their IC50 results were 31.64; 171.2; 367.7; 436.9; 445.0; 819.3; and 887.4 µg/mL, respectively.

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Keywords: Microalgae. Chagas disease. *Trypanosoma cruzi*

THE IL-33/ST2 AXIS IN *Trypanosoma cruzi* EXPERIMENTAL INFECTION: IMPLICATIONS FOR EARLY MYOCARDITIS AND BEHAVIORAL CHANGES

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Chagas disease (CD), caused by the protozoan *Trypanosoma cruzi*, leads to severe tissue dysfunction, with the host immune response playing a dual role in parasite control and tissue damage. Interleukin-33 (IL-33), an alarmin released upon cellular injury, signals through the ST2 receptor with protective or detrimental effects, but its role in *T. cruzi* infection remains understood. This study aimed to elucidate the impact of IL-33/ST2 signaling on CD pathogenesis. Female BALB/c wild-type (WT) and ST2 knockout ($ST2^{-/-}$) mice were intraperitoneally infected with 1000 *T. cruzi* Y strain trypomastigotes, and parasitological, immunological, and behavioral parameters were assessed. In the acute phase, despite similar parasitemia kinetics, WT infected mice exhibited severe clinical manifestations, including weight loss, reduced food intake and hypothermia. In contrast, $ST2^{-/-}$ infected mice showed an increased leukocyte count, mainly monocytes. Early myocarditis (7 dpi) was observed in $ST2^{-/-}$ mice, characterized by extensive inflammatory infiltrates, whereas WT mice displayed minimal or no cardiac involvement. By 20 dpi, $ST2^{-/-}$ mice developed cardiomyocyte degeneration and fibrosis. In the brain, both infected groups developed multifocal inflammatory foci. However, WT infected mice presented numerous amastigote nests in the cerebral cortex and cerebellum, whereas $ST2^{-/-}$ mice exhibited parasite nests only in the cortex. In the chronic phase (100 dpi), WT mice demonstrated persistent feeding suppression, clinical impairment, and behavioral dysfunction, including anxiety- and depressive-like behaviors. ST2-deficient mice, however, sustained normal feeding behavior and did not develop cognitive defects, suggesting a role of the IL-33/ST2 pathway in behavioural dysfunction during CD. These findings highlight the IL-33/ST2 axis as a critical regulator of immune-mediated pathology in *T. cruzi* infection, with distinct tissue-specific effects across acute and chronic disease phases.

Keywords: Chagas disease; Alarmin; Immunity

Financial support: CNPq; CAPES; FAPEMIG

LEISHMANICIDAL POTENTIAL DIARYLPENTANOIDS: *In vitro* EVALUATION AGAINST *Leishmania amazonensis* AND *Leishmania infantum*

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Leishmaniasis is a disease caused by protozoa of the genus Leishmania spp. Current treatments are limited due to significant side effects and the emergence of resistant strains. Curcumin has shown promising activity against these parasites in in vitro studies; however, its low bioavailability in biological systems compromises its in vivo efficacy. Consequently, the development of analogues has become a major focus of research. In this context, this study evaluated the in vitro leishmanicidal activity of diarylheptanoids (DH) and diarylpentanoids (DP) against Leishmania amazonensis and Leishmania infantum. Initial screening was conducted using 14 compounds against promastigote forms of L. amazonensis. The compounds that exhibited more than 60% lysis were further tested to determine the effective concentrations (EC50) against both promastigote and axenic amastigotes forms. Cytotoxicity was assessed by the XTT method in J774A murine macrophages and by hemolysis assays using defibrinated sheep blood. Among the compounds evaluated, DP6 showed potent leishmanicidal activity against promastigote forms of *L. amazonensis* and *L. infantum*, with EC50 values lower than 10 μM. Compound DP5 demonstrated efficacy against amastigote forms of both species, with EC50 values ranging from 5.01 to 38.43 μM. In addition, both compounds exhibited low cytotoxicity, with cell viability rates above 90% in concentrations up to 100 μM, and with no hemolytic effects in tested concentrations. These results indicate that diarylpentanoids are promising candidates for the in vivo studies against leishmaniasis, contributing to the advancement of more effective, safe and accessible therapeutic alternatives.

KEYWORDS: Diarylpetanoids; Diarylheptanoids; Leishmaniasis.

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ASSOCIATION BETWEEN TOXOPLASMOSIS AND DEPRESSION IN WOMEN IN THE GESTATIONAL AND POST-GESTATIONAL PERIOD

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Toxoplasmosis is a disease caused by the protozoan *Toxoplasma gondii*. Given that latent toxoplasmosis is associated with behavioral changes and neuropsychiatric disorders, the present study aimed to evaluate the relationship between *T. gondii* infection and depression before and after childbirth. To this end, a cohort study was conducted with women aged between 15 and 45 years. The Edinburgh Postnatal Depression Scale (EPDS) was used to assess depressive symptoms. A questionnaire containing 15 questions was used to identify the serological profile and epidemiological aspects related to toxoplasmosis, which covered personal data, economic and housing situation, and educational and health/disease aspects. Of the 78 volunteers analyzed in the first interview, 24 (30.8%) had positive serology for toxoplasmosis and 54 (69.2%) had negative serology, indicating a large percentage of women susceptible to infection by the protozoan. The result of the EPDS scale showed that 12.8% of the volunteers were predisposed to depression before delivery. It was observed that this percentage increased to 19.1% during the postpartum period, with an incidence of 33.3%. To verify the association between depression and infection by *T. gondii*, Pearson's chi-square test (X^2) was performed. In our study, an association was observed between the predisposition to develop postpartum depression and infection by the protozoan *T. gondii* (p=0.046), but this factor is not the only factor responsible for the disorder. An association was also observed between the use of licit drugs and the predisposition to depression during pregnancy (p=0.004). Therefore, further studies on mental disorders associated with the influence of pathogens are necessary to determine risk factors related to the development of depressive symptoms in pregnant women and in the puerperium.

Keywords: Toxoplasmosis, postpartum depression, risk factor.

STRUCTURAL ANALYSIS AND GENETIC EXPRESSION OF LIPIN AND KINASE OF LIPID DROPLETS OF *LEISHMANIA SPP*

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Leishmania parasites display organelles called lipid droplets (LD), which are known as factors of virulence. However, few enzymes responsible for LD biogenesis are known, and it is not yet determined whether there is a difference in genetic expression between the species of *Leishmania* and the structure of these proteins. This study involved an *in silico* analysis of lipin (LPN) and lipid droplet kinase (LDK) protein structures and gene expression, both those are related to the biogenesis of CLs in different species of *Leishmania*. For this, the gene sequences were compared *in silico*, such as alignment, phylogenetic analyses, construction of three-dimensional structures of the proteins encoded by these and structural alignment for identification of structural alterations of proteins. In addition, we performed the macromolecular docking of LDK protein. A comparative analysis of the expression of two genes identified was done by means of the RT-qPCR technique from mRNA samples previously extracted from axenic cultures of *L. amazonensis, L.braziliensis and L. infantum*. Thus, we confirmed that there is a statistically significant difference in the levels of genetic expression of *ldk* and *lpn* in *L. braziliensis* compared to *L. amazonensis* and *L. infantum*. Furthermore, we model the three-dimensional structures of LDK and LPN and the regions of LDK-LDK interaction, so as to be proposed for a model of LD biogenesis in *Leishmania*. The data suggest differences in the levels of genetic expression of these proteins possibly related to the LD formation by different *Leishmania* species, since the similar mutations were present in the *Leishmania* species with the same tissue tropism. These data bring new insights on the different species of the parasite that can be related to clinical forms of leishmaniasis and increase understanding of the biology of the parasite.

Supported by: CNPq.
Keywords: RT-qPCR, *Leishmaniasis*, Lipid droplets, Gene expression.

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INHIBITORY POTENTIAL OF ISOPRENOID BIOSYNTHESIS IN *TOXOPLASMA GONDII* BY LIMONENE: AN *IN SILICO* STUDY

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Abstract

Toxoplasmosis is a pathology caused by the apicomplexan *Toxoplasma gondii*, and in pregnant women or immunocompromised individuals, it can cause severe symptoms, including mental confusion, lack of coordination and seizures. Protein prenylation is a ubiquitous covalent post-translational modification found in all eukaryotic cells, comprising the attachment of a farnesyl or a geranylgeranyl isoprenoid. It is essential for the proper cellular activity of numerous proteins in apicomplexans. Considering the inhibition of prenylation already documented for *Plasmodium* sp. by the monoterpene limonene, the present study aimed to determine the inhibitory potential of this terpene also on the activity of the farnesyl/geranylgeranyl diphosphate synthase of *T. gondii* through modeling of this protein and subsequent docking procedures. The modeling of the protein of interest was performed in the *AlphaFold 2* software, a program for modeling protein structures based on artificial intelligence available in Colab. The entire amino acid sequence provided in the ToxoDB database (identifier: TGME49_224490) was used for structural prediction. After modeling, the model was refined in the YASARA server and used for simulations (in triplicate), with magnesium divalent cations (*MIB2*); and with small molecules in the SwissDock server (*Autodock Vina*): dimethylallyl diphosphate (DMAPP), DMAPP+isopentenyl diphosphate (IPP), limonene, and DMAPP+limonene. The conformations most similar to the 1rqi model and stable were subsequently analyzed. The 2D interaction diagram generated by the *Discovery Studio* software shows that the stability of the DMAPP+limonene model (-5.278 kcal/mol) is quite similar to that of DMAPP+IPP (-5.550 kcal/mol), which may indicate an inhibitory potential due to competition at this binding site. Future *in vitro* results should further clarify the mechanism associated with the effect of limonene on isoprenoid synthesis in *T. gondii*.

Keywords: Toxoplasmosis, Prenylation, Inhibition.

DETECTION OF WATERBORNE PROTOZOA IN RAW OR TREATED RECREATIONAL WATERS AND HELMINTHS IN SANDY ENVIRONMENTS, DURING PROFESSIONAL OR AMATEUR SPORT COMPETITIONS IN DIFFERENT STATES OF SOUTHERN BRAZIL

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A wide variety of sport modalities are practiced in water or sandy environments. The assessment of the sanitary quality of these places is not routinely performed outside the summer season. Indeed, the investigation of waterborne or soilborne parasites has never been carried out at the exact time of official sport competitions. The main goal of this study was to perform the detection of protozoa and helminths during professional and amateur water and land sport competitions in the states of Paraná and Santa Catarina, Brazil. Between November 2023 and December 2024, water (10L) and sand (1kg) samples were collected for parasitological analysis from different matrices: seawater - triathlon, crossing, surfing (harvested 10 minutes after the start of competition), dam (stand up paddle, swimming), water park (triathlon, pool volleyball) (n=29), and sand courts, from beach tennis championship or from beach soccer, at intervals (n=95). Water samples were concentrated by the membrane filtration technique. The sediments were purified by immunomagnetic separation (IMS) and visualization performed by direct immunofluorescence assay to search for *Cryptosporidium* and *Giardia*. The supernatant discarded from IMS was used for the detection of *Toxoplasma gondii*. DNA amplification by nested-PCR or qPCR was used for detecting protozoa, using specific molecular markers: *bg* (*Giardia*), *18S* (*Cryptosporidium*) and *REP-529* (*T. gondii*). Sand was homogenized with glycine or Triton 100 X, followed by sequential flotations in sucrose to isolate helminth eggs. *Giardia* and *Cryptosporidium* were detected in 41.3% of seawater or pools. *T. gondii* was isolated for the first time directly from seawater in 6.9% samples during water crossing and Surfing competitions. Sand samples (13.6%) harbored nematode larvae, *Ascaris* sp. and hookworms eggs. The results indicate the need to monitor robust stages of parasites in outdoor areas used for sport competitions to ensure the safety and health of athletes.

Supported by: UFPR, CAPES.

Keywords: Athletes; Intestinal parasites; *Toxoplasma gondii*.

ATYPICAL STRAIN OF TOXOPLASMA GONDII LEADS TO LUNG INFLAMMATION AND WORSENS PULMONARY PHYSIOLOGICAL CHANGES

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Toxoplasma gondii exhibits a genetically diverse population structure, particularly in Latin America, where atypical strains have been associated with more severe disease outcomes. Pulmonary complications have been reported in toxoplasmosis, yet the impact of different strains remains underexplored. This study aimed to evaluate the pulmonary effects of *T. gondii* infection in mice infected with atypical strain TgCkBrRN2 (CK2), isolated in Rio Grande do Norte/Brazil, and the clonal strain ME49. Mice were euthanized in acute (15 dpi) and chronic (60 dpi) phases to assess lung histopathology and pulmonary function via spirometry. During the acute phase, the CK2-infected group showed a more pronounced increase in pulmonary resistance compared to other groups, persisting up to 60 dpi. Both dynamic and static compliance were impaired throughout infection, with strain-specific differences notable in the acute phase, indicating CK2 induces more severe pulmonary physiological alterations. Inspiratory capacity findings were consistent with previous observations, and the Tiffeneau index suggested a restrictive disease pattern in chronic infected animals. Histopathological evaluation aligned with these observations, revealing CK2-infected animals exhibited more severe pathology with higher scores of airway, perivascular, and parenchymal inflammation in both acute and chronic phases compared to ME49 strain. These findings underscore how *T. gondii* infection disrupts pulmonary homeostasis and function, particularly with atypical strains inducing more severe inflammatory responses. Such disruptions may contribute to long-term respiratory impairments, emphasizing the need for further investigation into underlying mechanisms and potential clinical implications.

Cnpq, capes e fapemig.

T. gondii, *atypical strains, inflammation*.

ASSESSMENT OF THE LEISHMANICIDAL POTENTIAL OF TWO MICROALGAL EXTRACTS AGAINST THREE *LEISHMANIA* SPECIES.

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Leishmaniasis, a neglected tropical disease caused by protozoa of the genus *Leishmania*, manifests in three clinical forms: cutaneous, mucocutaneous, and visceral. Despite existing therapies, limitations such as adverse effects, invasive administration, treatment duration, and sometimes high costs highlight the need for new therapeutic alternatives. In this scenario, microalgae emerge as promising sources for biomolecule discovery due to their high biotechnological potential. Thus, this study evaluated the leishmanicidal potential of extracts from two microalgae species (IBLC015 and IBLC007) against the axenic promastigotes of *L. braziliensis*, *L. infantum*, and *L. amazonensis*. The microalgae were cultivated, centrifuged, and lyophilized. Their dry biomass was extracted with ethanol, followed by rotary evaporation and resuspension in DMSO. Leishmanicidal activity was assessed by direct counting in a Neubauer chamber, while the cytotoxicity of the extracts on macrophage cell lines (RAW) was determined using the alamarBlue™ cell viability indicator, both exposed to varying concentrations of the crude extracts. The IBLC015 extract exhibited IC_{50} values below 100 µg/ml for all tested species: 58.75 µg/ml against *L. amazonensis*, 54.5 µg/ml against *L. braziliensis*, and 76.62 µg/ml against *L. infantum*. In contrast, the IBLC007 extracts showed IC_{50} values of 368.6 µg/ml, 112.3 µg/ml, and 90.2 µg/ml, respectively, with only the last value below 100 µg/ml. Additionally, IBLC007 exhibited lower cytotoxicity against RAW cells (CC_{50} of 368.5 µg/ml), whereas IBLC015 showed a CC_{50} of 188.8 µg/ml. The highest selectivity index (SI = 4.08) was observed for IBLC007 against *L. infantum*, the species associated with the most severe form of the disease, followed by IBLC015 against *L. amazonensis* (SI = 3.46) and IBLC007 against the same species (SI = 3.44). Overall, all algal extracts exhibited antileishmanial activity, making them promising sources of biomolecules for future investigations.

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Keywords: Bioprospection; Leishmaniasis and Treatment.

Evaluation of the Trypanocidal Activity of Freshwater Microalgae Extracts

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Keywords: *Trypanosoma cruzi*, screening, microalgae

Abstract

Microalgae are a diverse biological group, including organisms such as cyanobacteria and protozoa, and are rich sources of bioactive compounds with potential pharmaceutical applications. Chagas disease, caused by the protozoan *Trypanosoma cruzi*, still lacks effective treatments, especially due to the scarcity of new drugs. This study investigated the potential of ethanolic extracts made from freshwater microalgae against *T. cruzi*, focusing on discovering new therapeutic alternatives.

Microalgae species (TO, AFA, KL, DB, AF, and CM) were cultured in Oligo medium, and the biomass was collected by centrifugation and lyophilized for storage. The ethanolic extracts were obtained by sonicating the biomass in absolute ethanol, followed by centrifugation to collect the supernatant and evaporation of the solvent. The extracts were dissolved in DMSO and evaluated for activity against the epimastigote forms of *T. cruzi* (Y strain) using the MTT cell viability assay.

The results showed that the extract from the TO species was the most effective, with an IC50 of 17.6 µg/mL, followed by AF (IC50 of 56.2 µg/mL) and KL (IC50 of 78.7 µg/mL). Despite a high IC50 (446.61 µg/mL), the DB extract induced up to 92.4% parasite mortality in the test with 550 µg/mL. The AFA and CM extracts also presented high IC50 values (259.6 µg/mL and 356.7 µg/mL, respectively) and lower efficacy at the highest concentrations tested.

These results highlight the potential of microalgae as a source of bioactive compounds for the development of new treatments against Chagas disease, emphasizing the importance of exploring this biodiversity for innovative therapies.

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LEISHMANICIDAL ACTIVITY OF ETHANOLIC EXTRACTS FROM IBLC105 AND IBLC017 MICROALGAE AGAINST *LEISHMANIA* SPECIES.

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Leishmaniasis is a neglected tropical disease whose etiologic agents are parasites of the genus *Leishmania*, which can manifest in cutaneous, visceral, and mucocutaneous forms. Currently used drugs have limitations regarding administration routes, high costs, and severe side effects. Therefore, there is a demand for new therapeutic options, and, due to their high productivity and biotechnological potential, microalgae are promising sources of new molecules with anti-*Leishmania* activity. The aim of this study was to evaluate the leishmanicidal activity of extracts of the microalgae IBLC105 and IBLC017 against Leishmania species. The microalgae IBLC105 and IBLC017 were cultivated in LC Oligo or Conway medium, to obtain ethanolic extracts, the cultures were centrifuged, lyophilized, extracted with absolute ethanol, and subjected torotary evaporation. The extracts were resuspended in DMSO. To evaluate the activity, promastigotes of *Leishmania amazonensis* (La), *L. brasiliensis* (Lb) and *L. infantum* (Li) were exposed to different concentrations of the extracts for 72h. The viability of the parasites was assessed by direct counting. The IC_{50} values obtained were 109.8 µg/mL (IBLC105) and 144.1 µg/mL (IBLC017) against *L. amazonensis*, 51.3 µg/mL (IBLC105) and 123.0 µg/mL (IBLC017) against *L. brasiliensis*, and 40.3 µg/mL (IBLC105) and 248.1 µg/mL (IBLC017) against *L. infantum*. Cytotoxicity tests with alamarBlue™ were carried out on RAW cells exposed to the extracts. The CC_{50} values were 311.5 µg/mL for IBLC105 and 248.1 µg/mL for IBLC017. The selectivity index (SI), calculated as IC_{50}/CC_{50} ratio, was greater than 4 for all *Leishmania* species. IBLC017 stood out with an SI of 4.02 against *L. amazonensis*, while IBLC105 showed the highest values, with an SI of 7.73 against *L. infantum* and 6.17 against *L. brasiliensis*. In conclusion, the tested extracts showed leishmanicidal activity with promising results and potential for future research.

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Keywords: leishmaniasis, bioprospecting, microalgae.

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The Impact of the *lpg2* Gene on *Leishmania amazonensis* Infection in Macrophages

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Abstract

INTRODUCTION: Leishmaniasis, caused by the protozoan *Leishmania*, is a significant global health health problem. In Brazil, *Leishmania amazonensis* is responsible for two clinical forms: Localized Cutaneous Leishmaniasis (LCL) and Diffuse Cutaneous Leishmaniasis (DCL). Macrophages play a crucial role in the immune response during *Leishmania* infection. Among the glycoconjugates on promastigote parasites surface are lipophosphoglycan (LPG), phosphoglycans (PG), proteophosphoglycans (PPG), and acid phosphatases (sAP). The *lpg2* gene encodes a GDP-mannose transporter, crucial for the synthesis of phosphoglycan (PG), a common domain for the surface molecules of these parasites. The research group used CRISPR/Cas9 to develop *Leishmania amazonensis* parasites deficient in *lpg2*. **OBJECTIVES**: This study aims to explore the inflammatory mechanisms triggered by the deletion of the *L. amazonensis lpg2* gene during the infection of murine macrophages. **METHODS**: Macrophages from C57BL/6 mice were cultured and infected with wildtype (*WT*) *L. amazonensis* promastigotes and *lpg2* knockout (*Δlpg2*) promastigotes. Infection ratios of 1:5 and 1:10 were used, and parasite load and viability were assessed. The culture supernatant was then analyzed using the Griess Reaction to detect nitric oxide (NO) and inflammatory mediators using ELISA. **RESULTS:** We observed a reduction in intracellular parasite load and viability of *Δlpg2* knockout parasites compared to *WT* parasites at both ratios tested. However, nitric oxide (NO) levels did not differ significantly between the two groups of parasites. Further assessment of cytokines, lipid mediators (PGE_2 and LTB_4) is necessary in this context. **CONCLUSION:** The study highlights the significance of PG-containing glycoconjugates as virulence factors in *L. amazonensis*. These initial findings hold promise for enhancing our understanding of inflammatory mechanisms during parasite-macrophage interactions in New World *Leishmania* species.

Keywords: Macrophage. *Leishmania amazonensis*. *lpg2*

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EVALUATION OF THE LEISHMANICIDAL POTENTIAL OF FOUR MICROALGAL ETHANOLIC EXTRACTS

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Leishmaniases are neglected tropical diseases caused by protozoa of the *Leishmania* genus, manifesting in cutaneous, mucocutaneous, and visceral forms. Current therapies present limitations, highlighting the need for novel therapeutic approaches. In this context, microalgae have emerged as promising sources of therapeutic biomolecules. This study evaluated the leishmanicidal potential of extracts from four microalgae species (IBLC 002, IBLC 103, IBLC 106, and IBLC 118) from the Microalgae Bank at Labbiotec, Federal University of Bahia, against three *Leishmania* species. The microalgae were cultivated in media Conway or LC Oligo, then centrifuged, lyophilized, and extracted using absolute ethanol. After rotary evaporation, the extracts were tested on *Leishmania* promastigotes cultured in Schneider and 199 supplemented media, and their leishmanicidal activity was evaluated by direct counting using a Neubauer chamber. The extracts demonstrated significant inhibitory activity. IC_{50}, CC_{50}, and selectivity index values were calculated, revealing differences in biological activity against axenic Leishmania promastigotes and variations in cytotoxicity in RAW cells. The IBLC 002 and IBLC 106 extracts showed IC_{50} values of 346.1 µg/mL and 82.02 µg/mL, respectively, against *L. infantum*; 161.9 µg/mL and 38.1 µg/mL against *L. braziliensis*; and 440.3 µg/mL and 181.9 µg/mL against *L. amazonensis*, with CC_{50} values of 537.8 µg/mL and 377.8 µg/mL for all tested extracts. On the other hand, IBLC 103 and IBLC 118 extracts showed greater selectivity, presenting IC_{50} values of 36.5 µg/mL and 118 µg/mL against *L. amazonensis,* 99.9 µg/mL and 43.6 µg/mL against *L. braziliensis*, and 18.7 µg/mL and 55.43 µg/mL against *L. infantum*, along with CC_{50} values of 755.1 µg/mL and 464.4 µg/mL, respectively. Thus, the results indicate that IBLC 103 and IBLC 118 exhibit the highest selectivity indices, highlighting them promising sources of bioactive molecules with leishmanicidal potential.

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Keywords: Biomolecules; Leishmaniasis and Treatment

TREATMENT OF *LEISHMANIA INFANTUM*-INFECTED HAMSTERS WITH A NEW IMMUNOCHEMOTHERAPY SCHEDULE: ANALYSIS OF INTRACELLULAR CYTOKINE PROFILE AND HISTOPATHOLOGY OF SPLEEN TISSUE

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Visceral leishmaniasis (VL) is a severe disease requiring new therapies due to current treatment limitations. Immunochemotherapy, which combines drugs and immunomodulators, emerges as a promising strategy to enhance therapeutic responses and reduce toxicity. Therefore, we aimed to evaluate the therapeutic efficacy of Chimera A adjuvanted with monophosphoryl lipid A (MPL) as immunotherapy or combined with Miltefosine as immunochemotherapy. Hamsters (*Mesocricetus auratus*) were infected with 5×10^7 stationary-phase *Leishmania infantum* promastigotes (MCAN/BR/2008/OP46) and received two treatment schedules. In the first approach, 60 days post-infection, the hamsters were treated with Miltefosine for 14 (M14) or 28 days (M28). In the second schedule, seven days after the chemotherapy treatment beginning, the animals submitted to immunotherapy (ChiA/MPL) and immunochemotherapy (ChiA/MPL +M14) received two series of five doses (ChiA/MPL) with 10-day intervals between series. Ninety days post-treatment, spleen samples were collected to assess intracellular cytokine profile (IFN-γ, TNF, and IL-10), parasite load, and histopathological analysis. Our study provides a robust evaluation of a specific compartmentalized immune response in the hamster model using multiparametric flow cytometry methodology. Chi A/MPL and ChiA/MPL+M14 treated hamsters showed increased IFN-γ and TNF-producing T cells and also reduced IL-10. Moreover, treated hamsters exhibited a remarkable reduction in the splenic parasite load. Considering the macroscopic structure of spleen tissue, we observed splenomegaly and thickening borders of the capsule of infected-nontreated hamsters. Treated hamsters presented a slight reduction in the follicle, suggesting efficient immune activation and contributing to parasite control. The results indicate that immunochemotherapy may be a promising alternative against VL while reinforcing the innovative potential of multiparametric flow cytometry in the hamster model.

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Keywords: Visceral Leishmaniasis, Immunochemotherapy, Syrian golden hamster

Molecular Analysis of the Immune Response and Parasite Control in Hamsters Immunized with Live-Attenuated *KHARON1*-Deficient *Leishmania infantum* as an Immunoprophylactic Strategy for Visceral Leishmaniasis

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Leishmanization, based on the inoculation of live parasites into the skin, was the only effective strategy against cutaneous leishmaniasis. With molecular advances, it has become possible to develop attenuated, non-pathogenic parasites capable of inducing protective immunity in a safe and long-lasting manner. The deletion of the *KHARON1* gene in *Leishmania infantum* resulted in an attenuated strain (LiKH) that can infect mice and trigger a robust immune response. In this study, we evaluated the immunogenicity and vaccine efficacy of LiKH in hamsters, a model more clinical representative of the disease. The animals were divided into groups that received one or two vaccine doses via subcutaneous or intradermal routes. They were analyzed one month after immunization and at one and eight months post-challenge with virulent *L. infantum*. Gene expression of immune cytokines (*TGF-β*, *TNF*, *IL-1*, *IFN-γ*, and *IL-10*) in the spleen was assessed by RT-PCR (Reverse Transcriptase Polymerase Chain Reaction), and parasite load in the spleen and liver was quantified by qPCR (Quantitative Polymerase Chain Reaction). Gene expression results indicate a strong immune stimulation in the subcutaneously vaccinated groups shortly after immunization. Eight months after the challenge, increased cytokine expression was observed in the intradermally vaccinated groups. Additionally, at both post-challenge time points (1 and 8 months), a significant reduction in hepatic parasite load was observed in animals that received two doses, regardless of the route. Those that received two doses via the subcutaneous route also exhibited a lower splenic parasite load compared to the control group. These findings suggest that immunization with LiKH is highly immunogenic, inducing a mixed Th1/Th2 response and promoting infection control, highlighting its potential as a promising immunoprophylactic strategy against visceral leishmaniasis.

Keywords: *Leishmania infantum*, leishmanization, KHARON1

Supported By: UFOP, Fiocruz Minas, CCA-UFOP, FINEP, FAPEMIG, Rede Imunobioleish-MG, CAPES, CNPq e INCT-DT.

INVESTIGATION OF THE RELATIONSHIP BETWEEN ADIPOSE TISSUE AND PARASITE BURDEN DURING LEISHMANIA INFANTUM INFECTION IN THE HAMSTER MODEL

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Leishmania infantum infection has been widely investigated in experimental models, mainly in hamsters, due to its high susceptibility to viscerotropic species and the ability to reproduce clinicopathological features of human (HVL) and canine visceral leishmaniasis (CVL). Although the effects on the main organs affected by the disease are well established, the involvement of adipose tissue (AT) is still poorly explored. Adipocytes can act as quiescent reservoirs for parasites, preventing repeated infectious cycles and providing a favorable energetic environment. Investigating the interaction between parasitism and AT may reveal new insights into the pathogenesis of the disease and its clinical manifestations. We focused on understanding the impact of infection on the white and brown AT in *L. infantum*-infected male and female Syrian golden hamsters (*Mesocricetus auratus*) during 90, 120, and 180 days. Our preliminary results have shown that infected male hamsters lost significant amounts of bodyweight, whereas there was no significant difference between the bodyweight of noninfected and infected female hamsters until 180 days. Our data suggest that weight loss is sex-dependent and may be related with changes in eating behavior. Hepatosplenomegaly was observed in male hamsters 90 days after infection. Meanwhile, infected female hamsters only presented hepatosplenomegaly from 120 days. Both males and females' infected hamsters showed kidney enhancement 120 days after infection. Infected male exhibited relative weight reduction in inguinal AT after 120 days and retroperitoneal, epididymal and brown AT after 180 days of infection. Similarly, infected female hamsters showed relative weight reduction in the white AT, retroperitoneal and periovarian, 180 days after infection. Literature is scarce about investigating the role of AT in the pathogenesis of VL. Hence, future investigations are needed to evaluate the maintenance of parasitism as well as the local immune response.

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Keywords: Visceral leishmaniasis; Syrian golden hamster; Adipose tissue.

SEROEPIDEMIOLOGICAL SURVEY AND PREVALENCE OF *TRYPANOSOMA CRUZI* INFECTION IN A COMMUNITY-BASED CARDIAC SCREENING INITIATIVE IN FEIRA DE SANTANA, BAHIA

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Chagas disease (CD) remains a significant public health concern in Latin America, contributing to high morbidity and mortality rates. In endemic regions such as Feira de Santana, Bahia, Brazil, Chagas-related mortality represents a substantial component of the local disease burden. This city was selected to participate in the *Every Heartbeat Matter* initiative, which integrates advanced diagnostic technologies, including telehealth-enabled electrocardiography and targeted echocardiographic screening, to improve the detection and management of structural heart disease in underserved populations.

The study enrolled 1,115 low-income individuals attending a health campaign for cardiological exams, of whom 78% (869) were female and 22% (246) male. Among them, 153 underwent blood collection for CD diagnosis, yielding a *T. cruzi* prevalence of 8.5% (13/153). The median age of seropositive individuals was 60 years (IQR: 39–69), with a female-to-male ratio of 5.5:1. Among *T. cruzi*-positive individuals, 10 (79.9%) were aware of their clinical status, while 3 (23.1%) were unaware of their infection.

Multivariate analysis identified household exposure to triatomine insects as the strongest predictor of *T. cruzi* seropositivity, with individuals reporting triatomine presence nearly nine times more likely to test positive (OR = 8.95, $p = 0.003$). No significant associations were observed for other variables, such as prior knowledge of the vector or the presence of a chicken coop, reinforcing the central role of direct vector exposure in transmission dynamics. Additionally, 70% of seropositive individuals were migrants from other endemic areas, highlighting the influence of population mobility on CD epidemiology.

These findings underscore the need to integrate advanced diagnostic tools and vector control strategies into community-based health programs to enhance early detection, reduce disease burden, and inform targeted public health interventions in endemic and underserved regions.

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Keywords: Chagas disease; screening; seroepidemiological survey

Reactivation of the Brazilian *Toxoplasma gondii* EGS Strain in an Immunosuppressed Murine Model.

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Toxoplasma gondii, an obligate intracellular parasite of the phylum Apicomplexa, is the etiological agent of toxoplasmosis. The clinical outcome of toxoplasmosis is associated with the parasite strains and the immunological status of the host. Strains of *T. gondii* isolated in Brazil exhibit significant genetic variability and deviate from the clinical patterns seen in other countries. The EGS strain, isolated in 1998 from the amniotic fluid of a patient in Minas Gerais/Brazil, presents a recombinant genotype (I/III). In the chronic phase of infection, tissue cysts form mainly in the central nervous system and muscle tissue. Immunosuppressed individuals, whether due to genetic factors, HIV infection, or immunosuppressive treatments are at risk of infection reactivation, which can culminate in fatal cases. Despite its clinical significance, the mechanisms underlying toxoplasmosis reactivation are poorly understood. Our study aims to establish *T. gondii* reactivation using a murine immunosuppression model infected with the EGS strain. C57BL/6 mice, 30 days post-infection, were treated with dexamethasone at 5 or 10 mg/kg. After 14 days, dexamethasone treatment led to a reduction in $CD4^+$ T, $CD8^+$ T, and B cells in the spleen, validating the immunosuppression status. Additionally, serological data indicated reactivation of the infection, with elevated IgM levels in the blood of treated animals. The treatment increased parasite load, as evidenced by higher numbers of tachyzoites in the ventral region assessed using the IVIS Lumina imaging system. The PCR analysis of the SAG-1 gene in the brain showed an increase in tachyzoites, particularly at the 5 mg/kg dose. These results demonstrate that DEX is capable of promoting immunosuppression in mice during infection with the EGS strain.

Keywords: EGS strain, immunosuppression, recrudescence.

SURVEY OF ANTIPARASITIC EVALUATION METHODS FOR *Trypanosoma cruzi*

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Chagas Disease, a Neglected Tropical Diseases group member, is caused by the trypanosomatids *Trypanosoma cruzi*. This disease is among the leading causes of morbidity and mortality worldwide. It is responsible for incapacitating and/or killing millions of people, representing an important medical need that remains unmet. The general objective of this project is to survey antiparasitic evaluation methods for the trypanosomatids *T. cruzi* for producing a review article and implementing laboratory techniques. In addition, it aims to assist in the implementation of an in vitro and in vivo testing platform for compounds and substances with antiparasitic activity, creating the possibility of generating data that will have a direct impact on the process of developing drugs for parasitosis, through the identification of new prototypes and/or drug candidates. Direct searches were performed in the scientific literature on antiparasitic evaluation with the parasite species, published between 2013 and 2022 in the PubMed database, using the term “anti-*Trypanosoma cruzi*”. A total of 203 articles were screened, of which 129 experimental studies were selected for detailed methods. The information collected included strain, parasitic form, interaction cell, and in vitro and in vivo evaluation methods. It was possible to observe that the analysis of parasite viability through methods such as Neubauer chamber counting, flow cytometry, and colorimetric techniques are still the most used in evaluations of trypanocidal activity. The Y and Tulahuen strains (including their variations) are the most used for experimentation with *T. cruzi*, varying between the three life stages of the parasite; the most used interaction cells continue to be macrophages and fibroblasts. It has been possible to observe that the simplest evaluation techniques are still the most used in the screening of compounds and substances to select promising compounds in the treatment of Chagas Disease.

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Keywords: Antiparasitic evaluation, literature review, *Trypanosoma cruzi.*

PANLEISH: A RT-qPCR PROTOCOL FOR DETECTION OF LEISHMANIA SPP IN BIOLOGICAL SAMPLES

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Leishmaniasis is a chronic progressive parasitic zooanthroponosis of high importance for public health, since it affects humans and domestic animals in its urban infection cycle. In addition, the disease can present lethal clinical manifestations, therefore deserving attention regarding prevention, diagnosis and monitoring. The diagnostic method for leishmaniasis may vary according to the clinical form and consequently the species of Leishmania responsible for the disease.The sensitivity and specificity of the tests may be low depending on the clinical form, with the gold standard for diagnosis being histopathological examination with direct identification of the parasite in biopsies of the lesion. In this study, we developed a unique RT-qPCR diagnostic test for the detection of *Leishmania spp* in the biological samples. For this, we identified consensus regions in the genomes of *Leishmania spp in silico* with a potential for designing primers for SYBR green-based RT-qPCR. DNA and RNA samples were obtained from the isolates from axenic culture of *L. infantum*, *L. braziliensis and L. amazonensis.* Futhermore, tissues of hamsters infected by *L. infantum* were used to validate the technique. We identified pairs of primers capable to detected 7 species of *Leishmania: L. donovani; L. major; L. amazonensis; L. mexicana; L. braziliensis e L. panamensis,* which are representatives of different clinical forms of the *Leishmaniasis*. In addition, our RT-qPCR test detected different levels of DNA/RNA from isolates of *Leishmania spp*. or the tissue samples of infected animals, but not in their blood. The test was specific for *Leishmania spp*, showing no nonspecific detection in *Trypanosoma cruzi* samples. Our data allow us *to state that we have established a unique molecular diagnostic method for different Leishmania* named here as “Pan-Leish”. However, further analyses are still needed to identify the sensitivity of the molecular test developed.

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Ethics committee: CEUA of Fiocruz (protocol of CEUA nº004, 2020).

Keywords: RT-qPCR, *Leishmaniasis*, Molecular diagnostic.

Effects of TcFLA-1BP and TcGP72 Deletion on the Infectivity and Survival of *Trypanosoma cruzi* in cell cultures

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Chagas disease, caused by the protozoan parasite Trypanosoma cruzi (Tc), is a neglected tropical disease with limited treatment options and no available vaccine. The parasite undergoes a complex life cycle with distinct morphological stages. Tc cell organization is supported by the cytoskeleton, where the flagellar attachment zone (FAZ) plays a central role in flagellum adhesion to the cell body, which is essential for parasite motility and host cell invasion. Despite the structural characterization of the FAZ in T. cruzi, its protein composition and dynamics throughout the biological cycle remain poorly understood. In this study, we characterized the role of the FAZ during T. cruzi infection in mammalian host cells using knockout parasites for FAZ-associated proteins (TcFLA-1BP$^{-/-}$ and TcGP72$^{-/-}$), generated via CRISPR/Cas9 editing. We observed that the absence of TcFLA-1BP led to heterogeneity in flagellar adhesion, with parasites displaying attached, partially detached, or completely detached flagella. In contrast, TcGP72$^{-/-}$ parasites exhibited a more homogeneous phenotype, with the flagellum consistently detached. Our findings demonstrate that TcFLA-1BP is crucial for efficient host cell infection in vitro, highlighting its essential role in parasite infectivity. Conversely, TcGP72 is nonessential for the infection process but significantly contributes to cytoskeletal remodeling during the parasite's life cycle. These results provide new insights into the distinct functional roles of FAZ proteins in T. cruzi. Furthermore, this study underscores the importance of TcGP72 in maintaining cellular architecture, reinforcing the relevance of FAZ proteins in parasite pathogenesis and structural integrity.

Supported by: FAPERJ and FINEP

Keywords: *T. cruzi,* flagellar attachment zone, infectivity

LEISHMANIA RNA VIRUS 1 AFFECTS PARASITE PROLIFERATION AND GENE EXPRESSION IN TEGUMENTARY LEISHMANIASIS PATIENTS

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Leishmania RNA Virus (LRV) has been associated with unfavorable clinical outcomes in infections with different *Leishmania* species. LRV1 is present in some species of the subgenus *Leishmania* (*Viannia*) and promotes an ineffective immune response in the infected host. Consequently, it leads to parasite persistence and is associated with the risk of developing mucosal leishmaniasis and cases of therapeutic failure when conventional treatment. We investigated the impact of LRV1 in samples from patients with tegumentary leishmaniasis (TL), its influence on the parasite load, and the expression of human genes of the immune response and *Leishmania* genes involved in the virulence of the parasite. We employed conventional PCR and qPCR to detect and quantify *Leishmania* and LRV1 loads, respectively, and used a large-scale qPCR assay (Fluidigm®) to analyze human and *Leishmania* gene expression in 96 clinical samples. Our main results highlight an association between LRV1 presence and samples with high parasite load ($p = 0.0016$). Gene expression analysis revealed that some human genes (IL21, IL12, NLRP3, and CASP1) and *Leishmania* genes (PRX and MAPK) were more expressed in samples from patients with low parasite load. The higher frequency of LRV1+ samples with a high parasite load suggests that the viral endosymbiont may promote increased parasite replication. When comparing LRV1+ and LRV1- samples, we identified distinct patterns of *Leishmania* gene expression: HSP60 and THIOL were more expressed in the LRV1+ group, while TRYR, GSH, PRX, and MAPK were overexpressed in the LRV1- group. Notably, the most expressed human genes are involved in critical signaling pathway of the immune response against infection, where the *Leishmania* genes appear to be linked to the mechanisms of resistance to host cell oxidative stress, potentially aiding the parasite in evading the immune defenses.

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Keywords: *Leishmania RNA Virus* 1; *Leishmania*; Gene expression; Parasite burden; Virulence factors.

Feline Visceral Leishmaniasis: Evaluation of a heterologous Protein-Based ELISA for Diagnosis

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Visceral leishmaniasis, caused by the protozoan *L. infantum*, is a zoonotic disease in which canines serve as the primary reservoirs. Consequently, most preventive and therapeutic measures target canine populations to reduce human infection risks. However, emerging evidence suggests that felines may also act as reservoirs, contributing to the persistence of the parasite in endemic regions. Despite this concern, feline leishmaniasis (FeL) remains underdiagnosed, underscoring the need for enhanced surveillance, prevention, and diagnostic approaches. Developing sensitive and specific diagnostic tests is crucial for improving FeL detection and refining control strategies. This study evaluated the performance of an enzyme-linked immunosorbent assay (ELISA) based on a heterologous protein (Protein D) for diagnosing FeL. The protein used in this study was obtained through bioinformatics analyses, with the sequence optimized by *in silico* tools. The epitopes composing the protein are derived from *L. infantum*. The sequence was inserted into a plasmid, which was produced by a commercial company. This plasmid containing the sequence was inserted into the *E. coli* Shuffle strain, responsible for protein expression. Serum samples from 16 cats in a community shelter in Aracaju-SE, Brazil, were analyzed. Results were compared with quantitative polymerase chain reaction (qPCR) and ELISA using crude *L. infantum* extract. The ELISA-Protein D assay successfully identified 100% (13/13) of positive cases detected by qPCR and crude extract ELISA. In the negative samples, its performance was similar to the crude extract ELISA, detecting 66.7% (2/3) of the negative cases. These findings suggest that the ELISA-Protein D assay has high sensitivity and potential as a valuable tool for FeL screening. However, additional studies involving a larger and more geographically diverse sample are needed to refine the assay's specificity, minimize false positives, and improve overall diagnostic accuracy.

