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PT.001

LONG COVID SYMPTOMS REPORTED YEARS AFTER SARS-COV-2 INFECTION: A CROSS-SECTIONAL STUDY IN ALAGOAS, BRAZIL

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COVID-19 may result in a broad spectrum of symptoms extending beyond the acute phase and persisting for four weeks or longer after initial clinical presentation, a condition known as Long COVID (LC). This study investigated LC symptoms reported by individuals previously diagnosed with COVID-19. A cross-sectional study was conducted among adults (≥18 years) who experienced mild, moderate, or severe COVID-19 between March 2020 and December 2022 in Alagoas, Brazil. The study was approved by the Research Ethics Committee under CAAE No. 68344823.2.0000.0155. Participants completed a structured questionnaire addressing demographic characteristics, clinical history, lifestyle habits, vaccination status, and post-COVID symptoms. The sample included 67 participants, of whom 47 had been hospitalized during the acute phase of infection (severe disease) and 20 had not required hospitalization (mild/moderate disease). Most participants were female (52.2%), aged 40-59 years (50%), self-identified as mixed race (54%), and 26.9% had received at least one COVID-19 vaccine dose before infection. Most infections occurred between 2020 and 2021 (86.57%). The most frequently reported LC manifestations were cognitive impairment (90%), fatigue (79.1%), psychiatric disorders (62.7%), sensory disturbances (64.2%), recurrent pain (54%), headache (53.7%), muscle weakness (47.8%), sleep disorders (44.8%), dyspnea (38.8%), cough (28.4%), persistent olfactory and gustatory dysfunction (25.4%), and gastrointestinal symptoms (20.9%). The interval between SARS-CoV-2 infection and study participation ranged from 22 to 63 months. These findings indicate that LC symptoms may persist for several years after the initial infection, highlighting the need for long-term monitoring and healthcare strategies, particularly among individuals with severe disease. Financial Support: CNPq/MCTI.

PT.002

SALIVARY SHEDDING OF BETA- AND GAMMAHERPESVIRUSES (CMV, HHV-6, HHV-7, EBV, AND HHV-8) IN NEW AND ESTABLISHED USERS OF HIV PRE-EXPOSURE PROPHYLAXIS

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Salivary beta- and gammaherpesviruses cause oral diseases like hairy leukoplakia and Kaposi sarcoma, but their behavior in HIV pre-exposure prophylaxis (PrEP) users is poorly characterized. We evaluated the prevalence and viral load of these salivary viruses among new and established PrEP users. Saliva from 130 PrEP users (65 initiating, 65 established; median duration 24 months). Saliva was collected with Salivette devices (Sarstedt, Germany); nucleic acids were extracted with the Extracta Kit DNA e RNA Patógenos on an EXTRACTA 32 extractor (Loccus, Brazil); and viral DNA was quantified by real-time PCR on a QuantStudio 6 Flex System with Design & Analysis Software v2.6 (Thermo Fisher, USA). Prevalence and viral loads were compared using Fisher's exact and Mann-Whitney tests in Jamovi. Participants were predominantly cisgender men who have sex with men, with frequent sexually transmitted infections (26% new, 42% established). HHV-7 was most prevalent (86% and 95%; p=0.127), followed by EBV (57% and 71%; p=0.144). Detection increased significantly among established users for HHV-6 (18% to 57%; p<0.001), CMV (8% to 26%; p=0.009), and HHV-8 (0% to 14%; p=0.003). EBV and HHV-7 showed the highest loads (~5 log10 copies/mL). Shedding was predominantly asymptomatic. Lesions were unrelated to herpesviruses. Salivary beta- and gammaherpesvirus shedding was highly prevalent and rose with PrEP duration. Funding Information: Sao Paulo Research Foundation (FAPESP) according to grant number 2025/05019-9; CNPQ grant number 407177/2023-0.

PT.003

SALIVARY SHEDDING OF ALPHAHERPESVIRUSES (HSV-1, HSV-2 AND VZV) IN HIV PRE-EXPOSURE PROPHYLAXIS USERS: A COMPARISON BETWEEN NEW AND ESTABLISHED USERS

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Alphaherpesviruses (HSV-1, HSV-2, and VZV) reactivate in saliva, driving oral disease and transmission, yet their shedding in HIV pre-exposure prophylaxis (PrEP) users remains poorly characterized. We evaluated the prevalence and viral load of salivary HSV-1, HSV-2, and VZV among new and established PrEP users. Saliva samples from 130 PrEP users were analyzed, including 65 initiating PrEP and 65 established users with a median duration of 24 months. Saliva was collected with Salivette devices (Sarstedt, Germany); nucleic acids were extracted with the Extracta Kit DNA e RNA Patógenos on an EXTRACTA 32 extractor (Loccus, Brazil); and viral DNA was quantified by real-time PCR on a QuantStudio 6 Flex System with Design & Analysis Software v2.6 (Thermo Fisher, USA). Detection frequency was compared using Fisher's exact test in Jamovi. Participants were predominantly cisgender men who have sex with men, and condom use during oral sex was rare (97% never). HSV-1 DNA was detected in 4.6% of both groups, with loads reaching 5.9 log10 copies/mL among established users. HSV-2 occurred in one new user (1.5%); VZV was undetected. No significant differences were observed between groups. Detection was almost entirely asymptomatic, with only one HSV-positive participant presenting a recurrent herpetic lesion. Salivary alphaherpesvirus shedding among PrEP users was infrequent but predominantly subclinical, supporting standard infection control and integrated oral and sexual health counseling in PrEP care. Funding Information: Sao Paulo Research Foundation (FAPESP) according to grant number 2025/05019-9; CNPQ grant number 407177/2023-0.

PT.004

Clinical Metagenomics for Diagnosis and Management of Persistent SARS-CoV-2 Infection in an Immunocompromised Patient: A Case Report

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Metagenomic sequencing is an untargeted approach that enables the identification of pathogens without requiring a prior diagnostic hypothesis, making it particularly valuable in complex clinical cases. In immunocompromised patients, persistent SARS-CoV-2 infection represents a significant diagnostic challenge and may promote intra-host viral evolution. This study describes a case in which metagenomics enabled the diagnosis of a persistent SARS-CoV-2 infection and guided therapeutic management.
A 46-year-old woman with Devic's syndrome receiving continuous rituximab therapy remained in the ICU for six months due to respiratory failure **associated with persistent severe COVID-19**. RT-PCR tests for SARS-CoV-2 remained negative throughout hospitalization. Metagenomic sequencing of nasopharyngeal swab, plasma, and bronchoalveolar lavage samples was performed using a hybrid-capture approach to investigate potential pathogens. Sequencing reads were processed through the CZID pipeline for taxonomic classification. Additional analyses were performed for viral genome assembly and characterization of the identified mutations.
Metagenomic sequencing enabled the identification of SARS-CoV-2 lineage B.1 in bronchoalveolar lavage. Genomic characterization revealed reversion mutations consistent with intra-host viral evolution during persistent infection. Following diagnosis, antiviral therapy with remdesivir was initiated, resulting in clinical improvement.
This case highlights the potential of metagenomic sequencing as a diagnostic tool in complex infectious cases with inconclusive conventional investigations, in the characterization of viral evolution, and in the clinical management of persistent infections.
This study is funded by the MCTI/CNPq Call No. 14/2023. Metagenomic reagents are provided by the Fiocruz Genomic Network (Rede Genômica Fiocruz).

PT.005

LONG COVID IN SARS-COV-2 POSITIVE INDIVIDUALS: SYMPTOMS, PREVALENCE, AND EVALUATION OF POTENTIAL PREDICTORS

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Long COVID is defined by the World Health Organization as symptoms beginning within three months after acute SARS-CoV-2 infection, persisting for at least two months, and not explained by another diagnosis. This study assessed the frequency, clinical manifestations, and potential predictors of Long COVID among individuals with SARS-CoV-2 infection in 2020?2021. A total of 474 individuals previously tested for COVID-19 were contacted and invited to complete a questionnaire on clinical and epidemiological characteristics. Samples had been collected in hospitals and primary healthcare units. Statistical analyses included Student?s t-test, Fisher?s exact test, and logistic regression. Sixty-nine individuals responded (14.6%); 41 (59.4%) reported prolonged symptoms, although only 23 (33.3%) fulfilled WHO criteria for Long COVID. The most frequent manifestations were fatigue and memory impairment (69.6%), followed by poor concentration, anosmia/dysgeusia, and headache (52.2% each). No significant associations were identified between Long COVID and age, sex, number of symptoms during the acute phase, or hospitalization. Logistic regression also showed no significant association of sex, age, or hospitalization with the outcome ($p = 0.70$). Among affected individuals, 56.5% sought medical care, indicating a relevant impact on quality of life and healthcare demand. Most respondents were non-hospitalized and younger than 50 years, contributing evidence from a population that remains comparatively understudied. Further analyses should expand the sample size and investigate viral variants and viral load as potential determinants of Long COVID.
Financial Support: CNPq and FAPEMIG

PT.006

Chikungunya virus infection under high glucose environment reverts insulin unresponsiveness and promotes cellular metabolic shift to increase anaplerosis and virion production

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Chikungunya virus (CHIKV) is a medically significant arbovirus responsible for Chikungunya fever (CHIKF), a debilitating disease marked by persistent joint and muscle pain. The most severe outcomes of CHIKF, including mortality, are frequently observed in individuals with comorbidities, like diabetes mellitus (DM). This study aimed to investigate how varying glucose concentrations influence CHIKV infection. Human umbilical vein endothelial cells (HUVEC) were cultured in normal glucose (NG – 5.5 mM glucose) or high glucose (HG – 25 mM glucose) to mimic normoglycemia and hyperglycemia conditions. Cells were infected with CHIKV and changes in host glucose metabolism were evaluated. Metabolic flux, viral replication, and cellular insulin sensitivity were assessed through biochemical, molecular analyses and metabolomics. CHIKV infection modulates host cell metabolism in a glucose-dependent manner. Under NG conditions, the virus regulates glucose metabolism to support replication and virion production. In contrast, HG environments enhance viral replication, exploiting the altered metabolic landscape. Notably, CHIKV restores insulin sensitivity in HG conditions, leading to increased glucose uptake. It also promotes anaplerotic reactions by diverting tricarboxylic acid (TCA) cycle intermediates toward amino acid synthesis and upregulates glycolytic flux into the hexosamine biosynthesis pathway (HBP). These findings provide mechanistic insight into how hyperglycemia associated with DM can exac erbate CHIKV pathogenesis. The virus's ability to hijack and redirect host metabolic processes in high glucose environments may underline the worsened disease severity observed in diabetic patients. Understanding these interactions could inform targeted therapeutic strategies for managing CHIKF in individuals with metabolic comorbidities