Supported by CAPES, CNPq, FAPEMIG

Keywords: Visceral Leishmaniasis, Feline Diagnosis, Recombinant proteins

IMMUNOGENICITY OF PvVir14-DERIVED PEPTIDES TO IMPROVE THE SEROLOGICAL DIAGNOSIS OF *Plasmodium vivax* INFECTION

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Malaria, caused by *Plasmodium* parasites, is a major public health issue, with *P. vivax* responsible for 90% of cases outside Africa. The differential diagnosis is crucial for the correct treatment. The Vir superfamily proteins of *P. vivax* relate to virulence and PvVIR14 circulates during the infection. This study assessed the reactivity of peptides derived from PvVIR14 and their potential as targets for a diagnosis test. We analyzed serum of 135 individuals from an endemic area of *P. vivax* malaria, and 153 from a non-endemic area with an outbreak of *P. vivax* malaria, categorized as infected and exposed. ELISA assays were performed to determine the presence of specific IgG and IgM against the protein and its derived peptides and a depletion ELISA identified the immunodominant peptides. In the endemic area, IgM reactivity to PvVir14, L06, H08, and G08 was observed in 27.3%, 22.2%, 10.2%, and 14.5% of *P. vivax*-infected individuals, respectively, while IgG reactivity was 72.2%, 80%, 68.1%, and 58.6%, respectively. IgM reactivity for PvVir14 was 33.3% for the exposed individuals and 29.3% for those who had two or more cases of malaria. Among individuals experiencing their first episode of malaria, the frequency of IgM reactivity was higher for the peptides L06 and G08. The frequency of specific IgG against PvVir14 and the three peptides was higher in individuals with the first or multiple cases of malaria. In the non-endemic area the highest IgM reactivity was found for the peptides H08 and G08 and IgG anti-PvVIR14 was detected in 20,3% of the individuals. The three peptides showed sensitivity and specificity above 80% for infected individuals against healthy controls and PvVIR14 was better in differentiation of

infected and exposed individuals. Moreover, the peptides exhibited immunodominance over the protein. These results represent an important perspective for developing new tools for the differential diagnosis of *P. vivax* malaria.

Supported by: FAPEMIG, CNPq

Keywords: *Plasmodium vivax*, PvVir-14, diagnosis

AMEBICIDAL EFFECTS OF ADAMANTANE-AZOLE GOLD(I) COMPLEXES: A STUDY OF CELL DEATH PATHWAY IN *Acanthamoeba castellanii* AND TOXICITY PROFILE

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Acanthamoeba spp. are protozoa that belong to the group of free-living amoebae, responsible for *Acanthamoeba* keratitis (AK), an ocular infection commonly associated with contact lens users, and granulomatous amoebic encephalitis (GAE), a brain infection with a high mortality rate. The lack of selective and effective treatments highlights the need for new molecules with biological activity against *Acanthamoeba.* Studies have demonstrated that gold complexes are capable of inhibiting selenoenzymes such as thioredoxin reductase, which are involved in the redox balance of *Acanthamoeba*. Therefore, this study aims to evaluate the activity of adamantane-azole gold(I) complexes (C1-C4) against *Acanthamoeba castellanii* (ATCC 50492) T4 genotype. It investigates the cell death pathway through changes in the cell cycle, mitochondrial activity and phosphatidylserine (PS) exposure, cytotoxicity against rabbit corneal cells (SIRC ATCC CCL-60), irritation potential on chorioallantoic membrane (HET-CAM), and in vivo toxicity in *Tenebrio molito*r larvae. Thus, the compounds C2 and C4 demonstrated high amoebicidal activity with 50% inhibitory concentration (IC_{50}) values of 0.12 µM and 6.23 µM, respectively. C2, C3 and C4 caused changes in the cell cycle and induced PS exposure, while C4 also induced mitochondrial depolarization. In the cytotoxicity assay, C3 was the least toxic complex, with a 50% cytotoxic concentration (CC_{50}) of 32.3 µM. C2 and C4 maintained over 70% cell viability at concentrations ≤ 25 µM. None of the compounds showed irritability on the chorioallantoic membrane of the hen's egg (SI<5) at 200 µM. Moreover, after 48 h of exposure at 200 µM, all gold complexes maintained a high larval survival rate (60%). In conclusion, the gold(I) complexes showed excellent results against *A. castellanii,* likely through apoptosis induction, and demonstrated low or absent toxicity in the tested models. These findings support the potential development of a new treatment for AK and GAE.

Supported by: CNPq and FAPESC

Keywords: *Acanthamoeba spp.,* gold(I) metal complexes; amebicidal activity;

Serological and Epidemiological profile of patients with megaesophagus followed up in a reference center in Bahia

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Chagas disease (CD), caused by Trypanosoma cruzi (T. cruzi), remains a significant public health challenge in Latin America, affecting over 70 million people. In Brazil, around 300,000 individuals present with chagasic megaesophagus and/or megacolon. Identifying T. cruzi infection in individuals with digestive manifestations is crucial for appropriate treatment and improving living conditions. This study aims to characterize individuals with megaesophagus, assess their risk history for T. cruzi vector transmission, and determine the seroprevalence of anti-T. cruzi antibodies.

This cross-sectional, descriptive study was conducted in a Gastroenterology Ambulatory Care at a Referral Hospital in Bahia, Brazil, using a convenience sample. Data collection included a pretested questionnaire and blood sampling for immunological analysis. Among 358 individuals diagnosed with megaesophagus, the majority were female (61.2%), of Black or mixed racial background (90.2%), and had incomplete primary education (50.7%). Despite exposure history, 94.5% recognized the triatomine bug, 87.3% had lived near forested areas, 72.3% had resided in mud houses, and 51.1% reported a family history of CD, primarily in siblings (64.3%). The seroprevalence of anti-T. cruzi antibodies was 61.9%.

The socioeconomic characteristics of the study population suggest conditions of poverty, reinforced by their residence in forested regions and substandard housing. The high prevalence of infected siblings strongly suggests vector-borne transmission through triatomine exposure. These findings underly the urgent need for

improved housing conditions, enhanced healthcare access, and strengthened public health policies to prevent and mitigate CD transmission. Addressing these determinants could significantly reduce the epidemiological and social burden of CD, ultimately improving patient outcomes and contributing to disease control efforts.

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Keywords: Megaesophagus. Chagas disease. *T.cruzi*

THE IL-33/ST2 AXIS MODULATES LIVER PATHOLOGY AND INFLAMMATION DURING *Trypanosoma cruzi* EXPERIMENTAL INFECTION

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Chagas disease (CD) is a neglected tropical disease that affects millions of people worldwide. The etiologic agent, *Trypanosoma cruzi*, leads to impairment of multiple organs, including the liver. The pathology of CD primarily results from the host immune response against the parasite. Recent studies indicate that interleukin-33 (IL-33) plays a key role in various inflammatory diseases. IL-33 is rapidly released into the extracellular space upon tissue injury or necrosis and exerts its biological effects by binding to its receptor, ST2. Whether IL-33 has protective or detrimental effects depends on the immune mechanisms underlying each disease. To investigate the impact of IL-33/ST2 signaling, female BALB/c wild-type (WT) and ST2 knockout ($ST2^{-/-}$) mice were intraperitoneally infected with 1,000 blood trypomastigotes of the *T. cruzi* Y strain. During the acute phase, WT-infected mice developed anemia, characterized by reduced hemoglobin, hematocrit, and erythrocyte counts, whereas $ST2^{-/-}$ infected mice exhibited leukocytosis, primarily driven by monocytes, along with thrombocytosis and increased macroplatelet production. $ST2^{-/-}$ mice also showed heightened hepatic macrophage activity, accompanied by elevated ALT and albumin levels, indicating liver damage. Histological analysis confirmed exacerbated hepatic inflammation, with increased inflammatory foci and hemorrhagic areas in $ST2^{-/-}$ infected mice. In the chronic phase, $ST2^{-/-}$ infected mice exhibited persistent hepatic inflammation, with increased eosinophil and macrophage activity. ALT and albumin levels remained elevated in $ST2^{-/-}$ mice, despite no significant differences in AST levels among infected groups, suggesting sustained liver dysfunction. Collectively, our findings indicate that the IL-33/ST2 axis plays a role in regulating chronic liver damage and inflammation. Understanding the role of the IL33/ST2 axis may pave the way for new therapeutic strategies to mitigate the damage during *T. cruzi* infection.

Keywords: Chagas disease; Hepatic Immune Response; IL-33/ST2 pathway

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FROM RESISTANCE TO RESURGENCE: HOW *LEISHMANIA INFANTUM* RECLAIMS ITS FITNESS AND METABOLIC EDGE AFTER ANTIMONY WITHDRAWAL

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Leishmaniasis, caused by the protozoan parasite *Leishmania*, continues to pose a major public health problem worldwide. Phenotypic shifts in this parasite related to genome plasticity contribute to resistance and complicate treatment. This study investigates the fitness cost of antimony resistance in *Leishmania infantum*. Ten SbIII-resistant clones were cultured under SbIII-free conditions for 18 passages, with most regaining sensitivity as indicated by their IC_{50} values. This resensitization was linked to decreased amplification of the *mrpA* gene, a key factor in resistance. Drug assays revealed the emergence of miltefosine resistance but not amphotericin B resistance after Sb^{III} removal. Resensitized clones exhibited significantly higher growth rates than resistant clones after eight drug-free passages, suggesting a fitness cost of resistance. Oxygen consumption assays showed increased O_2 consumption between passages P3 and P8, followed by a decrease after P8, indicating metabolic adaptations. Infection assays with bone marrow-derived macrophages showed reduced infectivity as the clones regained sensitivity, potentially linked to increased metacyclic differentiation, suggesting a trade-off between drug resistance and infectivity. Additionally, a significant decrease in MRP-A protein abundance was observed, with increases in AQP1, tryparedoxin, HSP70, PCNA, and tyrosine phosphatase at different time points. These changes indicate dynamic reprogramming of protein expression with the reversal of resistance. Our findings suggest that antimony resistance in *L. infantum* imposes a fitness cost, driven by genomic and proteomic adaptations that influence metabolism and infectivity. A comprehensive understanding of these mechanisms is essential for the development of effective strategies to manage drug-resistant *Leishmania* populations and improve relapse therapies.

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Keywords: *Leishmania*, drug-resistant, resensitization

ENZYMATIC ACTIVITY OF TRYPANOTHIONE REDUCTASE IN PROMASTIGOTES AND AXENIC AMASTIGOTES OF *Leishmania (Viannia) braziliensis*

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American tegumentary leishmaniasis is a neglected disease caused by *Leishmania* parasites. The enzyme trypanothione reductase (TR) is essential for the redox balance of these organisms, making it a strategic target for innovative therapies and studies on antimonial resistance, a critical phenomenon that may negatively impact the current first choice treatment for such intentions. This study aimed to explore TR enzymatic activity in promastigotes and axenic amastigotes of 10 *L. (V.) braziliensis* isolates, classified as responders and non-responders to meglumine antimoniate treatment. The parasites were maintained *in vitro* as promastigotes and induced to undergo amastigogenesis by altering pH (7.2 to 5.5) and temperature (26°C to 32°C). Parasite samples were treated with lysis buffer for total protein extraction, and protein concentration was measured using the Qubit™ Protein Assay Kit. TR activity was assessed in the presence of NADPH, $T[S]_2$, and DTNB by spectrophotometry (412 nm, 35 min, 27°C). Results showed a time-dependent effect on TR activity stabilization. Kinetic curves reached stability between 14 and 23 minutes for promastigote proteins and between 16 and 25 minutes for axenic amastigote proteins, defining a 15-minute reaction interval for subsequent analyses. The lowest activity was observed in Isolate 6, which then was used as a reference sample for normalizing results. An additional analysis compared isolates as groups based on their treatment response phenotype. While isolates showed statistically significant differences when compared individually, no significant difference was found when comparing groups based on the resistance phenotype. These results suggest that variation in enzymatic activity may reflect differences in TR expression, with potential implications for the parasite's ability to cope with oxidative stress and adapt to the host environment.

Keywords: *Leishmania (Viannia) braziliensis*, trypanothione system, trypanothione reductase, enzymatic activity, antimony

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THE EFFECT OF *TOXOPLASMA GONDII* STRAIN VARIABILITY ON PULMONARY IMMUNOPATHOLOGY

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Toxoplasma gondii exhibits a genetically diverse population structure, particularly in Latin America. In Brazil, atypical strains have been identified, characterized by key virulence factor polymorphisms. Most immunological studies focus on archetypal strains, leaving atypical effects unexplored. This study aimed to compare the impact of infection with different *T. gondii* strains in mice. Male BALB/c mice were infected with either the atypical strain TgCkBrRN2 (CK2), isolated in Rio Grande do Norte, Brazil, or the archetypal strain ME49. Throughout the infection, animals were monitored for mortality and morbidity. Blood samples were collected for cytokine quantification, and bronchoalveolar lavage (BAL) fluid was analyzed using multiparametric flow cytometry. Additionally, BAL supernatant was used for surfactant protein quantification via ELISA. Infection with the atypical strain CK2 induced a more inflammatory profile, characterized by elevated IL-12 and TNF levels and reduced systemic IL-10 compared to infection with the clonal ME49 strain. During the acute phase, alveolar macrophages underwent apoptosis, accompanied by pronounced inflammatory infiltration. Dimensionality reduction and machine learning analyses revealed a lack of alveolar macrophage repopulation in the chronic phase, a phenomenon more pronounced in CK2-infected mice. Functionally, infected mice exhibited surfactant accumulation in the lungs, with more severe alterations observed in those infected with the atypical strain CK2. Our findings demonstrate that strain-specific differences can significantly influence disease outcomes. The exacerbated inflammatory response and persistent lung dysfunction observed in CK2-infected mice suggest that atypical strains may contribute to more severe respiratory complications in toxoplasmosis. Understanding these strain-specific effects is crucial for refining disease models and developing targeted therapeutic strategies.

Supported By: CNPq, FAPEMIG

Keywords: *Toxoplasma gondii*; Atypical strains; Inflammatory response

VALIDATION AND APPLICATION OF BIOANALYTICAL METHOD FOR TISSUE QUANTIFICATION OF BENZNIDAZOLE

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Chagas disease is caused by the protozoan *Trypanosoma cruzi*. Benznidazole (BNZ) is the only drug available for its treatment in Brazil, and with low therapeutic efficacy, especially in the chronic phase of the disease. The variability of the therapeutic response in the different phases of the disease may be related to the unfavorable pharmacokinetic properties of this compound, and studies to quantify BNZ in biological samples are essential to better understand this correlation. This study aimed to validate an analytical method for quantifying BNZ according to ANVISA recommendations for bioanalytical methods, applicable in the study of biodistribution of Chagas disease. The specific objectives were: (i) to evaluate the parameters linearity, residual effect, matrix effect, selectivity and quantification limit of the method; (ii) to perform a stability study after freezing/thawing cycles, short/long term under refrigeration and in the freezer. (iii) To apply the validated methodology to quantify benznidazole in heart and colon samples from dogs infected with the Berenice-78 strain of *T. cruzi* during the chronic phase of infection. The method developed was linear in the concentration range of 0.1 to 100.0 µg/mL for the colon and heart, and presented sensitivity with precise and accurate results. In addition, the stability of BNZ was verified under different handling conditions required by the method. Tissue concentrations of BNZ in the heart ranged from 4.73 µg/g to 6.42 µg/g; in the colon, the concentrations found were lower, with values of 0.18 to 0.65 µg/g. Studies involving the heart and colon are essential to understanding Chagas disease, since *T. cruzi* directly affects these organs, leading to serious complications such as chronic Chagas cardiomyopathy and megacolon, respectively. Such changes may interfere with the pharmacokinetics and biodistribution, not only of BNZ, but of other drugs used concomitantly in the pharmacotherapy of Chagas disease.

Keywords: chagas disease; biodistribution; bioanalythical method.

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ANTI-*TRYPANOSOMA CRUZI* ACTIVITY OF THE MARINE MICROBIAL ALKALOID (R)-SALSOLINOL: CRUZIPAIN AS A POTENTIAL TARGET

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Chagas disease is a potentially fatal disease with only one available drug in Brazil. Over the past 50 years, the marine environment has provided more than 20,000 inspiring small molecules for drug discovery studies, with a myriad of biological activities including parasites. In this work, we aimed to isolate, characterize and evaluate the anti-*Trypanosoma cruzi* activity of secondary metabolites from the marine bacteria *Bacillus altitudinis*. The microbial secondary metabolites were fractionated by HPLC and the chemical elucidation of compounds was performed by Nuclear Magnetic Resonance (^{1}H and ^{13}C NMR) and HRLC/MS. The evaluation of the EC_{50} was determined in trypomastigotes and intracellular amastigotes and the mammalian cytotoxicity was carried out in human-derived monocytes (THP-1). *In silico* studies were performed for drug-like properties and protein-ligand interactions to assess the binding stability and molecular recognition of the active compound within cruzipain. The active compound was characterized as the alkaloid (R)-salsolinol (MW 260 Da), which showed activity against *T. cruzi* trypomastigotes and intracellular amastigotes, with EC_{50} values of 78 µM and 110 µM, respectively, without toxicity to THP-1 cells (CC_{50} >200 µM). The *in silico* pharmacokinetic predictions indicated a high gastrointestinal absorption and low blood-brain barrier permeability. Additionally, (R)-salsolinol showed no inhibitor characteristics for the five main P-450 cytochrome isoforms and it was approved by four pharma filters as an oral candidate. The interaction profile with cruzipain revealed that the key residues involved in binding were CYS25, TRP26, GLY66, LEU67, MET68, ASN69, VAL132, ALA133, VAL134, GLN156, LEU157, ASP158, HIS159, GLY160, GLU205, and SER207 as primary contributors. Targeting this enzyme with novel small-molecule inhibitors could offer a viable strategy for therapeutic intervention.

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Keywords: Trypanosoma cruzi, therapy, natural products

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THE ROLE OF M1 AND M2 MACROPHAGES IN THE CONTROL OF AMERICAN CUTANEOUS LEISHMANIASIS

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Abstract

INTRODUCTION: The main causal agent of cutaneous leishmaniasis (CL) in Brazil is *L. braziliensis*. Macrophages (Mϕ) play a dual role in this infection and are classified into M1 and M2 types. M1 Mϕ can kill *Leishmania*, while M2 Mϕ are more permissive to infection and facilitate proliferation. AIMS: 1. To determine the frequency of M1 and M2 Mϕ in the peripheral blood of CL patients. 2. To assesss whether *L. braziliensis* predominantly infects M2 Mϕ derived from peripheral blood monocytes of CL patients. 3. To evaluate whether the frequency of M2 Mϕ from peripheral blood monocytes in CL patients is related to disease severity and treatment failure. METHODS: Monocytes from patients with CL (N=20) were obtained from peripheral blood mononuclear cells and incubated to develop into macrophages. The Mϕ were infected with *L. braziliensis* and characterized using surface markers: $CD86^{hi}$ and TNF for M1 Mϕ and $CD206^{hi}$ and IL-10 for M2 Mϕ, using confocal microscopy. We also evaluated the presence of parasites in M1 and M2 Mϕ. To correlate the frequency of these cells with disease severity and response to treatment, we evaluated whether the frequency of M2 Mϕ was related to ulcer size and failure to treatment with antimonial and disease duration. RESULTS: In patients with CL, M1 Mϕ predominate (70%) in the peripheral blood compared to M2 Mϕ (30%). M2 Mϕ show a significantly higher infection rate than M1 Mϕ ($p<0.005$). Additionally, healing time in patients with a predominance of M2 Mϕ was longer (115 days vs. 77 days) than in those with a predominance of M1 Mϕ, though the difference was not statistically significant. CONCLUSION: The study of M1 and M2 Mϕ in the peripheral blood of CL patients reflects findings observed in dermal Mϕ. M1 Mϕ are more frequent, while M2 Mϕ are more permissive to infection, and these differences may influence the response to CL treatment.

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Keywords: Cutaneous Leishmaniasis; *Leishmania braziliensis*; M1 and M2 macrophage

ABSTRACT – PARASITO 2025

THERAPEUTIC POTENTIAL OF EXTRACELLULAR VESICLES FROM MESENCHYMAL STEM CELLS IN CUTANEOUS LEISHMANIASIS

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Cutaneous leishmaniasis (CL), caused by *Leishmania braziliensis*, is characterized by chronic ulcers and intense inflammation. Current treatments have limitations, including severe side effects and increasing parasite resistance. Extracellular vesicles derived from human umbilical cord mesenchymal stem cells (HuMSCs-EVs) offer immunomodulatory and regenerative potential, raising the possibility of using them in a combination treatment with Meglumine Antominate, the standard chemotherapy. We propose that HuMSC-EVs can regulate the overt immune response associated with CL pathogenesis and facilitate wound healing. To this end, we investigated the effects of exposure of L. braziliensis-infected macrophages to HuMSCs-EVs and their wound healing potential. BMDM infected with *L. braziliensis* and exposed to HuMSCs-EVs showed reduced infection rates, decreased parasite load (39% *vs* 23%), and a shift toward the anti-inflammatory M2 phenotype. *In vitro* cytokine analysis revealed increased levels of TNF-α, IL-10 and IL-1β, highlighting the immunomodulatory role of EVs. In keratinocyte wound-healing assays, HuMSCs-EV accelerated closure rates and promoted cell migration at different time points. Employing a pre-clinical model of CL, BALB/c mice infected with *L. braziliensis* and treated with HuMSCs-EV (applied intravenously) displayed smaller lesions without significant changes in parasite burden. These results indicate that HuMSC-EV promotes immune regulation and tissue repair, presenting a novel therapeutic approach for CL.

Supported by: INOVA Fiocruz

Key Words: Cutaneous leishmaniasis, Extracellular vesicles, Immunomodulatory

EVALUATION OF THE PROTECTIVE CAPACITY OF KHARON-DEFICIENT *Leishmania mexicana* AGAINST CHALLENGE WITH *Leishmania braziliensis*

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Abstract

Leishmaniasis is still a serious public health problem worldwide. There are currently no safe and effective vaccines available for humans, and disease control depends exclusively on chemotherapy, which, in addition to being toxic, has high rates of therapeutic failure. Thus, the search for vaccine development remains urgent. It is known that developing protective immunity in patients cured of leishmaniasis is possible, which shows the importance of an active infection in inducing protective immunity against *Leishmania*. The use of live attenuated parasites of the genus *Leishmania* as vaccine candidates has been resumed and widely studied, as they mimic natural infections without causing the disease, allowing interaction with essential antigens in the development of immunity. Previous studies have shown that deletion of the kharon1 gene results in defects in the development of the amastigote form, leading to intracellular death and rendering the parasites incapable of sustaining the infection. We previously showed that *L. infantum* deficient in Kharon (Δ*Likh1*) induces protection against a challenge with virulent *L. infantum*. In this work, we evaluated the ability of a Kharon-deficient *L. mexicana* strain (Δ*Lmexkh1*) to induce cross-protection against *L. braziliensis*, Brazil's leading etiological agent of Cutaneous Leishmaniasis. Our results show that Δ*Lmexkh1* cannot survive in infected macrophages, although promastigote forms showed normal growth. We will evaluate the effect of immunization with Δ*Lmexkh1* against a challenge with *L. braziliensis* in a preclinical infection model.

Keywords: live attenuated *Leishmania* vaccine, leishmanization, kharon

HISTOPATHOLOGICAL EVALUATION OF THE LIVER IN HAMSTERS IMMUNIZED WITH GENETICALLY ATTENUATED *Leishmania infantum* DEFICIENT IN KHARON1 AS A PROPHYLACTIC STRATEGY AGAINST VISCERAL LEISHMANIASIS

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Visceral Leishmaniasis (VL) is one of the most concerning neglected diseases worldwide, with the number of cases continuously increasing. This rise is linked to parasitic resistance to drugs, failures in vector control, co-infections, and the lack of effective vaccines. Despite scientific efforts, there are still no licensed vaccines for humans, and those available for dogs have limited efficacy. Therefore, new prophylactic strategies are essential. Among the most promising approaches is the use of live genetically attenuated parasites, such as *Leishmania infantum* KHARON1 (*Li*KH), which promotes a broader antigenic spectrum and stimulates a protective immune response. The present study evaluated histopathological alterations in the liver of hamsters (*Mesocricetus auratus*) immunized with *Li*KH. The animals received one or two doses of the vaccine via subcutaneous or intradermal administration and were analyzed one and eight months after the challenge with virulent *L. infantum* promastigotes. The results indicated significant differences in body and organ weights, which were more evident in animals that received two doses, regardless of the administration route. Histopathological analyses revealed a more intense inflammatory process in the liver of animals evaluated eight months after the challenge, suggesting an active long-term immune response. These findings reinforce the potential of *Li*KH immunization as a promising approach against VL. Future studies may further explore these findings, consolidating the hamster model and driving the development of live attenuated parasite-based vaccines for dogs.

Keywords*: Leishmania infantum*, hamster, KHARON1

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AVALIAÇÃO DO TRATAMENTO NA REATIVIDADE SOROLOGICA POR IMUNOCROMATOGRAFIA PARA IGG ANTI *T.CRUZI* EM PACINTES PORTADORES DE CHAGAS TRATADOS COM BENZONIDAZOL

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Abstract

Introduction: Chagas disease (CD), caused by the protozoan *Trypanosoma cruzi*, is classified as a neglected tropical infectious disease. The primary treatment available is Benznidazole, a drug provided by the Brazilian Unified Health System (SUS). However, treatment adherence remains suboptimal due to the high prevalence of adverse effects, which negatively impact patient adherence and follow-up. Despite challenges in treatment adherence, recent studies suggest that when treatment is completed, it significantly influences disease progression by reducing parasite load, decreasing the production of specific antibodies against *T. cruzi*, and lowering the risk of developing chronic forms of CD. **Objective:** This study aimed to evaluate the impact of Benznidazole treatment on serological parameters and the risk of developing cardiac complications in patients with CD. **Methodology:** Whole blood samples were collected from NN patients, who were categorized based on clinical presentation: indeterminate (X), cardiac (X), and digestive/cardiodigestive (X) forms. Immunochromatographic lateral flow tests were performed, and clinical data were retrieved from medical records. Patients were stratified according to serological reactivity and treatment adherence. Fisher's exact test was used to compare means and medians, assessing differences between groups. **Results:** Statistical analysis revealed a significant difference in serological reactivity between treated and untreated patients with chronic CD. Patients who received Benznidazole exhibited lower serological reactivity, often classified as non-reactive or weakly reactive. No difference in %LVEF or echocardiographic parameters was observed between treated and untreated cardiac patients. **Conclusion:** Adherence to Benznidazole treatment is essential for reducing the humoral immune response to the parasite, which may be associated with an improved prognosis in patients at risk of developing chronic forms of Chagas disease.

Keywords: Chagas disease, Benznidazole, Humoral response.

DEVELOPMENT OF MOLECULAR TEST BASED ON UNUSUAL ISOTHERMAL MULTIMERIZATION AND AMPLIFICATION (UIMA) FOR THE DIAGNOSIS OF TEGUMENTARY LEISHMANIASIS (TL)

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Tests based on isothermal amplification of nucleic acids have the potential to facilitate the decentralization of molecular diagnosis of tegumentary leishmaniasis (TL). Previous studies have shown that Bst DNA polymerase enzymes with reverse transcription activity can be used to amplify DNA by multimerization (UIMA), using a single primer or primers for different genomic regions. The aim of this study was to evaluate the use of sense (150) and antisense (152) primers for *Leishmania* kDNA in the UIMA technique for the diagnosis of TL. The selected primers were individually evaluated using different concentrations of the enzyme 2.0 Bst DNA polymerase WarmStart (4 to 8 U), reaction temperature (63ºC) and reaction time (30 to 180 min) in a water bath. The results were visualized “with the naked eye” using Sybr Green I 10,000X/DMSO. Furthermore, 4 µL of the UIMA products were visualized in a 6% polyacrylamide gel (6% PAGE) stained with silver. After standardization, the detection limit (1 ng to 1 fg of DNA) of the kDNA-UIMA assay was evaluated in comparison with the kDNA-qPCR assay. *Leishmania* DNA detection was only possible using primer 152 (1.5 µM) and the reagents at the following final concentrations: 2.0 Bst DNA polymerase WarmStart (8U), Bst enzyme buffer (1X), MgSO4 (6 mM); dNTPS (2 mM each dNTP). The 6% PAGE analysis revealed the presence of amplicons only in the positive controls for 120 and 180 minutes. The results obtained by visual interpretation were the same as those obtained by 6% PAGE analysis. The detection limit of kDNA-UIMA assay was 100 pg, lower than that of kDNA-qPCR (1 fg). Although the detection limit of kDNA-UIMA was lower than that of kDNA-qPCR, other primer sets will be evaluated, which may also be evaluated in association with primer 152, to increase the detection limit of the UIMA for the diagnosis of TL. The parameters of analytical specificity and clinical accuracy will also be evaluated.

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Keywords: UIMA, *Leishmania*, molecular diagnosis

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IMIQUIMOD AS AN IMMUNOMODULATORY THERAPEUTIC ALTERNATIVE FOR CHAGAS DISEASE

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Abstract

The treatment of Chagas disease, caused by the parasite *Trypanosoma cruzi* (*T. cruzi*), is still based on drugs licensed over 50 years ago: nifurtimox and benznidazole (BNZ). The only ones with proven efficacy against Treatment with BNZ is effective in the acute phase but has limitations in the chronic phase, with a high rate of adverse events. Thus, new compounds have been tested to improve current treatment options. Imiquimod (IMQ), an imidazoquinoline, has immunomodulatory action by acting as an agonist of the Toll-like receptor 7. Its activation stimulates the production of cytokines such as IFNγ and TNF, which could enhance the immune system's ability to eliminate the parasite. Thus, this study aimed to evaluate the in vitro efficacy of IMQ in protecting against *T. cruzi* infection. Treatment durations of 2 hours and 4 hours were tested in L929 fibroblast cell lines cultured in complete RPMI medium. Trypomastigotes were maintained in L929 cell cultures under the same conditions. 1×10^5 L929 cells were cultured for 24 hours and then treated with IMQ at concentrations of 10, 20, 50, and 100 µg/ml. After drug exposure, trypomastigotes (1×10^6) were added and maintained for 24 hours before staining the coverslips with Rapid Panoptic stain. The most promising results were observed in the group treated with 50 µg/ml (2h), showing significant differences compared to the untreated group and the 50 µg/ml 4h group, with a lower infection rate (20%) and reduced intracellular parasite development over time. The group treated with 100 µg/ml (2h), although not showing significant differences, had similar results to the 50 µg/ml (2h) group. Thus, cells treated with 50 µg/ml (2h) of IMQ demonstrated more efficient immunomodulation. However, further experiments are needed to confirm these findings. New tests are underway for confirmation and future in vivo studies. This study was supported by the Coordination for the Improvement of Higher Education Personnel (CAPES) – Funding Code 001.

Supported by: CAPES, FAPEMIG, UFOP
Keywords: Chagas disease, *Trypanosoma cruzi*, Imiquimod

EVALUATION OF THE *IN* VITRO SYNERGISTIC ACTION OF ALDIMINE AND HANTZSCH ADDUCT COMPOUNDS IN THE TREATMENT OF VISCERAL LEISHMANIASIS

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Visceral leishmaniasis (VL) is a neglected tropical disease caused by *Leishmania infantum*, which can result in severe complications and death if untreated. Treatment is challenged by high costs, adverse effects, and limited efficacy. In this context, combination therapy has emerged as a promising alternative to address these issues. In preliminary screening, the compounds T5, T9 and 8A4, from the Aldimine and Hantzsch Adduct chemical groups, showed individual leishmanicidal activity. Based on the initial results, the combinations T5+8A4 (C1) and T9+8A4 (C2) were selected to test their synergistic action against both evolutionary forms of *L. infantum*. The combinations were evaluated at two concentrations: C1-120µg/mL+150µg/mL, 60µg/mL+75µg/mL, and C2-100µg/mL+150µg/mL,50µg/mL+75µg/mL. Leishmanicidal activity against promastigote forms was tested in *L. infantum* (OP46) transfected with GFP, using propidium iodide (PI) to label non-viable cells. Activity against amastigote forms was assessed in RAW 264.7 murine macrophages infected with the same promastigote species. The evaluation was performed by flow cytometry (FACS Calibur) at 24, 48 and 72 hours, with Amphotericin B as the positive control (PC) and the untreated group as the negative control (NC). For promastigote forms, both combinations significantly reduced the viability, particularly C2, after 24 and 48 hours. For amastigote forms, a reduction in the percentage of infected macrophages was observed, with C1 decreasing by 53.8% and C2 by 57.5% after 24 hours of treatment compared to NC. The parasite load analysis, measured by mean fluorescence intensity (MFI), showed a reduction at all treatment times, especially at higher concentrations. The reduction values were: C1- 89.7%, 85.5% and 78.5%, and C2- 83.9%, 86.1%, and 80.1%, respectively, compared to NC. These results suggest that the combinations of these molecules exhibit in vitro leishmanicidal activity against *L. infantum*, although further studies are needed.

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Keywords: leishmaniasis, treatment, cytometry.

PROBIOTIC AS A VACCINATION PLATFORM AGAINST VISCERAL LEISHMANIASIS: CONSTRUCTION, COMPARISON WITH THE RECOMBINANT APPROACH AND IMMUNE RESPONSE ANALYSIS IN A MURINE MODEL

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Visceral leishmaniasis (VL) is a severe zoonosis, potentially fatal if untreated. Despite efforts to develop VL vaccines, improving antigen presentation remains crucial. Probiotics like *Lactococcus lactis* are promising as delivery systems. This study aimed to develop and evaluate a probiotic-based vaccine against *Leishmania*, comparing its immunogenicity to a recombinant approach. Recombinant vectors pEXU-LALU:TryPan and pET-28a-TEV:TryPan were synthesized and transformed into *L. lactis* FnBPA+ and *Escherichia coli* Shuffle, respectively. Clone identity was confirmed via PCR, and the chimera was expressed and purified for cromatography. To determine the appropriate vaccine dose, growth curves of vaccine and control strains were performed, establishing the correlation between OD, CFU/mL, and cultivation time. A transfection assay in CHO cells with pEXU-LALU:TryPan assessed vector functionality. BALB/c mice were orally immunized with the probiotic, TryPan protein + MPLA, or both approaches combined. Antibody responses were analyzed via ELISA. PCR confirmed the vaccine probiotic and *E. coli* constructs, with 220 bp and 2000 bp fragments, respectively. The chimera was successfully expressed, purified, and identified immunologically. Growth curves showed cultures reached ~1×10^9 CFU/mL in 2–2.5 hours. Transfection success was validated by ELISA, indicating constitutive chimera production in CHO cells. ELISA from immunized mice showed recombinant immunization induced a strong systemic antibody response, unlike the oral probiotic. However, combining both strategies with a protein booster significantly enhanced specific antibody production. Future steps involve in vivo testing and assessing the cellular immune response to VL.

Supported by: FAPEMIG; CNPQ; CAPES.

Keywords: oral vaccine; probiotics; visceral leishmaniasis.

BIOSYNTHESIS OF VITAMIN K1 (PHYLLOQUINONE) IN *LEISHMANIA (L.) AMAZONENSIS*

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Leishmaniasis is one of the main neglected diseases in Brazil and worldwide. Study of the biology and biochemistry of the parasite and the search for new targets for the development of antileishmanial drugs are necessary. New metabolic pathways of the parasite, such as the Mevalonate pathway, has proven to be an alternative for the development of new drugs. It is important to better understand the physiology and survival mechanisms used by this parasite. The biosynthesis of isoprenoids in *Leishmania* spp. has not yet been well studied. The aim of this study was to investigate whether Vitamin K1 biosynthesis occurs in promastigote forms of *Leishmania (L.) amazonensis (L. amazonensis*), and whether this pathway could be a good target for the development of new drugs. Vitamin K1, also known as phylloquinone, is the most common form of Vitamin K. It is found in green leafy vegetables, such as spinach, collard greens, and broccoli. The main function of vitamin K1, in the human host, is to assist in blood coagulation. The presence of Vitamin K1 in promastigote forms of *L. amazonensis* was identified by the RP-HPLC technique using radioactive precursors [1-(n)-3H]-phytol. We confirmed the presence of vitamin K1 in the parasite by gas chromatography-mass spectrometry. Treatment with Warfarin, a drug that inhibits the VKOR enzyme, was able to inhibit the growth of the parasites at a concentration in the micromolar range and induced the production of *ROS* (reactive oxygen species) in promastigotes of *L.* amazonensis. The presence of exogenous phylloquinone reduced the production of ROS, indicating a possible antioxidant function of phylloquinone, and was able to recover the parasites treated with Warfarin. We describe a "putative" sequence of the VKOR enzyme of *L. amazonensis*, as well as the identification of the active site. This study presented evidence of Vitamin K1 biosynthesis in *L. amazonensis* and suggests that inhibitors of the VKOR enzyme may be a leishmanicidal drug.
Supported By: CNPq and FAPESP.
Keywords: *Leishmania (L.) amazonensis*; Phylloquinone; Warfarin.

THE IMPACT OF SARS-COV2 CO-INFECTION ON CHRONIC CHAGAS DISEASE IN A MURINE MODEL: SOCS-2 AS A THERAPEUTIC MOLECULE

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Abstract

The balance of the immune response (IR) is essential for the survival of Trypanosoma cruzi and the host during the acute phase of Chagas disease (CD). Infection with Sars-cov2 can cause an unbalanced immune response associated with systemic damage in its host. Suppressor cytokine signaling (SOCS) 2 is an important regulatory protein of the innate and adaptive response, but its role during chronic infection by CD and betacoronavirus MHV-3 (virus that mimics Sarscov-2 in a murine model) is unknown. Our objective was to evaluate the role of SOCS2 during chronic CD, acute MHV3 infection and chronic CD/acute MHV3 co-infection in a murine model. C57BL/6 (WT) and SOCS-2 (KO) animals were infected with trypomastigote forms (Y strain), and after reaching chronic CD (100 days), they were co-infected or not with MHV-3. We performed clinical, physiological, histopathological, immunobiochemical, parasitism and microbiota analyses. Our data demonstrated a systemic alteration in all infections, but mainly in the groups coinfected with betacoronavirus, where cardiac and pulmonary dysfunctions occurred, associated with fibrotic processes in the tissue, and a higher viral load detected in SOCS2 KO mice when compared to WT counterparts. In addition, reduced mucus production and muscle degeneration were observed in the colon due to high parasitism and dysbiosis. SOCS2 deficiency also resulted in lung and intestinal tissue with a higher pro-inflammatory profile, increasing TNF, IFN-g and reducing IL-10 production by innate and adaptive immune cells. In summary, SOCS-2 is essential to modulate the immune response and the progression of pathogenesis, especially in coinfection with betacoronavirus MHV-3 in the chronic phase of CD, being a possible therapeutic target not only in CD but also in Sarscov-2 infections. Financial support: INCT-Dengue and Host Microorganism Interaction, Fapemig, Capes and Cnpq.

Supported by: Capes, Cnpq, Fapemig

Keywords: Chagas disease, Covid-19, SOCS-2

IMMUNE RESPONSE AND HISTOLOGICAL ANALYSIS OF *kh1*-DEFFICIENT LIVE-ATTENUATED *Leishmania infantum* IN MURINE MODEL

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Abstract

Live-attenuated vaccines expose the host immune system to a broad antigen repertoire, which is important for protective immunity. Our group selected *Leishmania infantum* strains lacking the *kharon* gene (*LiKh1*$^{-/-}$), essential for parasite virulence. We previously demonstrated their immunogenicity and ability to induce a protective adaptive response against virulent *L. infantum*. In this study, we assess the immunogenicity through innate immune response profiling and histological analysis in murine models. Macrophages, *Leishmania*'s primary host cells, play a key role in immunity. Since their activation profile affects infection outcomes, we assessed nitric oxide (NO) production from peritoneal macrophages through Griess assay. NO levels were undetectable after *L. infantum* stimulation and very low after IFN-γ pre-stimulation, possibly influenced by TLR2. However, post-infection, LPS+IFN-γ co-stimulation induced NO, suggesting innate immune modulation. Live-attenuated strains remained infective but failed to sustain infection *in vitro*. Understanding the local immune mechanisms required for the control of infection is essential for developing effective vaccines. To further evaluate their impact, BALB/c mice were infected with 1×10^7 *L. infantum* (*LiKh1*$^{-/-}$ or WT) via IP and analyzed 30 dpi. Liver and spleen sections were stained with HE for histopathology and anti-mTXNPx mAb for IHC. Splenic architecture was preserved in all groups, though mild white pulp disorganization was observed in WT. WT induced mild focal liver inflammation, while the attenuated strain apparently caused less damage, consistent with antigen labeling, RT-PCR, and LD. However, IHC revealed strong splenic staining in both groups. These findings highlight the attenuated strain as a promising vaccine candidate, inducing an immune response with less tissue damage. Further studies are needed to confirm its efficacy and to assess its impact on parasite differentiation.