PT.007

RAPID DIAGNOSIS OF CANINE DISTEMPER INFECTION USING NEAR-INFRARED SPECTROSCOPY AND MACHINE LEARNING

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Canine distemper infection is among the most important infectious diseases affecting dogs worldwide, causing high morbidity and mortality, particularly in puppies and unvaccinated animals. Early and accurate diagnosis is essential for appropriate clinical management, isolation measures, and epidemiological surveillance. However, conventional diagnostic methods, including antigen tests, serology, and molecular assays, are often limited by reagent dependency, laboratory infrastructure requirements, and associated costs. In this study, we investigated the potential of near-infrared (NIR) spectroscopy combined with machine learning as rapid and reagent-free diagnostic tools for canine distemper using blood serum samples. Spectra were acquired in the 1000–2700 nm region. NIR spectra were treated using second-derivative transformation to enhance spectral features and reduce baseline effects. Principal component analysis (PCA) was employed for exploratory analysis and dimensionality reduction, followed by support vector machine (SVM) algorithms for supervised classification. Spectroscopic analyses revealed biochemical alterations associated with proteins, lipids, metabolites, and immune-response-related molecular components, reflecting host–pathogen interactions induced by viral infection. PCA demonstrated clustering tendencies among healthy animals and distemper-positive dogs. The optimized machine learning models achieved classification accuracies above 85% during validation, demonstrating the feasibility of vibrational spectroscopy for diagnosis of canine viral diseases. The proposed methodology offers rapid analysis, minimal sample preparation, low operational cost, and independence from specific molecular reagents, highlighting its potential for veterinary point-of-care testing and disease surveillance in resource-limited settings.

PT.008

BOVINE LEUKEMIA VIRUS, BOVINE IMMUNODEFICIENCY VIRUS, AND BOVINE HERPESVIRUS-6 MOLECULAR DETECTION IN CATTLE WITH LYMPHOPROLIFERATIVE LESIONS

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Retroviruses and herpesviruses are among the main viral agents associated with neoplasia in animals. Bovine lymphomas are relevant diseases in cattle due to their economic impact. Although Bovine leukemia virus (BLV) is considered the primary etiological agent associated with these neoplasms, other viruses, such as Bovine immunodeficiency virus (BIV) and Bovine herpesvirus type 6 (BoHV-6), have been widely suggested as potential cofactors in their pathogenesis. In this study, 122 bovine tissue samples from 106 animals originating from the states of Minas Gerais and Pará, with lymphoproliferative lesions suggestive of lymphoma, were analyzed. Samples included 72 lymph nodes from different anatomical sites and 50 bone marrow and other tumor tissues. Conventional PCR for BLV based on the gene *tax*, and semi-nested PCR for BIV (gene *pol*) and the *DNA polymerase* (*DPOL*) gene of BoHV-6 were performed. Detection of BLV and BIV proviral DNA and BoHV-6 DNA was observed in 38.7% (41/106), 17.0% (18/106), and 62.3% (66/106) of animals, respectively. Coinfection with two viruses was observed in 34.9% (37/106) of animals, while triple viral coinfection was detected in 6.6% (7/106). Multiple Correspondence Analysis suggested associations between BLV and BIV positive animals and double or triple coinfection profiles, whereas lymph node samples were associated with BoHV-6 detection. The study highlights a high frequency of viral infections and coinfections in bovine lymphoproliferative lesions and supports the hypothesis that viruses other than BLV may act as cofactors in lymphoma pathogenesis.

Financial support: CAPES and CNPq

PT.009

CANINE PAPILLOMATOSIS REFRACTORY TO AUTOGENOUS VACCINE THERAPY: A CASE REPORT

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Canine papillomatosis is an infectious-contagious viral disease characterized by benign cutaneous or mucosal papillomas. This report describes a refractory case of extensive oral papillomatosis in a two-year-old male canine admitted to a veterinary teaching hospital. The animal exhibited numerous firm, rough, and friable cauliflower-like masses varying from gray-white to black distributed across the oral cavity. Due to the lack of spontaneous regression and persistence of clinical signs, an immunotherapy protocol using an autogenous vaccine was indicated as a cost-effective alternative. The papillomas were surgically excised under general anesthesia, following standard aseptic techniques and processed at the Laboratorio de Virologia Veterinária to formulate the autogenous vaccine. Following the complete administration of the vaccine protocol, no significant regression or dropping of the lesions was observed, with most papillomas persisting in the oral cavity. This outcome demonstrates the refractory nature of the clinical case against autogenous immunotherapy, highlighting the potential limitations of this treatment in certain presentations and emphasizing the need for alternative multi-modal therapeutic strategies.

***Keywords*:** dog, oral lesions, therapeutic resistance, *Papillomaviridae*.

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PT.010

Impact of HPV and HHV-6A/B Infections in Semen on Sperm Quality and Inflammation in Argentine Men

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Introduction: Male urogenital infections are key, albeit overlooked, factors in male infertility. Objective: In this study, we present data on the prevalence and impact of human papillomavirus (HPV) and human herpesvirus 6 (HHV-6A/B) in male genital samples. Materials and Methods: Semen samples and urethral swabs from sexually active or infertile men in Córdoba, Argentina—all of whom were unvaccinated against HPV—were analyzed using PCR/nested PCR and standard semen analysis. Results: The prevalence of urogenital HPV was 19%, predominantly high-risk genotypes (HR-HPV, mainly HPV-16) with frequent coinfections (74.4%), notably with *Chlamydia trachomatis* (CT, 52%). While isolated HPV infection did not alter standard sperm parameters and reduced inflammatory cytokines (IL-6, IL-1β), HR-HPV selectively increased sperm necrosis and oxidative stress. HPV–CT coinfections significantly reduced sperm concentration and motility while increasing IL-6 levels. Regarding HHV-6, a 28.5% prevalence was found in infertile men, with HHV-6B (76.7%) being significantly more frequent than HHV-6A (23.3%) and showing a low coinfection rate (23%). Isolated HHV-6B infection was significantly associated with altered seminal volume and sperm morphology compared to HHV-6A. In conclusion, although single infections with HPV or HHV-6 cause subtle, genotype-specific alterations, HPV–CT coinfections and HHV-6B significantly impair sperm quality and induce inflammation. This underscores the need for comprehensive urological screening and robust vaccination policies to preserve reproductive health. Financial support: Proyecto Consolidar SeCyt-UNC 2018-2027.

PT.011

AVIAN ADENOVIRUS AND INFECTIOUS BRONCHITIS VIRUS CO-INFECTION IN BROILER CHICKENS

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Co-infection with multiple pathogens can exacerbate clinical manifestations and complicate disease diagnosis in poultry. This study characterized a concurrent infection involving Fowl Aviadenovirus (FAdV) and Infectious Bronchitis Virus (IBV) in a broiler farm in Paraná State, Brazil, in 2021. Tracheal samples from broilers presenting respiratory signs, airsacculitis, and elevated mortality were analyzed by RT-qPCR, genotyping, and viral isolation in embryonated chicken eggs. Embryos showed high mortality and lesions consistent with IBV (dwarfing, curling, and hemorrhages). RT-qPCR amplified a 143-bp fragment of the IBV 5' UTR and Spike S1 genotyping identified the GI-23 lineage. Chorioallantoic fluid (CAF) was screened for FAdV, Infectious Bursal Disease Virus, Avian Reovirus, Chicken Anemia Virus, Newcastle Disease Virus and Mycoplasmas using hemagglutination and molecular assays. Both IBV and FAdV were detected. To selectively isolate FAdV, CAF was treated with chloroform to inactivate IBV, enabling FAdV isolation, which reproduced characteristic embryonic lesions, including hemorrhages, chorioallantoic membrane thickening, and hepatic alterations. Phylogenetic analysis of the partial hexon gene identified the isolate as *Aviadenovirus ventriculi*, serotype 1. *A. ventriculi* is associated with gizzard erosion syndrome, reduced performance, and increased culling, although infections may also occur subclinically. In this study, clinical signs were predominantly consistent with IBV GI-23 infection, while the contribution of FAdV remained unclear. These findings highlight the importance of investigating both viruses individually and in combination to clarify the role of FAdV in mixed infections affecting commercial poultry.

Financial Support: Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina (FAPESC).

PT.012

CHARACTERIZATION OF HUMAN T-LYMPHOTROPIC VIRUSES 1 AND 2 (HTLV-1/2) INFECTION IN A COHORT RESIDING IN NORTHERN BRAZIL

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Introduction: Human T-lymphotropic viruses 1 and 2 (HTLV-1/2) are retroviruses transmitted through blood exposure, sexual contact, shared needles, and vertical transmission, and remain neglected endemic infections. **Objective:** To assess the prevalence of HTLV-1/2 infection and identify related behavioral and epidemiological factors in a cohort under care at the Service for the Care of People Living with HTLV (SAPEVH) at the Federal University of Pará. **Methods:** This cross-sectional observational study included patients assisted at SAPEVH between 2021 and 2025. Venous blood samples were screened for anti-HTLV-1/2 antibodies using ELISA tests and confirmed by qPCR or Western blot. Participants completed an epidemiological questionnaire. Statistical analyses were performed using GraphPad Prism 8.0 and BioStat, with significance set at $p < 0.05$. **Results:** In the SAPEVH cohort, HTLV-1/2 infection prevalence was 10% (131/1,309); of those, 86.26% had HTLV-1 and 13.74% had HTLV-2. Most infected individuals were women (69.47%), identified as mixed race (63.36%), and lived in Belém's metropolitan area (88.55%). Over half were married or cohabiting (55.73%), and about a third completed high school (32.06%). Significant associations were found with marital status (higher positivity among married), blood transfusion history, and lower income (notably among those earning three minimum wages). Additional links were seen for single status and regular health follow-up. **Conclusion:** The infection was linked to marital status, low family income, and a prior history of blood transfusion. These results confirm that HTLV remains common in the area and underscore the importance of improved monitoring, early detection, and targeted public health efforts.