Keywords: *Leishmania infantum*, Attenuated Vaccine, Innate Immune Response

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HIGH FREQUENCY OF *Giardia lamblia* ASSEMBLAGE A SUGGESTS ANTHROPOZOONOTIC PARTICIPATION IN TRANSMISSION CYCLES IN THE EXTREME NORTH OF BRAZIL

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Giardia lamblia is a globally distributed intestinal protozoan that spreads through water or direct contact among hosts. Children under five years old are the most affected by the infection due to age-related factors such as oral exploration phase, beginning of autonomous locomotion, direct contact with the ground, developing hygiene practices, and an under-stimulated immune system. The species *G. lamblia* is phylogenetically divided into assemblages A to H. Assemblages A and B are expected in human infections but also have a high zoonotic potential. In this context, the objective was to determine the frequency of *G. lamblia* and the circulating assemblages in children from Boa Vista, RR. The study was conducted with preschoolers attending a school in the Caranã neighborhood in Boa Vista/Roraima (a state in the extreme north of Brazil(. Stool samples were collected and subjected to parasitological examination using the Ritchie and Kato-Katz methods. Molecular diagnosis of *Giardia* was also performed, and all samples had their DNA extracted using the QIAamp Fast DNA Stool Mini Kit and were subjected to amplification of a fragment of the B-giardina gene by polymerase chain reaction (PCR). Real-time PCR with High Resolution Melting (qPCR-HRM) was used to differentiate the assemblages of *G. lamblia*. Of the 168 samples collected, 76 were positive in the parasitological diagnosis and 56 in the molecular diagnosis. Genotyping identified 36 isolates of assemblage A, four of assemblage B, and 16 could not be differentiated. The positivity of *Giardia* infection was considered high compared to other waterborne protozoa. This suggests the possibility of direct transmission involving other hosts, which is corroborated by the high frequency of assemblage A. Knowing that *G. lamblia* can cause nutritional deficits and delays in physical and cognitive development, the implementation of control strategies is urgent to reduce giardiasis cases.

Supported by: CAPES, CNPQ, PAEF-IOC, UFRR.

Keywords: *Giardia lamblia*, assemblage, qPCR-HRM.

INVESTIGATING FUNCTIONAL DISTINCTIONS FOR TWO *LEISHMANIA INFANTUM* HOMOLOGUES OF THE POLY-A TAIL BINDING PROTEIN, PABP2 AND PABP3, THROUGH A COMPARATIVE ANALYSIS INVESTIGATING PABP2 PARTNERS IN THE PRESENCE AND ABSENCE OF THE NON-ESSENTIAL PABP3

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Abstract

Leishmania and related protozoa are unique in having their gene expression mainly regulated by post-transcripitional mechanisms acting upon their mRNAs. These likely involve the poly(A) binding protein (PABP), found at the 3' ends of most eukaryotic mRNAs. Three PABP homologs are found in *Leishmania* species, with PABP1 found associated with unique target mRNAs and protein partners, including translation factors, while PABP2 and PABP3 coprecipitate together and bind to a similar set of mRNAs and protein partners, distinct from those found with PABP1. PABP3 is the only one not essential in *Leishmania* and it is not found in *Trypanosoma* species. In order to better define roles for the *Leishmania* PABPs, we sought here to identify proteins bound to PABP2 in the absence of PABP3. Promastigotes from *L. infantum* cell lines lacking PABP3 were transfected with a plasmid allowing the ectopic expression of PABP2 having a C-terminally tagged HA epitope. PABP2-HA was then immunoprecipitated followed by identification of co-precipitated protein partners through mass spectrometry. The results were compared with PABP2-HA data obtained in the presence of PABP3, and with data from similar experiments with PABP1 and PABP3. Overall, a large set of protein partners were found with greater association with PABP2 when PABP3 is missing. These include PABP1 and several of its known partners, including the EIF4E4 and EIF4G3 translation factors, the RNA binding protein RBP23 and a NTF2 domain-containing protein (LINF_180008000), some not previously detected with PABP2. Nevertheless, several known PABP2 partners remain associated with the tagged proteins including other RNA binding proteins, such as DRBD2 and ZC3H39, and another NTF2 domain protein (LINF_210009700). PABP2 does not then seem to require PABP3 in order to associate with its most abundant protein partners, but PABP3 is needed to prevent its association with PABP1 partners and might be involved in modulating the PABP2 interactions.

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Keywords: RNA binding protein, eIF4F complex, gene expression regulation, translation initiation.

MOLECULAR DETECTION OF *Plasmodium spp* INFECTION IN NON-HUMAN PRIMATES FROM THE SOUTH OF THE BRAZILIAN ATLANTIC FOREST

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Malaria is a disease caused by protozoa of the genus *Plasmodium* and transmitted to the vertebrate host by female *Anopheles* mosquitoes during a blood meal. Although the endemicity of the disease is restricted to the Amazon region, autochthonous malaria cases also occur in the extra-Amazon region. Some of these cases are related to the Atlantic Forest regions, where malaria parasites primarily infect non-human primates (NHPs): *Plasmodium simium* and *P. brasilianum*. *Plasmodium simium* is confined to the Atlantic Forest regions of southern and southeastern Brazil, capable of infecting species from three of the five families of neotropical primates, while *P. brasilianum* has a broader distribution, from Mexico to southern Brazil, and can infect species from all five families of neotropical primates. Although originally infecting NHPs, both species are also capable of infecting humans. Given this zoonotic transmission scenario and knowing that Brazil harbors the greatest biodiversity of NHPs in the world, the role of these species as sources of *Plasmodium* infections for humans presents a significant challenge for malaria elimination. In this context, the study aimed to detect *Plasmodium* infection in samples from wild NHPs collected between 2004 and 2006 in a region on the border between Mato Grosso do Sul and Paraná. To achieve this, DNA extracted from 57 blood samples was subjected to conventional and real-time PCR diagnostics, with different targets: ribosomal (18S SSU rRNA), mitochondrial (*cox1*), and specific families of *P. vivax* and *P. falciparum* (*Pvr47, Pf364*). The results showed that 38.6% (n=22) of the samples were positive, with mitochondrial target-based diagnostics being more sensitive compared to ribosomal target-based diagnostics. These results emphasize the importance of advancing more sensitive diagnostic methods as an essential factor for detecting the parasite in NHP samples, as parasitemia in these animals tends to be lower than in humans.

Supported by: CNPq, Fapemig

Keywords: malaria; zoonotic transmission; reservoirs

CERATOMYXA SPP. PARASITIZING FISH OF THE FAMILY PIMELODIDAE FROM THE AMAZON

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Pimelodids, commonly known as catfish, are fish of the order Siluriformes with significant importance for commercial, ornamental, and sport fishing in South America. Myxozoa is a class within Cnidaria that includes species adapted to endoparasitism, characterized by significant simplifications and reductions in body size compared to free-living cnidarians during their evolutionary transition to parasitic life. South America, known for its rich freshwater ichthyofauna, has seen growing interest in the study of Myxozoa parasites of fish in this region.

In a study of fish parasites from the Amazon Basin, in the waters near the confluence of the Tapajós and Amazon Rivers, in Santarém-PA, specimens of the following pimelodid species were examined: *Brachyplatystoma filamentosum*, *B. vaillantii*, *B. rousseauxii*, *B. juruense*, *B. platynema*, *Platynematichthys notatus*, and *Phractocephalus hemioliopterus*. Myxozoans infecting the gallbladder were observed in some specimens of all these species. The parasites were characterized through light microscopy, transmission electron microscopy, and small subunit ribosomal DNA (SSU rDNA) sequencing.

Light microscopy revealed vermiform plasmodia with motility, containing myxospores characteristic of the genus *Ceratomyxa*. In *B. filamentosum*, *B. vaillantii*, *B. rousseauxii*, and *P. notatus*, the analyses identified the parasite as *Ceratomyxa gracillima* Zatti, Atkinson, Maia, Bartholomew & Adriano, 2018, originally described parasitizing *B. rousseauxii*. In *B. juruense* and *B. platynema*, morphological and molecular analyses revealed an undescribed species, provisionally identified as *Ceratomyxa sp. 1*. Similarly, for *P. hemioliopterus*, the analyses indicated a second undescribed species, provisionally named *Ceratomyxa sp. 2*.

This study highlights the diversity and distribution of different *Ceratomyxa* lineages among Amazonian pimelodid species.

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Keywords: Myxozoans, *Ceratomyxa*, Pimelodids

PIONEERING DETECTION OF *Toxoplasma gondii* IN GREEN TURTLES (*Chelonia mydas*): IMPLICATIONS FOR MARINE ECOEPIDEMIOLOGY

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Toxoplasma gondii is a zoonotic protozoan parasite known to cause several implications for human and animal health. The surface runoff and transport of infective structures from the terrestrial environment to the sea, as well as the detection of this parasite in marine invertebrates, such as shellfish, have already been observed. However, the knowledge of its ecoepidemiology in marine environments and its possible hosts are incipient or poorly explored in many areas worldwide. The main goal of this study was to investigate the prevalence of *T. gondii* infection in green turtles (*Chelonia mydas*) found along the northern coast of Santa Catarina State, Brazil, as an additional strategy to track environmental contamination by this protozoan. Additionally, the study examined pathological changes and the presence of other pathogens in turtles. The turtles analyzed were found dead or weakened along different beaches of the study area. A variety of tissues from eight green turtles were examined. DNA amplification was performed by nested-PCR using *B1* gene as molecular marker for *T. gondii* detection and histological sections were prepared and stained using the hematoxylin-eosin (HE) method. *T. gondii* DNA was detected in the brain and liver of two individuals (25%). The histopathological examinations revealed alterations in animals' respiratory, hepatic, and nervous systems and the presence of Trematode eggs from the Spirorchiidae family. This study provides the first evidence of marine turtles infection by *T. gondii*, contributing to the growing knowledge of its ecoepidemiology, highlighting the importance of considering marine turtles as potential hosts for the parasite. This expanded perspective enhances our understanding of the dynamics of toxoplasmosis in nature. It has significant implications for developing effective conservation and management strategies for this vulnerable species, which was classified by the IUCN Red List of Threatened Species in 2004 as Endangered.

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Keywords: marine environment; toxoplasmosis; zoonosis.

ANTIBIOFILM ACTION OF ANTIFUNGALS AND CHLORHEXIDINE AGAINST CO-CULTURES OF *Acanthamoeba* spp. AND *Candida* spp.

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Severe infectious keratitis is a major cause of corneal transplantation and vision loss, often caused by the protozoan of the *Acanthamoeba* genus and fungi such as *Candida* spp. These organisms can adhere to contact lenses, lens cases, and the patient's cornea, forming polymicrobial biofilms. Improper use of contact lenses and poor storage and cleaning of the cases are the main factors contributing to biofilm formation. Studies on the adhesion potential of *Acanthamoeba* spp. and the effectiveness of commercial drugs against these biofilms are scarce. Thus, this study evaluated the adhesion potential and biofilm formation of *Acanthamoeba* spp. and *Candida albicans*, both alone and in co-culture. In addition, the efficacy of natamycin, voriconazole, and chlorhexidine against these microorganisms was tested in vitro. The minimum antibiofilm concentration (MAC) and the minimum biofilm-eradicating concentration (MBEC) were determined for $8x10^5$ *Acanthamoeba* trophozoites (ATCC 50492) and a clinical isolate (R40). The same concentration was used for *C. albicans*. The treatments were carried out over 48 hours. Both *A. castellanii* and the clinical isolate demonstrated a high adhesion capacity after 48 hours. At 200 µM, all drugs showed promising antibiofilm activity and were effective in eradicating *C. albicans* and *Acanthamoeba* spp. biofilms, both alone and in co-culture. Natamycin was particularly effective in reducing *C. albicans* biofilms, both alone and in co-culture with *Acanthamoeba* spp. Chlorhexidine was highly effective in eradicating *Acanthamoeba* spp. This study highlights the importance of further research to optimize antibiofilm treatments for managing infections caused by both *Acanthamoeba* spp. and *Candida* spp. A better understanding of the adhesion behavior of *Acanthamoeba* spp. and the evaluation of treatment efficacy could contribute to the development of new treatment and multipurpose solutions for cleaning contact lens cases and preventing infections.

Keywords: biofilms keratitis; *Acanthamoeba*; *Candida.*
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HOST IMMUNE SYSTEM IMPACT ON THE FITNESS OF *LEISHMANIA AMAZONENSIS*

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Abstract

Leishmania parasites face environmental changes throughout their life cycle that require rapid biological adaptation. Metacyclic promastigotes and amastigotes have developed several strategies to evade and survive the immune system of their vertebrate hosts. In a successful infection, *Leishmania* causes a wide spectrum of diseases, ranging from skin to visceral lesions. Our group has previously described the infection course of *Leishmania amazonensis* LV79 strain in BALB/c and BALB/c nude mice, which have distinct T-cell dependent responses. In this model, lesion-derived amastigotes exhibited differential protein expression in a host-dependent manner. Here, we evaluate the impact of the immune system of these strains on parasite fitness. For this, BALB/c and BALB/c nude mice were infected with promastigotes of the *L. amazonensis* LV79 strain, and after 13-weeks of infection, amastigotes were isolated from the lesion footpads. We compared the cell viability of the lesion-derived amastigotes by MTT assay and analyzed the conversion of these amastigotes to promastigotes in M199 medium. The amastigotes recovered from BALB/c mice were more viable and generated cultures with a higher number of promastigotes than those from BALB/c nude mice. Next, we used a cross-infection model to analyze the virulence of these amastigotes in both mouse strains. We observed that amastigotes recovered from BALB/c caused larger lesions with a higher parasite load in both BALB/c and BALB/c nude mice, compared to those recovered from BALB/c nude mice. Amastigotes recovered from BALB/c nude led to larger lesions only in BALB/c nude mice. Our findings show that the host immune system modulates parasite fitness, directly influencing the survival and virulence of amastigotes in the next infection.

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Key words: *Leishmania amazonensis*, lesion-derived amastigotes, mouse model.

DISRUPTION OF TRYPANOSOMA CRUZI GENES ENCODING ACTIVE TRANS-SIALIDASES RESULTS IN A HIGHLY ATTENUATED STRAIN THAT PRESENTS IMPAIRED PARASITE EGRESS FROM HOST CELL

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Trans-sialidases (TS) are encoded by the largest gene family in the *T. cruz*i genome in which only few sequences encode enzymatically active TS (aTS). aTS roles during infection have not been fully characterized due to multiple copies of the aTS genes, along with repetitive sequences. Using CRISPR/Cas9 technology, we generated aTS knockout parasites (aTS-KØ) that display undetectable levels of TS activity. Although aTS-KØ parasites can infect cell *in vitro*, they are unable to establish infection *in vivo* even in the highly susceptible IFN-γ knockout mice. Moreover, data showed that the highly attenuated aTS mutants are capable of generating protection in immunized mice that were subsequently challenged with a virulent strain. *In vitro* infection assays with wild type (WT) and aTS-KØ parasites in different cell models: HeLa (non-phagocytic), THP1 and peritoneal macrophages (phagocytic) confirmed that the lack of aTS does not limit parasite internalization and amastigote multiplication in these cell types, but drastically affect release of trypomastigotes. Addback cell lines in which the aTS genes were re-expressed, showed that this *in vitro* infection phenotype is partially restored. HeLa cells infected with aTS-KØ have significantly lower mRNA transcript levels of inflammatory cytokines such as IL-1-β, IL-1-α, IL-6 and CXCL8 compared to WT parasites. The differential cytokine induction may be associated with a persistent MEK/ERK protein kinase activation axis during aTS-KØ infection. We also showed that the activation of anti-apoptotic pathways (PI3K/AKT) by *T. cruzi* is independent of aTS. Infection in the insect vector is also independent of aTS activity since no significant differences in total parasite numbers were observed in the digestive tract or urine of infected *Rhodnius prolixus*. These data highlight aTS as an essential virulence factor for *T. cruzi* survival in mammalian that could be used as attenuated vaccine.

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Keywords: Active *trans*-sialidases (aTSs); multigenic family knockout; parasite attenuation.

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CASPASE-8 ACTIVATION IS REQUIRED FOR CONTROL OF *LEISHMANIA AMAZONENSIS* REPLICATION IN MACROPHAGES

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Abstract

Leishmaniasis is a disease caused by the protozoan *Leishmania spp.*, considered a significant global health challenge. Macrophage-mediated innate immunity is essential for the parasite replication control. Central to this process is the NLRP3 inflammasome, which activates caspase-1, forming a protein complex that induces pyroptosis, an inflammatory form of cell death critical for parasite elimination. Caspase-1 activation drives the production of IL-1β, enhancing iNOS expression and nitric oxide synthesis, both essential for *Leishmania* elimination. Traditionally described as an initiator caspase involved in apoptotic cell death, caspase-8, in coordination with or via the activation of caspase-1, has also been shown to be important in controlling pathogen replication in macrophages infected by *Legionella* and *Yersinia,* and is associated with pyroptotic cell death. In this study, we explored the role of caspase-8 in regulating pyroptosis-related inflammatory processes during *Leishmania amazonensis* infection in macrophages. Using bone marrow-derived macrophages, we employed a combination of molecular and immunological approaches to uncover its function. Immunoblotting with a cleaved caspase-8-specific antibody, along with immunofluorescence microscopy, demonstrated that *L. amazonensis* infection induces caspase-8 activation in macrophages independently of pathways mediated by Ripk3 and caspase-1. Furthermore, flow cytometry analysis of macrophages from caspase-8/ripk3 double knockout mice revealed significantly increased susceptibility to *L. amazonensis*-RFP compared to wild-type controls after 24 hours of infection. These findings highlight the pivotal role of caspase-8 as a critical regulator of macrophage activation during *Leishmania* infection, enhancing their capacity to restrict parasite replication. By elucidating this pathway, our study provides valuable insights into the molecular mechanisms driving macrophage-mediated immunity against *Leishmania* infection.

Supported by: CNPq, CAPES, FAPESP

Keywords: *Leishmania*; Caspase-8; Macrophages.

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2'-HYDROXYFLAVANONE ACTIVITY AGAINST WILD-TYPE AND ANTIMONY-RESISTANT *Leishmania infantum* AND ITS PROOXIDANT PROPERTY AS A POSSIBLE MECHANISM OF ACTION

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Abstract

Pentavalent antimonials, the first line choice of Leishmaniasis treatment, are becoming ineffective due to parasitic resistance, the main limitation associated with its use. Visceral Leishmaniasis (VL), the most severe clinical manifestation of Leishmaniasis can be fatal in 95% of untreated cases. Therefore, the search for new alternatives for Leishmaniasis treatment is crucial. Natural products, such as flavonoids, are known for their prooxidant properties, inducing reactive oxygen species (ROS) that can be associated with cellular damage and death, and have been described as potent antileishmanial candidates. 2'-hydroxiflavanone (2HF) had already been described as a promising molecule against antimony-sensitive and -resistant *L. amazonensis* in a murine model of Cutaneous Leishmaniasis. This study evaluated the 2HF *in vitro* activity and its prooxidant effect as a possible mechanism of action against wild-type (WT), antimony-sensitive (S), and -resistant (R) *L. infantum*. WT, S, and R promastigotes were incubated with 2HF (0–96µM) for 72h. 2HF inhibited WT, S, and R promastigotes in a concentration-dependent manner, demonstrating an IC_{50} of 6.9; 11.57, and 20.24µM, respectively. To evaluate if the 2HF action is linked to ROS generation, promastigotes were treated with 2HF and incubated with 20µM of H_2DCFDA. 2HF promoted an increase in ROS levels in a concentration-dependent manner, reaching respectively a 1.9, 1.7, and 1.6 fold increase at the highest concentration of 2HF (96µM) compared to control. In the antiamastigote assay, WT *L. infantum* infected macrophages were treated for 72h with 2HF, showing a concentration-dependent reduction in the infection index, with an IC_{50} of 4µM. The selectivity index was 21,87. These results indicate 2HF as a potential candidate for VL treatment, reinforcing its ability to overcome resistance issues and its prooxidant activity, highlighting the need for further studies exploring mechanisms of action and *in vivo* efficacy.

Supported by: CNPq, CAPES, FAPERJ, IOC/FIOCRUZ

Keywords: Visceral leishmaniasis, resistance, and reactive oxygen species (ROS)

Besifloxacin is active against *Toxoplasma gondii* infection *in vitro*

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Ocular toxoplasmosis (OT) is a disease caused by *Toxoplasma gondii*, a protozoan belonging to the Apicomplexa phylum. Currently, the standard treatment for OT involves a combination of trimethoprim and sulfamethoxazole with corticosteroids. However, this therapy is only effective during the acute phase of the disease and can cause hypersensitivity reactions and severe adverse effects. Recently, antimicrobial repositioning has garnered significant attention as a promising therapeutic strategy, particularly for drugs targeting essential pathways located within the apicoplast. This is a prokaryotic-derived organelle that is important for the survival and replication of *T. gondii*. Besifloxacin (BSX) is a fluoroquinolone in Besivance® eye drops, previously used for bacterial conjunctivitis. Previous studies have shown that fluoroquinolones impair endodyogeny of *T. gondii*, leading to the formation of masses of parasites non individualized. Thus, we investigated the cellular effects of the antibacterial BSX on *T. gondii* infection *in vitro* to evaluate its potential as an alternative drug for OT. Cytotoxicity assays showed CC_{50} values > 100 µM in retinal epithelial (ARPE-19), kidney epithelial (LLC-MK2) and fibroblast (HFF) cell types. In antiproliferative assays using tachyzoites from the RH strain, BSX demonstrated high selective activity against *T. gondii*, with IC_{50} values of 5 µM and 2.8 µM after 24h and 48h of infection, respectively. Lysis plate analyses demonstrated a 65% reduction in HFF monolayer damage after 20 µM (IC_{90}) of BSX treatment, even post-removal on day 5. Immunofluorescence revealed BSX induced parasite mass formation, apicoplast loss/fragmentation, centrosome dispersion, and mitochondrial alterations in ARPE-19 cells infected with RH-ACP-YFP. Transmission electron microscopy confirmed endodyogeny arrest and failed daughter cell individualization in BSX-treated cells. These findings suggest BSX has potential for in vivo testing and repositioning for OT treatment.

Supported by FAPERJ, CNPq, CAPES

Keywords: Toxoplasma gondii; drug repurposing; besifloxacin

OCCURRENCE OF *Neorickettsia risticii* IN A TROPICAL-EQUATORIAL CLIMATE REGION, BAIXADA MARANHENSE IN MARANHÃO - BRAZIL

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Neorickettsia risticii is the causative agent of Potomac Fever, an acute disease characterized by fever and colonic lesions in horses. The bacterium's life cycle involves the trematode *Pleurolophocercus*, which parasitizes *Heliobia* snails commonly found in marshy regions with tropical Atlantic or subtropical climates. The Baixada Maranhense region consists of low, flood-prone plains and has a tropical-equatorial climate with distinct rainy and dry seasons and higher average temperatures. Although *Heliobia* snails, the known intermediate host of *N. risticii*, have not been recorded in this region, the presence of other snail species suggests the possibility of alternative hosts. This study aimed to investigate the occurrence of *N. risticii* in equines from Baixada Maranhense, Maranhão. Blood samples were collected from 184 horses across 21 municipalities in Baixada Maranhense, along with two additional locations with similar bioclimatic conditions. Blood smears were prepared for direct parasite detection, stained with Panótico Rápido, and examined under a light microscope using a 100x objective lens. For molecular detection, DNA was extracted using the Wizard® Genomic DNA Purification Kit (PROMEGA®), followed by nested PCR targeting the 16S ribosomal RNA region of *N. risticii*, according to the protocol established by Barlough (1998). One sample exhibited cytoplasmic inclusions in monocytes consistent with *N. risticii*, while molecular testing detected the pathogen in seven samples. This study provides the first evidence of *N. risticii* circulation in horses from Baixada Maranhense, emphasizing the need for further research to elucidate its transmission pathways in equatorial tropical environments.

Supported by: FAPEMA; CAPES.

Key-words: Horses; Potomac Fever; Snails

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FIGHTING LEISHMANIASIS WITH NUTRITION: MALNUTRITION IMPAIRS TREATMENT AND ALTERS DISEASE PROGRESSION

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Abstract

Malnutrition and visceral leishmaniasis (VL) are major public health challenges, contributing to significant morbidity and mortality worldwide. The progression of leishmaniasis is closely linked to the host immune status, and malnutrition can severely compromise immune function, altering disease susceptibility and organ tropism. This study aimed to validate a dietary model that closely mimics marasmic malnutrition, assessing its impact on nutritional, biochemical, and hematological parameters and investigating how malnutrition influences the establishment, progression, and treatment response of *Leishmania infantum* BALB/c mice infection. Animals were fed either a restricted diet (36.26% carbohydrate, 8.79% protein, 4.95% fat, 7.62 kJ/100 g) or a control diet (65.93% carbohydrate, 24.18% protein, 9.89% fat, 15.24 kJ/100 g). Some malnourished mice underwent a refeeding process with the control diet. Nutritional status was evaluated through phenotypic, hematological, and biochemical markers. The restricted diet, induced mild malnutrition, resulting in 12% weight loss, reduced growth rates, lower BMI, a 47.5% decrease in leukocyte counts, and a two-fold increase in cholesterol levels. Following *L. infantum* infection and meglumine antimoniate treatment revealed that malnutrition significantly impaired splenic infection but enhanced hepatic parasite burden. Refeeding restored hepatic infection levels but did not fully reverse splenic impairment. While meglumine antimoniate effectively reduced liver parasite loads in control mice (10^5-fold reduction), its efficacy was significantly affected in malnourished mice (10^3-fold reduction). However, refeeding successfully restored treatment effectiveness in the liver, reaching a 10^5-fold decrease in liver parasite load. These findings suggest that marasmic malnutrition alters VL pathogenesis and reduces treatment efficacy, emphasizing the importance of nutritional interventions in managing VL in malnourished populations.

Supported by: FIOCRUZ, IOC, CAPES, CNPq, FAPERJ.
Keywords: marasmic malnutrition; visceral leishmaniasis; host immune status; treatment; Leishmania infantum infection; refeeding intervention

ADVANCEMENTS IN EFFICACY TESTING OF TOPICAL NYSTATIN FOR TREATING *Leishmania (L.) amazonensis* INFECTIONS IN MICE

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Leishmaniasis is recognized by the World Health Organization as a neglected tropical disease. Given the challenges associated with its treatment, there is a pressing need for studies exploring new therapeutic options that offer improved efficacy and lower toxicity for patients. This study presents an *in vivo* efficacy evaluation of topical antifungal Nystatin cream (125 IU/mouse) as combination therapy with meglumine antimoniate in the BALB/c–*Leishmania (L.) amazonensis* infection model. The analyses included assessments of size lesion progression, parasite load in paw lesion, and parasite load in the draining lymph node. A significant reduction in paw lesion was observed in all treated groups compared to controls (untreated, placebo cream and treated with PBS) (p=<0,0001). Furthermore, parasite load in paw infection showed a significant reduction in the groups treated with Nystatin monotherapy compared to controls (p=0,0007). Similarly, parasite load in the lymph nodes demonstrated a significant reduction in the treated groups compared to controls (p= 0,001). The combined findings from this study suggest that Nystatin administrated topically has potential as a repositioned drug for the treatment of cutaneous leishmaniasis. These results indicate that Nystatin cream when combined with meglumine antimoniate could progress along the Technology Readiness Level continuum and is currently positioned at the preclinical mice study phase.

Keywords: *Repurposing, Nystatin, Combination therapy, Cutaneous Leishmaniasis.*

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Ultrastructure analysis and molecular characterization of *Trichomitus batrachorum* (Phylum: Parabasalia; Ordem: Hypotrichomonadida) isolated from liver of *Ameiva ameiva* (Reptilia: Squamata).

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Abstract

Trichomitus batrachorum, a species of trichomonad, has gained attention for its ecological significance and potential interactions with various hosts, particularly poikilothermic organisms like amphibians and reptiles. Molecular studies have placed this flagellated protist within the Metamonada clade, offering valuable insights into its evolutionary relationships with other trichomonads. Unlike parabasalids that inhabit endothermic mammals in relatively stable temperature conditions, protists associated with ectothermic reptiles, are subject to significant temperature fluctuations. The ability of *T. batrachorum* to thrive in the variable temperatures encountered by reptiles suggests that its parasitism may remain largely unaffected by climate change. This could have profound implications for the composition and interactions of these microbes with vulnerable animal species. In our study, we successfully detected and characterized *T. batrachorum* from the liver tissue of the lizard species *Ameiva ameiva* ((Reptilia: Squamata), collected in Presidente Figueiredo, Manaus state, Brazil. The identification of *T. batrachorum* was confirmed through culture, light microscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM) for ultrastructural analyses, and sequencing of the 18S rRNA and ITS genes. This successful detection and characterization of *T. batrachorum* provides a solid foundation for further research. One potential interpretation for this finding is that the flagellates may have migrated from the intestine to the bile duct, ultimately reaching the liver. Further research is needed to elucidate the potential pathogenicity of this flagellate and the role of *Ameiva ameiva* in the epidemiology of parabasalids in other animal species.

Keywords: Biodiversity, Amazon rainforest, Trichomonadids.

Funding agency: Fapeam, Inova Amazônia, Fiocruz, ILMD, CAPES

EFFECT OF THE ASSOCIATION OF CHLORPYRIFOS+CYPERMETHRIN®

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The dog tick or popularly known as the "red tick" is an ectoparasite found in tropical and subtropical regions that infests dogs and transmits diseases, and is often controlled with the use of commercial tickicide. The objective of this study was to evaluate the commercial combination of Chlorpyrifos+Cypermethrin® in *Rhipicephalus sanguineus*. Teleogynes of *R. sanguineus* were collected from two domestic dogs, raised in the city of Santa Inês-BA. They are two-year-old Blue Heeler breed dogs, which live at a house with access to a backyard and ocasional outings to the streets. Tickicides have never been used on these dogs. The collected teleogynes were taken to the Parasitology Laboratory of IF Baiano *campus* - Santa Inês, and the most active of them were selected, identified and separated into two groups of 10 units each: a control group and a test group. The product used was a commercial combination of Chlorpyrifos + Cypermethrin®. The teleogynes of the control group were submerged for five minutes in running water, while those of the test group were submerged for the same time, but in the dilution value of the tested product as indicated by the manufacturer. After immersion, the teleogynes of the two groups were dried with an absorbent paper and placed in Petri dishes. Daily observations were made, and the teleogynists in the control group were already laying eggs. The teleogynes in the test group did not lay eggs and remained hypoactive until the confirmation of the death of all those in group 34 after challenge with tickicide. Therefore, it is imperative to evaluate whether there is resistance to tickicide and respect the recommended dosage by the manufacturer when using commercial tickicide on animals.

Keywords: Tickicide, Teleogynes, Resistance.

CHAGAS DISEASE PREVENTION AND CONTROL STRATEGIES IN THE MUNICIPALITY OF SANTA INÊS-BA

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Chagas disease is an endemic parasitic infection that necessitates ongoing surveillance to minimize the incidence of new cases. This study aimed to delineate strategies for the prevention and control of Chagas disease within the municipality of Santa Inês, Bahia. The research is being conducted in Santa Inês, located in the Jiquiriçá Valley in the southwestern region of Bahia. This area has historically been considered a silent zone for Chagas disease, as there were no official reports of Triatominae, the Trypanosoma cruzi vector, until 2022. However, in that year, the first report of a triatomine insect was described, after biting a child in a rural area of the municipality. In response to this incident, the Parasitology Laboratory at the Instituto Federal Baiano - Campus Santa Inês, in collaboration with the Municipal Health Department and the Pathology and Molecular Biology Laboratory of Instituto Gonçalo Moniz/FIOCRUZ, initiated several actions aimed at preventing Chagas disease. To date, the following activities have been implemented: frequent lectures for community health agents and residents; the establishment of a triatomine information hub; improvement of the epidemiological surveillance system; and improvement of housing conditions through efforts by the municipal government (including the plastering of homes). Furthermore, materials such as a book addressing the topic in the region, a scientific article, and an educational pamphlet have been produced. With the surveillance system now operational and prevention strategies in place, it is crucial to intensify focus on human health units and to investigate potential non-human reservoirs to effectively manage and control any emerging cases of the disease.

Keyword: Parasitology, Educational actions, *Trypanosoma cruzi*

LONGEVITY OF RHIPICEPHALUS SANGUINEUS LARVAE

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Rhipicephalus sanguineus is a dog tick that can cause various health problems, including *Babesia* and *Ehrlichia*. The aim was to analyze the longevity of R. *sanguineus* larvae kept in vitro. Teleogens were collected from two two-year-old Blue Heeler dogs that had never been treated with a tick spray, raised in Santa Inês, Bahia, a municipality located in the Jiquiriçá Valley, in the southwest of Bahia. These teleogines were taken to the Parasitology Laboratory at Instituto Federal Baiano - *Campus* Santa Inês. After separating the largest and most active teleogynes, they were washed in running water and placed in Petri dishes to be laid. After laying, the eggs were separated into test tubes sealed with absorbent cotton to allow gas exchange, in order to monitor longevity. Eight days after the teleogynes were placed in the Petri dish to monitor the reproductive cycle, the first eggs were observed, a process that lasted thirty-four days, with daily observations. After this period, the eggs were transferred to a test tube and the larval stage began nine days later. From then on, observations were made every two days, until the larvae reached total mortality, 99 days between the start of hatching and death. The ticks were kept at room temperature throughout the observation period. The longevity of tick larvae is related to the risk of infestation in dogsThe longevity of ticks is directly associated with the risk of infestation, even when the larvae are transferred from the initial site.

Keywords: *Babesia; Ehrlichia*; Teleogynes.

Morphological, histological, and SSU rDNA data of a *Henneguya* species infecting an Amazonian fish of the family Cynodontidae

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Myxozoans are cnidarian endoparasites, mainly found in fish, with some species causing significant diseases. The genus *Henneguya* comprises more than 200 species, most commonly found in freshwater environments. The Amazon Basin covers an area of approximately 6.2 million km² and is one of the most productive ecosystems on the planet, with a vast diversity of fish. Among the fish species found in the Amazon are members of the family Cynodontidae, which are highly prized for sport fishing. In this study, we report the first record of a *Henneguya* sp. infecting the gills of *Cynodon gibbus*, a cynodontid species commonly used as food by riverside dwellers. Local fishermen collected specimens from the Rio Capim River, Pará State, Brazil. Twelve *C. gibbus* specimens were captured alive and examined for myxozoan infections. Parasite analyses were based on morphology, histology, small subunit ribosomal DNA (SSU rDNA) sequencing, and phylogenetic analysis. Cysts of *Henneguya* sp. were observed at the tips of the gill filaments. The myxospores measured 37.2 µm (36.0–39.4) in total length, 10.0 µm (9.4–10.9) in body length, 3.8 µm (3.83–4.34) in width, and 27.0 µm (25.4–29.36) in caudal appendage length. The nematocysts were of equal size, measuring 4 µm (3.43 – 4.47) in length, 1 µm (0.93 – 1.36) in width, and containing tubules with 3 to 4 turns. Histological analyses revealed that the parasite develops in the connective tissue of the gill filaments, causing structural deformation and disorganization of structure of the gill filaments. Phylogenetic analysis showed that *Henneguya* sp. parasite of *C. gibbus* grouped as a sister species to *Henneguya piaractus*, a gill parasite of the characid *Piaractus mesopotamicus*. The combination of morphological, molecular, and biological data allowed us to identify the myxozoan studied here as a putative new species, further increasing the documented biodiversity of Myxozoa in this poorly sampled region.

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Keywords: Cynodontidae, Myxozoa, Histopathology.

PROTEOMIC PROFILING OF THE MITRAL VALVES IDENTIFIES PROTEIN TARGETS ASSOCIATED WITH IMMUNOLOGICAL PATHWAYS IN CHRONIC CHAGAS CARDIOMYOPATHY

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Chronic Chagas Cardiomyopathy (CCC), caused by infection with the intracellular protozoan *Trypanosoma cruzi*, represents the most severe form of Chagas disease. Although CCC primarily affects the myocardium, clinical manifestations of mitral regurgitation suggest functional and structural changes in the mitral valves of affected patients. In this study, we analyzed the protein profile of mitral valves from CCC patients (n=7) using liquid chromatography coupled with mass spectrometry to identify potential targets associated with valvular immunopathology in CCC. We compared these profiles to healthy donor samples (HC, n=6) and those from patients with Idiopathic Dilated Cardiomyopathy (IDC, n=6), a cardiac condition that affects the myocardium and clinically resembles CCC. Our data showed an increased abundance of proteins involved in biological processes related to immune response activation in CCC mitral valves compared to HC. Notable proteins include PYCARD, a molecule implicated in inflammasome pathway activation; IGHG3, an immunoglobulin previously associated with the humoral immune response in Chagas disease; and the transcription factors STAT1, STAT2, and STAT5B, which are linked to cytokine-mediated inflammatory responses. Additionally, gene ontology analysis revealed associations between highly expressed molecules in CCC mitral valves and immune-related biological processes, including the humoral immune response and B cell activation. The protein expression profile observed in IDC was consistent with the less inflammatory nature of the disease; the increased abundance of proteins involved in energy metabolism and the decrease in proteins associated with immune activation may contribute to preventing immune-mediated damage to valve tissue in IDC. Our findings highlight proteins potentially involved in immunological pathways in CCC, providing new insights into the mitral valve as a target for cardiac immunopathology.

Key Words: Chronic Chagas Cardiomyopathy, Immunopathology, Proteomics

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AN IMMUNOCHEMISTRY TECHNIQUE BASED ANTI-*LEISHMANIA*-ACTIVATED C-KINASE MONOCLONAL ANTIBODY FOR CUTANEOUS LEISHMANIASIS DIAGNOSIS

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We developed an immunohistochemistry technique using an anti-*Leishmania*-activated C-kinase monoclonal antibody for the diagnosis of cutaneous leishmaniasis-CL (IHQ anti-LACK). For that, *Leishmania* (*Viannia*) *braziliensis* LACK sequence (A4HGX7_LEIBR) was submitted to epitope prediction and a small gene containing three antigenic regions was constructed and inserted in pET28a. Recombinant *Leishmania*-LACK was produced and used as an immunogen in BALB/c mice for mAb production. The capacity of the anti-LACK mAb to recognize native *Leishmania*-LACK was assayed by Western blotting and IHQ technique, using soluble antigens and histologic sections of skin biopsies from hamsters experimentally infected with *Leishmania (Leishmania) amazonensis, L. braziliensis* and *Leishmania* (*Viannia*) *guyanensis,* main causer parasites of CL in Brazil. In a next step, IHC anti-LACK was validated on skin lesion samples from 105 patients with suspected CL, attending the outpatient clinic of the Municipal Polyclinic of Teófilo Otoni between 2019 and 2020, having PCR as reference test. The performance of IHC anti-LACK was compared to direct examination (DE) and histopathological (HE). The anti-LACK mAb successfully recognized native LACK in soluble antigens and detected amastigotes of the three *Leishmania* spp. in histological sections from experimentally infected hamsters. Moreover, IHC anti-LACK presented sensitivity of 58.2% (CI95%: 45.0 – 70.3), specificity of 98% (CI95%: 89.5 – 99.7), and accuracy of 77.1% (CI95%: 68.2 – 84.1), higher than presented by DE and HE techniques, but there was no significant statistical differences. In a cross-table analysis, the IHQ anti-LACK presented Kappa index of 0.57 (CI95%: 0.40-0.74) and 0.62 (CI95%: 0.456 – 0.787) with the results from DE and HE, respectively. In this initial study the IHC anti-LACK demonstrated applicability for CL diagnosis and further improvement can will enhance diagnostic performance of the IHQ anti-LACK technique.

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Keywords: cutaneous leishmaniasis, laboratorial diagnosis, immunohistochemistry

EVALUATION OF MACROSCOPIC CHANGES, CYTOKINE LEVELS AND PARASITE LOAD IN HETEROLOGOUS PROTECTION MEDIATED BY RECOMBINANT PROTEINS IN HAMSTERS AGAINST VISCERAL LEISHMANIASIS

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The lack of an effective vaccine for the prophylaxis and control of leishmaniases makes them more serious, especially visceral leishmaniasis (VL), due to the severity of its clinical picture. The vaccines with the greatest immunogenic potential are based on the construction of recombinant proteins, which have shown most efficacy in experimental models. In this context, the proteins Enolase and MORN repeat-contaning 1 (MORN1) were evaluated in murine model, showing immunogenic potential and a significant reduction in parasite load in the organs most affected by VL (spleen and liver). Assays on hamsters (*Mesocricetus auratus*) are recommended for a more robust evaluation of these proteins, as this model shares clinical characteristics of the disease observed in dogs and humans. The aim of this study was to evaluate the efficacy of a vaccine composed of recombinant *Leishmania braziliensis* proteins against experimental visceral leishmaniasis in hamsters, focusing on the production in cytokines intracytoplasmic evaluated through of flow cytometry and quantification of parasite load in the animals by qPCR (*Quantitative Polymerase Chain Reaction*). The cellular response indicated an increase in CD4+ cells producing the cytokines IFN-γ and TNF, accompanied by a reduction in IL-10 levels in the immunized groups. Macroscopic analysis of the spleen revealed splenomegaly in the control groups compared to the other experimental groups. These results were associates with a reduction in the hepatosplenic parasite load, particularly in the groups immunized with the complete vaccine compositions (Enolase, MORN1 and Saponine adjuvant). Thus, the results show significant immunogenicity, especially in the groups immunized with the complete vaccine compositions, highlighting the vaccine relevance of the two proteins in a model of susceptibility to VL.

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Palavras-chave: *Leishmania braziliensis*, Enolase, MORN1.

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EPIGENETIC MODIFICATIONS IN *LEISHMANIA AMAZONENSIS*-INFECTED MACROPHAGES: THE ROLE OF THE ENZYME ENHANCER OF ZESTE HOMOLOG 2

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Abstract

Parasites of the Leishmania genus possess an arsenal of molecules that modulate the epigenetic state of the host, and the release of these molecules through Extracellular Vesicles (EVs) may be a strategy to ensure the survival of the parasite. Previous studies have shown that histone deacetylase 1 (HDAC1) plays a crucial role in the epigenetic regulation of macrophages during *Leishmania amazonensis* infection. In addition, the enzyme EZH2, responsible for the repressive epigenetic marker H3K27me3, regulates essential biological processes; however, its role during *L. amazonensis* infection, its relationship with HDAC1 and its impact on pathogenesis remain unknown. Thus, we propose to test the hypothesis that *L. amazonensis* infection induces repressive epigenetic modifications in macrophages, evaluating the role of EZH2 and the contribution of EVs. For this purpose, EVs were purified from *L. amazonensis* promastigotes at 35°C and, by microscopy, we observed the budding of these EVs. The predominant size was around 100 nm, and the concentration of 2.6x10e7 particles/mL was determined by NTA. Using the Western blotting technique, an increase in H3K27me3 was observed in *L. amazonensis* infection and also by treatment with EVs for 5 hours. In a period of 18 hours, it was possible to observe the decrease of this mark. Notably, the expression of EZH2 increased during infection and after treatment with EVs. Inhibition of EZH2 by GSK-343 for 24 hours in THP1 macrophages led to a decrease in the parasite load and the H3K27me3 even after infection. Significantly, that EVs were able to neutralize the inhibitory effect and recover the parasite load. HDAC1 and DNMT1 levels increased upon infection and after EV treatment. It is possible that EZH2 recruits DNA methyltransferases and histone deacetylases to silence genes that allow subversion of the macrophage response. Therefore, our data may provide new insights into the epigenetic modifications that occur after infection.

Supported by: CNPq, FAPERJ.

Keywords: epigenetics, methyltransferase, infection.

HIGHLY SELECTIVE THIAZOLE DERIVATIVES AS PROMISING ANTI-*TOXOPLASMA GONDII* AGENTS

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Toxoplasmosis, caused by the protozoan *Toxoplasma gondii,* is a major global health concern, affecting nearly one-third of the population. While often asymptomatic, the infection can be severe or even fatal in immunocompromised individuals. Current treatment relies on pyrimethamine and sulfadiazine, which present limitations such as potential toxicity, restricted efficacy, and the emergence of resistant parasite strains. To address the urgent need for new therapeutic options, this study investigated the antiparasitic potential of compounds with privileged structures, specifically thiazole derivatives. These compounds offer key advantages over existing treatments, including chemical versatility, distinct mechanisms of action, and the potential for targeted structural modifications, making them promising candidates for drug development. In this work, six 2-pyridyl 2,3-thiazole derivatives previously recognized for their potent antitumor activity were evaluated. Importantly, their anti-*T. gondii* activity had not been reported before. *In vitro* assays were conducted against intracellular *T. gondii* tachyzoites (RH strain) and human foreskin fibroblasts to assess compound selectivity. The half-maximal effective concentration (EC_{50}) ranged from 212 to 490 nM, while the half-maximal cytotoxic concentration (CC_{50}) ranged from 1.5 to 48 µM. Notably, one compound presented a selectivity index (SI) of 183, underscoring their potential as promising candidates for further investigation in the development of novel toxoplasmosis therapies. Future studies will focus on elucidating the mechanism of action of these compounds, optimizing their pharmacokinetic properties, and validating their efficacy in *in vivo* models.

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Keywords: Toxoplasmosis, treatment, drug

Lidocaine Reduces the Proliferation of *L. (L.) amazonensis* Promastigotes through Induction of Oxidative Stress, Damage to the Parasitic Membrane, and Cell Cycle Arrest

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Abstract

Due to the toxicity and increasing failure rates of conventional treatments for tegumentary leishmaniasis (TL), the search for new therapeutic approaches has been strongly encouraged. In this context, we recently investigated the antiparasitic effect of lidocaine (Lid), a local anesthetic of amide class, on *Leishmania (Leishmania) amazonensis* promastigotes and infected human macrophages. We observed a dose-dependent reduction in promastigote proliferation and in the macrophage infection index following treatment with Lid. Currently, we are exploring the mechanisms underlying the antiproliferative effect of Lid. To this end, L. (L.) amazonensis promastigotes were incubated with 0.625 mg/mL of Lid—the maximum non-cytotoxic concentration for human macrophages—for 24, 48, and 72 hours at 26 °C. After incubation, we assessed parasite viability by optical microscopy, as well as the production of reactive oxygen species (ROS), membrane integrity, and the distribution of promastigotes across cell cycle phases (SubG0/G1, G0/G1, S, and G2/M) using flow cytometry. Our results demonstrated that Lid prevented an increase in the number of viable parasites at all analyzed time points, confirming its antiproliferative effect. Furthermore, treated parasites exhibited a sustained increase in ROS production throughout incubation. We also observed a higher frequency of parasites with compromised membrane integrity at 48 and 72 hours. Finally, cell cycle analysis revealed that most promastigotes remained in the G0/G1 phase, suggesting that Lid blocks cell cycle progression. Thus, we conclude that the antiproliferative effect of Lid results from the induction of oxidative stress in the parasites, leading to reduced viability in part of the parasite population and inhibition of cell division in the remaining ones. These findings are promising and indicate that Lid may represent a potential therapeutic alternative for TL. However, additional studies are necessary to validate its efficacy and safety in *in vivo* models.

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Key Words: Lidocaine, Leishmania, Proliferation

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TRYPANOSOMA MINASENSE, T. CRUZI AND *T. RANGELI* INFECTING GOLDEN LION TAMARINS (*LEONTOPITHECUS ROSALIA*) (MAMMALIA; PRIMATES) FROM THE ENVIRONMENTAL PROTECTED AREA OF BACIA DO RIO SÃO JOÃO, RIO DE JANEIRO STATE.

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Abstract

Endemic to the Atlantic Forest, the golden lion tamarin (GLT, *Leontopithecus rosalia*) is an international symbol of conservation and described as a reservoir of *T. cruzi* since 1990, based on the molecular characterization of parasites isolated in blood cultures. The possibility of performing molecular diagnosis in blood clots without parasite isolation may reveal previously undescribed *Trypanosoma* species in these primates. The study aims to describe trypanosomatid infections in blood clots of GLTs collected in Bacia do Rio São João, a protected area of Rio de Janeiro state. Blood clot samples (n=133) from 98 GLTs were collected between 2019 and 2021, including recaptures (2 to 5 sampling events) at intervals ranging from 3 to 26 months. DNA was extracted (DNeasy Blood and Tissue - Qiagen®), measured (Qubit®, Invitrogen) and the diagnosis performed through Nested-PCR using the 18S rDNA target. Positive samples were subjected to Sanger sequencing and the sequences obtained were analyzed and compared with GenBank sequences, considering 99% as the minimum percentage of identity and coverage for taxonomic identification. Mixed infections were considered when two parasite species were identified in two capture events or when two electrophoresis bands from the same capture event were sequenced separately. We observed 50 GLTs positive for trypanosomatids (51%; 50/98), totaling 65 positive samples (48.9%; 65/133). *Trypanosoma minasense* was the most prevalent parasite infecting GLTs (41.8%; n=41), followed by *T. cruzi* (6.1%; n=6) DTUs TcII (n=5) or TcIV (n=1) and *T. rangeli* lineage D (1%; n=1), including two mixed infections by *T. minasense* and *T. cruzi* TcII. This is the first description of *T. rangeli* in GLT and mixed *Trypanosoma* infections in this species. The finding of *T. minasense* in three capture events of the same individual over a 26-month interval and of *T. cruzi* in another GLT for up to 16 months suggests the long-term maintenance of these infections in nature.

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Keywords: Trypanosomatids, blood clots, golden lion tamarin.

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Repurposing Memantine: A Novel Therapeutic Strategy for Leishmaniasis

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Abstract

Leishmaniasis is a neglected tropical disease caused by Leishmania spp. Its treatment comprises a limited selection of drugs, often associated with therapeutic failure, high toxicity, elevated costs, and the emergence of resistance. To overcome these challenges, combination therapy and drug repurposing have emerged as promising alternatives. In this context, this study evaluated the efficacy of memantine (MEM), a drug currently used to treat Alzheimer's disease, for drug repurposing as a monotherapy and in combination with reference drugs for leishmaniasis treatment, and its potential immunomodulatory effects. *Leishmania infantum* promastigotes were incubated to varying MEM concentrations for 24 and 72 h, leading to a concentration-dependent inhibition of cell viability. Against intracellular amastigotes, MEM reduced the infection index in a concentration-dependent manner after 72 h, with an IC_{50} of 5.49 µM, while exhibiting no toxicity to macrophages. When combined with miltefosine, MEM demonstrated an additive effect in both evolutionary forms, with a fractional inhibitory concentration index (FICI) of 1.67 and 1.38 for promastigotes and intracellular amastigotes, respectively. In a murine model of visceral leishmaniasis, BALB/c mice were infected with *L. infantum* promastigotes for 7 days and subsequently treated for 5 days with different doses of MEM, meglumine antimoniate, or vehicle control. Two experimental approaches were employed: mice were euthanized either immediately after treatment (short-term) or 18 days later (long-term). MEM reduced liver parasite load by 99.9% in both experimental approaches compared to the control group. Serological toxicity markers showed no significant alterations, suggesting MEM's safety. Additionally, immunological assays revealed its immunomodulatory potential. These results suggest memantine as a promising candidate for drug repurposing in leishmaniasis chemotherapy.

Supported by: FIOCRUZ, IOC, CAPES, CNPq, FAPERJ.

Keywords: Repurposing, visceral leishmaniasis, combined treatment, immunomodulatory effect.

Investigating the impact of Leishmania mexicana NAT10 acetyltransferase in gene expression and chromatin structure regulation

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Leishmania spp. is a parasite that causes leishmaniasis and has a complex life cycle, requiring sophisticated gene regulation mechanisms to adapt to different environments. *Leishmania* primarily regulates gene expression at the post-transcriptional level, but epigenetic factors and chromatin organization may also influence this process. The N-acetyltransferase NAT10 is responsible for adding acetylation in citidine in RNA molecules (ac4C) and also has a role in processes like modulating DNA accessibility,influencing gene expression. In this study, we investigated the role of NAT10 in chromatin structure and cellular responses in *Leishmania mexicana* through functional and omics-based approaches. Functional complementation assays using the *Saccharomyces cerevisiae* model revealed partial phenotypic complementation. *In vitro* activity assays with recombinant wild-type and mutant proteins confirmed its acetyltransferase activity. Phenotypic analyses of *L. mexicana* strains overexpressing wild-type and mutant NAT10 showed that the presence of the mutated gene led to reduced proliferation and morphological alterations. Electron microscopy images revealed higher chromatin compaction in the wild-type strain compared to the NAT10 single knockout. Additionally, ATAC-seq and RNA-seq experiments were performed, showing that in strand switch regions, chromatin is more accessible in the wild-type strain (T7/Cas9) compared to the NAT10 single knockout. RNA-seq analysis comparing the wild-type and NAT10-mutant overexpressing strains revealed that the most affected genes are involved in post-transcriptional regulation and RNA processing, chromatin organization and epigenetics, DNA repair, autophagy and protein degradation, as well as reduced metabolism and transport. The results of this study will complement ongoing research on this parasite and contribute to characterizing the role of NAT10 in *Leishmania* biology, contributing to our understanding of how it regulates gene expression.

Keywords: *L. mexicana*; chromatin structure, gene expression.

Impact of Dexamethasone on the Conversion of *T. gondii* within Neural Cells

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Toxoplasma gondii is an obligatory intracellular parasite and the causative agent of toxoplasmosis. The parasite exhibits two distinct infectious forms within hosts: tachyzoites, present in the acute phase of infection, and bradyzoites, which form tissue cysts in the chronic phase of infection. The EGS strain, isolated from amniotic fluid in MG, exhibits a recombinant genotype, both reflecting a phenotype of acute infection and the ability to form cysts spontaneously. Dexamethasone (DEX) is an immunosuppressive drug that can induce changes in lymphoid organs and, in patients chronically infected with *T. gondii*, the reactivation of the acute phase. This study aims to evaluate the conversion of the EGS strain *in vitro*. Initially, we analyzed the spontaneous or alkalinization-induced encystment of *T. gondii* in mouse neural cell lines. Neuro-2a cells were plated and infected with EGS strain at a MOI: 3, cultured in a medium at neutral or pH 8.0. Subsequently, they were fixed at 96-216 hours p.i. and incubated, with DBA-FITC (cyst wall marker), SAG-1 (tachyzoites maker) and BAG-5 (bradyzoite marker). We identified the predominance of bradyzoites mainly in samples cultured at alkaline pH. The effect of DEX [10μM] on the expression of stage-specific genes, SAG-1 and BAG-1, and on the egress of *T. gondii* was also evaluated. In the preliminary RT-qPCR results, no significant alterations were identified between the treated and untreated samples. However, the increase of extracellular parasites amount in the treated samples suggests that DEX induced the egress of the parasite from the host cell. Taken together, our early data suggest that alkalinization of the medium promoted an increase in the conversion to bradyzoites in Neuro-2a. In addition, the egress of *T. gondii* promoted by DEX treatment may provide clues about the cellular mechanism of action of DEX in the reactivation of the infection.

Supported by CAPES, CNPq and FAPERJ.

Keywords: *Toxoplasma gondii*, Encystment, Dexamethasone

INTRADERMAL VACCINATION WITH A CHIMERIC VACCINE TRIGGER POTENT HUMORAL AND CELLULAR IMMUNE RESPONSES AGAINST VISCERAL LEISHMANIASIS

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Canine visceral leishmaniasis (CVL) is considered a significant public health issue that is difficult to control due to the high prevalence of asymptomatic infected dogs and its expansion in Brazilian urban centers. Additionally, there is currently no licensed vaccine for visceral leishmaniasis in humans and the only available veterinary vaccine licensed in Europe is considered unsuitable for use in humans. Thus, developing an immunogenic, effective, and safe vaccine for prophylactic use is essential as an alternative for disease control. Therefore, we aimed to evaluate the immunogenicity of a chimeric vaccine adjuvanted with Hiltonol (ChiA/Hiltonol) as a candidate against VL. BALB/c mice received three doses intradermally of ChiA/Hiltonol using microneedles, with 15-day intervals between doses. Humoral (IgG, IgG1, and IgG2a) and cellular (cytokines-producing T cells) responses were assessed 15 days after the third immunization. We observed an increase of serum immunoglobulins IgG and IgG1 subclass anti-ChiA following vaccination with both ChiA and ChiA/Hiltonol. Moreover, ChiA/Hiltonol also triggered the IgG2a subclass production, suggestive of a balanced T_H1/T_H2 response. ChiA/Hiltonol vaccination led to increased IL-2, TNF, and IFN-γ-producing $CD4^+CD44^+$ and $CD8^+CD44^+$ T cells. It is known that IgG subclass switches are regulated by interaction with CD4 T cells. In mice, a T_H1-associated cytokine such as IFN-γ influences IgG2a subclass secretion. Spearman's *r* test reveals a strong positive correlation between IgG2a production and the percentage of IFN-γ-producing $CD4^+CD44^+$ T cells, suggesting that class switch is highly related to vaccination regimen with Chi-A/Poly ICLC. Our findings highlight the potential of the chimeric vaccine adjuvanted with the Hiltonol to induce protective immunity, with emphasis on the production of pro-inflammatory cytokines, TNF and IFN-γ, and IgG2a subclass.

Keywords: Visceral leishmaniasis; Vaccine; Protective immunity.
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CONGENITAL TRANSMISSION OF *Trypanosoma cruzi* (TcIII) IN THE CHRONIC PHASE IN AN EXPERIMENTAL MODEL

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Abstract

Trypanosoma cruzi, the etiological agent of Chagas disease, is primarily transmitted by triatomines. However, vertical transmission is epidemiologically significant as it can occur in both the acute and chronic phases of the infection. The chronic phase is characterized by a positive conventional serology and subpatent parasitemia. The aim of this study was to detect vertical transmission in the offspring of Swiss mice (*Mus musculus*) females in the chronic phase of *T. cruzi* infection. In this way, seven females mice (8 weeks old) were infected with 1×10^4 blood trypomastigotes of the KK (TcIII) isolate of *T. cruzi* and after 35 days, they were mated with healthy male breeders in a harem system (one male to two females) for seven days. The animal's ´parasitemia was monitored by direct examination using the Pizzi-Brener technique on blood samples from the females for 35 days, and in the offspring, it was observed until adulthood (60 days). Parasitemia was detected in 100% of females (7/7) from the fourth to the thirteenth day post-infection (DPI), and became subpatent after the fourteenth DPI. All females produced an average of 8.4 (59 in total) offspring per female. Of these, vertical transmission was detected in 1.69% (1/59) of the offspring. The parasitemia of this pup persisted until adulthood (52 days), with a peak parasitemia of 1,060 parasites/5µL on the 22nd day of life. These data suggest the ability of vertical transmission of *T. cruzi* (TcIII) by females in the chronic phase of the infection, emphasizing the need for early diagnosis in pregnant women and newborn.

Supported by: CNPq, CAPES
Keywords: American trypanosomiasis. Vertical transmission. Genotype-TcIII

A CHIMERIC VACCINE ADJUVANTED WITH POLY-ICLC ELICITS A ROBUST IMMUNE RESPONSE AND PROTECTION AGAINST EXPERIMENTAL VISCERAL LEISHMANIASIS

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Over the past years, several studies have been carried out to achieve an ideal vaccine for visceral leishmaniasis (VL). Many *Leishmania* antigen candidates have been purified and evaluated based on the development of a T_H1-type immune response and the induction of a protective response against *Leishmania* infection. The design of leishmaniasis vaccines has been constantly advancing. The biotechnology applied to vaccinology is a suitable approach that optimizes data analysis and computational recognition of vaccine targets. The ability to predict T cell-specific epitopes makes immunoinformatic an even more necessary approach, as in VL an efficient immune response against the parasite is triggered by T cells in response to *Leishmania spp*. immunogenic antigens. Vaccine adjuvants can be added to improve the immunogenicity and efficacy of vaccines against VL. Poly-ICLC is a synthetic adjuvant that has been successfully tested for safety and immunogenicity in phase I clinical trials in patients with various types of cancer. Using Poly-ICLC may contribute to the development of vaccines against human VL (HVL). The present study aimed to evaluate the Chimera A adjuvanted with Poly-ICLC (Chi-A/Hiltonol) as a vaccine candidate against VL. BALB/c mice were vaccinated with 3 doses subcutaneously with 15-day intervals between doses. Immunogenicity and efficacy were assessed 45 days after the *L. infantum* challenge. Our results revealed that chimera can elicit T cells producing T_H1-type cytokines in the vaccinated mice's spleen. Vaccination led to increased IL-2, TNF, and IFN-γ-producing $CD4^+CD44^+$ and $CD8^+CD44^+$ T cells and reduced IL-10-producing $CD4^+$ and $CD8^+$ T cells, reinforcing the establishment of T_H1 response. Lastly, ChiA/Hiltonol vaccinated mice exhibited a remarkable reduction in the splenic and hepatic parasite burden. Our candidate shows promise against VL and could be considered for further investigations in phase I and II clinical trials in dogs and/or humans.

Keywords: Visceral leishmaniasis; Vaccine; Efficacy.
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Survey of the occurrence of *Sarcocystis* spp. in cattle from the state of São Paulo, Brazil

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Sarcocystosis is a disease of Public Health relevance and of great economic importance. However, in Brazil, studies on the occurrence of *Sarcocystis* spp. in bovines are scarce, with few reports in the literature. Given the above, we aimed to survey the occurrence of *Sarcocystis* spp. in cattle from the state of São Paulo, Brazil. A total of 336 samples (esophagi and hearts) from 168 male, aged between 18 and 30 months, from four municipalities (Araçatuba, Monções, Ouro Verde and Pongaí) in the state of São Paulo, Brazil, were investigated in this study. The parasitological investigation of *Sarcocystis* spp. was performed by scarification of the muscles. The prevalence with a 95% confidence interval was calculated using the Wilson score method and the diagnostic agreement between the evaluated samples was obtained using Cohen's Kappa test. Of the animals investigated, 150 were positive, resulting in an occurrence of *Sarcocystis* spp. in 89.29% (CI 83.70% - 93.11%). Regarding the samples analyzed, we observed a higher occurrence of sarcocysts in the cardiac muscle 140/168 (83.33% [CI 76.97% - 88.21%]) when compared to the esophageal muscle 119/168 (70.83% [CI 63.56% - 77.18%]), being statistically significant (P = 0.0092). Cohen's kappa test (0.324) demonstrated reasonable agreement (76%) between the positivity and negativity of the samples used in the diagnosis of bovine sarcocystosis. Of the four properties evaluated in the municipalities of Araçatuba, Monções, Ouro verde and Pongaí, the occurrence of *Sarcocystis* spp. was, respectively, 95% (CI. 76.39% - 99.11%), 100% (CI. 95.47% - 100%), 77.78% (CI. 61.92% - 88.28%) and 70.97% (CI. 53.41% - 83.90%). In this study, we identified *Sarcocystis* spp. in 89.29% of the cattle investigated, with an infection rate of 100% in a herd in the state of São Paulo, Brazil. The high percentage of tissue cysts, mainly in bovine esophageal fragments, may suggest infections caused by *Sarcocystis hominis*, which has zoonotic potential. However, molecular studies will still be performed to confirm the species, but it is worth highlighting that only the macroscopic diagnosis of Sarcocystosis is carried out by federal inspection services.

Keywords: slaughterhouse, protozoosis, diagnosis.

CHARACTERIZATION OF A CHIMERIC RECOMBINANT PROTEIN BASED ON THE VACCINE CANDIDATES PvCyRPA, PvRipr AND PvGAMA AND EVALUATION OF ITS POTENTIAL AS A VACCINE CANDIDATE AGAINST ERYTHROCYTIC FORMS OF *P. vivax*.

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P. vivax is one of the most difficult *Plasmodium* species to eliminate and represents a major public health challenge, especially in countries like Brazil. In this scenario, the development of vaccines with chimeric recombinant proteins can contribute to malaria control. However, specific *P. vivax* antigens under investigation are still scarce. The proposal of this work is to develop a chimeric recombinant protein containing multiple blood-stage epitopes selected from molecular, biochemical and immunological aspects of PvCyRPA, PvRipr and PvGAMA proteins. Therefore, plasma and PBMCs from 300 Brazilian Amazon individuals with previous malaria episodes were used in ELISA (IgM, IgG and subclasses) and ELISPOT (INF-γ) assays. *In silico* prediction of B and T cell epitopes was performed in all proteins. The results showed that PvCyRPA and PvRipr are naturally immunogenic (22.2% and 35% of individuals with IgM antibodies, 56.7% and 44% of individuals with IgG antibodies against recombinant proteins, respectively). PvCyRPA had a higher frequency of IgG1 responders (70.6%), while PvRipr had similar frequencies of IgG1, IgG2 and IgG4 (46%, 44% and 50%, respectively). A total of 7 B cell epitopes were found in PvCyRPA, 4 epitopes in PvRipr and 3 epitopes in PvGAMA. The most promising epitopes were T312-G330-PvCyRPA (39.2% of responders), K879-G888-PvRipr (47%) and P497-T505-PvGAMA (26%). A total of 10 T cell epitopes were identified in PvCyRPA and used in Elispot assays. Pools containing E306-K320 were able to induce more INF-γ SFUs in the exposed group (43 and 29.75/250,000 cells for pools 4 and 5, respectively). In PvCyRPA the most promising B and T epitopes overlapped in 9 amino acids. As a preliminary conclusion, the proteins have already confirmed epitopes, inserted in promising regions in other *Plasmodium* species. The observed humoral and cellular immune responses reinforce their potential as vaccine candidates, especially as a chimeric recombinant blood-stage protein.

Keywords: *P. vivax,* recombinant chimeric protein and vaccine.

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Benzothiazole Derivatives as ligands of *Leishmania braziliensis* Heat Shock Protein 83 (*Lb*HSP83)

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Introduction: Cutaneous leishmaniasis (CL) is a neglected tropical disease caused by *Leishmania braziliensis*, with an estimated 700,000 to 1 million new cases reported annually. Current treatment options suffer from high toxicity and limited efficacy, underscoring the urgent need to develop novel drugs and treatment strategies. The molecular chaperone heat shock protein 83 (Hsp83) plays a critical role in the survival of *Leishmania* species by regulating key processes during phase transitions in the parasite's life cycle. **Hypothesis**: Structural modifications of benzothiazole derivatives, known inhibitors of human Hsp90, can yield ligands for *L. braziliensis* Hsp83 (*Lb*Hsp83). **Objective**: To identify potential inhibitors of *Lb*Hsp83. **Methods**: Recombinant full-length (*Lb*Hsp83) and N-terminal (*Lb*Hsp83N) *L. braziliensis* Hsp83 were expressed and purified using chromatographic methods. These proteins were then used in Differential Scanning Fluorescence (DSF) assays with 3 benzothiazole derivatives. **Results**: At a concentration of 500 µM, three compounds caused a significant ($p < 0.05$) ΔTm shift (-0.6 to -3.0 °C) in *Lb*Hsp83. For *Lb*Hsp83N, the ΔTm shift ranged from -0.4 to -2.31 °C. The most promising compound, N-(benzo[d]thiazol-2-yl) cyclohexane carboxamide (TJL068), exhibited a concentration-dependent response for both *Lb*Hsp83 and *Lb*Hsp83N, with a greater shift observed for *Lb*Hsp83 (ΔTm: -3.0 °C) compared to *Lb*Hsp83N (ΔTm: -1.9 °C). This result showed that TJL068 binds to the C-terminal domain of Hsp83, consistent with the binding behavior observed in other benzothiazole derivatives.. Notably, TJL068 also demonstrated potent leishmanicidal activity against *L. infantum* amastigotes with an effective concentration (EC50) of 5.5 µM. It exhibited high selectivity in cytotoxicity tests, with a concentration causing 50% cell death (CC50) in THP-1 cells at 203 µM, yielding a selectivity index of 37.. **Conclusion**: The leishmanicidal efficacy of TJL068 appears to be mediated through its binding to *Lb*Hsp83. Ongoing studies aim to further delineate the compound's effects on enzyme kinetics and explore potential therapeutic applications.

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Keywords: *Leishmania braziliensis*, Heat Shock Proteins (HSP), potential inhibitors

EXPRESSION AND PURIFICATION OF *Cryptosporidium parvum* COWP1 RECOMBINANT PROTEIN IN *Escherichia coli*

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Recombinant proteins are potential candidates for the development of new drugs, vaccines and new diagnostic and treatment methods, being obtained by inserting the target gene into expression vectors, which are molecularly cloned in heterologous systems to be translated and be produced in large quantities, thus improving sensitivity in diagnostic tests. Normally, its production is carried out in *Escherichia coli* expression systems, allowing the rapid expression of heterologous genes. The use of IgY is still poorly explored, having advantages such as: large quantity production, high affinity and specificity of antibodies and lower cost, among others. Oocyst wall proteins (COWP) are a group of proteins that are in the wall of oocysts and are involved in their structure and resistance under environmental conditions, which makes them a potential target for cloning in *E. coli* heterologous expression system. Thus, the development of diagnostic methods using chicken IgY and using the COWP1 protein as a template provides the design of a promising diagnostic tool, in addition to expanding knowledge for future studies. The full-length nucleotide sequence of *C. parvum* COWP1 gene was synthesized in PUC57 plasmid by GenScript (USA). The gene sequence was inserted, cloned and expressed in *E. coli* M15 (pREP4). After the expression, products were submitted fo SDS-PAGE 16%, for viewing induced and non-induced cultures. After the protein purification was performed by affinity chromatography using Hi-Trap HP column for ÄKTA™ Pure System. The recombinant COWP1 resulting product can be useful for antibodies production and developing diagnostic assays to monitor cryptosporidiosis outbreaks.

Keywords: *Cryptosporidium*, recombinant protein, COWP.

NOVEL INDOLE-BASED MARINOQUINOLINES AS PROMISING CANDIDATES AGAINST *TOXOPLASMA GONDII*

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Toxoplasma gondii, an obligate intracellular parasite, is widely distributed globally, with higher prevalence in warm and humid regions. This protozoan, the causative agent of toxoplasmosis, infects approximately 30% of the population, representing a significant public health challenge. The main drugs used to treat the disease, such as pyrimethamine and sulfadiazine, have notable limitations, acting only during the acute phase of infection and often causing allergic reactions and severe side effects. Marinoquinolines (MQs), a class of compounds originally isolated from marine microorganisms, have demonstrated significant pharmacological potential, including anti-*T. gondii* activity in both *in vitro* and *in vivo* studies. Given the limited therapeutic options for toxoplasmosis, this study aimed to explore new derivatives with inhibitory activity against the parasite. Sixteen novel synthetic MQs were evaluated, distinguished from previously reported compounds by the incorporation of indole derivatives linked to the marinoquinoline scaffold. The effective concentration (EC50) against intracellular *T. gondii* tachyzoites (RH strain) and cytotoxic concentration (CC50) against human foreskin fibroblasts (HFF) were determined for all derivatives. Selectivity indexes (SI) were calculated as the CC50/EC50 ratio. Among the compounds, one derivative stood out with an SI value of 170. Additionally, two derivatives (MQ-262 and MQ-263) exhibited SI values greater than 60, along with favorable ADMET predictions, including high gastrointestinal absorption, blood-brain barrier permeability, and no predicted toxicity. These findings highlight indole-based MQs as promising candidates for further evaluation in experimental models of toxoplasmosis, suggesting their potential as an effective treatment for this significant parasitic disease.

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Keywords: Toxoplasmosis, treatment, drug discovery.

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INVESTIGATING *LPG2* FUNCTION IN DENDRITIC CELL MIGRATION DURING *LEISHMANIA INFANTUM* INFECTION

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Abstract

Leishmaniasis is a zoonotic disease caused by *Leishmania* protozoa and can manifest in various clinical forms. The dissemination of the cells containing *L. infantum* are crucial for the parasite survival and establishment of the disease. Studies suggest that *Leishmania* lipophosphoglycan (LPG) plays a key role in host cell interactions; however, its relationship with dendritic cells (DCs) remains unclear. The objective of this work is to evaluate the role of *lpg2* in migration of human DCs infected with *L. infantum.* To investigate these interactions, human DCs were infected with wild-type *L. infantum*, Δ*lpg2,* or Δ*lpg2* + *lpg2* and analyzed using a Transwell system. Immunostaining was performed with antibodies against pFAK and pPaxillin to assess adhesion complex formation, Talin and Vinculin to evaluate podosome formation, and Rac1, Cdc42, Gelsolin, RhoA, and phalloidin to examine actin polymerization via confocal microscopy. CCR7 expression in DCs infected with different *Leishmania* strains was assessed by flow cytometry. Additionally, ERK1/2 pathway activation was evaluated by analyzing ERK1/2 expression using western blotting.The results showed that DCs infected with *L. infantum* Δ*lpg2* exhibited reduced migration compared to those infected with wild-type *L. infantum* or Δ*lpg2* + *lpg2*. This data was associated with the reduction in the formation of adhesion complexes, podosome and actin polymerization. Additionally, lower CCR7 and ERK1/2 expression was observed in DCs infected by *L. infantum* Δ*lpg2* compared to cells infected with the other strains. The data presented in this study suggest that *L. infantum* LPG modulates positively migration of DCs by enhancing adhesion complexes formation, podosome assembly, actin dynamics, as well as CCR7 expression and ERK1/2 pathway activation. Further experiments are needed to better elucidate the mechanisms by which LPG regulates DCs infected with *L.infantum.*

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Keywords: *Leishmania*, dendritic cells, migration.

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TISSUE DISTRIBUTION OF A MULTIDRUG RESISTANT STRAIN OF *Trypanosoma cruzi* IN THE FACE OF BENZNIDAZOLE TREATMENT

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Chagas disease is a major medical and social problem that affects approximately 7 million people. The reference drug for the treatment of this parasitosis, benznidazole (Bz), has an unsatisfactory toxicity and efficacy profile, resulting in therapeutic failures of up to 80%, particularly in the chronic phase of the disease. To understand the dynamics of the resistance mechanism, this study evaluated the tissue distribution of a Bz-resistant *T. cruzi* strain and the impact of treatment with this drug on parasitism maintenance and intensity. Four-week-old BALB/c mice were inoculated with blood trypomastigote forms of the Colombian strain. On the 10th day after infection, after confirmation of parasitemia, treatment was initiated orally, with a daily dose of 100 mg/kg of Bz for 5 days (n=6). Infected and untreated mice (n=9) and uninfected mice (n=3) were used as controls. The animals were euthanized 24 hours after the last dose, and several organs and tissues were collected. The samples were subjected to genomic DNA extraction using a commercial kit, and the parasite load was quantified by *qPCR*, expressed as parasite equivalents/25 ng of DNA (*pe*). The results showed a wide strain distribution, with higher parasitism in skeletal muscle and adipose tissue (~200 *pe*), followed by the heart (76 *pe*). Less than 10 *pe* were detected in the spleen, liver, lungs, and kidneys; the brain and small intestine had lower parasitism concentrations (<1 *pe*). Bz significantly reduced infection in all organs, particularly in the heart, where a 2000-fold reduction in the parasite load was observed. No parasite DNA was detected in the lungs of the treated animals. The data suggest that adipose tissue is an important niche for the Colombian strain in the acute phase of infection and point to differential activity of Bz in different tissues under the experimental conditions used.

Supported by: CNPq, CAPES, UNIFAL

Keywords: Chagas disease, drug resistance, parasite load.

Arachidonic Acid-Derived Eicosanoids in Visceral Leishmaniasis: Imbalance Between HETEs and Prostaglandins

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Visceral leishmaniasis (VL) is characterized by hepatosplenomegaly and a high parasite burden in these organs. Eicosanoids have been implicated in the regulation of inflammation, a hallmark of the disease. This study aimed to evaluate eicosanoid production during chronic VL in *Mesocricetus auratus* (Golden Syrian hamster). To achieve this, hamsters were infected via intraperitoneal injection with *Leishmania infantum* and analyzed five months post-infection. Parasite load in the spleen and liver was assessed by qPCR, while arachidonic acid (AA) -derived eicosanoids were quantified in tissue extracts using LC-MS/MS. These findings were correlated with clinical, laboratory, and histopathological parameters. Chronic VL in hamsters resembled human disease, presenting with splenomegaly, increased creatinine levels, and elevated liver transaminases. Histopathological analysis revealed granuloma formation, white pulp hypoplasia, and portal infiltrates in the spleen and liver. AA mobilization was elevated in these organs but remained unchanged in plasma. Among AA-derived eicosanoids, hydroxyeicosatetraenoic acids (HETEs) were significantly increased in the spleen but not in the liver or plasma. In contrast, prostaglandins PGE_2, 2-keto-PGE_2, and PGD_2 were reduced in the spleen. Notably, splenomegaly, elevated HETEs, and decreased PGE_2 levels correlated with parasite load, suggesting that *L. infantum* modulates these mediators in vivo. Our results indicate that *L. infantum* alters the local balance between HETEs and prostaglandins, fostering an inflammatory environment that may contribute to parasite persistence. The predominance of HETEs over prostaglandins seems to play a central role in VL progression. Further research is needed to clarify the mechanisms by which HETEs influence the pathogenesis of the disease.

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Key words: Visceral Leishmaniasis, eicosanoids, HETEs.

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SYNERGISTIC STRIKES: A PROMISING RATIONAL DRUG COMBINATION AGAINST *Leishmania amazonensis* AMASTIGOTES

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Drug combinations offer a strategic approach to cutaneous leishmaniasis treatment by targeting multiple pathways to enhance efficacy and selectivity while reducing toxicity and resistance. Here, we evaluated the *in vitro* activity of POD-1 combined with MB, NMB, and its derivates, NMB-B and NMB-P (under patent secrecy) against *ex vivo* amastigotes of *Leishmania amazonensis* (LTB0016 strain, 10^6 parasites/mL) searching for synergistic effects. Assays began from the $IC_{25,50,75}$ values of isolated compounds, followed by 24-hour exposures to fixed-ratio combinations (4:1; 3:2, 2:3, and 1:4) to determine the ICs within the combinations. FIC at 25%, 50%, and 75% were calculated, along with their mean sums ($\overline{x}\Sigma FIC_{25,\ 50,\ and\ 75}$), to classify interactions as synergistic (≤ 0.5), additive (> 0.5 and ≤ 4.0), or antagonistic (> 4.0). All combinations exhibited dose-dependent efficacy, with 81% inducing > 80% mortality and 50% reaching complete inhibition at maximum concentrations. ICs values decreased up to 26-fold compared to isolated compounds, with 90% ≤ 5 µM, 71% submicromolar, and 13% nanomolar. POD-1:NAM-P showed ICs of 0.02 µM for POD-1 (1:4 ratio) and 0.04 µM for NMB-P (4:1 ratio). Combinations significantly reduced POD-1's IC_{75} ($p < 0.05$). FIC analysis indicated 60% synergy, 40% additive effect, and no antagonistic, with POD-1:NMB-P exhibiting 92% synergy, including FIC values < 0.1 µM, a pattern also observed for POD-1:MB and POD-1:NMB. The $\overline{x}\Sigma FIC$ revealed 58% synergistic combinations, 42% additive, and no antagonism, highlighting POD-1:NMB-P (67% synergy), similar to POD-1:NMB. Results underscore the potential of synergistic combinations, especially POD-1:NMB-P, to enhance efficacy at nanomolar concentrations against clinically relevant *Leishmania* form, supporting further investigations.

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Keywords: cutaneous leishmaniasis; drug repurposing; synergistic leishmanicidal effects

COMPARING FOUR DNA EXTRACTION METHODS FOR FIELD-READY MOLECULAR DETECTION OF *Leishmania* USING THE COLORIMETRIC LAMP-BASED LeishID TEST

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Abstract

Early diagnosis of leishmaniasis is crucial for disease control. The loop-mediated isothermal amplification (LAMP) method has shown great potential for point-of-care (PoC) molecular diagnostics. However, the success of the diagnosis relies on obtaining high-quality DNA, making DNA extraction a critical step. In this study, we evaluated 4 different DNA extraction methods, followed by LAMP on 84 samples from various tissues of small mammals, including liver, skin, tail tip, and ear tip. The extraction protocols used were: (i) Wizard® Genomic DNA Purification Kit, (ii) SwiftX™ DNA Kit, (iii) non-commercial boiling method, and (iv) commercial DNA extraction reagent. LAMP reactions were performed using WarmStart colorimetric master mix (NEB) that turns from pink to yellow when positive. The amplified products were also analyzed by agarose gel electrophoresis. According to the 260/280 and 260/230 absorbance ratios, the DNA obtained by method (i) showed the best quality, with values ranging from 1.1-2.9 and 0.7-3.1, respectively. Method (ii) had ratios of 0.5-1.5 and 0.2-0.8, method (iii) ranged from 1.3-1.9 and 0.4-1.3, and method (iv) had ratios of 0.9-1.6 and 0.2-0.8. Method (i) led to better DNA quality, but with a lower yield, while method (ii) had lower DNA quality and the second highest yield. Method (iii) was the most promising, offering good yield and good purity. Method (iv) had the highest yield but lower quality compared to methods (i) and (iii). Method (iii) resulted in correct amplification, suggesting that this method is promising for PoC diagnosis of leishmaniasis. Based on this, we propose a field-deployable diagnostic solution. The boiling extraction and amplification reaction will be carried out on the miniPCR device. This will be part of a portable laboratory where all equipment and supplies will fit inside a Pelican-like rigid case, enabling point-of-need molecular tests. The proof-of-concept is being performed at a veterinarian clinic.

Keywords: DNA extraction, LAMP, *Leishmania*
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REFINING PRECLINICAL EVALUATION IN CHAGAS DISEASE: PROLONGED TREATMENT MODEL FOR FEXINIDAZOLE

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Therapeutic failures observed in clinical studies with drug candidates for Chagas disease highlight the need to refine preclinical evaluation strategies to identify models with greater translational value. Fexinidazole, a candidate with excellent antiparasitic activity and tolerability demonstrated in preclinical models of *T. cruzi* infection, resulted in alarming rates of hepatotoxicity and neutropenia in up to 30% of patients when evaluated for the treatment of chronic Chagas disease. In this context, this study aimed to assess the toxicity profile of fexinidazole and benznidazole (reference drug) in prolonged *in vitro* treatment models. HepG2 cells were incubated for 192 hours in the presence of 0.25mM to 2mM of benznidazole (Bz) and fexinidazole (Fx-sulfone). The culture medium was refreshed every 48 hours, and after 96 and 192 hours of incubation with the drugs, the cells were quantified by flow cytometry. The proliferation of treated and control cells (incubated with DMSO) was measured by calculating the CPD (cumulative population doubling). Results showed that cell proliferation was maintained during the first 96 hours of treatment, although significantly reduced in the presence of Fx-sulfone starting at 1mM and Bz starting at 0.5mM. However, a pronounced reduction (Fx-sulfone at 1mM and Bz at 0.5mM) or complete interruption of proliferation (Fx-sulfone at 2mM and Bz at 1mM and 2mM) was observed at 192 hours. These findings highlight the importance of exploring alternative models for evaluating the cytotoxicity of trypanocidal drugs.