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PT.013

LONG-TERM DECLINE IN NEUTRALIZING ANTIBODY RESPONSES AFTER 17DD YELLOW FEVER VACCINATION REVEALED BY CPE-VNT ANALYSIS

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Yellow fever vaccination is highly effective; however, the long-term persistence of neutralizing immunity after different 17DD vaccination regimens remains incompletely understood. This study evaluated neutralizing antibody responses after yellow fever vaccination using a cytopathic effect-based virus neutralization test (CPE-VNT). The assay was initially validated using sera from C57BL/6 mice experimentally immunized with 17DD vaccine virus. Subsequently, human serum samples from individuals vaccinated before 2017 with full-dose regimens (n=17) or after 2018 with fractional-dose regimens (n=15) were analyzed. The CPE-VNT successfully discriminated immunized from non-immunized animals, confirming assay applicability for yellow fever neutralization analyses. In human samples, individuals vaccinated many years earlier showed heterogeneous and frequently low neutralizing responses, with approximately 47% (8/17) lacking detectable neutralizing antibodies. Among positive individuals in this group, neutralizing titers were generally low, predominantly ranging from 1:20 to 1:80. In contrast, recently vaccinated individuals consistently exhibited detectable neutralization, frequently with higher titers ranging from 1:320 to 1:1280. These findings suggest a substantial decline in long-term neutralizing antibody responses after 17DD vaccination and reinforce the usefulness of CPE-VNT for monitoring vaccine-induced immunity. Further studies with larger cohorts are necessary to better define the durability of protection and the potential need for booster immunization in specific populations.

Keywords: yellow fever vaccine, neutralizing antibodies, CPE-VNT, waning immunity.
Financial Support: CAPES, FINEP, and FAPESB.

PT.014

Rabies virus pathogenesis in the kidney: potential involvement of the renal system in viral dissemination

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The rabies virus (*Lyssavirus rabies*, RABV) is a neurotropic pathogen that primarily infects the central nervous system (CNS), causing a fatal disease in almost all cases. Previous studies have suggested renal involvement during RABV infection, indicating that the kidney may contribute to viral pathogenesis. Therefore, we investigated viral kinetics and renal pathology in a murine model of wild-type RABV infection. Albino Swiss mice (3-4 weeks old; CEUA/IEC no. 25/2017) were inoculated intracerebrally (i.c., n = 8) or intramuscularly (i.m., n = 29) with AgV-3 (5.4 LD_{50}/0.02 mL) or vehicle (n = 9). Brain and kidney samples were collected from 5 to 30 days post-infection (dpi). RABV antigens were detected by immunohistochemistry, and histopathological changes were assessed by hematoxylin and eosin staining using a semiquantitative approach. RABV antigens were detected in the brain from 5 dpi after both i.c. (control: 0.0 vs. infected: 1280.0 cells/mm²) and i.m. inoculation (control: 0.0 vs. infected: 274.1 cells/mm²). In the kidney, viral antigens were detected throughout the experimental kinetics (control: 0.0 vs. infected: 178.4 cells/mm²) and were associated with edema, tubular vacuolization, vascular congestion, and glomerular involution. These findings demonstrate renal tropism during experimental rabies and support the kidney as a potential site of viral dissemination.

PT.015

PREVALENCE AND MOLECULAR SURVEILLANCE OF ANTIVIRAL RESISTANCE-ASSOCIATED MUTATIONS IN CYTOMEGALOVIRUS FROM TRANSPLANT RECIPIENTS

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Cytomegalovirus (CMV) infection remains a major cause of morbidity and mortality in solid organ and hematopoietic stem cell transplant recipients, driven by its ability to establish latency and reactivate under immunosuppression. Antiviral resistance is an emerging and underrecognized threat, particularly in settings with limited molecular surveillance data. This study aimed to investigate resistance-associated mutations in clinically relevant CMV genomic regions and to contribute to the epidemiological understanding of viral resistance in a high-risk population. A total of 47 clinical samples from transplant recipients (19–70 years) with detectable CMV DNAemia were analyzed. Target regions of UL54 (DNA polymerase) and UL56 (terminase complex) were amplified using optimized polymerase chain reaction (PCR) protocols with validated primers, followed by electrophoretic confirmation and Sanger sequencing. Successful amplification was achieved in 16 samples (34%) for UL56 and 7 samples (14.9%) for UL54. Preliminary sequencing analyses did not identify mutations associated with resistance to first-line antivirals, including ganciclovir, foscarnet, and letermovir. Although no resistance mutations were detected, these findings provide important baseline data in a context where regional molecular epidemiology remains scarce. The study highlights the feasibility of implementing genotypic resistance testing in routine clinical samples and reinforces the critical role of early molecular surveillance, particularly in cases of persistent viremia or suspected therapeutic failure. By addressing a relevant gap in local data, this work supports the development of evidence-based strategies for antiviral management and contributes to improving outcomes in transplant medicine.

PT.016

HIGH SALIVARY EPSTEIN–BARR VIRUS AND HUMAN HERPESVIRUS 8 DNA LOADS IN AN HIV-INFECTED PATIENT WITH OROPHARYNGEAL DIFFUSE LARGE B-CELL LYMPHOMA

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Diffuse large B-cell lymphoma (DLBCL) is the most common aggressive non-Hodgkin lymphoma subtype, strongly associated with immunodeficiency and oncogenic viral infections. Simultaneous salivary detection of Epstein–Barr virus (EBV) and human herpesvirus 8 (HHV-8) is rarely described in HIV-associated DLBCL. We report a rare case of advanced HIV infection presenting with oropharyngeal DLBCL and concurrent salivary detection of EBV and HHV-8. A 66-year-old man presented with progressive odynophagia, dysphagia, weight loss, and night sweats. Histopathological examination of an oropharyngeal lesion confirmed DLBCL. Staging revealed previously undiagnosed advanced HIV (CD4: 32 cells/mm³) and hepatitis B virus infections. Salivary real-time PCR detected high loads of EBV (1.28×10^8 copies/mL) and HHV-8 DNA (8.07×10^3 copies/mL). Despite CHOP chemotherapy, antiretroviral therapy, and supportive care, the patient developed recurrent febrile neutropenia, requiring hospitalization and broad-spectrum antibiotics. His condition deteriorated, and he died three months after diagnosis without evidence of lymphoma recurrence or metastasis. This case highlights simultaneous salivary shedding of two oncogenic gammaherpesviruses during profound HIV-related immunosuppression and aggressive lymphoma. While the clinical significance of salivary EBV and HHV-8 remains unclear, these findings support saliva as a promising, noninvasive specimen to monitor oncogenic herpesvirus activity in immunocompromised patients. This reinforces the need for studies exploring the relationships between viral shedding, immune status, and lymphoma outcomes.

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PT.017

Neuroinflammatory Biomarkers in HTLV-1 Infection: An Analysis of Cerebrospinal Fluid and Plasma

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Human T-cell lymphotropic virus type 1 (HTLV-1) infection is associated with chronic inflammatory diseases, particularly HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). However, most people living with HTLV-1 (PLHTLV-1) remain asymptomatic or present mild inflammatory manifestations, being classified as oligosymptomatic or having intermediate syndrome. Although elevated levels of pro-inflammatory cytokines and chemokines, including IFN-γ, TNF-α, IL-6, and CXCL10, have been reported in PLHTLV-1, reliable biomarkers capable of distinguishing clinical outcomes or predicting disease progression remain unavailable. Therefore, this study aimed to characterize cytokine profiles associated with different clinical presentations of HTLV-1 infection. A total of 40 cerebrospinal fluid (CSF) and 36 plasma samples were obtained from PLHTLV-1 followed at a referral center in Bahia, Brazil. Levels of IL-12p70, TNF-α, IL-6, IL-1β, IL-12p40, IL-23, IFN-γ, and CXCL10 were quantified by flow cytometry using a FACS Fortessa (BD Biosciences) and the LEGENDplex™ (BioLegend) assay. Data were analyzed using GraphPad Prism 5. Normality was assessed by the Shapiro–Wilk test, group comparisons were performed using the Kruskal–Wallis test with Dunn's post hoc test, and correlations between CSF and plasma biomarkers were evaluated using Spearman's correlation. IL-6, TNF-α, and CXCL10 were detected in both CSF and plasma. No significant differences or correlations were observed among clinical groups or between biological compartments. These findings indicate that these markers alone cannot discriminate HTLV-1 clinical outcomes, highlighting the need for additional biomarkers to improve understanding of HTLV-1 immunopathogenesis and disease progression.
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PT.018

CHICKEN EMBRYONATED EGGS AS A MODEL FOR STUDYING THE PATHOGENESIS OF INFLUENZA A VIRUS (A/H5)

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Since 2020, the world has experienced a panzootic caused by highly pathogenic avian influenza (HPAI) viruses. Human infections with avian influenza viruses are associated with high case-fatality rates, and the molecular mechanisms underlying disease pathogenesis remain incompletely understood. Therefore, this study aimed to develop an in vivo model to investigate the mechanisms involved in Influenza A/H5 pathogenicity. To evaluate the interaction between Influenza A/H5 and host cells, we used SPF and conventional chicken embryonated eggs and a vaccine strain of Influenza A/H5N8 (A/Astrakan/3212/2020-IDCDC-RG71A). Following inoculation into the allantoic cavity, eggs were incubated for 24 h at 35°C. The allantoic fluid was subsequently collected and subjected to mass spectrometry-based proteomic analysis.The infection of the embryonic gastrointestinal tracts was confirmed by immunofluorescence staining using anti-NP antibody. Preliminary results revealed distinct proteomic profiles between the two egg types. In infected SPF eggs the expression of SPINK7(4.53-fold), LysozymeC (2.60-fold) and Ovalbumin (2.32-fold) was significantly increased compared with uninfected SPF control eggs. Furthermore, infected SPF eggs presenting hemolytic signs exhibited exclusive expression of Cathelicidin-2 and Serpin-B1, suggesting a differential host response associated with infection. In contrast, conventional eggs showed increased expression of ovotransferrin, a protein recognized for its immunomodulatory properties and its ability to downregulate the production of pro-inflammatory cytokines, including TNF-α and IL-6. Overall, these findings demonstrate that SPF and conventional embryonated eggs exhibit distinct proteomic responses to Influenza A/H5N8 infection, suggesting that each model activates different host defense and inflammatory regulatory mechanisms. The embryonated eggs showed a valuable model for evaluating pathogenicity mechanisms.

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PT.019

Characterization of a cell-to-cell communication mechanism in amoebas of the genus Acanthamoeba in response to infection by the giant virus *Tupanvirus*

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Free-living amoebae of the genus *Acanthamoeba* are ubiquitous environmental protists that play important ecological roles and serve as natural hosts for giant viruses, including *Tupanvirus* (TPV). Previous studies have shown that *Acanthamoeba castellanii* employs encystment as a defense mechanism against giant viruses. When encystment precedes infection, viral replication is impaired, however, when infection is established, viruses inhibit encystment favoring viral production. This host–pathogen interaction suggests a temporal dynamic in which the outcome of infection depends on early host sensing. Therefore, we investigated whether A. *castellanii* engages in a cell-to-cell communication mechanism of defense against TPV infection. Conditioned media and cell lysates were collected from A. *castellanii* cultures infected with TPV at 8 and 16 hours post-infection. These fractions were used to treat fresh amoebae cultures for 24 hours, followed by viral challenge with TPV. Morphological analysis revealed that cells previously exposed to conditioned media or lysates derived from infected cultures exhibited reduced cytopathic effects compared to untreated controls, suggesting increased resistance to viral infection. These findings indicate that infected amoebae release factors capable of modulating susceptibility to viral infection, supporting the existence of an intercellular communication mechanism. Current efforts are focused on identifying the molecular nature of these factors through transcriptomic and metabolomic analyses, as well as assessing their impact on viral replication. Overall, this study provides evidence for a previously unexplored antiviral defense strategy in unicellular eukaryotes, contributing to the understanding of amoeba–giant virus interactions.

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PT.020

ELASTASE-INDUCED EMPHYSEMA EXACERBATED BY INFLUENZA A (H1N1): IMPACT OF MICROBIAL METABOLITE ACETATE ON MORBIDITY AND MORTALITY IN A MURINE MODEL

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Chronic obstructive pulmonary disease (COPD) is among the leading causes of death worldwide, and acute exacerbations—often triggered by viral infections—significantly increase morbidity and hospitalization rates. This study aimed to characterize a murine model of elastase-induced emphysema followed by influenza A infection and to evaluate whether modulation of inflammation by the microbial metabolite acetate affects disease progression. C57BL/6 mice received porcine pancreatic elastase (PPE) or PBS. After 28 days, mice were infected with 10^2 PFU of influenza A/PR/8/34 (H1N1) (FLU) or remained uninfected; emphysema-infected mice composed the exacerbation group (EXAC). Body weight and survival were monitored for 49 days. On day 6 post-infection, viral titers and lung leukocyte profiles were assessed. Acetate was administered either preventively or therapeutically. FLU and EXAC groups showed significant and sustained weight loss ($p < 0.01$). Mortality reached 28.5% in FLU and 100% in EXAC, while PBS and PPE showed no lethality. EXAC exhibited increased total leukocytes, mononuclear cells, and neutrophils compared to controls and FLU, whereas viral loads were similar between infected groups. Preventive acetate supplementation completely prevented mortality, while therapeutic administration improved survival to 86% compared to 43% in untreated EXAC. These findings demonstrate that exacerbation increases morbidity and mortality independently of viral load and that acetate confers significant protection, highlighting its potential as a therapeutic strategy targeting host inflammatory responses.

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PT.021

Impact of Influenza A Virus Infection on Synaptic Plasticity in Human iPSC-Derived Neurons

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Recent researchers have reported a marked increase in the prevalence of psychiatric and neurodevelopmental disorders. Although this rise may be partially explained by improvements in diagnostic methods, environmental factors, such as viral infections, may also contribute to these outcomes. Despite its predominantly respiratory tropism, Influenza A virus (IAV) can affect the central nervous system (CNS) and has been associated with neurological manifestations, including encephalitis and cognitive impairment. Nevertheless, the mechanisms through which this infection interferes with neurodevelopment, neural function, and synaptic plasticity remain poorly understood. Based on this context, this study aimed to investigate the effects of IAV infection on the CNS, with an emphasis on cellular and molecular alterations related to synaptic plasticity. Cortical neurons derived from induced pluripotent stem cells (iPSCs) obtained from neurotypical individuals were cultured and subjected to viral infection. Furthermore, synaptic parameters were analyzed in neurons derived from individuals previously diagnosed with autism spectrum disorder (ASD) and congenital Zika syndrome (CZS). The comparison among these models enabled the assessment of whether pre-existing genetic and cellular alterations may be associated with different degrees of vulnerability of the developing CNS to the effects of IAV infection. Preliminary evidence indicated that the H1N1 subtype can enter the neurons and promote the production of viral RNA and proteins, although it appears to have a limited capacity to complete multiple replication cycles. Nevertheless, a restricted or abortive infection may be sufficient to induce alterations in neuronal excitability, synaptic transmission, neural network activity, and mechanisms of synaptic plasticity.

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PT.022

DENDRITIC CELL PROFILE IN HUMANS: PERSPECTIVES FOR STUDYING PATIENTS WITH CHRONIC INFECTION BY HUMAN T-CELL LYMPHOTROPIC VIRUS TYPE 1 (HTLV-1)

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Human T-cell lymphotropic virus (HTLV) infects 10-20 million individuals worldwide. Brazil has one of the highest absolute numbers of cases, with approximately one million infected individuals. HTLV-1 is associated with severe clinical manifestations, particularly HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). However, the reasons why only 2–10% of people living with HTLV-1 (PLWHTLV-1) develop complications remain poorly understood. Dendritic cells (DCs) are professional antigen-presenting and important cellular targets of HTLV-1. Accordingly, the disease progression may be associated with alterations in DC phenotypic and functional profiles, which remain poorly characterized. Therefore, the present study aimed to evaluate the DC profile in peripheral blood mononuclear cells (PBMCs) from patients progressing to HAM/TSP and asymptomatic PLWHTLV-1. Blood samples were obtained at the Ambulatório Orestes Diniz, an outpatient clinic of Hospital das Clínicas at the Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Brazil. Phenotypic characterization and evaluation of activation and functional parameters were standardized for the assessment of $CD1c^+$ and $CD141^+$ DC subsets by spectral flow cytometry using a BD FACSymphony A5 SE cell analyzer at the UFMG flow cytometry facility, employing a panel of 13 immune parameters. Our findings indicated that $CD141^+$ DC populations constitute the majority of DCs within the myeloid compartment ($CD11c^+HLA\text{-}DR^+$). In contrast, $CD1c^+$ cells demonstrated high sensitivity to stimuli that polarize immune responses toward a Th1 or pro-inflammatory profile. These results suggest that the panel developed herein may be a valuable tool for investigating DC subsets in chronic HTLV-1 infection, a retrovirus of significant public health relevance.

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PT.023

LONGITUDINAL IMMUNE SIGNATURES OF LATE-RELAPSING HEPATITIS FOLLOWING YELLOW FEVER

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Late-relapsing hepatitis after yellow fever (LHep-YF) is a recently described complication characterized by recurrent jaundice and elevation of liver enzymes during convalescence. Here, we investigated the longitudinal systemic immune response associated with LHep-YF following natural yellow fever virus (YFV) infection. This observational study included 20 patients infected during the 2017-2018 outbreak in Minas Gerais, Brazil, evaluated at the acute (1–15 days post-symptom onset (DPSO)), early convalescent (30–60 DPSO), and late convalescent (85–120 DPSO) phases. Patients were classified as developing LHep-YF (n=11) or not (NLHep-YF, n=9). Neutralizing antibodies against wild-type and 17DD vaccine strains were measured by PRNT50, and 48 serum immune mediators were quantified using a multiplex luminex assay. Comparative statistics, fold-change, signature profiling, and correlation network analyses were performed. Neutralizing antibody titers were comparable between LHep-YF and NLHep-YF patients at all timepoints, indicating that humoral immunity alone did not explain relapse outcome. In contrast, LHep-YF patients displayed broader and more sustained systemic immune activation across all phases, with increased inflammatory, regulatory, and growth mediators. During the acute phase, G-CSF, IL-3, IL-4, MIF, and GM-CSF emerged as early discriminators. At 30–60 DPSO, IL-4, IL-5, IL-12p40, and G-CSF were prominent, whereas at 85–120 DPSO, IL-10, IL-13, IL-4, CCL2, and IL-12p40 defined a persistent late-phase signature. Correlation network analyses demonstrated greater immune connectivity and stronger pro-inflammatory coordination in LHep-YF patients. Together, these findings indicate that LHep-YF is associated not with impaired antibody responses, but with prolonged immune dysregulation marked by early hyperactivation, sustained cytokine production, and incomplete inflammatory resolution.
Financial support: Fiocruz Minas, CNPq, NIH, CAPES, FAPEMIG.

PT.024

BIOLOGICAL CHARACTERIZATION AND ANTIBIOFILM ACTIVITY OF A BACTERIOPHAGE INFECTING *Serratia marcescens*

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Serratia marcescens is a ubiquitous Gram-negative bacterium that colonizes hosts and forms biofilms that favor persistence. We characterized a bacteriophage infecting an *S. marcescens* strain associated with the gut microbiota of a lepidopteran pest and assessed its biotechnological potential. The phage was isolated from urban sewage using the double-layer agar method. Plaque and virion morphology, adsorption kinetics, host range against 20 isolates at 30 and 37 °C, stability from -80 to 80 °C and pH 1-13, solvent sensitivity, and antibiofilm activity were evaluated. Biofilm inhibition was assessed at MOIs of 0.01 and 0.1, whereas preformed biofilms were treated at MOIs of 1-1000 for 24 and 48 h. The phage formed translucent plaques of 0.5-0.8 mm, with 58% exhibiting diffuse halos of 1.7-2.5 mm. Transmission electron microscopy revealed an 80-nm icosahedral capsid and a 100-nm contractile tail. The phage infected lepidopteran-associated and environmental *S. marcescens* isolates. Diffuse halos occurred at 30 °C, whereas only non-haloed plaques formed at 37 °C. Chloroform and ethyl ether did not significantly affect viral titers. Infectivity was maintained from -80 to 60 °C and between pH 3 and 11, with reduced infectivity at 70 °C, pH 2, and pH 12, and no plaques at pH 1 or 13. Treatment of preformed biofilms significantly reduced biomass after 24 and 48 h at all MOIs, with no differences among them. The phage also significantly inhibited biofilm formation when applied preventively. Overall, its broad physicochemical stability and antibiofilm activity support its potential as a biocontrol agent against *S. marcescens*.