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Keywords: Chagas disease; fexinidazole; preclinical, toxicity

LIPID MEDIATORS IN HOST-PATHOGEN INTERACTIONS DURING LEISHMANIA INFECTION: AN INTEGRATIVE REVIEW

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Lipid mediators can control the inflammatory response in several infectious diseases, including leishmaniasis. However, these mediators may promote antagonistic roles in the *Leishmania*-host interaction depending on the *Leishmania* species involved in the infection. Herein, we analyzed the role of mediators in the *Leishmania*-host interaction in the experimental and clinical context, aiming to identify the main cell types studied, as well as the main eicosanoids and their influence during the infection. For this, we performed an integrative review including studies that evaluated the role lipid mediators in vivo and in vitro experimental models of *Leishmania* infection, as well as in clinical studies up to the present date. We identified 41 in vitro experimental articles, 13 in vivo experimental articles and 6 clinical studies. The main lipid mediators studied in the pathogen-host interaction were the eicosanoids LTB_4 and PGE_2, which are related to the inflammatory response in cutaneous and visceral leishmaniasis. In vitro models using macrophages and neutrophils infection reveal that LTB_4 plays a fundamental role in reducing the parasite load, while PGE_2 and $PGF_{2\alpha}$ promote the suppression of the cellular response, favoring the survival of the parasite in the host. In the in vivo infection, PGE_2 is related to the visceralization process of the disease and the persistence of tegumentary lesions. An emerging role in pathophysiology has been pointed out for mediators of the HETE class and for Resolvin D1, which act favoring *Leishmania* infection and are associated with more severe cases of the disease. Thus, it can be concluded that lipid mediators play crucial roles in the *Leishmania*-host interaction, modulating the inflammatory response and disease progression. Studies exploring the contribution of intervention in the production of lipid mediators during the course of the disease are still needed.

Supported by: CNPq.

Keywords: Lipid mediator, *Leishmania*, host-pathogen interaction.

IMMUNOMETABOLISM OF LEISHMANIASIS, HOW DO THE PARASITES AFFECT THE METABOLISM OF MACROPHAGES FROM SUCCEPTIBLE BALB/C MICE?

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It is well established in the scientific community that certain intracellular parasites can modulate host cell metabolism as a strategy to promote their survival and replication among the parasites of the genus *Leishmania*. Leishmaniasis, a significant and persistent public health challenge in Brazil and other developing countries, encompasses a broad spectrum of clinical manifestations that depends on both the host's immune status and the infecting *Leishmania* species.

Given that *Leishmania amazonensis* and *Leishmania braziliensis* induce distinct clinical forms with different immunological profiles, we sought to determine whether the metabolic alterations induced by these two parasite species differ in macrophages from BALB/c mice, which is a susceptible model for Leishmaniasis

To address this, we pretreated macrophage cultures with metabolic inhibitors prior to infection, thereby preventing parasite-driven metabolic modulation. Our findings indicate that *L. amazonensis* exhibits a more quiescent profile, relying less on host cell metabolism for survival. In contrast, *L. braziliensis* displayed a distinct pattern, as metabolic inhibitors from all tested classes significantly reduced both the infection rate and parasite burden.

Furthermore, real-time metabolic assays revealed that infection did not significantly alter glycolysis or basal respiration rates. However, mitochondrial fitness parameters were substantially affected in infected groups compared to controls. Notably, previous studies in resistant macrophage lineages have shown that *L. amazonensis* and *L. braziliensis* infections enhance both glycolytic and respiratory metabolism, contrasting with our findings in the susceptible lineage.

These results reinforce the direct link between cellular metabolism and functional capacity while providing a framework for further investigation into the leishmanicidal mechanisms triggered by metabolic pre-treatment.

FUNDING: This study was supported by the Coordination for the Improvement of Higher Education Personnel (CAPES) – Finance Code 001, the National Council for Scientific and Technological Development (CNPq), and the Bahia State Research Support Foundation (FAPESB).

KEYWORDS: LEISHMANIA, IMMUNOMETABOLISM, MACROPHAGE

DETECTION OF *Leishmania infantum* DNA DIRECTLY FROM CLINICAL SERUM SAMPLES VIA LAMP WITHOUT DNA EXTRACTION

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Abstract

The implementation of precise, reliable, and efficient diagnostic tools is crucial for controlling leishmaniasis. The loop-mediated isothermal DNA amplification (LAMP) technique is compatible with the point-of-care scenario. It is fast and easy to perform, eliminating the need for expensive equipment, presenting a promising alternative for molecular diagnosis. Employing serum as a biological sample for the LAMP reaction simplifies the pre-analytical step, allowing for precise and minimally invasive detection of *Leishmania* DNA, which facilitates diagnosis in areas with limited infrastructure. In this study, we validated the use of serum from dogs naturally infected with *Leishmania infantum*, confirmed by DPP and ELISA, from an endemic area in Ouro Preto, Minas Gerais, Brazil. We utilized species-specific primers developed by our team, integrated into LeishID—a LAMP-based molecular diagnostic solution that demonstrated high sensitivity and specificity at clinically relevant concentrations. We analyzed 69 blood samples from dogs, including 23 symptomatic, 23 asymptomatic, and 23 uninfected dogs. For the LAMP assay, we employed a colorimetric detection using hydroxynaphthol blue (HNB), where a positive reaction is indicated by a blue color change, while negative samples remain purple. LAMP reactions were conducted at 65 °C for 40 min, and the results were validated by agarose gel electrophoresis. The colorimetric test correctly identified all positive (symptomatic) and negative samples, achieving 100% sensitivity and specificity. In positive asymptomatic dogs, electrophoresis identified 16 positive samples, resulting in a sensitivity of 76.67% (95% CI = 57–90%) and a specificity of 100% (95% CI = 85–100%). The use of DNA extraction-free crude serum, combined with the LeishID platform, enabled molecular detection of *Leishmania*, ensuring high sensitivity and specificity. This approach is particularly suitable for field applications in resource-limited endemic regions.

Keywords: *Leishmania;* LAMP; serum
Financial Support: Fapemig, CAPES, CNPq, Fiocruz.
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METABOLIC REPROGRAMMING IN HUMAN MONOCYTES: UNVEILING THE ROLE OF CELLULAR METABOLISM IN *LEISHMANIA SPP.* SURVIVAL AND IMMUNE EVASION

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Different *Leishmania* species have developed distinct immune evasion mechanisms, such as interference with cellular signaling and metabolic processes, to achieve effective infection in the host's mononuclear cells. This study aims to investigate the metabolic signature of *Leishmania amazonensis* and *Leishmania braziliensis* during human monocyte infection.

Monocytes were isolated from peripheral blood and infected with *Leishmania spp.* for 18 hours. Extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) were measured using Seahorse. To assess the impact on metabolic pathways, the cells were pre-incubated with specific inhibitors, and parasite load was quantified. Mitochondrial mass and membrane potential were analyzed using MitoTrackers, and reactive oxygen species production was monitored using MitoSox and Cellrox. Cytokine levels were measured by Luminex.

Our data indicate that monocytes infected with *L. amazonensis* and *L. braziliensis* display a similar metabolic profile, with reduced ECAR and OCR rates. Treatment with inhibitors of glycolytic, mitochondrial metabolism, β-oxidation, and fatty acid synthesis resulted in a significant decrease in parasite load. A reduction in cytokine production was also observed in the C75 treatment, both in *L. amazonensis* and *L. braziliensis* infections. Additionally, an increase in mitochondrial mass and membrane potential was identified, and interference with monocyte metabolism was associated with increased oxidative stress, elevating cellular and mitochondrial ROS production.

This study demonstrates that infection with *L. amazonensis* and *L. braziliensis* induces similar metabolic changes in host cells, and that modulation of cellular metabolism is essential for parasite elimination.

FUNDING: This study was supported by the Coordination for the Improvement of Higher Education Personnel (CAPES) – Finance Code 001, the National Council for Scientific and Technological Development (CNPq), and the Bahia State Research Support Foundation (FAPESB).

KEYWORDS: LEISHMANIA, IMMUNOMETABOLISM, MONOCYTE

miR-205 Suppression in Human Keratinocytes by *Leishmania braziliensis* Exposure Delays Skin Wound Repair Through Cell Adhesion Pathways

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ABSTRACT

In inflammatory skin diseases, keratinocyte migration plays a crucial role in re-establishing the epithelial layer, a fundamental process in wound healing. Cutaneous leishmaniasis (CL), a chronic inflammatory skin condition marked by ulcerated cutaneous lesions and persistent inflammation, lead to a reprogramming of host cell transcripts. This reprogramming facilitates the modulation of gene expression related to the response to the parasite. Consequently, post-transcriptional regulation mediated by microRNAs (miRNAs) can adjust the molecules involved in attenuating the damage caused by injury. Thereby, the aim of our study was to explore the role of miR-205 in the migration of human keratinocytes exposed to *Leishmania braziliensis* (Lb) within a 2-dimensional environment. Our research unveiled a consistent downregulation of miR-205 in bioinformatics analyses performed using transcriptomic data from biopsies of patients with various inflammatory skin diseases, including cutaneous leishmaniasis, psoriasis, lupus, and leprosy. Intriguingly, we found that, irrespective of the disease, miR-205 was consistently inhibited in inflammatory skin conditions. We validated these findings through biopsies from CL patients and by exposing human HaCaT keratinocytes to Lb. In both ex vivo and in vitro assays, the expression of miR-205 was downregulated in the presence of Lb, and our experiments showed that miR-205 promotes keratinocyte migration in a wound scratch model, while Lb delays wound healing. The scratch area was quantified, and the results revealed a dynamic interplay between miR-205 and Lb in wound closure. Additionally, we assessed cell adhesion molecules, observing a reduction in one of the essential proteins involved in adhesion complex formation, focal adhesion kinase (FAK), in response to parasite exposure. Furthermore, we noted alterations in actin dynamics in the groups exposed to Lb. Importantly, the inhibitions observed in the presence of Lb were reversed in groups treated with miR-205. Finally, Transwell assay

reinforces that Lb delays the migration of keratinocytes and that the presence of miR-205 reverses this effect. Our findings underscore the significance of regulating miR-205 in migrating keratinocytes, highlighting its fundamental role in the re-epithelialization process and a promising candidate for application in clinical practice.

Keywords: miR-205; wound healing; Cutaneous Leishmaniasis.

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EXPLORING HYPOTHETICAL PROTEINS TO UNVEIL NOVEL TARGETS ASSOCIATED WITH Sb^V-RESISTANCE IN *Leishmania* PARASITES

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Abstract

In many countries, the first-line drugs to treat leishamniasis rely on antimony (Sb^V)-based medicines. However, factors such as high cost, difficulties in administration, severe toxicity, and the emergence of resistant strains highlight the need for new therapeutic strategies. *Leishmania* parasites exhibit high genome plasticity that favors the selection of Sb^V-resistant strains (SbR). Studies revealed that *Leishmania* spp. genomes code for approximately 60% of hypothetical proteins, which includes those differentially expressed in SbR mutants. In this sense, it is crucial to understand the role of these protein-encoding genes in the context of Sb resistance. Elucidating the role of these proteins may reveal new therapeutic targets for leishmaniasis treatment. This study aimed to identify differentially expressed genes (DEGs) encoding hypothetical proteins in *Leishmania* resistant to Sb and to perform computational function prediction based on their structure. To achieve this, we evaluate publicly available data on DEGs in SbR *Leishmania*. 204 out of 603 DEGs were classified as hypothetical or functionally unknown proteins and were present in at least two independent studies. Among these genes, 28 exhibited a *cutoff* greater than 1.5, providing further strong evidence of their potential role in resistance. Some known markers harbored in chromosome 23 were found to be upregulated in half of the analyzed studies, reinforcing its established role in Sb resistance phenotype. These 204 targets are being analyzed to predict their structure using AlfaFold and compare it on PDB through the FoldSeek tool to propose and validate their functions. Our findings highlight the importance of these proteins in Sb resistance and suggest potential new therapeutic targets. The functional characterization of these proteins is essential for developing effective alternatives for leishmaniasis treatment.

Keywords: Leishmaniasis, antimony resistance, hypothetical proteins, structural prediction
Support: Fiocruz, Fapemig, Capes, CNPq

EXPERIMENTAL INFECTION OF *Hepatozoon* spp. FROM THE SNAKES *Boa constrictor* AND *Eunectes murinus* IN colonized *Aedes aegypti, Ae. Albopictus, Anopheles aquasalis* and *Culex quinquefasciatus* MOSQUITOES

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Hepatozoon (Apicomplexa, Hepatozoidae) is a genus of hemoparasites commonly found in snakes worldwide. Its life cycle requires vectors, such as mosquitoes, in which sporogony occurs, producing sporozoites—the infective form for vertebrates. The present study aimed to experimentally determine the sporogonic development of *Hepatozoon* parasites of snake *Eunectes murinus* and *Boa constrictor* in laboratory-reared mosquitoes(Diptera: Culicidae - *Aedes aegypti*, *Aedes albopictus*, *Anopheles aquasalis*, and *Culex quinquefasciatus*). The snakes positive for *Hepatozoon* were kept at the CIGS zoo in Manaus-AM and approximately 3 ml of blood was collected by caudal puncture. The blood was stored in heparin tubes at 31ºC in a water bath, the average body temperature of the snakes. Females of mosquitoes were fed this blood through an experimental system consisting of glass feeders covered with an artificial Parafilm® membrane. The engorged individuals were maintained separated by groups of snakes in screened entomological cages and dissected 6h, 12h, 18h, 24h, 48h, 72h, 6th, 9th, 13th, 16th and 20th hour/days after feed; the intestinal contents were smeared and impressions of this and the hemocoel were made. The parasitic forms found were photographed and characterized throughout the dissection period. In the first hours after infection, free and intraerythrocytic gamonts were observed in intestines smears of the *Cx. quinquefasciatus* mosquitoes from both groups of snakes and in *A. aegypti* that were fed on *E. murinus* blood. The dissected mosquitoes of all species began to show immature ovoid or rounded oocysts from the 9th day and sporulated oocysts from the 12th day, with formation of sporozoites. From the 9th to the 20th day, oocysts were present throughout the hemocoel of the thorax, abdomen and head of the mosquito, with no presence in the intestines. On average, the intestines presented 73 oocysts, ranging from 3 to 178 (n = 19) and each oocyst with 89 sporozoites, ranging from 64 to 231 (n = 9), containing 22sporozoites ranging from 15 to 101 (n = 38). This is the first study of experimental infection with the genus *Hepatozoon* through membrane feeding and the first record of Culicidae experimental infected with *Hepatozoon* from the snake *E. murinus*.

Supported by: CAPES, CNPq, FAPEAM, INOVA FIOCRUZ

Keywords: hemoparasites, sporogony, reptiles, vectors.

Occurrence of congenital toxoplasmosis in the state of Maranhão in 2023.

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Congenital toxoplasmosis is a parasitic infection caused by Toxoplasma gondii, via transplantation to the fetus as a result of maternal infection during pregnancy., which can lead to: fetal or neonatal death, those who survive can be asymptomatic, or symptomatic; with congenital toxoplasmosis characterized by significant neuro-ocular alterations, such as chorioretinitis, cerebral calcifications that can lead to sequelae with impairment of the nervous system and eyes that can appear in the early years or in adulthood with impairment in motor and cognitive development and vision in those affected. This is a descriptive study that aims to find out about the occurrence of congenital toxoplasmosis. We used data from the Disease Notification System: SINAN NET. 140 cases were reported in the state of Maranhão in 45 municipalities in the state; the largest number of cases were in the municipalities of: São Luís (48), Timon (11), Colinas (09), Açailândia (04), Caxias (03), Codó (03), Imperatriz (03) and Pinheiro (03) with 81- 62.03% of the cases. Laboratory confirmation was possible in 83 cases and 05 clinical epidemiological, males with 61 cases (43.57%), females 79 (56.43%). all were under one year of age, as for race: unknown (07), white (07-) black (02), brown (118). As for progress, the following data was obtained: unknown (29), cure (52) and death (1). It can be seen that congenital infection still occurs in the state of Maranhão, especially in the state capital São Luís. In addition, more attention needs to be paid to pregnant women who have not had the disease or who may acquire it during pregnancy. Considering the importance of congenital Toxoplasmosis infection for the quality of life of those affected, and emphasizing the importance of carrying out serological screening for Toxoplasmosis in pregnant women during prenatal care and for early diagnosis and treatment

Keywords: Congenital , Toxoplamosis, pregnancy

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DETECTION OF ANTI-*LEISHMANIA INFANTUM* ANTIBODIES IN DOMESTIC CATS IN THE MUNICIPALITY OF RIO DE JANEIRO

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Abstract

Several studies have suggested a potential role of cats in the transmission cycle of zoonotic visceral leishmaniasis (ZVL). In this context, it is crucial to understand the role of these animals in the cycle of urban leishmaniasis to improve disease management and control. This study investigated the presence of anti-*Leishmania infantum* antibodies in cats treated at the Jorge Vaistman Municipal Veterinary Medicine Center, Rio de Janeiro (RJ), with the aim of identifying the exposure of these animals to the parasite. For this purpose, the LVC-BioManguinhos diagnostic kit was used. This test was adapted and standardized with the anti-IgG antibody for felines (Goat anti-Cat IgG – Fc HRP Conjugated). In this study, 278 blood samples from domestic cats, which showed no clinical signs of leishmaniasis, were collected between 2017 and 2018, from regions with a high incidence of ZVL. Positive and negative controls were provided by the team at the Federal University of Piauí. Anti-*L. infantum* antibodies were found in 48 (17.2%) of the 278 sera analyzed. The presence of seropositive animals indicates the need to acquire knowledge about the current prevalence of feline infection in the region, in order to prevent the transmission of this neglected zoonosis, which poses a potential risk to both human and animal health. To control cross-reactivity with *Sporothrix* spp., a highly endemic fungal infection in domestic cats in the city of Rio de Janeiro, an ELISA test was performed with 10 serum samples. Of these, 8 (80%) were seroreactive for *L. infantum*. These results may represent cases of cross-reaction or subclinical coinfection in the animals investigated for *L. infantum* and the fungus causing sporotrichosis. The possibility of cross-reactivity with *Sporothrix spp.* highlights the need for more specific serological tests to allow a more accurate seroepidemiological study.

Supported by: IOC/FIOCRUZ; CAPES

Keywords: leishmaniosis, cats, serology

The Immunomodulatory Potential of Oral Administration of *Lactococcus lactis* Producing Hsp65 in the Treatment of Cutaneous Leishmaniasis Caused by *Leishmania major*

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INTRODUCTION: Leishmaniasis is a complex of neglected diseases caused by protozoan parasites of the genus *Leishmania*, endemic in more than 90 countries worldwide. Cutaneous leishmaniasis (CL) is the most common clinical form of the disease, with *Leishmania major* as the main species responsible for CL in the Old World. CL manifests as ulcerative lesions with nodular or thick borders, resulting from an imbalance between the inflammatory and immune responses, emphasizing the importance of developing therapeutic alternatives that modulate inflammation. In this context, a previous study performed by our group, based on the use of *Lactococcus lactis* producing HSP65 for the treatment of cutaneous leishmaniasis caused by *Leishmania braziliensis*, demonstrated the ability to modulate the immune response and attenuate inflammation caused by the infection. **OBJECTIVE:** To evaluate the immunomodulatory potential of oral administration of *Lactococcus lactis*-producing Hsp65 as an inducer of oral tolerance in the treatment of CL caused by *L. major*. **METHODOLOGY:** BALB/c and C57BL/6 mice were treated with *L. lactis* expressing Hsp65 before or after infection with *L. major*. We assessed lesion thickness, parasite load, and cytokine production. **RESULTS:** Mice were treated with *L. lactis*-producing Hsp65 before or after the infection with *L. major,* and did not result in significant differences in lesion development or parasite load. Furthermore, there were no significant differences in the production of IFN-γ, IL-10, and IL-4, among the different groups evaluated. **CONCLUSION:** The use of *L. lactis*-producing Hsp65 shows promising prospects as a treatment for cutaneous leishmaniasis caused by *L. braziliensis*. However, further investigations are necessary to better understand the role of this therapy in modulating the inflammatory and immune responses during CL caused by *L. major*.

Supported by: Foundation for the Support of Research in the State of Bahia (FAPESB)

Keywords: Leishmaniasis; Oral Tolerance; *Lactococcus lactis*, HSP65

Metabolic Modulation in Macrophages During *Leishmania* Infection: Impacts on Parasite Survival and Therapeutic Potential

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Macrophages are crucial players in innate immunity, recognizing and responding to pathogens. *Leishmania* spp. infections trigger significant metabolic alterations, increasing the energy demands of infected cells in a process closely linked to host mitochondrial integrity. However, the mechanisms by which different *Leishmania* species manipulate host cell metabolism remain unclear. In this study, we investigated key metabolic processes essential for *Leishmania* persistence in macrophages, focusing on pathways exploited by the parasite.

Bone marrow-derived macrophages (BMDMs) from C57BL/6 mice were infected with *Leishmania* spp., and metabolic activity was assessed through extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) using the Seahorse system. Mitochondrial function was analyzed with MitoTracker probes, and parasite burden was quantified after treatment with metabolic inhibitors. Additionally, we examined parasite adhesion, phagocytosis, and mitochondrial reactive oxygen species (ROS) production using MitoSOX. Our findings reveal that despite distinct immune evasion mechanisms, *L. amazonensis* and *L. braziliensis* induce similar metabolic changes in macrophages. Infected cells displayed increased glycolysis and oxygen consumption, accompanied by enhanced proton leak and decreased ATP production. Infection also led to elevated mitochondrial mass and membrane potential.

Notably, inhibitors of oxidative phosphorylation and glycolysis significantly reduced parasite burden, reinforcing the crucial role of metabolic pathways in host-parasite interactions. Collectively, our results suggest that *L. amazonensis* and *L. braziliensis* exploit host metabolism in a similar manner to ensure intracellular survival. Despite species-specific immune evasion strategies, both parasites consistently impact host bioenergetics. Importantly, targeting these metabolic pathways pharmacologically significantly impairs parasite survival, offering promising therapeutic strategies against leishmaniasis. This study advances our understanding of host-parasite interactions and provides valuable insights for developing novel treatments for this neglected disease.

Supported by: CAPES, Funding Code 001

Keywords: Metabolic modulation, Macrophages, *Leishmania spp.*

REPOSITIONING HDAC AND KINASE HYBRID INHIBITORS AGAINST MALARIA PARASITE *PLASMODIUM FALCIPARUM*

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Malaria disease is still one of the significant public health problems worldwide, resulting in thousands of deaths per year. The emergence of parasites resistant to classical antimalarials indicates the urgent need for repositioning drugs with different pharmacophores. Due to the complex life cycle of the malaria parasite, regulation of cellular processes is essential for parasite survival and infection progression. Epigenetic regulation such as histone modifications are associated with the regulation of key processes in the life cycle of the parasite. Moreover, post-translational modifications such as protein phosphorylation by kinases have been implicated in essential events for parasite development such as in invasion and egress from erythrocytes. This study tested a series of hybrid kinase and histone deacetylase (HDAC) inhibitors, bearing anticancer activity, in *Plasmodium falciparum in vitro*. To evaluate the effect of compounds in parasite development, infected red blood cells were incubated with varying concentrations of compounds for 72h, and final parasitemia was obtained by flow cytometry using double staining to check mitochondria viability with MitoTracker Deep Red and nucleus staining with SYBR Green. Our data show that these compounds impair the parasite development, with IC_{50} values in the nanomolar range against wild type 3D7 and parasites resistant to chloroquine, Dd2. Moreover, cytotoxicity data show that at least four compounds present high selectivity towards the malaria parasite, identifying them as potential hits for developing new antimalarials.

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Key words: Malaria, antimalarial, *Plasmodium*

IN VITRO ANTI-*TOXOPLASMA GONDII* ACTIVITY OF *OCOTEA DAPHNIFOLIA* EXTRACT

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Toxoplasmosis, caused by the protozoan *Toxoplasma gondii*, is a global public health and animal health issue. Current treatments have limited efficacy and can cause adverse effects, highlighting the critical need for new antiprotozoal agents. This study evaluated the effects of the ethanolic extract of *Ocotea daphnifolia* (EEOD) on the *in vitro* multiplication of *T*. *gondii*. The MTT assay was used to determine the cytotoxic effects of ethanol (as the solvent) and EEOD on VERO cells. VERO cells were infected with the RH strain of *T*. *gondii* tachyzoites for antiparasitic testing, followed by treatment with EEOD concentrations ranging from 0.49 to 500 µg/mL for 24 hours. The antiparasitic activity was assessed by counting the number of intracellular tachyzoites among 200 viable cells using optical microscopy. Ethanol exhibited toxicity to VERO cells at concentrations above 2.5%, with an IC50 value of 5.49%. EEOD was safe for VERO cells, with viability above 78% even at the highest concentrations tested. The concentrations that resulted in the highest inhibition of parasite replication were 500 µg/mL (53.63% inhibition), 31.25 µg/mL (28.59% inhibition), and 125 µg/mL (22.68% inhibition), respectively. Notably, no significant difference was observed between the positive control (200 µg/mL sulfadiazine) and the negative control (0.5% ethanol). In conclusion, EEOD showed low toxicity to host cells and may contain bioactive compounds with potential antiparasitic activity. However, further studies are necessary to explore its therapeutic potential against toxoplasmosis.

Supported by: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)

Keywords: Antiprotozoal, ethanolic extract, toxoplasmosis.

MICROPARTICLES OF RIFAMPICIN FOR PULMONARY ADMINISTRATION IN TUBERCULOSIS: INSPECTION ON CELL UPTAKE

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Tuberculosis is an infectious, respiratory and chronic disease caused by *Mycobacterium tuberculosis (M. tuberculosis)*. Among the available treatment, Rifampicin is an antibiotic and a first line treatment. It presents a low water solubility and conversely, a high permeability property. Polymeric nanoparticles can be useful for a controlled drug delivery to improve drug efficacy in a tuberculosis setting. Here, a nano-embedded microparticles (NEMs) were designed to bypass pulmonary barriers. To address this, a Cashew gun was modified into phthalated cashew gum (PCG), which targets alveolar macrophages, by increasing hydrophobicity and ultimately improving drug encapsulation efficiency. Polymeric nanoparticles blank PN-PCG and (PN)-PCG-RF were successfully obtained by nanoprecipitation method, and their performance were investigated. The PN-PCG exhibited a particle size of 69,46 ± 1.11 nm and 112.9 ± 0.61 nm for the (PN)-PCG-RF. Negative zeta potentials were observed for both PN-PCG and (PN)-PCG-RF (-40.0 and -27.5, respectively). Scanning electron microscopy revealed NEMs-RIF in the form of spherical agglomerates. To address the possibility of PN-PCG targeting TB bacterium residing in the granuloma, we employed the phagocytosis ratio of PN-PCG probed with a fluorescent tag into macrophages as a surrogate of PN-PCG availability and effectiveness. Therefore, the cellular uptake of these materials was studied at 3 and 24 hours after treatment demonstrated efficient cellular internalization of the rhodamine nanoparticles (Rho-PN-PCG) in J774 macrophages, which was attributed to the PCG composition binding to the galactose-type lectin C receptor (MGL-2/CD301b) and indicating a time-dependent uptake pattern. RIF-NEMs were successfully developed from PN-PCG-RIF, having potential for the treatment of tuberculosis. In conclusion, PN-PCG provided to be a powerful tool for the treatment of TB.

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Keywords: *Mycobacterium tuberculosis*, rifampicin, pulmonary drug delivery.

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DIFFERENTIAL GENE EXPRESSION IN BONE MARROW CELLS CLASSIFIES DOGS DIFFERENT CLINICAL OUTCOMES NATURALLY INFECTED WITH *LEISHMANIA INFANTUM*

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INTRODUCTION: Visceral leishmaniasis (VL) is the most severe form of leishmaniasis, affecting internal organs like the spleen, liver, and bone marrow. It is endemic in 76 countries and present in at least 12 in the Americas, with Brazil accounting for 90% of Latin American cases. Dogs serve as reservoirs, transmitting VL to humans via *Lutzomyia longipalpis* bites. In a cohort study in an endemic area of Camaçari, Bahia, Brazil, dogs were classified as resistant or susceptible based on clinical, immunological and parasitological evaluations. **OBJECTIVE:** Given the critical role of bone marrow in the persistence of parasites in infected animals, this study aims to assess the immunocellular responses of bone marrow cells in naturally infected dogs from the cohort study using RNA sequencing (RNAseq). **METHODOLOGY:** RNAseq was conducted on bone marrow cells from dogs classified as susceptible or resistant. We compared global gene expression by bone marrow cells of infected dogs versus healthy controls to identify differentially expressed genes (DEGs) and biomarkers. Machine learning identified four predictive genes—TINAGL1, FOS, ADCY9, and EGR2—that can distinguish susceptible and resistant from healthy dogs. RT-qPCR was used to validate the RNAseq findings. For this process, RNA was extracted, converted into cDNA, and then analyzed through RT-qPCR using specific primers. In addition, pathway enrichment analyses were conducted and identified in bone marrow cells from susceptible dogs, reduction in the expression of genes involved in DNA double-strand break repair. These alterations will be validated in cells from susceptible dogs. **RESULTS:** *TINAGL1* was more expressed in susceptible dogs, while *FOS* was higher in controls; *ADCY9* and *EGR2* showed no significant differences. Future analyses will include western blotting to confirm gene expression. Genomic DNA will be extracted to assess double strand breaks in susceptible dogs. **CONCLUSION:** *TINAGL1* and *FOS* may play key roles in VL infection.

KEYWORDS: Visceral Leishmaniasis, genes, dogs, biomarkers, bone marrow.

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ISOLATED CONDENSED TANNINS FROM *Mimosa tenuiflora*: POTENTIAL PRECURSORS FOR THE DEVELOPMENT OF ANTILEISHMANIAL DRUGS

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Leishmaniases are neglected diseases with a wide geographical distribution, and the available treatment presents certain limitations. The drugs used exhibit high toxicity, severe side effects, and limited efficacy, making the development of new, effective drugs necessary. In this context, natural products emerge as promising sources for new drugs. *Mimosa tenuiflora* contains phytoconstituents, such as condensed tannins, with the potential to inhibit pathogens through interaction with membrane proteins. This study investigated the antileishmanial activity of condensed tannins isolated from the bark of *M. tenuiflora*.

Leishmania amazonensis promastigotes were used to evaluate in vitro antiparasitic activity, while NIH/3T3 cells were used for cytotoxicity analysis. Molecular docking assays assessed the interaction of condensed tannins with the *Leishmania mexicana* L-arginase enzyme (PDBID: 4IU0).

The results demonstrated that condensed tannins significantly inhibited parasite growth at higher concentrations (IC_{50} = 24.59 µg/mL), with low toxicity (CC_{50} > 1,000 µg/mL) and high selectivity (SI > 40.65). In molecular docking, tannins interacted with residues in the active site of L-arginase, suggesting an enzymatic inhibition mechanism.

Based on these findings, it is possible to suggest that condensed tannins compromise parasite survival by blocking essential metabolic pathways. This is the first report on the antileishmanial activity of purified condensed tannins from *M. tenuiflora*, highlighting their biotechnological potential. The high selectivity and safety observed reinforce the viability of these molecules as drug candidates. This study paves the way for further research using this molecule as a potential precursor for new drugs.

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Keywords: Biotechnology, Leishmaniases, Condensed Tannins

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IMPACT OF *IN VIVO L. BRAZILIENSIS* INFECTION ON BONE MARROW-DERIVED MACROPHAGES (BMMΦ) MIGRATION

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The Dissemination of *Leishmania*-infected cells is crucial for the establishment of the disease. However, the mechanisms driving cell migration post-infection remain underexplored. Studies on different *Leishmania* species have shown that macrophage migration is reduced following infection, a process linked to decreased adhesion complex formation and altered actin dynamics. Despite these findings, the impact of *Leishmania* infection on bone marrow cell recruitment *in vivo* remains poorly understood. This study investigates the migration of bone marrow-derived macrophages (BMMΦ) infected *in vivo* with *L. braziliensis*. Mice were either infected with *L. braziliensis*, and BMMΦ were isolated at 2, 5, and 10 weeks post-infection. These BMMΦ underwent directional migration assays using a transwell system with the chemoattractant MCP-1. Adhesion complex formation was assessed through immunostaining for phosphorylated focal adhesion kinase (p-FAK) and paxillin (p-Paxillin), alongside actin cytoskeleton analysis using markers such as phalloidin, Cdc42, RhoA, and Rac1. Immunofluorescence was analyzed via confocal microscopy. Results showed a significant reduction in BMMΦ migration at all post-infection time points. This reduction was associated with decreased p-FAK and p-Paxillin expression, both essential proteins for adhesion complex formation. Infected BMMΦ also exhibited altered actin dynamics, with increased Rac1 and Cdc42 expression at 2 and 5 weeks, along with decreased RhoA expression, indicating reduced actin cytoskeleton contraction. Understanding how *Leishmania* infection affects bone marrow cell recruitment *in vivo* is essential for elucidating the pathogenesis of cutaneous leishmaniasis. Further research on the bone marrow changes induced by *L. braziliensis* infection and their role in modulating BMMΦ migration is crucial. These insights could contribute to the development of new therapeutic strategies and preventive measures against severe leishmaniasis.

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Keywords: Macrophages; Cell migration; *Leishmania*; Bone marrow.

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DEVELOPMENT AND CHARACTERIZATION OF POLY (L-LACTIC ACID) NANOPARTICLES CONTAINING 17-DMAG, AN HSP90 INHIBITOR, AS A POTENTIAL CHEMOTHERAPY AGENT FOR CONTROLLING *LEISHMANIA BRAZILIENSIS* INFECTION *IN VITRO*

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ABSTRACT

INTRODUCTION: Heat shock protein 90 (Hsp90) plays a crucial role in the life cycle of *Leishmania*. 17-DMAG, an Hsp90 inhibitor, was tested in BALB/c mice infected with *L. braziliensis*, resulting in reduced lesion size, pro-inflammatory cytokine production, and parasite burden. However, prolonged treatment with 17-DMAG led to symptoms of toxicity. To address this issue, 17-DMAG was encapsulated in nanoparticles (NPs) to facilitate controlled drug release. **OBJECTIVE:** To develop and characterize polymeric nanoparticles (NPs) containing 17-DMAG as a potential chemotherapeutic agent for the treatment of THP-1 cells infected with *L. braziliensis in vitro*. **METHODOLOGY:** NPs were synthesized using the double emulsion method, with poly (L-lactic acid) (PLLA) as the polymer to encapsulate 17-DMAG (PLLA-17-DMAG-NPs). Encapsulation efficiency (EE%) was determined by high-performance liquid chromatography. Cytotoxicity was assessed by CC_{50} in THP-1 cells. Physicochemical properties were analyzed by Dynamic Light Scattering, Transmission Electron Microscopy and Scanning Electron Microscopy. **RESULTS:** PLLA-17-DMAG-NPs exhibited spherical morphology and nanometric size. The encapsulation efficiency varied between 2% and 74%, with no significant differences in particle size or polydispersity index. In cytotoxicity assessments, PLLA-17-DMAG-NPs at a concentration of 2 µM showed no cytotoxicity effects, while free 17-DMAG exhibited a CC_{50} of 2.6 µM. **CONCLUSION:** PLLA nanoparticles encapsulating 17-DMAG represent a promising strategy for treating *Leishmania* infections. PLLA-17-DMAG-NPs showed high encapsulation efficiency, maintained nanometric size, and exhibited reduced cytotoxicity compared to free 17-DMAG. These findings suggest that encapsulation effectively minimizes toxicity while preserving the therapeutic potential of the drug. Further studies are planned to determine the IC_{50} of both free and encapsulated 17-DMAG and to optimize nanoformulation parameters for enhanced efficacy.

KEYWORDS: Nanoparticle; 17-DMAG; *Leishmania braziliensis*.
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SPECIES-SPECIFIC *Leishmania* DNA DETECTION USING THE LAMP-BASED DIAGNOSTIC TOOL LeishID

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Abstract

Sensitive, reliable, and rapid diagnostic tools are crucial to tackle leishmaniases. Recent reports have revealed *Leishmania* species associated with dermotropic forms causing visceral leishmaniases, and *vice versa*. This poses a problem for epidemiology and a challenge to better understand the dynamics of the pathophysiology of leishmaniases. Although several molecular methods detect *Leishmania*, most do not identify it at the species level. The loop-mediated isothermal DNA amplification (LAMP) technique stands out for its ease of execution, and high sensitivity, without the need for a thermal cycler. In this context, here we developed LeishID, a LAMP-based diagnostic method to specifically identify *L. infantum*, *L. braziliensis*, and *L. amazonensis.* We selected species-specific targets from 26 publicly available *Leishmania* genomes, covering at least 16 different species. The accessory genome was filtered to obtain unique sequences for each species. LAMP primers were designed and the displacement probe technique was applied for multiplex assay purposes, using fluorophore-labeled loop primers. For the LAMP assay, we used the WarmStart Colorimetric LAMP mix (NEB). Depending on the primers used, 15 min of incubation is enough to detect *Leishmania* DNA, although 40 min offers greater sensitivity, with detection limits ranging from 0.01 fg to 1 pg. The use of specific primers ensured accurate identification of the analyzed species, without cross-reactions. Clinical validation was performed on 47 spleen biopsy specimens obtained from dogs with canine leishmaniasis and on 43 clinical specimens derived from skin lesions of patients with cutaneous leishmaniasis from Minas Gerais, Brazil. LeishID was able to identify *L. infantum* and *L. braziliensis*, in spleen and skin lesions, respectively, with a sensitivity of 97.9% and a specificity of 100%. Thus, LeishID is rapid, specific, and sensitive in differentiating *Leishmania* species at clinically relevant concentrations.

Keywords: *Leishmania*; LAMP; diagnostics
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COMPARATIVE ANALYSIS OF INITIAL EVENTS OCCURRING DURING MACROPHAGE INTERACTION WITH *LEISHMANIA BRAZILIENSIS* ISOLATES CAUSING DISTINCT CLINICAL FORMS

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Abstract

INTRODUCTION Cutaneous Leishmaniasis (CL) is caused by *Leishmania braziliensis* (*Lb*) and can manifest as localized cutaneous (LCL), mucosal (ML), or disseminated leishmaniasis (DL). The interaction between *Leishmania* parasites and macrophages, the primary host cells, plays a crucial role in determining the establishment and outcome of the disease. Although the involvement of phospholipase C (PLC) and Akt in phagocytosis has been extensively studied, their specific role in *Lb* phagocytosis remains unexplored. **OBJECTIVE** This study aims to characterize the initial events of *Lb* entry into macrophages using isolates obtained from patients with LCL or DL. **METHODOLOGY** Genetically characterized *Lb* isolates from LCL or DL patients were utilized. Parasite intracellular viability was assessed at 12 h post-infection in THP-1 cells to examine potential differences in infectivity between *Lb*-LCL and *Lb*-DL. The impact of PLCγ and Akt on infection outcomes was evaluated by treating THP-1 cells infected with *Lb*-LCL or *Lb*-DL with specific inhibitors (U73122 and GSK 690693, respectively), and the parasite viability was measured at 4 and 12 hours post-infection. To assess free radical production, THP-1 cells were labeled with the superoxide probe H2DCFDA, incubated with Lb-LCL-19432 or Lb-DL-18211, and analyzed under fluorescence microscopy. **RESULTS** *Lb*-DL isolate showed lower viability compared to *Lb*-LCL after 4 and 12 hours of infection. Inhibition of PLCγ significantly reduced parasite viability in cells infected with both *Lb*-LCL and *Lb*-DL, while Akt inhibition did not affect the viability of any isolate tested. In addition, *Lb*-LCL promastigotes induced greater production of reactive oxygen species (ROS) compared to *Lb*-DL. **CONCLUSION** PLCγ appears to play a role in *Lb* phagocytosis for both LCL and DL tested isolates. Further experiments are ongoing to compare the influence of initial events in the outcome of infection caused by LCL and DL isolates in THP-1 cells.

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Keywords: Leishmaniasis; *Leishmania braziliensis*; phagocytosis.

CANINE VISCERAL LEISHMANIASIS IN THE MUNICIPALITY OF GOIANA/PE: EPIDEMIOLOGICAL, CLINICAL AND LABORATORY CHARACTERIZATION

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Visceral leishmaniasis (VL) is a zoonosis of great importance to public health that has serious clinical repercussions. VL is caused by protozoa of the genus Leishmania sp., which is transmitted by the bite of females of the species Lutzomyia longipalpis. Dogs play an important role in the transmission cycle of VL, being considered the main domestic reservoir of the parasite, since due to their cutaneous parasitism they favor the infection of the sand fly. Thus, controlling the disease in the canine population makes it possible to reduce the occurrence of human visceral leishmaniasis (HVL). This study aims to identify dogs that are reactive to Canine Visceral Leishmaniasis (CVL) and identify areas at risk for the transmission of CVL in Goiana/PE. An active search was carried out through a draw in 09 locations in Goiana-PE, with a history of HVL. After the guardian signed the informed consent form (CEUA: 187/2023), the dogs were registered, clinically evaluated, and blood samples were collected for laboratory diagnosis. A Dual Path Platform Rapid Test (TR-DPP®) was performed as screening and an Enzyme-Linked Immunoassay (EIE-ELISA) as confirmatory. Molecular diagnosis (PCR) will be used to identify the parasite's DNA. The geographic coordinates of the dogs' residences were obtained to build the thematic map. As preliminary results, 78 residences were visited with 87 registered dogs, all of which were of mixed breed and aged between 7 months and 13 years, 43 (49.4%) males and 44 (50.6%) females. None of the animals showed clinical symptoms characteristic of CVL. Of the 80 samples submitted to the DPP, 32 (40%) samples were reactive, while 12 (15%) were reactive in the ELISA. Only 06 (7.5%) samples were reactive in ELISA and DPP. These data indicate the maintenance of CVL in the areas considered endemic in Goiana-PE, requiring the reassessment of control measures aimed at reducing the number of CVL cases in the municipality.

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Keywords: Visceral leishmaniasis . dogs . diagnosis.