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PT.025

ALTERED ECTONUCLEOTIDASE EXPRESSION AND PURINERGIC SIGNALING IN NEWBORNS WITH PERINATAL HIV EXPOSURE

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Ectonucleotidases and purinergic receptors modulate inflammatory responses by regulating the balance between extracellular ATP and adenosine. Alterations in this signaling pathway contribute to immune activation in individuals living with HIV. Newborns with perinatal HIV exposure exhibit increased morbidity and mortality from infectious diseases, yet the mechanisms underlying their impaired immune responses remain poorly understood. Here, we explored the role of purinergic signaling pathways in shaping the immune responses of infants exposed to HIV. For this, umbilical cord blood samples were collected from parturients living with HIV and clinically healthy mothers. The expression of ectonucleotidases and purinergic receptors in whole blood was evaluated by real-time PCR while the surface expression of the ectonucleotidases CD39 and CD73 was assessed by flow cytometry. Our data indicate that maternal presence of HIV increases the expression of CD39 in the whole cord blood. Notably, the frequency of CD4+ T cells expressing CD39 was higher in cord blood from infants exposed to HIV. In contrast, a reduced frequency of CD73+CD8+ T cells, as well as decreased CD73 expression on CD8+ T cells and CD19+ B cells, was observed in newborns from mothers living with HIV. Additionally, increased expression of the purinergic receptor P2X1 was detected in cord blood from newborns with perinatal HIV exposure. Together our findings suggest that altered ectonucleotidases expression may contribute to the accumulation of inflammatory purinergic mediators in the cord blood of infants exposed to HIV, favoring a state of immune activation associated with dysfunctional immune responses.
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PT.026

EVALUATION OF THE EFFECTS CAUSED BY FATTY ACIDS IN MACROPHAGES INFECTED WITH DENGUE VIRUS

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Dengue is among the most prevalent arboviral diseases worldwide. In parallel, the association between obesity and infectious diseases has gained attention, especially after the COVID-19 pandemic. Evidence suggests that metabolic alterations associated with obesity may modulate the immune response during Dengue virus (DENV) infection, contributing to more severe disease outcomes. Therefore, this study aimed to evaluate the effect of fatty acids, such as palmitic acid and sodium oleate (P/O), on viral replication in DENV-infected macrophages.Peritoneal macrophages were obtained after induction with sterile 3% thioglycollate in C57BL/6 mice. After collection, cells were treated in vitro with P/O for 24 h at a concentration of 0.5 nM and subsequently infected with DENV-2 at MOI 1. After 48 h, supernatants and macrophages were collected for quantitative real-time RT-PCR (RT-qPCR) analysis. RNA from supernatants was automatically extracted, whereas cellular RNA was extracted using TRI Reagent®. RT-qPCR was performed using DENV-2 primers and a positive sample with known viral titer. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$). Results were expressed as mean ± standard deviation. Viral load in macrophages and supernatants did not show changes after fatty acid treatment, with values of 18.3 ± 24.73 and 3.34 ± 0.183 viral copies/mL, respectively. In groups infected only with DENV, values were 4.38 ± 0.71 and 3.26 ± 0.12, respectively. These findings suggest that fatty acids do not modulate DENV replication in peritoneal macrophages.

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PT.027

Development and Genomic Validation of a Capsid-Tagged Mayaro Virus Infectious Clone via CPER for Virus-Host Interaction Studies

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Mayaro virus (MAYV) is an emerging alphavirus causing acute febrile illness and chronic arthralgia in Latin America, yet the molecular mechanisms of its replication and capsid-mediated virus-host interactions remain unclear. In this study, we established a reverse genetics platform using Circular Polymerase Extension Reaction (CPER) to generate recombinant infectious MAYV. We engineered a wild-type virus (MAYV WT) and a capsid-tagged version carrying a FLAG (MAYV FLAG). Viral genomes were assembled in a plasmid under a CMV promoter and a downstream HDV ribozyme. Infectious particles were rescued by transfecting HEK293T cells; viral particles were recovered 9 days post-transfection from cell culture supernatants and amplified in Vero cells. Viral fitness was evaluated through plaque assays and viability assays. Capsid-tagged expression was verified by western blotting two days after infection. Genomic stability across serial passages was assessed via Illumina metagenomic sequencing. Both recombinant viruses replicated efficiently, displaying kinetics and cytopathic effects similar to the reference virus, demonstrating that the FLAG insertion did not compromise viral fitness. Metagenomic sequencing yielded over 750,000 reads (50–231 bp, 50% GC content), confirming successful tracking of the viral genome. Two mutations were identified into the recombinant genome but both reflect natural polymorphisms present in other circulating MAYV strains rather than passage-induced artifacts. In conclusion, we generated a genetically stable, functionally validated capsid-tagged MAYV infectious clone. This recombinant virus serves as a tool for protein-protein interaction assays, enabling future mapping of how the viral capsid interacts with host cellular factors. Financial Support: CNPq, Serrapilheira, and FAPESP

PT.028

MONKEYPOX VIRUS INDUCES A PROINFLAMMATORY RESPONSE WITH LIMITED TYPE I INTERFERON AND INFLAMMASOME-ASSOCIATED CYTOKINE INDUCTION IN PBMCS FROM HEALTHY DONORS.

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During natural monkeypox virus (MPXV) infection, viremia is well documented and precedes systemic viral dissemination. The balance between antiviral signaling and inflammatory activation may therefore influence disease progression. This study characterized viral load and cytokine gene expression in primary human peripheral blood mononuclear cells (PBMCs) infected with MPXV. PBMCs were isolated from five healthy donors by Ficoll density-gradient centrifugation, infected with MPXV at a multiplicity of infection of 1, and collected up to 48 hours post-infection. Total nucleic acids were extracted using TRIzol. Viral DNA load was quantified by TaqMan quantitative polymerase chain reaction, whereas IL1B, IL6, IL18, IFNA1, and IFNB1 expression was evaluated by reverse-transcription quantitative polymerase chain reaction using the 2^−ΔΔCt method. At 48 hours post-infection, MPXV DNA was detected in PBMCs from all five donors. Viral loads ranged from approximately 3.5×10^4 to 1.4×10^5 genome-copy equivalents, demonstrating marked inter-donor variability. MPXV infection was also associated with increased IL1B and IL6 expression, indicating activation of a proinflammatory response. In contrast, IFNA1 and IFNB1 showed limited induction, and IL18 did not exhibit a comparable increase. These findings reveal a dissociation between proinflammatory, antiviral, and inflammasome-associated cytokine responses in MPXV-infected PBMCs and support further investigation of viral immune modulation in primary human mononuclear cells.
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PT.029

SARS-CoV-2 Infection Has the Potential to Promote HIV-1 Latency Reversal in Human Microglia: Insights from 2D Microglial Cell and 3D iPSC-Derived Brain Organoid Models

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HIV-1 establishes persistent latent reservoirs in microglia, contributing to viral persistence within the central nervous system. Given the neurotropic potential of SARS-CoV-2 and its ability to induce hyperinflammatory responses, it is pertinent to investigate its influence on HIV-1 latency. This study investigated the impact of SARS-CoV-2 infection on HIV-1 latency reversal in human microglial cells. Latently HIV-1-infected HC69.5 human microglial cells were exposed to lentiviral pseudoviruses expressing the Spike proteins of the Wuhan, Gamma, Delta, and Omicron BA.5 variants (MOI 2). Pseudovirus entry and HIV-1 latency reversal were evaluated by confocal microscopy followed by quantitative image analysis. Human iPSC-derived brain organoids containing either latently HIV-1-infected HC69.5 microglia or HIV-negative HMC3 microglia were additionally infected with live SARS-CoV-2 Omicron BA.5 (MOI 1) and analyzed by immunofluorescence. Exposure to Spike pseudoviruses increased the proportion of GFP-positive cells from 11.5% in mock-treated cultures to 38.0–56.4%, suggesting induction of HIV-1 latency reversal. Omicron BA.5 produced the highest proportion of pseudovirus-positive cells (45.1%), whereas Wuhan induced the highest proportion of GFP-positive cells (56.4%). Spike variants also elicited distinct cytokine profiles, with the Wuhan Spike inducing the strongest TNF-α response, whereas Gamma and Delta induced the highest IL-1β levels. In brain organoids, Omicron BA.5 infection increased TNF-α levels approximately 4.5-fold in organoids containing HC69.5 microglia, whereas only a modest increase was observed in HIV-negative HMC3 control. These findings suggest that SARS-CoV-2 has the potential to promote HIV-1 latency reversal in human microglia and highlight a possible mechanism by which viral coinfections may influence HIV persistence in the central nervous system.

PT.030

Response in Alphavirus infection: Do CHIKV and MAYV trigger distinct immune profiles?

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Inflammation is a protective response triggered by harmful stimuli. Infection with alphaviruses, such as Chikungunya (CHIKV) and Mayaro (MAYV), elicits a robust inflammatory response, characterized by high concentrations of pro-inflammatory cytokines. Among these, IL-1β, which is produced by inflammasome activation in response to viral infection and could induce pyroptosis, stands out as an important marker of inflammation induced by these arboviruses. The aim of this project was to evaluate IL-1β secretion during CHIKV and MAYV infection, thereby contributing to a better understanding of the inflammatory responses triggered by these alphaviruses. Human monocytes were cultured and differentiated into macrophages with PMA (5 ng/mL) for 48 hours prior to infection and then infected with a 5 M.O.I. of CHIKV and MAYV. 24 hours after infection, supernatants were collected, and TRI Reagent was added directly to the remaining cells, which were then collected. The following analyses were performed: ELISA to quantify IL-1β secretion; qPCR to assess gene expression of ASC, NLRP3, CASP1, and AIM-2; PCR to determine viral load; and Western blot to analyze the protein expression. IL-1β secretion was significantly elevated in MAYV-infected cells compared to both the control group ($p < 0.01$) and CHIKV-infected cells ($p < 0.01$). Additionally, infection with both CHIKV and MAYV upregulated AIM2 gene expression. The elevated IL-1β secretion observed after MAYV infection helps explain differences in immune response activation among these alphaviruses, contributing to a better understanding of the mechanisms associated with pathogenesis and the host response during these infections.

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PT.031

DENGUE VIRUS NS3 PROTEIN ALTERS MITOCHONDRIAL BIOENERGETICS AND IMPACTS RESPIRATORY COMPLEX ORGANIZATION IN HUMAN CELLS

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Dengue is an arboviral disease of major global impact caused by dengue virus (DENV), with Brazil reporting high and recurrent numbers of cases. Previous studies, including those from our group, have shown that DENV infection alters mitochondrial structure and bioenergetics, although the underlying molecular mechanisms remain poorly understood. We hypothesize that modulation of mitochondrial respiratory activity by DENV proteins contributes to these alterations. We previously demonstrated that the DENV non-structural protein 3 (NS3) reduces mitochondrial respiratory chain complex I (CI) activity in a protease activity-dependent manner. Moreover, *in silico* analyses identified putative NS3 cleavage sites within CI subunits, suggesting that NS3 may directly target the complex and impair its function. However, these experiments relied on isolated mouse liver mitochondria and recombinant NS3 produced in *E. coli*, which lacks the post-translational modifications present in human cells. Here, we investigated the effects of NS3 expression in HEK293T cells. High-resolution respirometry showed that NS3 expression reduced complex I-linked oxygen consumption, consistent with previous findings. We further assessed mitochondrial complexes and supercomplexes by BN-PAGE and observed an altered banding pattern, including an apparent reduction in supercomplexes containing complexes I, III, and IV. These findings suggest that NS3 may affect supercomplex stability, contributing to impaired complex I-linked respiration. To investigate the molecular basis of these alterations, we are performing proteomic analyses of native gel bands to characterize changes in mitochondrial complex composition. Together, our findings provide new insights into DENV-induced metabolic remodeling and support a role for NS3 in modulating mitochondrial function during infection.
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PT.032

Genome-wide CRISPR/Cas9 screening identifies host pathways associated with Oropouche and Mayaro virus infection

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Introduction: Emerging arboviruses, including Oropouche virus (OROV) and Mayaro virus (MAYV), pose an increasing public health threat due to their expanding geographic distribution, neuroinvasive potential, and the absence of licensed antiviral therapies. Identifying host factors required for viral infection is essential to better understand virus–host interactions and to identify novel therapeutic targets. **Methods:** A genome-wide CRISPR/Cas9 loss-of-function screen was performed using the Brunello sgRNA library in permissive cells infected with OROV and MAYV. An additional screen was conducted using a recently identified recombinant OROV lineage associated with the 2024 outbreak in the Brazilian Amazon. Candidate genes were prioritized using MAGeCK enrichment analysis followed by Gene Ontology (GO) annotation. Functional validation was initiated using CRISPR/Cas9-mediated gene knockout and RNA interference (RNAi) after optimization of transfection conditions. **Results:** Genome-wide screening identified multiple host genes involved in OROV and MAYV infection. Enrichment analysis revealed pathways related to vesicular trafficking, cytoskeleton organization, cellular metabolism, protein homeostasis, and immune responses, highlighting their importance during arbovirus infection. Screening with the recombinant OROV strain identified additional host factors potentially associated with viral adaptation, whereas MAYV infection revealed distinct candidate genes converging on similar biological pathways, suggesting shared host mechanisms. Optimized RNAi conditions established a robust platform for functional validation. **Conclusions:** These findings demonstrate the utility of genome-wide CRISPR/Cas9 screening for identifying host determinants involved in emerging arbovirus infections. The identification of conserved host pathways shared by OROV and MAYV advances our understanding of arbovirus biology and provides promising targets for future mechanistic studies and host-directed antiviral strategies.

Financial Support: FAPESP, CAPES.

PT.033

ANALYSIS OF INHIBITORS APOPTOSIS PROTEINS OF *ALPHABACULOVIRUS CHRINCLUDENTIS* AND THEIR INFLUENCE ON HOST RANGE

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Anticarsia gemmatalis and *Chrysodeixis includens* are economically important agricultural pests that affect soybean and various vegetable crops. Controlling these pests is vital for agricultural productivity. Consequently, baculoviruses like *Alphabaculovirus chrincludentis* (formerly Chrysodeixis includens nucleopolyhedrovirus, ChinNPV) show great promise due to their high specificity and minimal environmental impact. ChinNPV belongs to Group II alphabaculoviruses and possesses a circular, double-stranded DNA genome. Various cellular processes are involved in viral replication. Among these, a critical mechanism is the virus's ability to modulate host cell apoptosis, which occurs through processes that include inhibitors of apoptosis proteins (IAP). This study aimed to investigate the role of IAPs genes of ChinNPV in the virus–host interaction. The objective was to evaluate the ability of two ChinNPV IAPs (IAP2 and IAP3) to inhibit apoptosis in *Anticarsia gemmatalis*, *Spodoptera frugiperda*, and *Trichoplusia ni* insect cells, using recombinant baculoviruses expressing these genes in the presence or absence of the known anti-apoptotic gene *p35*. To support this investigation, caspase-3 and -7 activity assays were performed alongside cell viability assays based on cellular ATP levels. The data revealed that the role of these IAPs is subject to different modulations depending on the host cells and the involvement of the *p35* gene. However, IAP3 was prominent due to its high inhibitory effect, being the only protein capable of inhibiting apoptosis in the absence of the *p35* gene. The findings expand the understanding of viral pathogenesis and open promising avenues for developing more efficient and specific biopesticides.

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PT.034

DENGUE VIRUS INFECTION DISRUPTS MITOCHONDRIAL RESPIRATORY SUPERCOMPLEX ASSEMBLY IN HUMAN CELLS

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Dengue virus (DENV) infection reprograms host cell metabolism and immune responses, including marked alterations in mitochondrial bioenergetics. Previous work from our group showed that DENV-infected human hepatocytes display enhanced fatty acid oxidation, an event potentially linked to reduced Complex I (CI) activity previously observed in mitochondria exposed to the viral non-structural protein 3 (NS3). Mitochondrial respiratory complexes can assemble into different combinations known as supercomplexes, optimizing electron transfer, oxidative phosphorylation efficiency and reactive oxygen species control. Since these arrangements are linked to metabolic and functional changes involving CI and Complex IV (CIV) in other liver conditions, we investigated whether DENV infection affects the assembly of distinct supercomplex combinations and individual complex activity. Human hepatocyte (HUH7) and lung epithelial (A549) cell lines were infected with DENV or mock-infected, and mitochondrial extracts were analyzed by native gel electrophoresis followed by in-gel enzymatic activity assays. Infected cells showed a preliminary reduction in respirasome formation (CI+CIII+CIV), together with changes in other supercomplex combinations and decreased in-gel CIV enzymatic activity, consistent with previous data from our group showing reduced CIV-linked oxygen consumption in infected hepatocytes. Ongoing work will use dodecyl-maltoside and different digitonin concentrations to determine, respectively, whether individual complexes are differentially affected by infection and how supercomplex stability influences DENV modulation, further exploring the structural-functional link between supercomplex organization and virus-induced metabolic reprogramming. Together, these preliminary findings points to a previously uncharacterized phenomenon: viral infections can impair mitochondrial supercomplex assembly, with potential implications for identifying novel targets in virus-host metabolic interactions.
Financial Support: CNPq.

PT.035

ARGINYL-tRNA TRANSFERASE CONFERS BROAD-SPECTRUM ANTIVIRAL ACTIVITY AGAINST PHYLOGENETICALLY DISTINCT PLANT VIRUSES

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Our group has previously shown that arginyl-tRNA transferase (ATE), encoded by the CBD2 open reading frame of cotton (*Gossypium hirsutum*), contributes to resistance against Cotton leafroll dwarf virus (CLRDV). ATE catalyzes arginylation — the addition of arginine residues to proteins bearing specific N-terminal residues (glutamic acid, aspartic acid, or oxidized cysteine) — thereby targeting them for ubiquitination and proteasomal degradation. Overexpression of ATE in *Arabidopsis thaliana* inhibits CLRDV replication and systemic spread, pointing to a role in antiviral defense. Whether this activity extends to viruses from other families, however, remained unknown. Here, we investigated the effect of ATE on Potato virus X (PVX) infection in *Nicotiana benthamiana* and *A. thaliana*. Plants were inoculated with *Agrobacterium tumefaciens* carrying a PVX-GFP construct, co-infiltrated with either the ATE construct or an empty vector control. Fluorescence analysis (UV imaging and ImageJ/FIJI quantification) at 4–6 days post-infiltration revealed a significant reduction in GFP fluorescence ($p < 0.01$) and a 24-hour delay in systemic viral spread in ATE-expressing plants. In transgenic *A. thaliana* (35S:ATE), systemic leaves showed reduced levels of viral movement protein transcript, consistent with decreased viral replication. Together, these findings suggest that ATE exerts broad-spectrum antiviral activity against both CLRDV and PVX — two viruses from unrelated families. Future work will test its efficacy against additional virus families, optimize ATE expression in crop species, and apply bioinformatics approaches to identify candidate PVX protein targets, shedding light on the molecular basis of ATE-mediated antiviral defense.

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PT.036

Time-Dependent Metabolic Reprogramming in Chikungunya and Mayaro Virus Infection: A Role for Osteopontin

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Osteopontin (OPN/SSP1) is a multifunctional glycoprotein central to immune modulation and inflammation, with a poorly understood role in arthritogenic alphavirus infections such as Chikungunya (CHIKV) and Mayaro (MAYV) virus, despite its potential involvement in chronic joint damage. This study evaluated OPN's influence on the antiviral response and metabolism of THP-1-derived human macrophages infected in vitro with CHIKV and MAYV, using siRNA-mediated OPN silencing followed by assessment of viral load, phenotype, metabolic activity, nitric oxide production (iNOS2), cytokines, and inflammatory/metabolic transcripts at 1, 3, and 6 hours post-infection. Both viruses induced SPP1 expression, more strongly for CHIKV, alongside a time-dependent, virus-specific shift toward aerobic glycolysis: MAYV triggered early, sustained HIF1A/PKM/HK2 induction, while CHIKV showed a delayed, multiphasic response peaking in HK2 (1h, 6h) and HIF1A/PPARGC1/PPARG (3h). OPN silencing consistently blunted these glycolytic and mitochondrial gene inductions, particularly HK2 at 6h and PPARGC1 at 3h in CHIKV, indicating a positive regulatory role for OPN in this reprogramming. Accordingly, OPN knockdown reduced viral load in both viruses and increased mitochondrial oxidative phosphorylation, suggesting that OPN loss impairs the glycolytic shift required for efficient viral replication, generating a hostile metabolic microenvironment. Silenced cells also displayed a hybrid macrophage phenotype (CD80/CD206 co-upregulation), reduced iNOS2 (with compensatory oxidative stress in CHIKV), reversal of early NFKB repression, and attenuation of the late inflammatory storm. These findings indicate that osteopontin facilitates alphavirus replication through glycolytic reprogramming of infected macrophages while promoting hyperinflammation, supporting its potential as both a disease severity biomarker and therapeutic target for these infections.