EVALUATION OF ANTI-*TOXOPLASMA GONDII* ACTIVITY OF ASPARTIC PROTEASE INHIBITORS

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Toxoplasmosis, caused by the protozoan *Toxoplasma gondii*, affects approximately one-third of the global population and can lead to severe complications, such as congenital, ocular, and neurological disorders. The treatment of toxoplasmosis is hindered by significant limitations, particularly concerning efficacy and safety, underscoring the urgent need for new drug development. Aspartic protease inhibitors are compounds with previously documented activity against protozoa and viruses, including *Plasmodium*, *Leishmania*, and HIV. In *T. gondii*, seven genes encode aspartic proteases, with TgASP1, TgASP2, and TgASP5 expressed during the parasite's infectious stage. This study aimed to investigate the anti-*T. gondii* activity of underexplored aspartic protease inhibitors. Seventeen inhibitors were evaluated in *in vitro* assays against intracellular tachyzoites of *T. gondii* (RH strain) and human foreskin fibroblasts (HFF). Effective concentration (EC_{50}), cytotoxic concentration (CC_{50}), and selectivity index (SI) values were determined to identify compounds with selective activity. Additionally, absorption, distribution, metabolism and excretion (ADME) predictions were performed to select compounds with favorable pharmacokinetic and pharmacodynamic profiles. The investigated inhibitors exhibited EC_{50} values ranging from 0.9 to 40 μM and CC_{50} values from 4.9 to 257 μM, resulting in SI up to 34. Among the evaluated inhibitors, twelve compounds demonstrated desirable ADME characteristics, including one compound with documented activity and efficacy against *Plasmodium*. To further explore the therapeutic potential of these inhibitors for toxoplasmosis, future studies should focus on elucidating their mechanism of action and validating their *in vivo* efficacy.

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Keywords: Toxoplasmosis; plasmepsins; preclinical assays

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EVALUATION OF THE ANALYTICAL SENSITIVITY OF PRIMERS DESIGNED FOR THE NADH 1 AND NADH 5 GENES IN POLYMERASE CHAIN REACTION (PCR) FOR THE IDENTIFICATION OF *Trypanosoma cruzi*

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Abstract

Chagas disease, also known as American trypanosomiasis, is an infection caused by *Trypanosoma cruzi* that affects humans and other mammalian species. Detection of the parasite's DNA through Polymerase Chain Reaction (PCR) can be crucial for diagnosing the chronic phase of the infection due to subpatent parasitemia and in cases where conventional serology yields inconclusive results. This study aimed to determine the analytical sensitivity of primers designed for the NADH 1 and NADH 5 genes using serial dilutions of *T. cruzi* DNA extracted from cultures. The primers were designed using Primer-Blast based on DNA sequences of reference strains, clones, and isolates of *T. cruzi* deposited in the NCBI GenBank and were tested both *in silico* and *in vitro*. Subsequently, analytical sensitivity was evaluated using strains and isolates from DTUs I, II, and III, with *T. cruzi* DNA concentrations ranging from 0.001 fg to 1000 fg. After PCR amplification, the primers targeting the NADH 1 gene (5' – AAGTCCAGCAACCAATTCACTT – 3' and 3' – CGTTACTCTGTGATGGCTTGA – 5') generated a fragment of approximately 71 bp, which was detected at a concentration of 1000 fg in DTU I. These primers failed to detect DTUs II and III at any tested concentration. Conversely, the NADH 5 primers (5' – AGAGTACACAGTTTGGGTTG – 3' and 3' – CCACATACAACTAACGTTGC – 5') generated a 100-bp fragment and detected *T. cruzi* DNA at 1000 fg in DTU I and from 10 fg to 1000 fg in DTUs II and III. Thus, the NADH 1 primers were effective for detecting *T. cruzi* DTU I at high DNA concentrations, while the NADH 5 primers were efficient in identifying the parasite in DTUs I, II, and III.

Supported by: CAPES, CNPq
Keywords: *Trypanosoma cruzi*, NADH 1, NADH 5

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The Icoaraci Virus (ICOV) Non-Structural Protein N (NSs) modulates the Infection of Macrophages by *Leishmania amazonensis*

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Abstract

Leishmania (L.) amazonensis is a causative species of Cutaneous Leishmaniasis and the main species found in cases of Anergic Diffuse Cutaneous Leishmaniasis, a more severe form of the disease. This species is accidentally transmitted to humans by *L. flaviscutellata*. Recently, we demonstrated that *L.amazonensis*/Amazonian Phlebovirus Icoaraci(ICOV) coinfection enhanced the parasite load in vivo and in vitro, along with the modulation of the expression of IFN1beta. Phleboviruses are RNA viruses organized into three segments. The S segment encodes the NSs protein, the main virulence factor of phleboviruses, which affects the expression of some antiviral-responsive genes and downregulates key factors of the host transcriptional machinery. Phlebotominae sand flies transmit Phleboviruses to vertebrates, including humans, and the cocirculation of Leishmania and Phlebovirus species has been widely reported. Given the importance of Leishmaniasis for public health and the known importance of viral coinfections, it becomes relevant to analyze the role of the ICOV Nss, considering that previous studies have observed the ability of ICOV infection to improve parasite load in a coinfection model. Due to the lack of information on the role of phlebovirus NSs in America's Phleboviruses, we decided to study the role of ICOV NSs. To this end, we obtained, through lentivirus-mediated transduction, macrophage cell lines stably expressing the ICOV/ NSs. Infection index assays with the NSS/ expressing macrophages showed a favorable effect on infection by *L. amazonensis*. In qPCR assays, a relative increase in INF beta and IL-10 mRNA was observed in cells expressing NSs and in the expression of genes related to the antioxidative response: NRF2, HO-1, and SOD-1. Western blot assays corroborated the augment of NRF2 in macrophages expressing NSs. Our data suggests that ICOV/NSs induce the antioxidative response, favoring the parasite infection.

Supported by: CNPq, CAPES, FAPERJ
Keywords: Co-infection, phlebovirus, *L.amazonensis*

INNOVATIVE LIPOSOMAL NANOFORMULATION CO-INCORPORATING AMPHOTERICIN B AND MILTEFOSINE FOR THE THERAPY OF VISCERAL LEISHMANIASIS

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Liposomal amphotericin B (AmB) or AmBisome® and oral miltefosine (Milt) are two major therapeutic options for visceral leishmaniasis (VL). Nevertheless, the clinical efficacy of these drugs is limited in severe cases of HIV/VL co-infection and cutaneous leishmaniasis (CL). Previously, our group introduced a novel method for encapsulation of AmB into pre-formed liposomes. Considering the great potential of drug combination for further improving leishmaniasis chemotherapy, the aim of this work was to develop a novel liposomal nanoformulation co-incorporating AmB and Milt and evaluate its therapeutic efficacy in a hamster model of VL. AmB was incorporated into pre-formed PEGylated liposomes containing Milt. The mixed PEGylated formulation showed vesicles with diameter of 135 (±14) nm and low polydispersity index (PI<0.3), with AmB encapsulation efficiency of 90%. The mixed PEGylated formulation was compared to PEGylated formulations containing solely AmB or Milt and to AmBisome® in *L. infantum*-infected hamsters for its effects on the parasite load in liver and spleen after 10 doses of treatment, given every day by IP route (5 mg/kg of AmB and 3.9 mg/kg of Milt). Animals, either untreated or treated IP with empty PEGylated liposomes, were used as negative controls. Treatments with mixed AmB/Milt liposomal formulation, liposomal AmB formulation and AmBisome®. promoted significant parasite supression in the liver in comparison untreated control. The mixed formulation showed the highest level of parasite reduction (94.3%), compared to Ambisome® (88.3%) and PEGylated liposomal AmB (84,2%). Significant reduction was also observed in the spleen, at a similar level, for all AmB/Milt liposomal formulations. This study supports the increased efficacy of the mixed PEGylated formulation and its potential for the treatment of severe cases of VL and for situations where there has been therapeutic failure with the use of Milt or AmB alone.

Supported by: FAPEMIG, CNPq and FINEP.

Keywords: Visceral leishmaniasis, Miltefosine, Amphotericin B.

IMMUNOGENICITY AND VACCINE EFFICACY OF A MULTIEPITOPE ANTIGEN DESIGNED FROM *Leishmania infantum* EXOANTIGENS

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In Brazil, visceral leishmaniasis (VL) is caused by *Leishmania* (*Leishmania*) *infantum* and primarily controlled through the euthanasia of seropositive dogs, a strategy with limited efficacy. In this context, prophylactic vaccines represent a promising alternative for disease control. This study evaluated the immunogenicity and vaccine efficacy of a multiepitope antigen, PQ-4, composed of sequences from main exoantigens (Exo-Ags) secreted by *L. infantum*. Exo-Ags were obtained from promastigotes and analyzed using two-dimensional electrophoresis and Western blotting with sera pools from mice immunized with Exo-Ags, patients with VL, control mice and healthy individuals. Immunoreactive proteins were identified by mass spectrometry, and their amino acid sequences were subjected to epitope prediction. Selected epitopes, combined with specific spacers, were used to design PQ-4, which was produced in *Escherichia coli*. BALB/c mice were immunized three times with PQ-4, with or without saponin, challenged with *L. infantum*, and evaluated 42 days post-challenge. PQ-4 was immunogenic, both alone and with saponin, inducing increased frequencies of naïve and effector $CD4^+$ and $CD8^+$ T lymphocytes, as well as total IgG. Afterward the experimental challenge, an increase in effector CD4+ T lymphocytes, effector memory $CD4^+$ and $CD8^+$ T lymphocytes, naïve $CD8^+$ T lymphocytes, and memory B lymphocytes was observed, along with elevated plasma interleukin-6 levels. Furthermore, the splenic parasite burden was reduced by 54.3% and hepatic burden by 37%, with no significant differences between PQ-4 alone or combined with saponin. These findings emphasize the potential of exoantigens and bioinformatics tools for the rational development of vaccines against VL, highlighting the efficacy of PQ-4 in inducing robust immune responses and reducing parasite burden in an experimental model.

Supported by: CAPES-Brazil; FAPEMIG-Brazil

Keywords: visceral leishmaniasis; vaccine; exoantigens

Assessing Toxoplasma gondii Exposure in Bats: A Comparison of Blood Collection Methods

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The interaction between parasites and hosts results from an evolutionary balance but can be affected by environmental changes. In Bahia, significant deforestation may influence the spread of zoonotic agents. Toxoplasmosis, caused by Toxoplasma gondii, affects various species, but the presence of antibodies in bats remains understudied. This study aimed to assess the frequency of anti-T. gondii antibodies in bats from Bahia and compare the efficiency of whole blood and filter paper blood collection for immunological testing. Bats were captured in enclosed environments and using mist nets. Blood was collected via intracardiac or venous puncture, with part of the samples deposited on filter paper. Liver samples were obtained from euthanized animals. Serological tests were performed using direct agglutination, and statistical analyses were conducted in EpiInfo 7 and DagStat, including chi-square tests, McNemar's test, and the Kappa coefficient. A total of 45 bats from different municipalities and habitats were analyzed. Nine animals (20%) tested positive for T. gondii antibodies, with seven positive in both whole blood and filter paper samples and one in the liver. Diagnostic analysis showed high specificity (92.31%) for filter paper but moderate sensitivity (60%), indicating a high number of false negatives. The Kappa coefficient (0.5231) indicated moderate agreement between matrices, and statistical analysis confirmed a significant association between results ($p = 0.0036$). Whole blood and filter paper tests demonstrated good specificity and negative predictive value, but the reduced sensitivity of filter paper limits its clinical applicability. Although it is a viable alternative, whole blood remains the most reliable matrix for detecting T. gondii antibodies in bats.

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Key-words: Toxoplasma gondii, bats, serological diagnosis.

EXPLORING CHILEAN PATAGONIA EXTREMOPHILES AND MARINE BACTERIA METABOLITES AS NEW ANTITRYPANOSOMAL COMPOUNDS

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Neglected Tropical Diseases as Chagas disease represents a public health challenge, affecting 7 million people worldwide. With an ineffective treatment, the search for new therapies is urgent. Microbial metabolites have been widely used as prototypes in FDA-approved drugs. Marine and extremophilic microorganisms produce an array of metabolites, depending on their adaptations to extreme conditions as those found in the glaciers and hypersaline lakes. Considering the huge chemodiversity of these microbial metabolites, we explored the anti-*T. cruzi* potential of extremophiles from sediments of the Chilean Glacier Grey Lake, the hypersaline Bitter Lagoon (Patagonia), and marine bacterial metabolites from Brazil. Sediments were plated in Marine agar and TSA agar medium, and the bacteria were isolated using conventional techniques. The identification of the bacteria was performed by MALDI-ToF/MS and 16S rRNA gene partial sequencing. The bacterial metabolites of ten Gram-negative bacteria were extracted with organic solvents and the anti-*T. cruzi* (Y strain) activity was evaluated against trypomastigotes at 150 µg/mL for 24h incubation, using resazurin. Chemical analyses were performed using Nuclear Magnetic Ressonance (^{1}H and ^{13}C NMR). The MALDI-ToF/MS analysis resulted the identification of only one bacteria, the *Planococcus maritimus*. The 16S rRNA sequencing identified three isolates as *Pseudoalteromonas rubra*, *Pseudoalteromonas piscicida* and *Cyclobacterium plantarum*. Our results demonstrated that all organic extracts killed 100% of the parasites at the highest tested concentration, with EC_{50} values between 3 and 62 µg/mL and benznidazole 6 µg/mL. Two potent extracts (PLASE1.1 and SMG37) were selected for NMR analysis and the results showed a mixture of compounds (< 1 kDa) containing aromatic rings. Marine microorganisms and those living in extreme environments represent new sources of small molecules with potential antitrypanosomal activity.

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Keywords: Trypanosoma cruzi, therapy, natural products

PRELIMINARY RESULTS ON THE IDENTIFICATION OF *BLASTOCYSTIS* SUBTYPES IN PATIENTS WITH PULMONARY TUBERCULOSIS FROM THE SOUTHERN REGION OF BRAZIL

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Abstract

It has been a great challenge to explain whether *Blastocystis* is a pathogen or a common organism of the intestinal microbiota. In recent years, several studies have focused on the diagnosis of *Blastocystis* in immunocompromised individuals. The aim of the present study was to analyze *Blastocystis* subtypes circulating in patients with pulmonary tuberculosis in an area of Southern Brazil. For this purpose, Genomic DNA was extracted of fecal samples from patients with diagnosed and undergoing treatment for pulmonary tuberculosis at the Reference Center for Tuberculosis Dr. Bruno Piancastelli Filho, city of Londrina, Paraná, Brazil. PCR amplification was performed using *Blastocystis*-specific SSU-rDNA primers and positive samples were subjected to sequencing. The sequences were aligned and compared with other sequences in GenBank. To date, only one subtype (ST1) was detected in the sequenced samples. We intend to sequence the remaining samples and further evaluate all the sequences obtained, as well as evaluate clinical data from these individuals. We recognize that studies evaluating the subtypes of *Blastocystis* circulating in the world are extremely important, especially in Brazil, where there is a shortage of studies with this purpose.

Keywords: *Blastocystis*, pulmonary tuberculosis, Brazil

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Giardiasis Infections in Preschoolers: An Analysis of Daycare Centers in Teresina, Piauí

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Abstract

Human infections caused by Giardia lamblia are common in children between the ages of two and five due to poor hygiene habits and ineffective immunity, with varied clinical manifestations: weight loss, diarrhea, epigastric pain and insomnia. The aim of this study was to survey the prevalence of giardiasis in preschoolers enrolled in public daycare centers in the municipality of Teresina, Piauí, Brazil, and to relate its epidemiological aspects and risk factors through serial coproparasitological examinations and a questionnaire applied to parents to find out about the socioeconomic and hygienic condition of each participant in the study. The research took place between 2014 and 2018. Initially, letters of consent from the schools were collected, along with the Free and Informed Consent Form. After this stage, fecal samples were collected from the students in containers with preservative (10% formaldehyde) and sent to the IFPI Parasitology Laboratory for analysis using the Hoffmann, Pons and Janer method. Of the 405 samples analyzed, 21.2% were positive for G. Lamblia (86/405), 51.8% were negative (210/405) and 26.9% (109/405) were positive for other enteric parasites. Of the 86 children with giardiasis, 84.9% (73/86) were from families with an income of less than one minimum wage and 15.1% (13/86) had an income of more than one minimum wage. The highest prevalence was observed among children aged 5 to 6, 54.1% (46/86), and the lowest among those aged 3 to 4, 45.9% (40/86). Giardiasis had a high prevalence in the study participants, this infection can cause impairment in the physical and cognitive development of these children. Giardiasis was associated with risk factors such as low parental income and schooling, consumption of untreated water and poorly sanitized food. Ongoing health education programs, good hygiene habits and regular parasitological tests are effective strategies for combating this parasitosis.

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Keywords: preschoolers, Giardiasis, Child health

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EPIDEMIOLOGICAL PROFILE OF INTESTINAL PARASITIC INFECTIONS IN A COMMUNITY ON THE OUTSKIRTS OF TERESINA – PI

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Abstract

Intestinal Parasitic Infections (IPIs) are caused by protozoa or helminths and represent a public health problem, being associated with social vulnerabilities such as low income, poor sanitation and housing, as well as limited access to health and education. The main symptoms include irritability, abdominal pain, diarrhea, malnutrition and anemia. This study aimed to assess the prevalence of IPIs in a community on the outskirts of Teresina-PI. From January to December 2019, fecal samples from 178 participants were analyzed using the Hoffman, Pons & Janer method at the Parasitology Laboratory of the IFPI - Teresina Central Campus. Among the participants, 52.2% (93/178) were female and 48.8% (85/178) male, distributed in the following age groups: 2 to 9 years (24.7%), 10 to 19 years (23.6%), 20 to 59 years (47.8%) and ≥ 60 years (3.9%). Of the 178 samples analyzed, 44.2% (79/178) were positive only for protozoa. The prevalence was 39.8% (37/93) in females and 48.2% (41/85) in males. With regard to prevalence by age group, the highest rate occurred in children aged 2 to 9 years (52.3%), followed by those aged 10 to 19 years (45.2%), 20 to 59 years (41.7%) and ≥ 60 years (28.6%). The non-pathogenic protozoa identified were Endolimax nana (18.5%), Entamoeba coli (17.4%) and Iodamoeba butschlii (2.4%), while the pathogenic ones included Giardia lamblia (11.8%) and Balantidium coli (2.8%). The results of this study are similar to those of other studies carried out in Piauí and other Brazilian regions, and reinforce the need for effective IPI control strategies, extending them to protozoa, including improvements in access to drinking water, proper solid waste management and mass treatment with drugs, preceded by parasitological tests. In addition, health promotion actions can strengthen skills that encourage behavioral changes and the adoption of preventive measures.

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Keywords: Intestinal Parasitic Infections. Protozoa. Public Health.

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HOTSPOT MAPPING AND THERMOSTABILITY ANALYSIS OF 17-DMAG BINDING TO *LEISHMANIA BRAZILIENSIS* HSP83

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Introduction: Previously, we have shown that human molecular chaperone heat shock protein 90 (*Hs*Hsp90) inhibitors, including 17-DMAG, exhibit both *in vitro* and *in vivo* leishmanicidal activity. It is reasonable to assume that 17-DMAG exerts its effects by inhibiting the parasite's Hsp (Hsp83), a key regulator of phase transitions in its life cycle. However, direct experimental evidence supporting this hypothesis is still lacking. This knowledge gap hinders the molecular simplification of 17-DMAG to reduce synthesis costs, limits the evaluation of critical hotspot residues (e.g., Asp90 in Hsp90), and the efforts to enhance its selectivity profile. **Hypothesis:** Evolutionary constraints have differently shaped the hotspot regions of *Hs*Hsp90 and *L. braziliensis* Hsp83 (*Lb*Hsp83). **Objective:** To confirm that 17-DMAG binds to *Lb*Hsp83 and to identify key hotspot residues involved in this interaction. **Methods:** Hotspot analysis was performed using the DRUGpy plugin along with FTmap and e-FTmap servers. Recombinant N-terminal *L. braziliensis* Hsp83 was expressed, purified via chromatographic methods, and analyzed in differential scanning fluorescence assays in the presence or absence of 17-DMAG. **Results:** The binding of 17-DMAG to *Lb*Hsp83 was confirmed by its large increase in melting temperature (ΔTm = 10.4°C) upon 17-DMAG addition (500 μM), which was consistent with high-affinity binding, further validated by concentration-response assays (Kd = 0.32μM). After establishing *Lb*Hsp83 as the molecular target of 17-DMAG, the hotspot profiles of *Hs*Hsp90 and *L. major* Hsp83 (*Lm*Hsp83), the closest homolog with 3D structure available were compared. The analysis revealed differences in hotspot strength (*druggable vs. borderline-druggable*), suggesting that inhibiting *Lm*Hsp83 may be more challenging than targeting *Hs*Hsp90. Additionally, this analysis shows a fraction of the *Lm*Hsp83 hotspot remained unoccupied by 17-DMAG, indicating potential for affinity enhancement. A comparison of atomic consensus sites from these structures highlighted that fine-tuning apolar and hydrogen donor interactions is crucial for designing selective inhibitors. **Conclusion:** Identifying *Lb*Hsp83 as the molecular target of 17-DMAG was paramount to carrying out hotspot analysis that offers valuable insights to guide potency and affinity optimization.

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Keywords: *Leishmania braziliensis*, Heat Shock Proteins (HSP), 17-DMAG, hotspot analysis, DSF

NEW PERSPECTIVES ON LEISHMANIASIS TREATMENT AND THE POTENTIAL OF NANOPARTICLES CONTAINING NATURAL EXTRACTS: A LITERATURE REVIEW

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Abstract

Introduction:Leishmaniasis is a significant public health issue, affecting around 12 million people in 88 countries. Caused by *Leishmania* spp., it manifests in cutaneous, mucocutaneous, and visceral forms, with the latter being the most severe. In Brazil, treatments include Glucantime, Amphotericin B, and Miltefosine, but these drugs have high toxicity and costs. The growing resistance of *Leishmania* spp. to conventional therapies and their limitations have driven the search for alternative treatments. Nanotechnology, combined with natural bioactive compounds, has emerged as a promising strategy to develop more effective and less toxic therapeutic options. **Objective** This review evaluates the therapeutic potential of nanoparticles with natural bioactive compounds for leishmaniasis treatment. **Methods:** A bibliographic search was conducted in PubMed, SciELO, and Google Scholar using the keywords "leishmaniasis," "nanoparticles," "natural compounds," and "medicinal plants," focusing on relevant studies in Portuguese, English, and Spanish. **Results and Discussion:** Nanoparticles with natural compounds show strong antileishmanial potential, reducing parasite load and improving therapy. Encapsulated lupeol enhances bioavailability and efficacy, while polymeric nanoparticles with 17-DMAG effectively target *Leishmania* with lower toxicity. *Libidibia ferrea* fractions in microemulsions also show promise. Nanotechnology offers better efficacy, reduced toxicity, and cost-effectiveness, but large-scale production, clinical validation, and regulatory approval remain challenges. **Conclusion**: Nanoparticles with natural bioactives offer a promising alternative for leishmaniasis treatment, enhancing efficacy and reducing toxicity. Further research should optimize formulations, validate clinical safety, and ensure accessibility in endemic areas. Integrating nanotechnology could revolutionize treatment and improve patient outcomes.
Keywords: Leishmaniasis, Nanotechnology, Phytotherapeutics

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CANINE VISCERAL LEISHMANIASIS: EVALUATION OF RESPONSE TO TREATMENT BASED ON CHEMOTHERAPY AND IMMUNOCHEMOTHERAPY

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Abstract

Canine visceral leishmaniasis (CVL) is an infectious, chronic, systemic disease caused by the protozoan *Leishmania infantum*, and dogs are considered the main reservoir of the parasite. Controlling the disease has been a challenge to public health, since there are few resources available and due to its geographic expansion. Treatment has limitations related to side effects, the possibility of resistant strains, and does not guarantee a parasitological cure. CVL is considered an immunomodulated disease, in which the animal's immune response is fundamental in the presentation of clinical manifestations and the course of the disease. Therefore, immunotherapy appears as a promising option, with the aim of restoring the Th1 cellular immune response, increasing levels of cytokines such as IL-2 and IFN-ɣ. The present study includes dogs naturally infected by L. infantum and aims to evaluate and compare the clinical and laboratory evolution of the treatments instituted. For this purpose, the animals were divided into three groups: one using miltefosine and allopurinol, the second using miltefosine, allopurinol and domperidone and the third using miltefosine, allopurinol and immunotherapy “A”. The protocol was established according to the staging of the animal (according to Brasileish) and three evaluation times were determined for analysis. For all times, clinical evaluation, blood collection for hemogram and biochemical examination, lymph node puncture for culture, biopsy of skin fragments to evaluate parasite load by PCR and histopathology were included. So far, it has been possible to observe the absence of adverse reactions to the application of the immunotherapy and a good clinical response.

Supported by: Fapesc, CNPq, Capes, UFSC.
Keywords: Canine visceral leishmaniasis, *Leishmania infantum*, immunotherapy

PHARMACOLOGICAL PROSPECTING OF THERMORECEPTORS IN *LEISHMANIA* SPP.

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Leishmania spp. are dimorphic parasites with a heteroxenic life cycle. In the invertebrate host, the promastigote form resides in the vector's digestive tract, where the pH varies from neutral to slightly acidic, and the temperature is around 26°C. Upon infecting the vertebrate host, the parasite encounters an acidic pH in the parasitophorous vacuole and a temperature of 35–37°C. Both forms have evolved to survive within these environments. In mammals, Transient Receptor Potential (TRP) channels detect environmental stimuli, including temperature changes. This study aims to functionally characterize the receptor used by *Leishmania* spp. to sense temperature fluctuations and trigger differentiation between promastigote and amastigote forms. We performed phenotypic screening using TRPV1 and TRPM8 receptor agonists and antagonists to "mimic temperature," assess morphological changes, and determine IC_{50} values. The key compounds and their IC_{50} values for promastigotes of *L. amazonensis* and *L. infantum* were: olvanil (>128 and 15.23 µM), capsazepine (22.9 and 43.37 µM), AMTB (6.64 and 15.28 µM), M8-B (21.15 and 19.38 µM), and icilin (15.97 and 53.30 µM). In initial scanning microscopy assays, *L. infantum* promastigotes treated with icilin (128 µM) displayed morphological changes similar to axenic amastigotes. Since icilin is a cold-sensitive channel agonist, we further examined the effects of low temperatures. After 72 hours at 16°C, promastigotes adopted a rounded morphology, distinct from their elongated shape at 26°C. These findings suggest that both icilin and cold conditions act as environmental stressors, triggering adaptive responses in promastigotes. Although preliminary, our results also indicate the possible presence of temperature receptors in Leishmania spp.

Suported by: CNE/Faperj, CNPq, Instituto Oswaldo Cruz – Fiocruz and CAPES.

Keywords: *Leishmania*, thermoreceptors, differentiation

Evaluation of the therapeutic potential of mesenchymal stem cells overexpressing lif in a model of chronic chagas cardiomyopathy

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Chagas disease, caused by the protozoan parasite Trypanosoma cruzi, poses a significant global health challenge, particularly in endemic countries. Chronic cardiomyopathy, a severe manifestation of the disease, is characterized by inflammation, fibrosis, and subsequent heart failure. Current treatments, such as benznidazole, have limited efficacy during the chronic phase, underscoring the need for innovative therapeutic strategies. We hypothesize that the administration of MSC-LIF promotes cardiac repair through anti-inflammatory and regenerative mechanisms. The expected outcomes may provide insights into novel therapies for Chagas disease and contribute significantly to the field of regenerative medicine.Therefore, the aim of this work was evaluate the therapeutic potential of mesenchymal stem cells (MSCs) that overexpress leukemia inhibitory factor (LIF) in a model of chronic Chagas cardiomyopathy.This study utilizes an experimental model where black C-57mice were infected with T.cruzi Colombian strain, and after 4 months of infection they were treated with the cells, wich was performed by weekly intravenous injections of cell suspensions containing $1x10^6$ MSCs or MSC_LIF. Post-treatment, Mice underwent ergometric tests to evaluate cardiac function, and after euthanasia the organs were collected for analysis. Although there was no improvement in the exercise capacity of mice infected with T. cruzi, the administration of LIF-overexpressing MSCs (MSC-LIF) and wild-type MSCs (MSC-WT) resulted in a significant reduction in inflammation when compared to the vehicle group, with no significant difference between MSC-LIF and MSC-WT. However, in terms of fibrosis analysis, MSC-LIF demonstrated superior outcomes compared to all other groups.

Financial Viability: The project has financing from Pronex (PNX 0002/2014).

Keywords: Chronic Chagas cardiomyopathy (CCC); cell therapy; mesenchymal stem cells;

TNF BLOCKERS ALONE AND ASSOCIATED WITH BENZNIDAZOLE IMPACT *IN VITRO* CYTOKINE DYNAMICS IN CHRONIC CHAGAS DISEASE

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Studies involving the immune response in Chagas disease suggest an imbalance in the immune response of symptomatic patients, with an inflammatory profile dominating in Chagas heart disease, mainly by tumour necrosis factor (TNF). TNF is considered a key cytokine in immunopathology in chronic carriers in several processes during the immune response. Our work aimed to evaluate regulatory (interleukin [IL]-4 and IL-10) and inflammatory (TNF, interferon-gamma [IFN-γ], IL-2 and IL-6) cytokines in peripheral blood mononuclear cells culture supernatants. of affected patients with undetermined clinical forms—IND (n = 13) mild heart form—CARD1 (n = 13) and severe cardiac form—CARD2 (n = 16), treated in vitro with two TNF blockers, Adalimumab (ADA) and Etanercept (ETA) alone or in association with Benznidazole (BZ). The results indicate that ADA was more competent in blocking TNF (compared to ETA) in all groups but with much lower levels in the CARD2 group. ETA statistically decreased TNF levels only in the CARD2 group. IFN-γ increased in the CARD2 group after treatment with ETA relative to ADA. IL-4 had its levels decreased when treated by both drugs. IL-2 was detected in cells from CARD2 carriers compared to the NEG group after treatment with both drugs. The association with BZ decreased levels of IL-2/TNF and increased IL-4. These data reinforce the participation of TNF in severe Chagas heart disease and bring perspectives on using these blockers in the immunological treatment of Chagas disease since the use of BZ is extremely limited in these patients.

Supported By: CNPQ, CAPES, FACEPE

Keywords: tumour necrosis factor inhibitors, Chagas cardiomyopathy, cytokines

ANTILEISHMANIAL ACTIVITY OF 4-QUINOLONE-3-CARBOXAMIDE DERIVATIVES

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Abstract

Among infectious and parasitic diseases, leishmaniasis is on the rise due to therapeutic limitations, such as issues with tolerability, toxicity, and loss of efficacy over time. To address this scenario, the present study focuses on the synthesis of quinolones that may offer a new treatment option for leishmaniasis. The quinolone class, commonly found in antibacterial drugs, has shown activity against protozoa through mechanisms that remain uncertain. A potential target for antiparasitic therapies is the mitochondrial electron transport chain, which facilitates electron transfer through electron donors such as NADH and FADH2, generating various catabolic processes. This pathway plays a crucial role in ATP production in many organisms, and its inhibition can lead to the generation of toxic reactive oxygen species (ROS), such as superoxide anion (O_2^-). In this study, a series of seven 4-quinolone-3-carboxamide derivatives (CR series) were synthesized, featuring substitutions in the benzene ring of the carboxamide group with CH_3, Cl, and NO_2 at the meta and para positions. The leishmanicidal activity was evaluated in *Leishmania amazonensis* promastigotes, with CR-mCl (IC_{50} = 20.7 µM) and CR-mNO2 (IC_{50} = 25.8 µM) being the most active compounds in the series. An analysis of the structure-activity relationship revealed that the CR-pCl and CR-pCH3 molecules, which contain Cl and CH_3 substituents at the para position, were inactive at the tested concentration. This suggests that the meta position may provide a larger surface area for interaction with the target. Further studies on the activity of these compounds in intracellular amastigotes will be conducted to better understand their structure-activity relationship, as well as their effects on mitochondrial membrane potential and ROS production. These investigations aim to elucidate the pharmacological mechanism of these compounds and explain their observed activity.

Supported by: CNPq, FAPERJ

Keywords: leishmania, quinolones, mitochondria

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Evaluation of the lpg2 gene during *Leishmania infantum* and host cell interaction

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Abstract

INTRODUCTION: Visceral leishmaniasis is considered very severe, with high mortality rates when not treated properly. Is characterized by hepatosplenomegaly and caused by the species *Leishmania infantum* in Brazil. The promastigote form of the parasite has molecules in their surface important for the host infection, the most abundant one is the Lipophosphoglycan (LPG). The *L. infantum* promastigote also has other phosphoglycans on its surface. In response to these virulence factors, macrophages activate their microbicidal mechanisms to combat *L. infantum* infection. In this context, parasites LPG deficient, obtained by the CRISPR/Cas9 system, represent an efficient tool to evaluate the parasite-host cell interaction. **OBJECTIVES**: This study aims to investigate how the presence or absence of lpg2 can alter the microbicidal functions of murine macrophages during infection by comparing *L. infantum* wild-type (WT), *L. infantum* knockout (Δlpg2), with the deletion of the lpg2 gene and the *L. infantum* genetically restored Add-back (Δlpg2 + lpg2). **METHODS**: Macrophages derived from the bone marrow of C57BL/6 mice were cultured and subsequently infected with *L. infantum* WT, Δlpg2 and Δlpg2 + lpg2 metacyclic promastigotes. After 4, 48 and 72 hours of infection the parasite load and viability were evaluated. The culture supernatant was collected to detect nitric oxide and inflammatory mediators. **RESULTS:** We observed a reduction in the Δlpg2 parasites survival compared to WT parasites. Furthermore, macrophages infected with the *L. infantum* Δlpg2 had a significant increase in nitrite production compared to the WT and Δlpg2 + lpg2 groups, suggesting a greater microbicidal potential of macrophages infected by the Δlpg2. **CONCLUSION:** *L. infantum* Δlpg2 parasites have lower virulence and stimulate a stronger macrophage response, highlighting LPG's role as a key virulence factor. Further research is needed to understand the inflammatory mechanisms and the role of LPG2 in macrophage activation.

Keywords: Macrophage. *Leishmania infantum*. *lpg2*

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MALARIA IN THE BRAZILIAN AMAZON: META-ANALYSIS OF THE DISTRIBUTION, EPIDEMIOLOGICAL PROFILE, RISK FACTORS, AND IMPLICATIONS FOR PUBLIC HEALTH

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Introduction: Malaria is an infectious, febrile, acute, and potentially severe disease caused by the parasite *Plasmodium* spp. It has a significant impact on morbidity in tropical and subtropical countries. In Brazil, *P. vivax*, the main species found in the Americas, has a heterogeneous geographic distribution, with temporal and spatial variations, including areas of high incidence, disease-free regions, and locations with low transmission risk**.**
Aims and scope: To determine an accurate meta-analysis of the epidemiological profile of malária in the Amazon Basin in Brazil.
Methods: This was a retrospective, descriptive, and cross-sectional epidemiological study based on the meta-analysis of secondary data on malaria in the Brazilian Amazon Region, between 2012 and 2023. The data were collected through the National Center for Epidemiological Intelligence and Genomic Surveillance (CNIE), available on the Ministry of Health's website, and included in the research were malaria cases resulting from *Plasmodium vivax* infection. Descriptive analysis was performed using the Stata software to assess the following variables: race/color, age, sex, and the parasitic incidence in the Amazon basin per 1,000 inhabitants.
Results and findings: During the analyzed period from 2012 to 2023, a total of 3,010,283 malaria cases were reported, of which 41,160 were in pregnant women and postpartum women. The majority of the reported cases were in males (61%), of mixed race/color (67.5%), and in the age group of 20–29 years (19.49%). The analysis conducted to evaluate the incidence per 1,000 inhabitants revealed that during the analyzed period, the incidence was 5.92 cases per 1,000 inhabitants. Geographical maps based on models indicate the highest concentrations of malaria cases per 1,000 inhabitants in the states of Acre (19.92), Roraima (10.86), Amazonas (9.30), and Amapá (9.28).
Conclusions: Malaria was highly prevalent. Our results can guide future health initiatives through public policies aimed at strengthening epidemiological surveillance, intensifying vector control measures, and expanding access to early diagnosis and treatment. Additionally, integrated actions between the health, environment, and education sectors are crucial to reduce malaria transmission and minimize its impact on the Amazonian population.

Keywords: Brazilian Amazon, Malaria, Public Health.

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INTRA-GENOTYPIC VARIABILITY IN TRYPANOSOMA CRUZI DTU TCII

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Abstract

Trypanosoma cruzi is a genetically diverse parasite grouped into seven Discrete Typing Units (DTUs): TcI to TcVI and TcBaT. Due to its association with Chagas disease (CD), studies on TcII in wild animals are rare, and its diversity is poorly understood. This study aimed to analyze TcII variability, testing the hypothesis of genetic variability and that primate and humans select the same sublineages. DNA from TcII isolates (marsupials n=12, primates n=80, humans n=55) were subjected to PCR targeting the 18S rDNA gene, followed by next-generation sequencing on Illumina platform. Sequences were identified by bioinformatics analysis. Genetic distances were analyzed using a matrix (MegaX) and a haplotype network (Network). Four DTU TcII sublineages were identified: alpha, beta, gamma and delta. Alpha was widely distributed and adaptable in all the samples. No genetic distance differences were observed with reference sequences Y, Esmeraldo and Basileu. It was the only sublineage identified in *Didelphis* sp., which may indicate that alpha is the oldest sublineages, because this mammal is considered the ancestor of *T. cruzi*. Beta was found in primate and human, making it the second most widely distributed. Gamma was found only in primates, and delta only in human samples. Gamma was closest genetic distance to alpha (0.002), followed by beta (0.008). Delta was genetically distant from alpha (0.015) and beta (0.023). Human samples showed mixed infections: alpha+beta (n=4) and alpha+delta (n=1). Primate samples showed alpha+beta (n=5) and alpha+gamma (n=1). Beta and gamma sublineages seem to be transmitted by vectors that do not interact with marsupials and delta transmitted by vectors interacting directly with humans. The differentiation between gamma and delta hosts may be due to limited environmental exposure, with gamma being restricted to primates. These findings offer new perspectives on the genetic variability of TcII and its interactions with hosts in nature.

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Keywords: Intra Genotypic Diversity, *Trypanosoma cruzi* TcII, Primate

Assessment of the clinical profile of patients with digestive impairment associated with Chagas disease in Bahia

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Chagas disease (CD) is caused by the protozoan Trypanosoma cruzi (T. cruzi), a neglected disease and persistent public health problem in Latin America, where an estimated 70 million people live with this disease. In Brazil, an estimated 300,000 people have Chagas megaesophagus and/or megacolon. Due to its magnitude, it is necessary to identify T. cruzi infection among people with digestive manifestations so that it is possible to treat and improve the quality of life of those with the disease. The objective of this study is to characterize people with megaesophagus, the history of potential risk for vectorial transmission of T. cruzi and the seroprevalence of anti-T. cruzi.

This is a cross-sectional, descriptive study carried out in the outpatient clinic of a gastroenterology reference hospital (HGRS) in the state of Bahia. For data collection, a previously tested questionnaire was used, applied in the anamnesis of each participating patient, which had questions about lifestyle habits, day-to-day life, diet and current stages of symptoms. Of the 363 people with megaesophagus interviewed, it was observed that they were female (61.2%), had absent dysphagia (30.5%) occasionally (26.6%) daily (10.0%) and in all meals (33.0%), ate consistent foods (74.5%) not consistent (25.5%) if they were solid (1.1%) pasty (57.6%) liquid (38.0%) or through a tube (2.2%), with absent regurgitation (50.4%) occasionally (28.5%) daily (7.5%) and in all (13.6%), salivating a lot (38.2%) little (61.8%), absent chest pain (61.9%) occasionally (26.9%) daily (5.0%) all (6.2%).

Given the clinical characteristics of the interviewees, a comparison of megaesophagus can be made between chagasic and idiopathic patients treated at the outpatient clinic, comparing clinical cases of both situations, corroborated by the patients' lifestyle habits. These findings highlight the need for improvements in screening tests, research on cases of idiopathic megaesophagus, and emphasis on adequate medical care, in addition to more effective public health policies to prevent and reduce the transmission of CD and its epidemiological and social impact.

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Keywords: Megaesophagus. Chagas disease. Clinical profile

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BODO SP. (KINETOPLASTEA; EUBODONIDA) IDENTIFICATION IN BLOOD SAMPLES FROM ANTEATERS AND ARMADILLOS FROM THE PANTANAL AND CERRADO OF BRAZIL

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Abstract

Among the organisms of the Kinetoplastea class (which includes the trypanosomatids), the genus *Bodo* is one of the most studied free-living protozoa, typically found in aquatic environments. Although described as non-parasitic, several studies detected bodonids in vertebrate samples. Armadillos and anteaters are animals rarely seen in nature but can be found in flooded fields, a typical habitat for bodonids. Molecular diagnosis in blood clots allows the detection of non-cultivable parasites and/or at low parasitic loads and may reveal undescribed kinetoplastid species. This study aimed to describe kinetoplastid infections in armadillos and anteaters from Pantanal and Cerrado of Brazil. Blood samples from four armadillo species and the giant anteater were collected in Mato Grosso do Sul between 2011 and 2021 in collaboration with the Wild Animal Conservation Institute. DNA from 144 blood clot samples was extracted using an ammonium acetate precipitation protocol. The diagnosis was performed by Nested PCR targeting the 18S SSU rDNA. Positive samples were subjected to Sanger sequencing and the characterizations were confirmed by phylogenetic analysis. Of the 144 samples tested, *Bodo* sp. DNA was detected in 22 (15.3%) samples of giant anteater and giant armadillo. Infection by trypanosomatids was reported in other 18 (12.5%) animals. In general, the bodonid sequences showed approximately 95% identity and were clustered close to *Bodo saltans* sequences. These infections were herein defined as *Bodo* sp. due to low identity value to define a species and the possibility of being a new species is not discarded due to poor knowledge of the *Bodo* species real diversity. The presence of *Bodo* sp. in four recapture events may represent the long-term maintenance of these infections (7-26 months). The high detection rate of bodonid DNA in armadillos and anteaters reinforces the putative capacity of free-living protozoa to infect vertebrates.