PT.037

Chikungunya and Mayaro viruses infect an embryonic heart cell line and induce a strong mRNA expression of antiviral and proinflammatory pathways

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Arthropod-borne viruses (arboviruses) constitute a significant ongoing public health threat. Since the beginning of the century, the number of notifications of arbovirus outbreaks and epidemics, including Chikungunya (CHIKV) and Mayaro (MAYV) alphaviruses, has been rising, leading to the emergence of more atypical manifestations, such as heart-related symptoms, including arrhythmias, myocarditis, and pericarditis. This study aimed to examine the dynamics of CHIKV and MAYV infections in H9c2 (rat cardiomyoblast) cell lines. The cells were infected with 1 MOI of the viruses, and infection progression was monitored for up to 48 hours post-infection (hpi). The cytopathic effects, vRNA, and viral particles in the supernatant were detected. Also, in the first 24 hours of infection, gene expression of an entry receptor (*Mxra8*), antiviral (*Ddx58, Ifih1, Mavs,* and *Ifnb1*), proinflammatory (*Tnf, Il6,* and *Cxcl1*), and pathogenic markers (*Cacna1c, Fbxo32,* and *Hmox1*) were analysed, assembling an expressive genetic panel about the infection of the cells. Overall, H9c2 cells proved to be an effective model for studying CHIKV and MAYV infections, showing high viral loads and upregulation of the analyzed pathways, with results that could contribute to understanding viral tropism and the development of new therapeutic strategies.

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PT.038

DISTINCT MITOCHONDRIAL AND ANTIVIRAL RESPONSES OF HUMAN LYMPHOID CELLS TO IN VITRO INFECTION WITH INFLUENZA A (H1N1) VIRUS AND SARS-COV-2

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Influenza A (H1N1) virus (IAV) and SARS-CoV-2 infect lymphoid cells in secondary lymphoid tissues, but the mitochondrial and antiviral responses induce are incompletely understood. We investigated comparatively the effects of IAV and SARS-CoV-2 on mitochondrial homeostasis and antiviral responses in cultures of lymphomononuclear cells from palatine tonsil and the modulation of these responses by the SphK/S1P/S1PR1 signaling axis. Cultures were pretreated for 24 h with W146 (S1PR1 antagonist) or SKI-II (SphK1/2 inhibitor) and infected with IAV or SARS-CoV-2 (MOI=1). At 24 and 48 hpi gene expression was determined by RT-qPCR. Both W146 and SKI-II reduced SARS-CoV-2 viral RNA load in cells and culture supernatants at 24 hpi, and this effect was maintained at 48 hpi. In contrast, during IAV infection, W146 increased intracellular and extracellular viral RNA load at early time points, whereas SKI-II increased intracellular viral RNA load and viral RNA detection in culture supernatants predominantly at 48 hpi. In IAV-infected cells SKI-II increased the expression of genes involved in mitochondrial dynamics and antiviral responses, such as DRP1, MFN1, MFN2, PINK1, and MAVS, predominantly at 48 h post-infection. In contrast, in SARS-CoV-2-infected cells SKI-II induced preferentially genes associated with mitochondrial biogenesis (TFAM and PGC1A) and immune responses (cGAS, STING, IFNA, IFNB, IFNG, IL10, and IL1B), predominantly within the first 24 hpi. Collectively, these findings suggest that IAV and SARS-CoV-2 elicit distinct responses in human lymphoid cells following modulation of the SphK/S1P/S1PR1 axis, highlighting the relevance of this pathway in regulating mitochondrial homeostasis and antiviral responses against pandemic respiratory viruses.

Influenza A virus
SARS-CoV-2
Mitochondrial homeostasis

Financial Support: FAPESP, CNPq, CAPES.

PT.039

THE SPHK/S1P/S1PR1 LIPID SIGNALING AXIS MODULATES VIRAL REPLICATION AND MITOCHONDRIAL HOMEOSTASIS DURING INFLUENZA A (H1N1) IN VITRO INFECTION OF PRIMARY HUMAN B LYMPHOCYTES

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We have previously reported that Influenza A virus (H1N1) (IAV) infects human B lymphocytes and here we investigated modulating roles of the lipid SphK/S1P/S1PR1 signaling axis on IAV viral load, mitochondrial homeostasis and antiviral responses in those cells. Cultures of B lymphocytes isolated from human palatine tonsils were pretreated for 24 h with W146 (S1PR1 antagonist) or SKI-II (SphK1/2 inhibitor) and then infected with IAV (MOI=1). Viral RNA load was quantitated by RT-qPCR in cells and supernatants at 24 and 48 hours post infection. Gene expression was quantitated by the $2^{-\Delta\Delta Ct}$ method. W146 caused significant increase in intracellular viral RNA load ($p = 0.0028$) at 48 h post-infection while reducing viral RNA load in supernatants ($p = 0.0003$). In contrast, SKI-II significantly reduced intracellular viral RNA load ($p = 0.0018$), with decrease DRP1 ($p < 0.0001$) and MFN1 ($p = 0.0013$) expression. SphK1/2 inhibition further reduced intracellular viral load ($p < 0.0001$) and increased the expression of genes involved in mitochondrial homeostasis (MFN1, MFN2, TFAM, and PINK1) and antiviral responses (MAVS, IFNA, and IFNB). In conclusion, the SphK/S1P/S1PR1 axis integrates mechanisms associated with viral replication, eggress, mitochondrial homeostasis, and antiviral responses in human B lymphocytes infected by IAV and indicate that this pathway is a regulator of virus-host interactions.

Influenza A virus
SphK/S1P/S1PR1 signaling
B lymphocytes
Mitochondrial homeostasis

Financial Support: CAPES, FAPESP (2019/26119-0), CNPq.

PT.040

MOLECULAR AND SEROLOGICAL INVESTIGATION OF CANINE VIRAL PATHOGENS IN RURAL DOGS FROM CENTRAL-WESTERN BRAZIL

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Free-roaming domestic dogs may contribute to the maintenance and transmission of canine parvovirus (CPV-2), canine distemper virus (CDV), and canine adenovirus (CAV) to susceptible dogs and wildlife at the domestic–wildlife interface. However, molecular, serological, and epidemiological studies involving domestic dogs and wildlife remain scarce. This study investigated molecular detection and serological evidence of exposure to CDV, CAV, and CPV-2 in domestic dogs from rural properties in three municipalities encompassing the Pantanal and Cerrado biomes, neighboring protected areas in Mato Grosso do Sul, Brazil. Samples were collected from 156 dogs in the Pantanal (n = 80) and Cerrado (n = 76) biomes. Blood samples were tested by qPCR for CPV-2 and CAV and by RT-qPCR for CDV, followed by Sanger sequencing of positive samples. Antibodies against CDV, CAV, and CPV-2 were assessed using a semiquantitative dot-ELISA assay (Canine VacciCheck, Biogal Galed Labs). CPV-2 was molecularly detected in two dogs (1.3%), both identified as the CPV-2b. CDV and CAV were not detected. Overall seropositivity was 77.6% for CDV, 62.8% for CPV-2, and 55.8% for CAV. Phylogenetic analysis showed that both sequences clustered with a CPV-2b sequence previously detected by our group in a jaguar (*Panthera onca*) from the same region. These findings document the detection of CPV-2b and widespread serological evidence of exposure to canine viral pathogens in rural dogs. The phylogenetic relatedness between CPV-2 sequences from domestic dogs and a jaguar highlights the importance of vaccination of domestic dogs and integrated viral surveillance at the domestic–wildlife interface.

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PT.041

Impact of arbovirus infection on the phenotypic profile of macrophages: primary cells versus cell lines

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Monocytic cell lines such as THP-1 and U937 are widely used in in vitro studies, however, they exhibit intrinsic biological differences and often diverge from the responses observed in isolated primary human macrophages. This study aimed to evaluate and compare the phenotype of different human macrophages following infection with Chikungunya (CHIKV) and Mayaro (MAYV) viruses. Human monocytes from different sources (isolated CD14+, THP-1, and U937) were differentiated into macrophages using 5 ng/µL of PMA. After 24 hours of resting, the cells were infected with CHIKV or MAYV at a multiplicity of infection (MOI) of 1. Flow cytometry was used to quantify the expression of surface markers (CD80 and CD206) and an intracellular marker (NOS2), comparing infected cells to uninfected controls. CHIKV infection reduced the expression of all markers in primary macrophages while inducing a slight increase in the THP-1 and U937 cell lines. In contrast, MAYV infection caused a strong upregulation (fold change > 1.7) of NOS2, CD206, and CD80 in primary cells and the U937 line. Interestingly, THP-1 cells showed an opposite response to MAYV, with a marked reduction in the expression of all markers (fold change < 0.8). These findings demonstrate that cell lines respond heterogeneously to arboviruses, and THP-1 diverges significantly from primary macrophages during MAYV infection.

PT.042

LATE REGULATORY T CELL RESPONSE IN RABIES: A POSSIBLE DOUBLE-EDGED SWORD BETWEEN NEUROPROTECTION AND VIRAL PERSISTENCE

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The rabies virus (RABV) subverts host immune responses to preserve neuronal architecture while inducing severe neuronal dysfunction. Because regulatory T cells (Tregs) may contribute to this process, we evaluated the temporal expression of $FOXP3^+$ cells, interleukin (IL)-10, and IL-6 in the spinal cord of experimentally infected mice (CEUA IEC nº. 12/2021). BALB/c mice were inoculated intramuscularly (n = 10) with RABV antigenic variant 3 (AgV-3) at 5.6 LD_{50}/0.02 mL and evaluated over 21 days post-infection (dpi). FOXP3, IL-10, and IL-6 *in situ* expression was assessed by immunohistochemistry (IHC) and compared with control (n = 8) and sentinel (n = 4) groups, whereas histopathological changes were evaluated by a semiquantitative hematoxylin and eosin (HE) staining. Differences among groups were analysed using one-way ANOVA or the Kruskal–Wallis test, as appropriate. RABV-positive cells increased from 7 dpi, peaking at 21 dpi ($\bar{x}$ = 2.26; p = 0.0006). The inflammatory marker IL-6 peaked at 11 and 15 dpi ($\bar{x}$ = 2.43; p = 0.0037), whereas FOXP3 ($\bar{x}$ = 2.64; p = 0.0003) and IL-10 ($\bar{x}$ = 2.16; p = 0.0022) showed delayed expression between 15 and 21 dpi. The histopathological analysis indicated that the RABV induces vascular congestion ($\bar{x}$ = 0.7), neuronal swelling ($\bar{x}$ = 1.9), neuronal degeneration ($\bar{x}$ = 2.7), and mild inflammatory infiltrates ($\bar{x}$ = 2.7) in the spinal cord. In summary, AgV-3 infection elicited an early inflammatory response followed by late Treg-associated modulation, supporting a possible double-edged role between neuroprotection and viral persistence.