Supported by: IOC/Fiocruz, CNPq, FAPERJ, Wild Animal Conservation Institute, Funbio, Santander Mobility Fund

Keywords: *Bodo* sp., molecular diagnosis, blood clot.

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IMPLICATION OF CLASSICAL PHAGOCYTOSIS IN THE INITIAL EVENTS OF LEISHMANIA BRAZILIENSIS INFECTION IN MONOCYTE-DERIVED MACROPHAGES

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ABSTRACT

INTRODUCTION:*Leishmania braziliensis* (*Lb*), the primary causative agent of cutaneous leishmaniasis in Brazil, results in localized, mucosal, diffuse or disseminated forms. The initial parasite-macrophage (MΦ) interactions are crucial for determining infection outcomes, but remain poorly understood for *Lb*. **OBJECTIVE:**This study investigates these interactions, particularly focusing on phagocytosis and parasitophorous vacuole (PV) formation, and their roles in parasite survival within MΦ. **METHODOLOGY:**MΦ were treated with the AKT inhibitor GSK 690693 and the Akt inhibitor, and the PLC inhibitor U73122 to study their effects on parasite internalization and subsequent intracellular events. Infection rates, reactive oxygen species (ROS) production using the H2DCFDA probe, and endosomal markers Rab5 and Rab7 by fluorescent antibodies were assessed in PVs of infected cells and in cells incubated with zymosan as phagocytic control. Hydrolytic activity and vacuole acidification were evaluated using DQ-BSA or Lysotracker (Lys). Quantification was performed by fluorescence microscopy for ROS and confocal microscopy for quantifying markers. Additionally, the effect of chloroquine (Chl) on vacuole acidification was examined. **RESULTS**:Initial hours of infection showed a rise in amastigotes per MΦ, stabilizing from 24 to 72 h. U73122 reduced infection rates, indicating its role in parasite uptake. ROS production was elevated in the U73122-treated group, but not in the GSK 690693 group. Rab5 expression was higher at 2 h, and Rab7 at 24 h in PVs. Chl treatment reduced Lys positivity, confirming acidification of *Lb*-induced PVs. CONCLUSION: PLC inhibition diminishes *Lb* uptake, suggesting its involvement in phagocytosis via PIP_2 hydrolysis. In addition, the enzyme increases ROS levels by mechanisms should be elucidated. Moreover, the differential expression of Rab5 and Rab7 and the impact of Chl on vacuole acidification show that *Lb* follows a classical phagosome maturation pathway in MΦ.

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Keywords: Phagocytosis, Macrophages, *Leishmania braziliensis*.

Development of amidoxime 5-nitrofuran-3-carboxylate derivatives bioactivated by *Leishmania infantum* nitroreductases

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The therapeutic arsenal for leishmaniasis has become outdated, with unacceptable side effects and increasing cases of resistance. Therefore, ongoing research to discover new low-cost antileishmanial compounds suitable for oral administration is of fundamental importance. Several studies have demonstrated that amidoxime-containing compounds exhibit leishmanicidal activity. In the group's previous work, a series of 4,5-dihydrofuran derivatives containing an amidoxime group was optimized against *Leishmania amazonensis* promastigotes, focusing on position 3 of the dihydrofuran ring by introducing aliphatic fractions containing an ester group, leading to the derivative OSC75 (IC_{50} = 6.3 µM). To further enhance activity while minimizing toxicity, an additional hit-to-lead optimization round was conducted. This study aimed to compare the antileishmanial activity of furan and thiophene derivatives with or without a nitro group (OSC245, OSC254, OSC255, and OSC256). Nitro derivatives have significant potential for drug development due to the presence of nitroreductases in trypanosomatids, which are not homologous to those in humans. This distinction enables selective bioactivation of drug candidates, leading to parasite-specific toxicity. Antileishmanial activity was evaluated in *L. amazonensis* promastigotes, with all derivatives showing activity and inhibiting parasite growth, with IC_{50} values ranging from 7.6 to 78 µM. The nitro-containing derivatives OSC254 (IC_{50} = 7.6 µM) and OSC256 (IC_{50} = 29.1 µM) were more potent than their non-nitro counterparts. To further investigate the nitro-activation hypothesis, the potency of these compounds will be compared in wild-type (*LiWT*) *Leishmania infantum* promastigotes and those overexpressing type 1 nitroreductase (*LiNTR1*).

Support by: CNPQ, FAPERJ/CNE
Keywords: *nitroderivatives, L. infantum, NTR1*

DIVERSITY OF PROTOZOAN PARASITES THAT INFECT ANURANS AND REPTILES IN THE BRAZILIAN AMAZON REGION

1LABORATÓRIO ECOLOGIA DE DOENÇAS TRANSMISSÍVEIS NA AMAZÔNIA, INSTITUTO LEÔNIDAS E MARIA DEANE – FIOCRUZ AMAZÔNIA, MANAUS, BRASIL; 2PROGRAMA DE PÓSGRADUAÇÃO EM BIOLOGIA DA INTERAÇÃO PATÓGENO-HOSPEDEIRO, INSTITUTO LEÔNIDAS E MARIA DEANE – FIOCRUZ AMAZÔNIA, MANAUS, BRASIL; 3LABORATÓRIO DE DOENÇAS PARASITÁRIAS- COLEÇÃO DE PROTOZOÁRIOS, INSTITUTO OSWALDO CRUZ, FIOCRUZ RJ, RIO DE JANEIRO, BRASIL; 4FIOCRUZ RONDÔNIA, PORTO VELHO, BRASIL

The Amazon is home to one-third of the world's biodiversity, housing 163 species of anurans and 315 of reptiles. These animals have significant zoonotic potential, as they are reservoirs for a wide variety of parasites, such as protozoa of the Trypanosomatidae family, obligate parasites of vertebrates, invertebrates and plants, and transmitted between hosts by insects. Of the 25 genera of the Trypanosomatidae family *Trypanosoma* and *Leishmania* have been the most studied because of their medical and veterinary importance. The other genera are few studied. Therefore, the project seeks to identify the trypanosomatid species that infect different reptile and anuran hosts, in order to fill gaps in knowledge of biodiversity. The collections were carried out in rural areas of the municipalities of Presidente Figueiredo (PF) and Urucurituba (UR), AM. In each municipality, two areas were selected, in each one a Pitfall Trap with Drift Fence (AIQ) was built. A blood sample was obtained from each captured animal by cardiac or caudal puncture for the preparation of a smear and subsequent observation of the parasites under optical microscope. At all 141 animals were captured, 67 belonging to the Amphibia Class and 74 to the Reptilia Class. In PF, 93 individuals were collected and in UR, 48. Trypanosomatids were observed in the anuran Pristimantis fenestratus, compatible with the species Trypanosoma fallisi; in Allobates femoralis and Rhinella marina, were observed trypanosomatids compatible with Trypanosoma rotatorium and T. tsunezomiytai. In addition, 50% of the reptiles and 11.4% of the amphibians presented protozoa of the phylum Apicomplexa (Hepatozoon, Hemogregarinas and Plasmodium). The identification of the parasite species is being confirmed by sequencing. The results of this work will contribute to reducing gaps in knowledge of the biodiversity of hemoparasites of wild animals, as well as to analyze their zoonotic potential and the risk to animal and human health.

Key words: Anuran, reptil, hemoparasites, Trypanosomatídae, Apicomplexa

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SULFONE-MODIFIED AMIDOXIMES AS POTENTIAL ANTILEISHMANIAL AGENTS AGAINST *LEISHMANIA AMAZONENSIS*

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Abstract

Current treatments for leishmaniasis rely on a limited therapeutic arsenal associated with severe side effects, resistance, and therapeutic failure. Therefore, the search for new therapies is essential. Our research group has been investigating the antileishmanial effects of various amidoxime derivatives, as these compounds can function as drugs or prodrugs due to their reduction to amidines and subsequent transformation by multiple enzymes into amides, leading to the release of NO. Additionally, amidoximes exhibit better bioavailability compared to amidines. These modifications were designed to enhance the physicochemical properties of these compounds. In this study, six derivatives (OSC series) were synthesized, allowing substitutions primarily at position 2 of the 4,5-dihydrofuran scaffold, for comparison with *4-(5-benzyl-3-(4-fluorophenyl)sulfonyl)-5-methyl-4,5-dihydrofuran-2-yl)-N'-hydroxybenzimidamide*, our HIT compound. At position 2, ortho-nitrobenzamide, picolinamide, N'-hydroxy-2-nitrobenzimidamide, and N'-hydroxypicolinimidamide were evaluated. Positions 3 and 5 were modified by introducing a pyridine moiety (OSC151, OSC258). Antileishmanial activity was assessed in *Leishmania amazonensis* promastigotes, with IC_{50} values ranging from 7.4 to 39.7 µM. Among the tested derivatives, OSC151 (IC_{50} = 30.7 ± 3.3 µM) and OSC258 (IC_{50} = 39.7 ± 9.6 µM) exhibited lower activity, suggesting high sensitivity to pyridine modifications at positions 3 and 5, which reduced efficacy compared to the HIT compound. Conversely, OSC260 (IC_{50} = 11.8 ± 1.3 µM), OSC275 (IC_{50} = 9.4 ± 0.6 µM), and OSC276 (IC_{50} = 11.3 ± 1.6 µM), which involved modifications at position 2, showed comparable activity. Notably, the picolinamide derivative OSC272 (IC_{50} = 7.4 ± 0.9 µM) was the most active in this series. Further studies will be conducted to evaluate the activity of these derivatives in intracellular amastigotes and to elucidate their structure-activity relationship and mechanism of action.

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Keywords: AMIDOXIMES, SULFONE-MODIFIED, *LEISHMANIA AMAZONENSIS*

SEARCH FOR LEISHMANIA CYP450 INHIBITOR DRUG AS A REPOSITIONING STRATEGY

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The treatment of leishmaniasis is primarily based on the use of pentavalent antimonials. Drugs such as pentamidine and amphotericin B, originally developed for other diseases, have been repurposed for this condition. However, these drugs are associated with side effects, high toxicity, and resistance. The development of new drugs requires significant investment, whereas drug repositioning offers a cost-effective alternative to address public health needs. Enzymes of the cytochrome P450 complex (CYP450) play a crucial role in the metabolism of xenobiotics and are involved in key metabolic processes, such as sterol and fatty acid biosynthesis. Some studies suggest that inhibitors of CYP51 in *Leishmania* spp. could serve as a viable therapeutic strategy. Based on this, our study hypothesizes that drugs inhibiting human CYP450 may exhibit leishmanicidal activity by targeting enzymes of the same complex in *Leishmania* spp., whether they have already been described or not. Recently, our group identified three additional enzymes belonging to this complex, annotated in the *Leishmania infantum* genome as putative CYP types. Using molecular biology techniques, we confirmed the presence of these transcripts in the *Leishmania amazonensis* genome. In this context, 27 licensed drugs from a library of CYP450 inhibitors were tested, and 11 demonstrated leishmanicidal activity in promastigotes. Among these, ritonavir, efavirenz, and saquinavir exhibited IC_{50} values of 3.1µM, 2.8µM, and 3.0µM in amastigotes, respectively. Cytotoxicity assays conducted in murine macrophages determined CC_{50} values of 69.2µM, 39.7µM, and 45.2µM for these drugs, respectively, indicating a selectivity index greater than 10 for all three. To confirm whether their mechanism of action involves CYP450 inhibition, these drugs will be analyzed using a commercial kit, with the microsomal fraction of *Leishmania* serving as the CYP450 source. The remaining selected drugs are currently undergoing testing in amastigotes.

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Keywords: Leishmaniasis, CYP450, repositioning.

DETECTION OF PHLEBOTOMINAE TRANSMITTED BUNYAVIRALES COINFECTIONS IN DOGS WITH VISCERAL LEISHMANIASIS IN RIO DE JANEIRO, BRAZIL

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Abstract

Leishmaniasis is a parasitic disease caused by protozoans of the Genus Leishmania. The disease is widespread worldwide, and in some countries, it is a significant healthcare concern. The Phebotominae vectors of Leishmania may transmit Phleboviruses and Pacuvirus from the Bunyavirales Order. These viruses also pose a health risk to humans and livestock, causing symptoms such as thrombocytopenia and fever. Previous studies in our lab have shown that coinfection, both in vitro and in vivo, increases both the parasite load and the viral titer of the sample, suggesting an association between more severe cases of leishmaniasis and coinfections with viruses from the Bunyaviricetes order. Dogs are the main reservoir for L. infantum transmission, and we hypothesized the existence of coinfection in transmission foci of Visceral Leishmaniasis with Leishmania and Bunyavirales. To test this hypothesis, we searched for sequences of segment L, the most conserved region of the genomes of both Phleboviruses and Pacuviruses. We focused on sequences found in the New World transmitted sand file Bunyavirales genomes and designed two sets of PCR primers to detect these viruses. We tested bone marrow and sera samples from 25 VL-positive dogs from the Municipality of Rio de Janeiro. We detected by RT-PCR in 8 animals, thus suggesting that dogs are coinfected with Bunyavirales and Leishmania. Our data show, for the first time in Brazil , the coinfection in VL foci adds a layer of complexity to the understanding of the factors enrolled in L. infantum transmission.

More samples from Rio de Janeiro and from other States in Brazil are currently in progress

Supported by: CNPQ, FAPERJ, FIOCRUZ

Keywords: Leishmania, coinfection, detection

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OCCURRENCE OF *Typanosoma cascavelli* IN SAMPLES OF BATS FROM THE ATLANTIC FOREST OF THE STATE OF ESPÍRITO SANTO

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Abstract

The genus *Trypanosoma* is constituted by parasitic protozoans capable of infecting several vertebrate hosts as mammals, fishes and reptiles. The trypanosomatid *Trypanosoma cascavelli* was initially described as a parasite of terrestrial snakes. However, this species has already been found infecting homeothermic animals like marsupials. *T. cascavelli* has two haplogroups, one is associated with marsupial infections and the other with infections of both snakes and marsupials. In spite of these findings, questions about hosts and how this species is capable of infecting poikilothermic and homeothermic animals are yet to be answered. This study had as objective to characterize trypanosomatid samples from bats of an Atlantic Forest area in the Southeast of Brazil. We obtained a total of 110 samples composed of 88 blood clots and 22 hemoculture of 16 bat species in three rural areas of Guarapari municipality. The samples were submitted to DNA extraction and polymerase chain reaction for the marker 18S rDNA for the Trypanosomatidae family. DNA libraries were prepared and sequenced on the MiSeq platform. Bioinformatic analysis was performed to identify the Operational Taxonomic Units (OTUs). A haplotype network was built to verify the *Trypanosoma cascavelli* intra-specific groups encountered. Among the total of bats captured (n = 103), 33 samples were submitted to the metabarcoding sequencing and taxonomic classification. We have identified for the first time in the Chiroptera order, an infection caused by *T. cascavelli* in *Artibeus* sp. and *Phyllostomus* sp. Infections by this species had previously been thought to be exclusive to snakes and marsupials. After the assembly of the haplotype network we verified that our samples corresponded to the *T. cascavelli* group found only in marsupial species. The detection of this specific haplogroup shows us the generalist potential of *Trypanosoma cascavelli*.

Supported by: FIOCRUZ, CNPq, FAPERJ, UERJ

Keywords: *Trypanosoma sp.;* Chiroptera; Atlantic Forest

EVALUATION OF U18666A EFFECTS ON THE STEROL METABOLISM OF *LEISHMANIA SPP.*

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Leishmaniasis is a neglected tropical disease that poses a major global public health challenge, particularly due to increasing resistance and therapeutic failures of current treatments. *Leishmania spp.*, the causative agent of leishmaniasis, relies on sterol metabolism for survival, making this pathway a potential therapeutic target. U18666A is known to inhibit cholesterol biosynthesis and transport in mammalian cells, but its effects on trypanosomatids remain unexplored. This study aimed to evaluate the impact of U18666A on the sterol metabolism of *Leishmania* spp. The compound demonstrated significant antiproliferative activity against *L. amazonensis* promastigotes and amastigotes, with IC_{50} values below 1 µM. Importantly, U18666A exhibited no toxicity in murine peritoneal macrophages at concentrations up to 24 µM. Ultrastructural analysis revealed progressive organelle alterations in treated parasites, suggesting inhibition of intracellular sterol trafficking. Additionally, gas chromatography-mass spectrometry (GC-MS) analysis suggested that U18666A disrupts sterol biosynthesis at multiple points, including sterol C14α-demethylase (CYP51) and sterol C4-methyl oxydase (CYP5122A1), the latter being a recently described enzyme in *Leishmania*. Furthermore, co-treatment with ezetimibe, a cholesterol absorption inhibitor, showed an additive effect, highlighting the potential of drug combination strategies against leishmaniasis. These findings enhance our understanding of parasite biochemistry and open new perspectives for the development of more effective therapeutic approaches for leishmaniasis

THERAPEUTIC POTENTIAL: CAN PEPTIDES FROM THE SNAKE VENOM (CROTALUS DURISSUS TERRIFICUS) BE AN ALTERNATIVE IN THE TREATMENT OF CHAGAS DISEASE?

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Chagas disease has more than 100 years, is a highly morbid and mortal parasitic disease that affects about 8 million individuals and is caused by the protozoan *Trypanosoma cruzi*. In Brazil, the only available treatment is Benzonidazole, which has several side effects reported in up to 98% of cases, requiring treatment interruption in up to 20%. In this sense, snake venoms have demonstrated inhibitory potential against pathogenic microorganisms and little cytotoxicity to cells. The venom of *Crotalus durissus terrificus* has two peptides, phospholipase A_2 (PLA_2) and crotamine (CTM), with several reported antiparasitic activities. This study aimed to evaluate the lethal (LC_{50}) and cytotoxic (CC_{50}) activities of PLA_2 and CTM against the trypomastigote forms of *T. cruzi* and human peripheral blood mononuclear cells (PBMC), respectively, and their immunomodulatory potential. For CC_{50}, blood was collected from three healthy individuals and PBMC were subjected to the MTT method in 96-well microplates containing PLA2 and CTM (100–1.56 µg/mL) for 24 to 72 hours. The LC_{50} was obtained after evaluation of surviving trypomastigote forms subjected to treatment with PLA_2 and CTM (100–3.12 µg/mL) for 24 hours. The culture supernatant of PBMC infected by the parasite and treated with LC50 of the peptides was subjected to flow cytometry analysis to evaluate cytokine production (IFN-γ, TNF, IL-10, IL-6, IL-4, IL-2) by the Cytometric Beads Array kit. The results did not demonstrate cytotoxicity of any of the peptides to PBMC, the LC_{50} for PLA_2 and CTM was 52.07 µg/mL and 0.248 µg/mL, respectively, and statistical differences were observed only in the increase of IL-10 and IL-6 for PLA_2 and CTM, and of IL-2 for CTM when in the presence of *T. cruzi*. We conclude that PLA_2 and CTM present an interesting alternative against trypomastigote forms of *T. cruzi*, not being cytotoxic to human PBMC, but do not have promising immunomodulatory activity at the doses evaluated in the present study.

Supported by: FACEPE

Key-words: Cytokines; Peptides; *Trypanosoma cruzi*

FELINE TRICHOMONIASIS: WHERE DOES THE DANGER LIVE?

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In recent years, the protozoan *Tritrichomonas fetus* has been identified as the cause of chronic colitis in felines in several regions of the world. Thus, the aim of this study was to investigate the occurrence of *T. fetus* in felines in the region of Araçatuba, São Paulo, Brazil, and to correlate it with the environment where these animals live. A total of 75 felines were investigated in this study. Of these, 35 were domesticated, 16 lived in a commercial cattery and 24 in a Non-Governmental Organization (NGO). Regarding age, 16 were kittens, 10 were juveniles and 49 were adults. Regarding breed, 58 were Mixed Breed (SRD), 16 Persians and one Siamese. Regarding sex, 27 were males, 37 females and 11 were unidentified. To diagnose the protozoan, the animals underwent colonic lavage with saline solution. After collecting, the samples were analyzed by optical microscopy at magnifications of 10 to 40X, to observe the T. fetus trophozoites. The analysis of the results consisted of descriptive statistics using the chi-square test, evaluating positivity and negativity, with the main variables (breed, sex, age group and location). In this study, of the 75 cats submitted to parasitological examination, five were positive. Regarding the analysis of the results, we observed a significantly higher occurrence of T. fetus in felines raised in NGOs, with no statistical significance in relation to breed, sex and age group. Thus, in this study, we observed a higher occurrence of the protozoan in adult animals (100% [5/5]), SRD (80% [4/5]) that live in environments with high population density (100% [5/5]). In our study, the occurrence of feline trichomoniasis was 6.66%, with adult animals, mixed breeds, that live in environments with high population density, being the most affected.

Keywords: diagnosis; colitis; *Tritrichomonas foetus*

AMPHODEPOT®: A NOVEL SINGLE DOSE INTRALESIONAL TREATMENT OF CUTANEOUS LEISHMANIASIS

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Abstract

Intralesional (IL) meglumine antimoniate is the gold standard for the treatment of uncomplicated cutaneous leishmaniasis (CL) in Brazil. However, due to the rapid absorption to the circulation, repeated doses are necessary. Thus, we proposed a new AmB formulation that could be effective with a single IL dose. First, biodegradable polymeric microparticles of PLGA loaded with AmB were designed for both phagocytosis and sustained extracellular drug release. They were effective but the drug load was low (0.7%), mainly due to the amphoterous AmB nature that makes polymeric encapsulation difficult. Here, we proposed to increase the drug loading using a modified emulsion and solvent evaporation method. Microparticles with smooth and spherical surface and average size of 3 µm were synthesized, showing 9 times higher drug load (7% AmB). Such AmB/PLGA formulation was registered as AmphoDepot®. For anti-parasitic activity, *Leishmania amazonensis* promastigotes were cultured with different concentrations (0.01-100 µg/mL) of AmphoDepot® or free AmB for 72 h. Cell viability assayed by resazurin showed that both AmB forms were similarly active (IC_{50} = 0.01 and 0.02 µg/mL, respectively). For cytotoxicity, bone marrow-derived macrophages ($2x10^5$/well) were treated with 1.23 – 300 µg AmB/mL for 48h. AmphoDepot® were less than 6-fold cytotoxic than free AmB (CC_{50} = 4.6 and 0.8 µg/mL, respectively). For in vivo studies, BALB/c mice were infected in the ear with *L. amazonensis* ($2x10^6$), and 21 days later they were given a single dose of 10 µg of AmB in AmphoDepot® or in the free form (Anforicin®). On day 56 of infection, parasites were measured in the ear by limiting dilution assay. AmphoDepot® was able to reduce the parasite load by 86% (without deoxycholate) whereas Anforicin® reduced only 32% in comparison with vehicle alone. In conclusion, AmphoDepot® is the first single-dose formulation of AmB that is safe and may greatly simplify CL treatment.

Supported by: CNPq, Faperj and Vale do Rio Doce.
Keywords: polymeric particles, amphotericin B, leishmaniasis.

EVALUATION OF CELL ADHESION MOLECULES LIKE POTENTIAL BIOMARKERS FOR CHRONIC CARDIAC PATIENTS WITH CHAGAS DISEASE

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Abstract
Chagas disease is a protozoan infection endemic to Latin America caused by *Trypanosoma cruzi*. Chagas cardiomyopathy is the most severe chronic manifestation and leading cause of mortality. Parasite persistence in the chronic phase triggers an inflammatory response, stimulating the secretion of soluble cell adhesion molecules (sCAMs), which participate in the recruitment of leukocytes to the infection site. The sCAMs have been investigated in other diseases as potential biomarkers for diagnosis and prognosis. This study aimed to evaluate sCAMs levels in chronic patients with different stages of Chagas heart disease progression. Sera from 303 individuals were classified based on cardiac involvement (infected asymptomatic; infected mild cardiac; infected severe cardiac; non-infected cardiac and non-infected asymptomatic). sCAMs (sP-selectin, sE-selectin, sL-selectin and sVCAM-1) were quantified. sCAMs levels were measured using the Cytometric Bead Array Flex and acquired on a FACSCalibur flow cytometer. The ability of sCAMs to discriminate among groups was assessed using Receiver Operating Characteristic (ROC) curve analysis, with the area under the curve (AUC) measuring test performance. sCAMs showed good performance (AUC > 0.8) in distinguishing chronic asymptomatic patients from those in the initial cardiac phase. Also, sL-selectin presents an ability to differentiate infected and non-infected individuals (AUC=0.88), despite both groups lacking clinical signs. Additionally, sL-selectin differentiated infected and non-infected individuals, both with heart disease (AUC=0.85). To differentiate mild from severe cardiomyopathy, sVCAM-1 and sP-selectin demonstrated good performance, with sL-selectin standing out with an excellent accuracy (AUC=0.92). Therefore, sCAMs may serve as potential biomarkers for the diagnosis and prognosis of chronic cardiac patients with Chagas disease.

Supported by: CNPq, CAPES.
Keywords: Selectins, *Trypanosoma cruzi*, VCAM-1.

ORAL LIPOSOMAL FORMULATION WITH AMPHOTERICIN B FOR THE TREATMENT OF CUTANEOUS LEISHMANIASIS IN HAMSTER MODEL

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Leishmaniasis is a neglected disease with few treatment options that are flawed or toxic. Liposomal amphotericin B is the most effective therapeutic agent for VL, but has limited efficacy in CL and HIV/VL coinfection, restricted to parenteral route. Our hypothesis is that PEGylated liposomes can improve therapeutic efficacy by prolonging blood circulation time and improving stability in gastric fluid. We evaluate the effect of PEGylation of liposomal AmB on its stability in simulated gastric fluid and oral efficacy in hamsters with CL. AmB formulations in conventional and PEGylated liposomes were characterized for particle size, encapsulation efficiency/quantification of AmB and AmB aggregation state at room temperature and after incubation for 2h at 37ºC in acidic solution. The release kinetics of formulations before and after heat curing were also compared. Hamsters were infected with *L. amazonensis* and treated with AmB liposomal orally at daily doses of 5 mg/kg for 10 days. Treatment efficacy was assessed by recording lesion size and parasite load in comparison to oral miltefosine. AmB formulations presented mean diameters between 110 and 130 nm, drug encapsulation efficiencies greater than 98% and CD spectra consistent with a non-aggregated form. PEGylated formulation showed greater stability than the conventional when exposed to acidic environment. Treatment of infected hamsters with PEGylated formulation promoted significant reduction in lesion size compared to untreated group at level more pronounced than with the conventional one and equivalent to miltefosine. Statistically significant suppression of parasite load was observed in lesions of animals treated with miltefosine and PEGylated formulation in relation to conventional, with this suppression greater in PEGylated formulation. This work demonstrates that PEGylation of liposomal AmB increases its stability in simulated gastric fluid and its oral efficacy in a hamster model of CL.

Supported by: PPGIT, FAPEMIG, CAPES.

Keywords: Amphotericin B, Liposomes, Leishmaniasis.

Decoding the Microbial Influence: How Bacteria from Gut Microbiota Modulates *Giardia intestinalis* Morphology and Pathogenicity

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Giardiasis, an intestinal disease caused by the protozoan parasite *Giardia intestinalis*, affects over 300 million people globally each year. *G. intestinalis* infections are linked to structural and compositional changes in mucosal bacteria, which are part of the stable microbiota colonizing human mucosal surfaces. Evidence indicates that human microbiota can significantly alter the course of giardiasis in hosts. However, the cellular and molecular mechanisms during interactions of *G. intestinalis*, host cells, and microbiota remain unclear. This study aimed to evaluate the cellular effects on *G. intestinalis* cells following interactions with bacteria characteristic of both eubiotic and dysbiotic microbiota. Cell cultures were incubated in two ways: first, with isolated bacteria in Giardia medium to observe the generation time, and second, by co-incubating the parasites with bacteria. The cellular morphology and ultrastructure were examined using Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). Co-incubation of the parasites with bacteria revealed alterations in both growth and morphology. Exposure to *Enterococcus lactis* induced parasite lysis, damaging the plasma membrane without altering the structure of the ventral disc. In contrast, interaction with *Lactobacillus casei* resulted in wrinkling of the plasma membrane in the dorsal region and distortion at the edge of the ventral disc. However, co-incubation with the commensal bacterium *Cronobacter sakazakii* did not significantly affect the parasite's morphology, and the interaction with *Escherichia coli* appears to stabilize Giardia's growth. These preliminary results confirm that bacteria exert different morphological effects on *Giardia*. Overall, our findings suggest that eubiotic bacteria may negatively impact *G. intestinalis*, while commensal bacteria do not substantially harm the parasites.

Supported By FAPERJ, INOVA/Fiocruz, Instituto Oswaldo Cruz.

Keywords: Giardia; Microbiota; Interaction

Cannabinoid-Driven Structural and Functional Changes in *Trichomonas vaginalis*

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Trichomonas vaginalis, the causative agent of trichomoniasis, is responsible for a widespread sexually transmitted infection affecting approximately 278 million people annually. Current treatment relies on metronidazole (MTZ) but increasing resistance and adverse effects present significant challenges. *Cannabis sativa*, which contains over 100 phytocannabinoids, has gained attention for its therapeutic potential, including applications in parasitic infections such as *Plasmodium* and *Trypanosoma cruzi*. Recent legal and commercial developments have further reinforced interest in its medical applications. This study investigates the anti-parasitic effects of cannabinoids, including WIN 55,212-2, cannabivarin (CBV), and natural extracts of *C. sativa* with distinct phytochemical profiles: EBDRS9, rich in cannabidiol (CBD); EBPWK12, rich in tetrahydrocannabinol (THC); EBCBG30, rich in cannabigerol (CBG); and EBTHCV1MIX, a combination of CBD and THC. Their impact on the growth and morphology of *T. vaginalis* was assessed, along with pharmacokinetic properties. Notably, CBV complies with Lipinski's rules, suggesting favorable characteristics for oral drug delivery. The tested compounds significantly inhibited *T. vaginalis* trophozoite growth, including both synthetic cannabinoids and natural extracts, and impaired parasite adhesion to host cells. Morphological alterations such as membrane projections, blebbing, autophagosome formation, and damaged hydrogenosomes were observed. These findings highlight the antiparasitic activity of cannabinoids and *C. sativa* extracts against *T. vaginalis*, inducing significant cellular alterations and warranting further investigation into their mechanisms of action.

We acknowledge APEPI for providing the *C. sativa* extracts used in this study.

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Keywords: Cannabis, Trichomoniasis, Ultrastructure

IN SILICO AND TRANSCRIPTIONAL ANALYSIS OF GENES THAT ENCODE ENZYMES RELATED TO EICOSANOID METABOLISM OF *LEISHMANIA SPP*

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Parasites of the *Leishmania* genus can synthesize eicosanoids, once they possess enzymes related to their metabolism. In turn, eicosanoids are bioactive lipids capable of controlling inflammatory response in vertebrate hosts. Recently, it was demonstrated that the profile of eicosanoids produced by *Leishmania* varies according to the parasite species and the clinical condition of the leishmaniasis associated with the disease. Herein, we analyzed the differences in the genes involved in eicosanoid metabolism, as well as the alterations in the proteins encoded by these, such as prostaglandin F synthase (PGFS) and cytochrome p450 oxidase 1 (CYP1). For this, we analyzed *in silico* the genes associated with the metabolism of eicosanoids in *Leishmania spp* by means of bioinformatics tools, such as: alignment, phylogenetic analysis of the two identified genes, modeling of proteins encoded by them, and structural alignment of proteins aimed at identifying polymorphisms in regions of interest. We also carried out the development of pairs of primers to subsequently carry out a comparative evaluation of genetic expression by means of the RT-qPCR technique in the different species of *Leishmania*. A reduction was observed in the levels of genetic expression of *cyp1* in *L. braziliensis* compared to *L. infantum* and *L. amazonensis*. Regarding the expression of *pgfs*, no significant differences in gene expression were observed between the *Leishmania* species studied. Furthermore, we identified that the same mutations in the genes and proteins are conserved in *Leishmania species* related with similar clinical forms of leishmaniasis. Then, the data indicate differences of *cyp1* expression between *Leishmania* species, which can be related to virulence during host-pathogen interactions to be investigated in the next studies.

Supported by: UFOB.

Keywords: *cyp1*, *pgfs*, *Leishmania*.

RECORDS OF THE BIOLOGICAL COLLECTION BIO COL-LLDC/INPA/MCTI, MANAUS, AM

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Biological collections are a set of biological materials stored and organized in specific locations and temperatures, which preserve the collected and studied specimens, providing information on biodiversity, its species, origin and geographic distribution.They are important for scientific research, biodiversity conservation and public decision-making.The Biobank of the Leishmaniasis and Chagas Disease Laboratory (LLCD) of the National Institute for Amazonian Research (INPA) is called Bio COL-LLDC, whose biological collection of trypanosomatids was started around 1975, when the Director of INPA at the time, Dr. Paulo A. Machado, hired entomologist Dr. Jorge Arias to form a group to study leishmaniasis.The main function of Bio COL-LLDC is the acquisition, preservation, identification, cataloging and distribution of species/strains of trypanosomatids, as well as a serum library, a deposit of extracted DNA, among other biological materials kept at ultra-low temperatures (-80ºC) and/or or cryopreserved (-196°C), authenticated to support scientific research, epidemiological studies, as well as the development and production of technological products and processes,in obtaining extracts and molecules bioactives, also acting as specialized service providers. All incoming samples are registered, with a specific nomenclature called Stock Code,with the prefix IM (INPA Manaus), followed by a number and its specificities,such as biological species,country of origin and registration number.Of the total information obtained from isolates,in the period between 1985 and 2020,totaling 35 years of work,with the exception of samples from reference strains, tissues, DNA and serum, a total of 4,115 samples were recorded. At least 2,686 were from patients (mostly isolates identified as *Leishmania guyanensis*) and 1,429 from others (insects, mammals and reptile).Biological collections, in addition to biological data, also integrate climatic and environmental information, essential for understanding life on the planet at different times, projecting future scenarios and understanding patterns of change in biodiversity. The relationship between biological and environmental data makes it possible to model phenomena, predict the emergence and spread of agricultural pests and diseases, and increase effectiveness in combating epidemics.

Supported by FAPEAM, CAPES and CNPq.

Keywords: Biological collections, *Leishmania*, Sandfly.

SOROEPIDEMIOLOGICAL SURVEY ON THE PREVALENCE OF CHRONIC CHAGAS DISEASE IN VILA DOS REMÉDIOS, NOVO HORIZONTE, BAHIA

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Chagas disease (CD), caused by the protozoan *Trypanosoma cruzi*, remains a neglected tropical disease with significant public health implications. Despite Bahia being an endemic region for CD, its true prevalence remains uncertain due to underreporting and limited surveillance efforts. This study aimed to estimate the prevalence of chronic CD through a seroepidemiological survey conducted in the village of Vila dos Remédios, Novo Horizonte, Bahia.

A structured questionnaire was administered to collect sociodemographic data, followed by screening with the TR Chagas Bio-Manguinhos rapid test. Individuals who tested positive, along with a subset of negative cases, underwent venipuncture for laboratory confirmation at LACEN-BA. A total of 912 individuals participated, with 454 (49.7%) residing in the Mercês rural region, 449 (49.2%) in the village of Vila dos Remédios, and 10 (1.1%) from other areas.

Among these, 247 individuals were referred for confirmatory testing, including 52 rapid test-positive cases and 195 randomly selected negatives. Laboratory testing identified seven confirmed cases of chronic CD, yielding a prevalence of 2.8% (7/247). Only two of these individuals (40%) were previously aware of their diagnosis. Additionally, two cases produced inconclusive results, necessitating further investigation. The median age among confirmed cases was 53 years (IQR: 47-60), with an equal female-to-male ratio (1:1).

Although Novo Horizonte is classified as an endemic area for chronic CD, the observed prevalence in Vila dos Remédios was relatively low compared to other endemic regions in Bahia. These findings emphasize the need for expanded epidemiological investigations and systematic screening across the city of Novo Horizonte to establish a more comprehensive understanding of disease prevalence and inform targeted public health interventions.

Funding: INOVA-FIOCRUZ, CAPES, CNPq, FAPESB.

Keywords: Chagas disease; screening; seroepidemiological survey

ANTI-*ACANTHAMOEBA* EFFICACY OF PHENDIONE-LINKED METALLOCOMPLEXES AS AN EXCELLENT ALTERNATIVE FOR THE TREATMENT OF SEVERE AMOEBIC KERATITIS

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Amoebic keratitis is a serious infection that affects the human cornea and can lead to loss of visual acuity and the need for corneal transplantation. *Acanthamoeba* genus is among its main causative agents. The use of contact lenses and lack of proper hygiene are major factors in contracting the infection. Available clinical therapies are scarce, ineffective, and present toxicity to the cornea. Therefore, the need for new, effective therapeutic targets is urgent. This study evaluated the efficacy of Ag(I), Cu(II), and Zn(II) metallocomplexes bound to phendione against *A. castellanii* ATCC 50492 - genotype T4, and a clinical isolate (R40), as well as the irritant potential of these compounds in an ex vivo model. The metallocomplexes and the phendione ligand were successfully synthesized in the interdisciplinary laboratory of medicinal inorganic chemistry and catalysis using synthetic routes described in the literature. The metallocomplexes were tested at concentrations ranging from 200 to 10 µM for 48 hours at 30°C against *Acanthamoeba* spp. trophozoites ($8x10^5$ cells/mL). Alamar Blue® was used as a viability dye, and fluorescence was read at 535 nm (emission) and 690 nm (excitation). The irritant potential was determined using the hen egg chorioallantoic membrane assay (HET-CAM). At 200 µM, Cu phen, Ag phen, and Zn phen showed high efficacy, inhibiting approximately 90% and 80% of the *A. castellanii* strain and R40 isolate trophozoites, respectively, after 48 hours. Better IC50 values were found against the ATCC strain: Cu phen (IC50 4.35 µM), Ag phen (IC50 12.2 µM), Zn phen (IC50 17.56 µM), compared to the R40 isolate: Cu phen (IC50 6.96 µM), Ag phen (IC50 22.4 µM), Zn phen (IC50 61.7 µM). At 200 µM, none of the metallocomplexes showed irritant potential, with an irritation score (IS < 5). Thus, the metallocomplexes linked to phendione represent excellent alternatives for the development of new ophthalmic formulations for the treatment of severe amoebic keratitis.
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Keywords: phendione metallocomplexes, *Acanthamoeba*; amoebic keratitis

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Introduction: The Leishmania-macrophage interaction is one of the most important and determining steps in the establishment of infection. In this sense, in vitro studies have been aimed at better understanding the mechanisms involved in this interaction. Objective: To evaluate in vitro the infectivity of amastigote forms of *L (L.) amazonensis*, isolated from different clinical forms of LTA in the infection of peritoneal macrophages from BALB/c mice. **Material and Methods**: 9 strains from clinical cases of patients with ATL treated at the Instituto Evandro Chagas (SVSA, MS), Ananindeua-Pará, were distributed into 3 groups: Localized Cutaneous Leishmaniasis (LCL), Borderline Disseminated Cutaneous Leishmaniasis (LCDB) and Diffuse Anergic Cutaneous Leishmaniasis (LCAD). *Leishmania* promastigote strains were cultivated in RPMI until the moment of axenic amastigote transformation to infect macrophages at a ratio of 4 parasites/macrophage. The cultures were incubated at 35ºC and 5% CO2 and after 24h, the coverslips were stained with Giemsa to count the number of infected macrophages, number of parasites and determination of the infection index (II). Statistics were performed in the STATISTICA program with ANOVA-MANOVA and Ducan correction test. **Results**: There was variation in the % of infected macrophages LCL (68%), LCDB (80%) for LCAD (87%) with a difference ($p < 0.05$) between them and a higher % in LCAD strains. In relation to the average number of parasites/macrophage of 5 parasites/macrophage with LCL and LCDB, in cases of LCAD the average was 11 with a difference between LCL and LCDB ($p = 0.003$). In II, it was observed: LCL (345), LCDB (400) and LCAD (779), a significant difference between LCL x LCDB ($p = 0.0001$), LCL x LCAD ($p < 0.0001$) and LCDB x LCAD ($p < 0.0001$). **Conclusion**: BALB/c macrophages are susceptible to infection by axenic amastigote of *L. (L.) amazonensis* with intra-specific variation, with greater infectivity for LCAD strains, the most severe clinical form of ATL.

Keywords: *L (L.) amazonensis,* axenic amastigote, Diffuse Anergic Cutaneous Leishmaniasis.

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TURNING UP THE HEAT: THERMAL PROTEOME PROFILING UNCOVERS HIDDEN MECHANISMS OF METRONIDAZOLE IN *GIARDIA*

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Giardiasis, a gastrointestinal infection caused by the protozoan parasite *Giardia*, is among the most widespread worldwide. Despite its global distribution, treatment options remain limited, and resistance to frontline drugs is an emerging concern. Metronidazole (MTZ) is the most widely used treatment for giardiasis, but relapse rates of up to 50% suggest potential resistance mechanisms. Therefore, a thorough understanding of the mode of action (MoA) of MTZ and its molecular targets is critical for developing novel therapeutic strategies. To address this challenge, we employed thermal proteome profiling (TPP), which combines cellular thermal shift assays with quantitative mass spectrometry to identify MTZ-target interactions in whole-parasite protein extracts. Bioinformatics analyses included data normalization, melting curve fitting, and identification of proteins exhibiting a temperature shift greater than 2°C upon MTZ interaction. Our approach identified 64 proteins, validating known MTZ targets like pyruvate:ferredoxin oxidoreductase (PFOR) and protein 21.1. Our study also identified novel potential targets within the *Giardia* proteome, including 14-3-3 proteins, giardins, and vacuolar protein sorting (VPS) components. Molecular docking revealed that VPS has a high binding affinity for MTZ, prompting further validation. Notably, exposure of *Giardia* cultures to MTZ resulted in significant alterations in extracellular vesicle size, providing the first evidence that VPS proteins are involved in the MoA of MTZ. Our findings offer novel insights into MTZ's molecular interactions in *Giardia* and highlight previously unrecognized drug targets. These results lay the foundation for developing new

diagnostic tools for antimicrobial resistance surveillance and the rational design of next-generation anti-*Giardia* therapies.

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Keywords: *Giardia,* Metronidazole, proteomics

IDENTIFYING POSSIBLE MISMATCH POINTS IN PRIMERS REGIONS OF HSP70-LAMP ASSAY FOR THE DIAGNOSIS OF TEGUMENTARY LEISHMANIASIS

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Nucleic acid tests (NATs) are fast and highly accurate tools for diagnosing infectious diseases. Loop-mediated Isothermal amplification (LAMP) is a promising NAT for diagnosing leishmaniasis, including tegumentary leishmaniasis (TL). In recent years, studies employing LAMP assays with high accuracy values for TL have been published. As a globally distributed genus Leishmania parasites show significant genetic variance inside and between species and strains, even in genomic regions generally considered conserved. This variance, if located in an assay's primer target region (TR), can result in decreased accuracy. The present study aims to identify genomic variations inside an assay's TR for detecting *Leishmania (Viannia) braziliensis*. We used primers previously published for an HSP70-LAMP assay. The sequences of primers were aligned to *L. braziliensis* (taxid:5660) to determine their TRs within the genome using primer-BLAST. Next, a nucleotide-BLAST of the TR was performed to identify all *L. braziliensis* sequences available in the NCBI's GenBank database. Only mutations overlapping the TRs of the primer set were considered. Mismatches occurring in the first 5 positions of the 3' end of the analyzed primers were designated as "high risk", and the remaining variants were assigned "moderate risk". Using Megablast, 145 nucleotide sequences related to *L. braziliensis* HSP70 were found, with only one showing 100% agreement with the TR. Six relevant TRs were identified, each containing at least four polymorphisms inside primer-complimentary regions for the HSP70-LAMP assay. Most mutations were located inside the F3 and B2 primer regions, with just one polymorphic locus inside the F2 region. No "high risk" alterations were found. The next step will be to synthesize whole TRs for testing them against their respective primer set. This analysis will allow us to know the real effect of mutations on the detection limit of the primers used in HSP70-LAMP assay.

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Keywords: LAMP, mutations, *Leishmania*

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POSITIVITY IN XENODIAGNOSIS OF DOGS WITH VISCERAL LEISHMANIASIS TREATED WITH MILTEFOSINE AND ALLOPURINOL AND ITS RELATIONSHIP WITH CLINICAL, LABORATORY PARAMETERS, AND PARASITIC LOAD IN THE SKIN.

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Abstract

Treatment of dogs with VL using Milteforan® was approved in Brazil in 2016. Our objective was to evaluate the infectivity of dogs for Lutzomyia longipalpis 12 months after treatment and the clinical status, laboratory response, and parasitic load in the skin. Thirty dogs seroreactive were treated for 28 days with Milteforan®(2mg/kg/SID) and Allopurinol (10mg/kg/BID) and fitted with repellent collars. They were divided into two groups according to the use of Allopurinol: G1 interruption of Allopurinol; G2 Allopurinol for 12 months. The dogs were monitored for one year with clinical and laboratory evaluations. Skin biopsies were taken to quantify the parasitic load, and at the end of the 12 months follow-up, xenodiagnosis was performed. We observed a significant reduction in the parasitic load of dogs in G2, at six and twelve months after treatment compared to baseline, while in G1 there was no variation. 60% of the infectious dogs were from G1. The severity of onychogryphosis and dermatitis was associated with increased parasitic load, and the increase in serum albumin concentration is associated with its reduction. These effects occurred regardless of the treatment time. Dogs that were in stages 1 or 2 of the disease before treatment showed reduction in the parasitic load over time, with lower values than those that started in stages 3 or 4. 90% of the infectious dogs were in stages 3 or 4 of the disease, with significant association. Dogs that started treatment with antibody titer up to 1/160 showed reduction in the parasitic load over time. At the end of the study, dogs with low titers had lower parasitic load than dogs with higher titers. All infectious dogs for Lu. longipalpis had antibody titer of 1/160 or higher, and this titer was associated with the infectivity of the dog. We can conclude that infectious dogs for the vectors have characteristics that serve as markers of infectivity, such as antibody titer of 1/160 or higher and staging 3 or 4 of the disease.

Supported by: CNPq; CAPES; IRR/FIOCRUZ; INOVA/FIOCRUZ
Keywords: canine visceral leishmaniasis; treatment; xenodiagnosis

HIGH PREVALENCE OF *Giardia lamblia* IN CHILDREN FROM A TOURIST CITY IN BRAZIL

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Giardia lamblia is an enteroparasite with global distribution, zoonotic in nature, transmitted through water or direct intra- and interspecific contact. Children are the primary group affected by this infection. It is estimated that the prevalence of *G. lamblia* infection ranges from 2% to 7% in developed countries and can exceed 50% in developing countries such as Brazil. Although the Lagos Region of Rio de Janeiro is known for its tourism significance, there are also pockets of poverty and inadequate water and sewage treatment, with the frequency of enteroparasites being unknown. In this context, the aim of this study was to assess the prevalence of *G. lamblia* infection among children attending daycare centers in the municipality of Armação dos Búzios. To this end, fecal samples were collected from children under 5 years old attending the 12 municipal daycare centers in the city of Armação dos Búzios/RJ. The samples were subjected to a parasitological stool examination using the Ritchie and Kato-Katz methods. Out of 1,227 preschool children enrolled in the municipality of Armação dos Búzios, 417 unique stool samples were collected on a voluntary basis. The parasitological examination revealed the presence of the following protozoan forms: *Giardia lamblia* (123/417, 29.5%), *Entamoeba histolytica complex* (3/417, 0.71%), *Endolimax nana* (107/417, 25.6%), *Entamoeba coli* (21/417, 5.0%), and the helminth *Ascaris lumbricoides* (6/417, 1.4%). The prevalence of *G. lamblia* is dynamic and can vary both spatially and temporally. Although no previous surveys are available, the frequency of this infection was considered high when compared to other regions of the state. Considering that Armação dos Búzios is the third most important tourist destination in Brazil and the possible transmission routes of *G. lamblia*, the identification of high infection rates points to the potential spread of the parasite to different municipalities along the tourist route.

Supported by: FAPERJ, CNPq, PAEF/IOC, FIOCRUZ, CAPES.
Partnership with the Municipal City Hall of Armação dos Búzios/RJ

keywords: *Giardia lamblia*, frequency, Armação dos Búzios.

DYNAMICS OF IGM AND IGG ANTIBODY RESPONSES AGAINST *PLASMODIUM* ANTIGENS IN NEOTROPICAL PRIMATES IN THE ATLANTIC FOREST

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Zoonotic transmission of malaria, especially in the Brazilian Atlantic Forest, poses a major challenge for disease control and elimination. However, there are few studies about the immune response of naturally infected neotropical primates (NP). This study evaluated the dynamics of the immune response in NP, analyzing the reactivity of IgM and IgG antibodies against circumsporozoite protein (CSP) peptides and blood-stage proteins (AMA-1, EBP-2 and DBPII) from different *Plasmodium* species at two time points. Samples from 28 NP collected in 2011 and 2019 from free-living individuals in Joinville/SC (n=6), captive individuals from the Rio de Janeiro Primate Center (CPRJ) in Guapimirim/RJ (n=9), and in 2015 and 2017 from captive individuals at the Indaial Biological Research Center (CEPESBI) in Indaial/SC (n=13) were analyzed by ELISA. In Indaial, the small number of responding animals at baseline showed an increase in IgM reactivity against CSP and a decrease against blood-stage proteins, over an 8-year interval. In addition, IgG reactivity against CSP and against blood-stage proteins also decreased. In the same interval, in Guapimirim/RJ, there was an increase in IgM reactivity against CSP and against blood-stage proteins. IgG reactivity against CSP also increased, but little IgG reactivity against blood-stage proteins was observed. In Joinville/SC, the vast majority of animals tested showed strong reactivity of both IgM and IgG against all proteins, with little variation between time periods, the interval of which was shorter. The data presented here suggests a large variation in malaria epidemiological conditions in each region, with emphasis on Joinville, which showed greater reactivity as a reflection of the high rates of animals positive for *Plasmodium* spp infection. These results confirm the intense circulation of *Plasmodium* species among NP, reinforcing the need for epidemiological surveillance in the Atlantic Forest regions.

Supported by: CNPq, Fapemig

Keywords: Malaria, immune response,

PARASITE INFECTION IN BLOOD AND TISSUES OF THREE FISH SPECIES FROM LAKES IN THE EASTERN AMAZON: FIRST CASE REPORT

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Parasitic communities in fish can be used as bioindicators of environmental quality, as they reflect the ecological conditions of aquatic ecosystems. Fish frequently serve as hosts to various parasites, including myxosporeans, which can cause irreversible tissue damage depending on the intensity of infection. This study identified hemoparasites in fish collected from the Açu and Viana lakes in the Eastern Amazon, Maranhão. Four sampling events were conducted (two during the dry season and two during the rainy season), totaling 240 specimens of the species *Hoplias malabaricus, Pygocentrus nattereri,* and *Prochilodus lacustris.* The fish were anesthetized with clove oil, and blood samples were collected via caudal puncture to prepare blood smears, which were subsequently stained with modified Rosenfeld. Tissue samples were fixed in 10% formalin for further histological processing. Three parasites were identified: *Trypanosoma* spp., *Myxobolus* spp., and *Henneguya* spp. During the dry season, *Trypanosoma* spp. exhibited a prevalence of 100% in *H. malabaricus* in Lago Açu and 70% in Lago Viana, whereas in the rainy season, the rates were lower (65% and 55%, respectively). In *P. lacustris*, prevalence was 55% in Lago Açu during the rainy season, increasing to 65% in the dry season. *Myxobolus* spp. showed higher prevalence in *H. malabaricus* in Lago Açu during the dry season (75%) and in *P. nattereri* in Lago Viana, both in the dry (85%) and rainy (48%) seasons. *Henneguya* spp. presented lower prevalence rates, with *H. malabaricus* in Lago Açu showing 50% in the rainy season and 43% in the dry season, while *P. nattereri* in Lago Viana exhibited 32% in the dry season. *P. lacustris* had the lowest prevalence, restricted to Lago Viana (25% in the dry season and 10% in the rainy season). The results indicate seasonal variations in parasite prevalence, highlighting the influence of environmental conditions on infection dynamics and reinforcing the importance of parasitological monitoring as an indicator of environmental quality in the studied lakes.

Keywords: Fish parasites, *Myxobolus*, *Henneguya*

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Education), FAPEMA (Maranhão Foundation for Scientific Research and Development), PPGECB/ UEMA.

INTEGRATED ANALYSIS OF WATER QUALITY IN COASTAL CITIES OF SANTA CATARINA: PHYSICOCHEMICAL, MICROBIOLOGICAL, AND PARASITOLOGICAL EVALUATION

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Water quality in coastal regions is a topic of great relevance for public and environmental health, especially in areas with intense tourist activity. Brazil, a popular summer destination, attracts many visitors to its beaches, however, the lack of sewage treatment in many citys causes contamination in these waters, affecting public health. Thus, this study aimed to comprehensively evaluate the water quality of rivers flowing into the sea and beaches of three tourist municipalities in southern Brazil: Balneário Piçarras, Balneário Camboriú, and Itapema, which have 20,23%, 95% and 76,55% urban sewage treatment, respectively, from June 2022 to May 2023. Water temperatures, turbidity, pH, salinity, and conductivity were analyzed. In addition, the occurrence of total coliforms, *Escherichia coli*, Enterococos, *Giardia* spp. and *Cryptosporidium* spp. were also evaluated. The physicochemical analyses were conducted *in situ* using a multiparameter meter, while the microbiological analyses were performed using the Quanti-Tray system with Colilert and Enterolert reagents, and the parasitological analyses were carried out using the Merifluor kit with identification of the structures of interest under an epifluorescence microscope, following standardized laboratory protocols. The results revealed that Balneário Camboriú showed the highest averages in microbiological parameters and *Giardia* spp. cysts, while Itapema showed the highest average regarding *Cryptosporidium* spp. oocysts. When comparing the different types of water matrices, the river samples showed higher concentrations of total coliforms and *E. coli.* The seasonal analysis showed the highest detection values of total coliforms and *E. coli* in the fall and Enterococos, *Giardia* spp., and *Cryptosporidium* spp. in the summer. It was also possible to observe a positive correlation between fecal indicator bacteria and enteric protozoa. These results highlight the need for more efficient and integrated management involving sewage treatment and tourism development in these regions.

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Keywords: Water quality; Coastal areas; Tourism.

Prevalence of cats carrying asymptomatic *Giardia* spp. in the municipality of Londrina, Paraná, Brazil

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Giardia duodenalis complex is a protozoan that parasitizes the intestinal tract of various hosts (animals and humans), making it a zoonosis of public health relevance. This study investigated the occurrence of asymptomatic cats carrying *Giardia duodenalis* in the municipality of Londrina/PR, to establish a preventive approach considering its implications for veterinary medicine and public health. A total of 272 fecal samples were collected from felines in various locations in Londrina, divided into kittens and adults, domiciled or not (strays or belonging to non-governmental organizations - NGOs), exclusively asymptomatic and who had not received giardicide treatment in the last three months. The techniques selected for evaluation were immunochromatography (rapid test) and the modified Faust methodology. Cats that tested positive for either technique were considered positive. All cats that tested positive using the zinc sulfate centrifugation method (Faust) also tested positive using immunochromatography. This resulted in a 96% agreement between the rapid test (immunochromatography) and Faust. The fact that only a single sample was positive corroborates the literature, in which felines tested positive on the first day. In this study, the rates of asymptomatic cats for *Giardia* spp. were estimated at 41.18% (domestic cats) and 84.80% (non-domestic cats), and there was a statistical difference ($P<0.05$) between adult cats and kittens, non-domestic cats and domestic cats. Therefore, adopting preventive measures to minimize the risk of transmission to other susceptible hosts is necessary. Immunochromatography can be recommended and implemented as a complementary test to the Faust technique, optimizing diagnosis, especially in asymptomatic cats, since it can eliminate and transmit genotypes F and A (zoonotic) in greater quantity and also genotypes C and B to a lesser extent. It is an important water and food borne protozoan.

Keywords: felines, domiciled, SNAP Test.

MONITORING OF MILTEFORAN® TREATED *Leishmania infantum* NATURALLY INFECTED DOGS THROUGH THE LAMP-BASED LeishID TEST

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Abstract

Dogs are the main urban reservoirs of *Leishmania infantum*, a pathogen commonly associated with canine visceral leishmaniasis (CVL). Given the lack of a definitive cure for this disease, the Brazilian Ministry of Agriculture, Livestock, and Supply (MAPA), in partnership with the Ministry of Health, approved the use of Milteforan® (miltefosine) to treat CVL. MAPA emphasizes that the decision to use the medication is the responsibility of the animal's tutor and highlights the need for parasitological monitoring of the treated dogs. Here we use LeishID - a LAMP (loop-mediated isothermal DNA amplification)-based method - for the molecular detection of *L. infantum* in clinical samples from naturally infected and treated dogs. These dogs lived with their tutors in Florianópolis, Santa Catarina, in southern Brazil. The DNA was extracted from skin samples of 11 dogs. The samples were collected at different time points: T0 (before treatment), T1 (one month after the start of treatment), T2 (six months after the end of treatment), and T3 (twelve months after the end of treatment). The LAMP reaction was performed using the WarmStart colorimetric master mix (NEB) and the amplified products were also resolved in agarose gel electrophoresis to confirm specific amplification. At time points T0, T2, and T3, 11 samples were identified as positive, while at time point T1, 10 samples tested positive. At T2, LAMP was able to detect *L. infantum* DNA in 100% of samples (11/11) while 54.5% (6/11) of samples were considered reagents by RT-qPCR. These findings indicate that LAMP demonstrates higher sensitivity in this context compared with RT-qPCR. The detection of *L. infantum* was observed at all points of all time, despite the administered treatment. Therefore, LeishID is a valuable tool for monitoring dogs undergoing treatment for visceral leishmaniasis, as it successfully detects the presence of the parasite's DNA in the animal's tissue even 12 months after the completion of treatment.

Keywords: *Leishmania infantum*; molecular diagnostics, LAMP, milteforan
Financial Support: Fapemig (RED-0032-22; RED-00196-23), CAPES, CNPq (312353/2023-5), Fiocruz

FIRST MOLECULAR CONFIRMATION OF ZOONOTIC CILIATE PARASITE IN BRAZILIAN TAPIRS

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Over the years, the mammalian fauna has been declining, with *Tapirus terrestris*, known as the tapir, standing out in this scenario. In Rio de Janeiro, the tapir became extinct, and its reintroduction began in 2017 at the Guapiaçu Reserve (REGUA). Among the various causes for the decline of fauna, parasitic infections are included. This study aimed to investigate gastrointestinal parasites in tapirs, with an emphasis on the Ciliophora Group. Between 2023 and 2024, 41 fecal samples were collected from tapirs kept under human care and in the wild. Of these, 21 samples were obtained from tapirs in six institutions in Rio de Janeiro, São Paulo and Minas Gerais. Furthermore, 20 samples were obtained from the REGUA. The feces were collected from enclosures, trails, and carcass and were subjected to microscopic examinations. Those that exhibited forms of ciliated were inoculated in culture medium and the isolates subjected to molecular analysis. For samples that were not isolated, molecular analysis was performed on DNA extracted from the feces. Samples obtained from the Reserve trails were also analyzed to confirm the host through molecular analysis. In total, 70.7% of the samples tested positive for parasites, with an emphasis on the Ciliophora Group (58.5%), followed by strongyle eggs (31.7%) and coccidian oocysts (19.5%). To date, 20 nucleotide sequences have been generated, all of which were identified as *Balantioides coli*. Of these, 11 were classified as A0, 8 as B0, and one as mixed. Both *B. coli* type A0 and B0 sequences were identified in tapirs in Rio de Janeiro, both under human care and in the wild. Only type A0 was identified in the samples from São Paulo. It is worth noting that the A0 variant of *B. coli* has been identified in humans, where it can cause serious dysenteric infections. The results of this study indicate, for the first time, that the tapir serves as a host for *B. coli* and may contribute to the maintenance of this zoonotic parasite in the wild.

Keywords: Wild animals, *Balantioides coli*, Intestinal parasite

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miR-193b Regulates Host Defense Against Leishmania braziliensis by Promoting Reactive Oxygen Species Production

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ABSTRACT

Leishmaniasis is one of the most neglected diseases in the world. Cutaneous leishmaniasis (CL) is the most common form of this disease, characterized by ulcerated skin lesions and chronic inflammation. The underlying mechanisms of its pathogenesis are not fully understood, and little is known about post-transcriptional regulation during CL. In this process, small non-coding RNAs play a crucial role. MicroRNAs (miRNAs) are small endogenous RNAs that modulate expression through translational repression or target RNA degradation. These findings highlight the potential to identify miRNA expression associated with disease prognosis or severity. Previously, we demonstrated that miR-193b, miR-671, and TREM1 show significant correlation only in patients with faster healing (up to 59 days) and not in those requiring longer healing times (over 60 days). These results suggest that this axis may be strongly associated with the healing time of patients with cutaneous leishmaniasis (CL). Therefore, the aim of this study was to investigate the role of miR-193b in macrophages infected with Leishmania braziliensis (Lb). For this, human macrophages were infected with Lb to validate the expression profile of miR-193b in CL at 30 minutes, 4 hours, and 12 hours. Then, the macrophages were transfected with miR-193b and infected by Lb for 4 and 12 hours to evaluate its role in infection control. Additionally, reactive oxygen species (ROS) were assessed in the presence of miR-193b overexpression. The expression profile of miR-193b was significantly inhibited in these cells after 30 minutes and 4 hours of infection; however, this expression was induced at the 12-hour time point. Macrophages transfected with miR-193b after 12 hours of infection, there was a significant reduction in parasitic load and the number of amastigotes per cell. The reactive oxygen species (ROS) assay revealed that miR-193b promoted an increase in ROS in macrophages infected for 12 hours, reforcing the potential of this miRNA in infection control. Conclusion: Taken together,

these analyses show that miR-193b promotes infection control by Lb in human macrophages after 12 hours through the induction of reactive oxygen species leading to a better disease outcome.

KEYWORDS: miR-193b, macrophage, Leishmaniasis.

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EVALUATION OF THE ANTI-*Leishmania amazonensis* AND CYTOTOXIC ACTIVITY OF THE AQUEOUS EXTRACT OF *Echinacea purpurea* (L.) MOENCH

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Leishmania amazonensis plays a fundamental role in the prevalence of leishmaniases, being involved in the manifestation of cutaneous leishmaniasis in 12% of cases in Latin America, with reports of cutaneous, diffuse, mucosal, and visceral involvement. Case studies report that patients infected with *L. amazonensis* may exhibit resistance to conventional treatments, raising the need for the development of new effective drugs. *Echinacea purpurea* is a medicinal plant explored due to its numerous phytoconstituents, which have already demonstrated dose-dependent activity against *L. donovani* and *L. major*. Although the biological activities of *E. purpurea* depend on the preparation method, it shows potential for biotechnological exploration and enhancement. Thus, the present study evaluated the anti-*Leishmania amazonensis* activity of the aqueous extract of *E. purpurea*.
The study consisted of an in vitro antiparasitic evaluation using *L. amazonensis* (PH8) promastigotes and NIH/3T3 cells for cytotoxicity.
Anti-*Leishmania amazonensis* activity was obtained only at concentrations of 500 and 1000 µg/mL, with an inhibitory percentage of 41.87%. Regarding the cytotoxic profile, the aqueous extract of *E. purpurea* did not present considerable toxicity (IC_{50} > 1,000 µg/mL).
The results obtained here support previous studies reporting a broad spectrum of action. The study found a limitation regarding the concentration range tested; however, the low cytotoxicity allows for the exploration of this extract at new concentrations. The findings of the present work encourage future studies investigating the phytochemical properties of *E. purpurea* and the compounds involved in anti-*Leishmania* activity.
In light of the above, it can be said that *E. purpurea* possesses promising molecules for the development of new drugs or the refinement of conventional ones.

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Keywords: Natural Products, Leishmaniasis, Botanic Extracts

LAMP-BASED *Leishmania* DNA DETECTION TO DIFFERENTIATE BETWEEN *L. braziliensis* AND *L. guyanensis* FOR THE FAST DIAGNOSTIC OF CUTANEOUS LEISHMANIASIS

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Abstract

Differential diagnosis is crucial in cutaneous leishmaniasis (CL) due to its resemblance to diseases such as sporotrichosis, lupus, and skin cancer, especially in overlapping endemic areas. Additionally, treatment outcome varies between *L. guyanensis* and *L. braziliensis*, considering their differences in therapeutic response and the risk of progression to more severe forms. Loop-mediated isothermal amplification (LAMP) is a fast and straightforward method for DNA amplification under constant temperature conditions, eliminating the need for complex equipment. Its simplicity makes it a valuable tool for the molecular diagnosis of leishmaniasis. In this study, we designed specific primers to differentiate *L. braziliensis* and *L. guyanensis*. These primers are a component of LeishID, a LAMP-based molecular diagnostic solution developed by our group, which demonstrated promising results with high sensitivity and specificity at clinically relevant concentrations. We selected species-specific targets from 26 publicly available *Leishmania* genomes, representing at least 16 different species. The accessory genome was filtered to identify unique sequences for each species. LAMP primers were then designed, and the LAMP assay was conducted using the WarmStart Colorimetric LAMP mix (NEB). The primer set demonstrated a detection limit of 0.1 pg and was able to accurately differentiate the DNA of *L. guyanensis* from *L. braziliensis* in 30 min., with no cross-reactivity observed with other trypanosomatid species, such as *T. cruzi*, *Crithidia*, *Endotripanum*, and *Herpetomonas*, nor with mammalian DNA. The primer set showed promising performance with high specificity in differentiating between *L. braziliensis* and *L. guyanensis*. This is a valuable tool for the accurate molecular diagnosis of cutaneous and mucocutaneous leishmaniasis

Keywords: Cutaneous leishmaniases; LAMP; diagnostics.
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SIMPLIFIED FLUORESCENT LABELING OF *LEISHMANIA* SPP. PARASITES AS A TOOL FOR STUDYING EARLY EVENTS OF PARASITE-HOST CELL INTERACTIONS

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ABSTRACT

INTRODUCTION The interaction between *Leishmania* and macrophages is crucial to understanding the mechanisms of cellular infection. Fluorescent labeling of parasites enables visualization through confocal microscopy or flow cytometry, allowing for detailed analysis. In this context, Oregon Green and Calcein Red are potentially cell tracers due to their high fluorescence intensity and low cytotoxicity. Given the limited literature, a detailed evaluation of their performance was conducted. **OBJECTIVE** Evaluate the effectiveness of Oregon Green and Calcein Red labelling on various *Leishmania* species as a tool for investigating early parasite-host cell interactions. **METHODOLOGY** *Leishmania braziliensis (Lb)* promastigotes were labeled with Oregon Green or Calcein Red, and their fluorescence intensity was assessed via flow cytometry over four days post-labeling. The growth of *Lb* cultures labeled with Oregon Green was monitored for six days. Promastigotes from *Lb, Leishmania amazonensis* and *Leishmania infantum* were labeled with concentrations of 1 mM, 5 mM or 10 mM of Oregon Green, and fluorescence intensity evaluated using fluorescence-activated cell sorting (FACS). **RESULTS** Oregon Green proved more effective for labeling *Leishmania* promastigotes than Calcein Red, showing decrease in fluorescence intensity from 24 h after labeling. Oregon Green does not hinder the development and growth of *Lb* promastigotes. A 10 mM concentration of Oregon Green exhibited the highest fluorescence intensity, making it the optimal concentration for evaluation via FACS and confocal microscopy, enhancing the visibility of the promastigotes and allowing more detailed and clear observation. **CONCLUSION** Oregon Green has proven to be an effective Cell-Trace for *Leishmania* promastigotes in studies involving confocal microscopy and FACS, with experiments extending up to 24 h post-infection. Further research is underway to evaluate the fluorescence of Oregon Green in the intracellular form of the parasite.

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Keywords: Cell-Trace; Oregon Green; *Leishmania* spp.

USE OF GLUCANTIME ASSOCIATED WITH A LESION CARE PROTOCOL: A NEW OPTION IN THE TREATMENT OF PATIENTS WITH CUTANEOUS LEISHMANIASIS WITHIN THE CONTEXT OF ONE HEALTH

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Cutaneous leishmaniasis (CL) is an endemic disease in 89 countries, affecting 12 million people. Brazil is among the nine countries with the highest number of reported cases. According to PAHO, early diagnosis is crucial for effective treatment, promoting healing of lesions, preventing disease progression, and improving the patient's quality of life. The objective of this study was to update health professionals, the population, and individuals with CL in Timbaúba-PE, on lesion care, disease control measures with education and health, and to strengthen existing protocols in a One Health vision of the SUS. Suspected cases of CL were identified by active search. After the project was clarified, patients who agreed to participate signed the TCLE (CAAE 59093316.0.0000.5190), underwent anamnesis, and blood was collected for serological diagnosis (RIFI). Confirmed cases were treated with Pentavalent Antimonial (Glucantime). At the same time, the wound care protocol was used, encouraging self-care regarding wound hygiene and daily dressing changes, with weekly monitoring. Training was provided to Community Health Agents and health professionals were updated on leishmaniasis, in addition to recreational activities for students. From 2018 to 2023, we collected 298 suspected CL samples. Of these, 105 (35%) were reactive for CL (clinical and IFI), 57 (54%) were male and 48 (46%) were female. The 105 patients received Glucantime/20 days and were monitored with the wound care protocol, where 100% evolved to cure and serological results. In the last six years, the rate of confirmed cases of CL followed a downward curve, starting at 54.1% in 2018 and reaching 13.3% in 2023. Therefore, we conclude that treatment with Glucantime, combined with the lesion care protocol, in addition to health education for health professionals and the population, is essential in controlling CL and can be used by the SUS as an effective strategy for successfully combating CL in endemic areas.

Supported by: IAM-FIOCRUZ-PE, INOVA-FIOCRUZ. iLIKA-UFPE and FACEPE

Keywords: Treatment with Glucantime . Skin lesion care . One health.

Survey of the occurrence of *Leishmania* spp. in horses from the state of São Paulo, Brazil

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Currently, the participation of horses in the zoonotic cycle of leishmaniasis is totally understood. However, they can act as reservoirs of the protozoan and an important source of infection, since they attract sandflies to feed on their blood. Therefore, this study aimed to investigate the occurrence and perform the molecular characterization of *Leishmania* spp. in horses from the northwest region of the state of São Paulo, Brazil. A total of 185 horses of different ages, sexes, breeds and functions were investigated in this study. Blood, serum and conjunctival swab samples were collected and stored at -20ºC. Antibodies against *Leishmania* spp. were detected using the Enzyme Linked Immuno Sorbent Assay (ELISA). Whole blood samples and ocular conjunctival swabs were subjected to genomic DNA extraction and then nested PCR of the 18S rRNA gene to amplify a fragment of ~355 bp of *Leishmania* spp. The analysis of the results consisted of interferential statistics using the Chi-square test to verify the positivity and negativity of the animals with each of the variables, such as sex, age group, breed and function. Of the 185 serum samples submitted to ELISA, 42 were reactive, resulting in a prevalence of 22.70% of horses with antibodies against *Leishmania* spp. However, through nested PCR in the whole blood and swab samples, there was no amplification of the genetic material of the aforementioned protozoan, all being considered negative. Regarding the statistical analysis, we observed a significantly higher occurrence of females with anti-*Leishmania* spp. antibodies. compared to males, with no significance in relation to the other variables (age group, function and breed). Thus, we found antibodies against *Leishmania* spp. in 22.70% of the horses in the Northwest region of the state of São Paulo, Brazil, and we did not detect the agent through nested PCR.

Key words: diagnosis, equines, protozoosis

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IDENTIFICATION OF *Trypanosoma cruzi* BY DNA AMPLIFICATION USING 18S PRIMER IN POLYMERASE CHAIN REACTION (PCR)

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Abstract

American trypanosomiasis/Chagas disease is an infection caused by the protozoan *Trypanosoma cruzi*. Parasitological methods are effective for identifying the parasite; however, in the chronic phase, they show low sensitivity due to subpatent parasitemia in the patients' peripheral blood. To overcome this limitation, Polymerase Chain Reaction (PCR) has emerged as an effective alternative for improving parasite detection and diagnosis. This study aimed to identify *T. cruzi* from different DTUs through DNA amplification using newly designed PCR primers targeting the 18S gene. The primers were designed based on the DNA sequences of *T. cruzi* reference strains, clones, and isolates deposited in the NCBI GenBank. The primers (5' – CAAGCGGCTGGGTGGTTATT – 3' and 3' – CACGGATTTCCCACAAAGGC – 5') were designed using Primer-Blast and tested both *in silico* and *in vitro*, yielding a 104-bp fragment. The standardized PCR conditions were: one cycle of initial denaturation (94ºC/4'), 35 cycles of denaturation (94ºC/30"), annealing (60ºC/1'), and extension (72ºC/1'). Next, the analytical sensitivity was evaluated across different DTUs, DNA concentrations, and parasite loads in the blood. Additionally, the primers were tested on blood samples from different host species. The primers detected the parasite at concentrations ranging from 0.1 fg to 1000 fg in TcI (Col 1.7), 1 fg to 1000 fg in TcII (Y), and 10 fg to 1000 fg in TcIII (CBS56). In blood, the primers detected *T. cruzi* DNA at concentrations of 1000 p/mL in TcI and TcII, and from 10 p/mL to 1000 p/mL in TcIII. Thus, the primers demonstrated high sensitivity in detecting low concentrations of *T. cruzi* from DTUs I, II, and III. Furthermore, parasite DNA was identified in all culture samples of triatomine isolates. However, *T. cruzi* identification was less efficient in human blood samples and in samples from domesticated animals such as *Canis familiaris, Ovis aries,* and *Capra hircus*.

Supported by: CAPES, CNPq
Keywords: *Trypanosoma cruzi*, Discrete Typing Units, Polymerase Chain Reaction

EFFICACY OF VACCINES BASED ON CHIMERIC OR MULTIEPITOPE ANTIGENS FOR PROTECTION AGAINST VISCERAL LEISHMANIASIS: A SYSTEMATIC REVIEW

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Leishmania (Leishmania) infantum and *Leishmania (Leishmania) donovani* are the primary causative agents of visceral leishmaniasis (VL). Vaccines that incorporate chimeric or multiepitope antigens have shown promise in triggering a potent and lasting immune response against this disease. This review systematically evaluates the efficacy of these antigens and identifies potential immunogenic targets for vaccine development. A systematic search was registered (PROSPERO: CRD42023449370) and conducted by independent reviewers across four databases to assess the efficacy of vaccines based on chimeric or multiepitope antigens against VL. The review included original studies that reported parasite load or positivity rates in animals immunized with these vaccines and subsequently challenged or exposed to *L. infantum* infection in preclinical and clinical studies. Key information was extracted, tabulated, and analyzed, with the risk of bias being assessed using the SYRCLE Risk Tool. A total of 22 studies were selected, with only one being a randomized clinical trial. Seventeen distinct antigens were constructed, including eleven multiepitope antigens, five chimeric antigens, and one pool of three synthetic peptides designed with selected epitopes from *L. infantum* proteins by epitope prediction analyses. Although there was no consensus on the evaluation methodology, the evaluated organs, or the best way to present the results, the reduction in parasite load varied from 14% to 99.6% and from 1.7 to 9.0 log orders. Limiting dilution was the most used method for assessing parasite load, followed by quantitative real-time polymerase chain reaction (qPCR). This systematic review underscores the promising potential of chimeric and multiepitope antigens as VL vaccine candidates and presents information to guide the rational development of new research for vaccination against VL.

Supported by: CAPES-Brazil; CNPq-Brazil

Keywords: visceral leishmaniasis; vaccine; multiepitope antigens

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PREVALENCE OF ENTERIC PROTOZOA IN PRESCHOOL CHILDREN IN THE CITY OF TERESINA, PIAUÍ

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Abstract

Intestinal infections caused by protozoa are strongly associated with a lack of basic sanitation and environmental contamination by fecal waste. Thus, their prevalence is more common in regions of socio-environmental vulnerability, which are more frequent in developing countries. This study aimed to estimate the frequency and risk factors associated with enteric protozoan infections in preschoolers in the rural area of Teresina - Piauí, by means of parasitological stool tests and a questionnaire applied to parents to find out about the socioeconomic and hygiene conditions of each participant in the study. From January to December 2018, fecal samples were analyzed from 137 children aged between three and six years old, 54.7% (75/137) of whom were male and 45.3% (62/137) female. The samples were processed using the Hoffman, Pons & Janer methods at the Parasitology Laboratory of the IFPI - Teresina Central Campus. The overall prevalence of protozoa was 53.3% (73/137), with 62.7% (47/75) and 41.9% (26/62) positive for males and females, respectively. 60.3% (44/73) of those infected were polyparasitic. Giardia duodenalis and Entamoeba histolytica/díspar were the pathogenic protozoa found, with 19.7% (27/137) and 1.5% (4/137) of cases. The non-pathogenic protozoa were: Endolimax nana 48.2% (66/137), Iodamoeba butschlii 19% (26/137), Entamoeba coli 15.3% (21/137). Low income, schooling and open defecation were associated with these infections. The presence of animals in the household was associated with Giardia duodenalis infections. Giardiasis is the main intestinal parasitosis in preschoolers at the daycare centers studied. The high prevalence of non-pathogenic protozoa suggests fecal contamination of water and/or food, representing a serious risk to the health of this population, since these same vehicles can transmit pathogenic parasites.

Supported by:

Keywords: Preschoolers. Protozoa. Basic sanitation.

IMMUNOMODULATION OF *TRYPANOSOMA CRUZI*-INFECTED ADIPOSE TISSUE TREATED WITH BENZNIDAZOLE: IMPACT ON INFLAMMATORY RESPONSE AND INTERACTION WITH IMMUNE CELLS

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Adipose tissue (AT) plays a crucial role in energy homeostasis and also exhibits refined immunological mechanisms, making it a promising target for parasites such as *Trypanosoma cruzi*. As an effective barrier to parasite evasion, the effectiveness of the recommended drug, Benznidazole (BZ), may be questionable. To address this, we evaluated the immunomodulation of *T. cruzi*-infected AT treated with BZ (1 µg/mL) in cell culture (n=4). Additionally, to assess the influence of immune response cells, we simulated a co-culture microenvironment (n=5) between infected and BZ-treated AT in indirect contact with peripheral blood mononuclear cells (PBMCs). In both culture models, after 72 hours, culture supernatants were collected to measure inflammatory mediators (cytokines, chemokines, and adipokines), AT was harvested to quantify parasite load, and PBMCs were collected to detect activation/inhibition markers. Our findings showed that in the model assessing only infected and treated AT, although BZ did not eliminate the parasite, it reduced the inflammatory state by lowering IL-6 compared to the untreated condition (p=0.0352). In contrast, in the model evaluating PBMC influence in this microenvironment, we observed that AT induces upregulation of IL-2, IL-6, and MCP-1 but downregulates TNF and IL-8 in the presence of PBMCs and *T. cruzi*. However, this phenomenon was not associated with BZ treatment, as observed in the previous model; this time, PBMCs were crucial for modulating the immune response. Additionally, we found that BZ decreases adipsin in *T. cruzi*-infected AT in the presence of PBMCs (p=0.0207), which may be beneficial, as this adipokine is linked to inflammatory conditions. Therefore, we propose that although BZ reduces inflammatory mechanisms, AT-associated cells can modulate the immune response in favor of the parasite, making AT a significant ally of *T. cruzi*.

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Keywords: Immunomodulation; Adipose Tissue; *Trypanosoma cruzi*.

Ultrastructural Identification of Intraflagellar Transport Trains-Like Structures in the human parasite *Trichomonas vaginalis*

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Trichomonas vaginalis (*Tv*) is a pathogenic protist, diffused worldwide, that infects the human urogenital tract, causing trichomoniasis [1]. *Tv* has five flagella: four anterior (AF) and one recurrent flagellum (RF) associated with the undulating membrane. In addition to motility, *Tv* flagella are important roles in pathogenesis, such as phagocytosis [3], and intercellular communication [1]. However, the biogenesis of this organelle in *Tv* is still unknown. Most eukaryotic flagella rely on intraflagellar transport (IFT): the bidirectional movement of multi-megadalton particles that organize themselves into train-like structures along the axoneme [4]. Studies on IFT in *Tv* are scarce, and the existence of this mechanism in the parasite need more investigation. This work aimed to identify IFT train-like structures in *Tv*. For this, *Tv* was cultured in TYM (tryptose, yeast extract and maltose) medium, as previously described [1]. Next, parasites were prepared for transmission electron microscopy (TEM), using negative staining and ultrathin section techniques, as previously described [1]. TEM revealed IFT train-like structures in the AF and RF of *Tv,* exhibiting typical morphological features of IFT trains previously described for other flagellated cells: a thin electrondense filament between the outer doublet of the axoneme and the flagellar membrane. Two types of IFT trains were identified: (a) compact short trains, with a mean thickness of 5 nm and a length up to 115 nm; and (b) long trains exhibiting a mean thickness of 28 nm and a variable length from 300 nm to 1.300 nm. Transversal ultrathin sections revealed that trains were almost exclusively along two sets of outer doublets (5-6 and 6-7). Thin electrondense links were seen connecting the trains to those doublets possibly representing the molecular motors (kinesin 2 and cytoplasmic dynein 1b) responsible for the movement of the trains. Our results provide the first ultrastructural evidence of IFT trains in *Tv*.

Keywords: Axoneme; Ciliogenesis; Electron Microscopy.

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A NOVEL VACCINE STRATEGY FOR CHAGAS DISEASE BASED ON RNA CONTAINING SEQUENCES FROM *TRYPANOSOMA CRUZI* TRANS-SIALIDASES

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Abstract

Trans-sialidases (TS) present on the surface of *Trypanosoma cruzi* are responsible for transferring sialic acid residues from host glycoconjugates to mucins on the parasite surface, a mechanism that is related to the parasite capacity to evade the host immune system. Present in over 1000 copies in the genome parasite, only 16 TS sequences encode proteins with catalytic activity that contain amino acids repeats known as SAPA (Shed Acute Phase Antigen). In addition, SAPA domain increases the stability of the enzyme in the bloodstream, which is considered a parasite virulence factor. Previously, our group showed that immunization of mice with recombinant TS induces the development of a protective Th1 response, essential for intracellular pathogen infection control, and that the presence of SAPA repeats in the recombinant protein results in the negative modulation of this protective response. Since RNA vaccines are based on intracellular antigen synthesis and, therefore, induces a potent Th1 response, we decided to compare immunization protocols based on recombinant protein and the corresponding mRNA encapsulated into lipid nanoparticles (LNPs). Sequences of a TS gene encoding the full-length protein and a version without SAPA were cloned into a mammalian expression vector and used for mRNA synthesis using *in vitro* transcription protocols. After cell transfection, the capacity of these mRNAs to produce the antigens were evaluated by western blot using antibodies generated against the recombinant proteins. Immunization with RNA/LNP formulations containing these sequences, induces similar humoral and cellular immune responses compared to immunization with recombinant proteins, as well as similar protection against challenge with a virulent *T. cruzi* strain, as shown by parasitemia and tissue parasitism. We are currently testing different prime-boost heterologous protocols in which immunization with recombinant protein and RNA/LNP formulations are combined.

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Keywords: RNA vaccine, *T. cruzi*, immune reponse