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PT.043

EX VIVO MONKEYPOX VIRUS INFECTION OF HUMAN TONSILLAR EXPLANTS: ESTABLISHING A LYMPHOID TISSUE MODEL

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Monkeypox virus (MPXV) shows tropism for lymphoid tissues, and lymphadenopathy is a hallmark of mpox. However, human lymphoid tissue models of MPXV infection remain limited. We established and validated an *ex vivo* human tonsillar explant model to investigate MPXV infection and replication. Palatine tonsils were obtained from five pediatric donors undergoing adenotonsillectomy (mean and median ages, 4 and 5 years, respectively). Explants measuring 5 mm³ (approximately 40 mg) were prepared using a sterile 4-mm biopsy punch and cultured at an air-liquid interface in Transwell inserts. Tissue architecture was evaluated by hematoxylin and eosin staining and immunohistochemistry for CD4 and CD20, while viability was assessed using Alamar Blue on days 3 and 7. Explants were inoculated on the epithelial surface with a West African MPXV strain (5×10^5 $TCID_{50}$/mL; MOI=0,01), while controls received mock inoculum. At 72 hours and 7 days post-infection, viral genomes were quantified by real-time PCR, and viral proteins were detected by immunohistochemistry using serum against UV-inactivated MPXV. Explants preserved lymphoid cytoarchitecture and remained viable through day 7. Viral genome loads increased significantly between 72 hours and 7 days in all donors (paired Student's t-test, $p<0.05$). Strong MPXV immunostaining was detected on the epithelial surface and throughout the lymphoid parenchyma at day 7, whereas mock controls remained negative. Galectin-3 staining was also increased in infected explants, possibly reflecting myeloid cell activation and necrotic cell clearance. This model supports MPXV infection, genome accumulation, and lymphoid tropism, providing a useful platform for pathogenesis studies and preclinical antiviral evaluation. Financial Support: [FAPESP grant 2025/09418-5]; [CNPq grant 404248/2025-0]

PT.044

PATHOGENICITY OF MAYARO VIRUS AND CHIKUNGUNYA VIRUS DURING INFECTION OF HUMAN ENDOTHELIAL AND OSTEOBLAST-LIKE CELLS

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The *Togaviridae* comprises a single genus*, Alphavirus,* which includes pathogens of major epidemiological importance in Brazil. Some *Alphaviruses* can cause inflammatory muscle and joint pain with persistent arthralgia. The most-studied arthritogenic viruses are the Chikungunya virus (CHIKV) and the Mayaro virus (MAYV), which cause sporadic outbreaks. As a result, increasing reports of atypical clinical outcomes, particularly endothelial and chronic inflammatory complications. Here, we aimed to establish infection of endothelial cells and osteoblasts by CHIKV and MAYV. For this, this study explored the *in vitro* pathogenic evolution of immortalized somatic cell hybrid/umbilical vein (Ea.hy926) cells and osteosarcoma (MG-63) cells, which were cultured and exposed to the viruses at a multiplicity of infection (MOI) of 1.0. Cells were infected and monitored for up to 72 hours post-infection. Cytopathic effects (CPE) were evaluated, and samples were collected at 6, 18, 24, 48, and 72 hours post-infection. Viral replication was quantified by RT-qPCR, and the expression of host-response genes was analyzed. Interestingly, we noted that all cell lines were permissive to infection, exhibiting prominent cytopathic effects and exponential replication curves, and that MAYV replicated to high titers in Ea.hy926 and upregulated RIG-I, MDA5, IL-6, IL-8, and IFN-β, whereas CHIKV maintained a lower viral titer and downregulated RIG-I and MDA5 expression. Our data support the conclusion that MAYV induced alterations in Ea.hy926 and that both alphaviruses may contribute to immune dysregulation and enhance osteoclast infection, which may contribute to chronic disease. This project received financial support from FAPESP (2025/10639-6).

PT.045

Highly polarized localization of viral factors in the midgut of Aedes aegypti mosquitoes infected with Zika virus

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Aedes aegypti mosquitoes are the main vectors of arthropod-borne viruses (arboviruses) such as dengue, Zika and Chikungunya. The mosquito midgut is the first target tissue for arbovirus infection after a blood meal. Thus, uncovering details of viral replication in the midgut can uncover potential control strategies. Here, we describe the subcellular localization of viral proteins and RNAs from dengue (DENV) and Zika (ZIKV) in midgut cells. We observed that viral non-structural (NS) proteins and the intermediate replicative double-stranded RNA (dsRNA) are localized in the apical (lumen) region of midgut cells. In contrast, viral structural proteins preferentially accumulate in the basal region of enterocytes, towards mosquito body cavity. This suggests that the infection in the midgut cells is polarized, which could facilitate viral systemic dissemination. Our data also show that the ZIKV RNA polymerase (NS5) has predominantly nuclear localization in mosquito midgut cells, although replication occurs in the cytoplasm. Finally, we observed that nuclear translocation of NS5 occurs without affecting the integrity of the nuclear envelope in infected cells. In addition, NS5 is not strongly associated with chromatin-enriched regions, suggesting that the viral polymerase may localize to regions of active transcription. Thus, our data suggest highly polarized localization of viral factors in midgut cells, which correlates with highly successful infection of this tissue.

PT.046

MOLECULAR INVESTIGATION OF RABIES VIRUS IN BROWN FAT FROM DIFFERENT BAT SPECIES

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Lyssavirus rabies (RABV), belonging to the family Rhabdoviridae, is the etiologic agent of rabies, a severe zoonosis primarily maintained by bats in Brazil. Although molecular diagnosis by RT-PCR is well-established in the central nervous system, investigation in extraneural tissues, such as brown fat, is essential to understanding the pathogenesis and infection dynamics in these reservoirs. The aim of this study was to investigate the presence of RABV in the brown fat of rabies-positive bats. A total of 49 bats collected in the state of São Paulo (2024) were analyzed. Following necropsy and RNA extraction from brown fat, RT-PCR targeting the nucleoprotein gene was performed using the Início and P784 *primers*, reducing the initial denaturation time to 3 minutes, adding 6 cycles to reach a total of 36, increasing the annealing temperature by 1°C, adding 15 seconds to the extension step, and decreasing the final extension time by 3 minutes, as well as increasing the $MgCl_2$ concentration. The optimization of the protocol increased detection from 42% (21) in the initial reaction to 63% (31) in the optimized version. Among the 31 positive samples, 55% (27) corresponded to non-hematophagous bats (*Artibeus lituratus*, *Myotis nigricans*, *Artibeus planirostris*, *Eptesicus furinalis*, *Nyctinomops laticaudatus*, and *Histiotus velatus*), 4% (2) to hematophagous bats (*Desmodus rotundus*), and 4% (2) to unidentified species. The detection of the RABV genome in brown fat confirms extraneural dissemination, suggesting that this tissue acts as an active reservoir or site of viral latency, particularly during periods of torpor or low metabolic activity.

PT.047

AIM2 inflammasome activation promotes disease severity during betacoronavirus infection

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Inflammasome activation has emerged as an important mechanism involved in SARS-CoV-2-induced pathology. Although previous studies suggest the participation of the AIM2 inflammasome during infection, the cellular sources involved and its contribution to disease progression remain poorly understood. To investigate AIM2 inflammasome activation during betacoronavirus infection and evaluate its contribution to disease outcome. K18-hACE2 transgenic mice were intranasally infected with SARS-CoV-2 (10^4 PFU), and the lungs were analyzed on day 3 post-infection. AIM2 activation was assessed by immunofluorescence through puncta detection in lung sections. In vitro assays were performed using human bronchial epithelial cells Calu-3 infected with SARS-CoV-2 at MOIs 0, 1, and 1. Additionally, WT and Aim2-/- mice were intranasally infected with MHV-A59 (10^4 PFU), a murine model of betacoronavirus infection, to assess survival, weight loss, and body temperature. Infection of K18-hACE2 mice with SARS-CoV-2 induced AIM2 activation in lung tissue, evidenced by puncta formation detected by immunofluorescence. Similarly, infected Calu-3 cells showed AIM2 activation 8 hours after infection. In experiments with MHV-A59, Aim2-/- mice showed greater survival compared to WT animals. Although no significant differences in weight loss were observed, WT animals showed a more pronounced reduction in body temperature on the 8th day post-infection. Experiments using K18-hACE2 Aim2-/- mice infected with SARS-CoV-2 are ongoing. Betacoronavirus infection activates the AIM2 inflammasome in lung epithelial cells, contributing to disease progression. Ongoing studies aim to define the underlying mechanisms and the cell subtypes involved.

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PT.048

HISTOPATHOLOGICAL ANALYSIS OF TONSILLAR EXPLANTS INFECTED WITH MONKEYPOX

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Our group established an ex vivo palatine tonsil model to investigate monkeypox virus (MPXV) replication, tissue injury, and inflammatory responses in secondary lymphoid tissues, which are important sites of viral amplification and systemic dissemination during MPXV infection. Here, we investigated whether ex vivo MPXV infection induces morphological alterations and activates specific cell death pathways in human tonsillar tissue. Palatine tonsil explants were obtained from five children undergoing adenotonsillectomy (mean age, 5.8 years) and inoculated on the epithelial surface with a West African MPXV strain (5×10^5 $TCID_{50}$/mL; multiplicity of infection, 0.01). Mock-inoculated explants were used as controls. At seven days post-infection, tissues were fixed in Carnoy's solution, paraffin-embedded, and processed for histological and immunohistochemical analyses. Hematoxylin and eosin staining revealed extensive necrosis, marked eosinophilia, loss of tissue architecture, and pronounced structural disorganization in MPXV-infected explants. No cleaved caspase-3 immunofluorecence was detected in infected tissues, suggesting that apoptosis was not prominently activated under these experimental conditions. Conversely, confocal analysis showed a 3.5-fold increase in mixed lineage kinase domain-like protein (MLKL), a key marker of necroptosis, in infected explants compared with mock controls. These findings indicate that MPXV infection causes substantial damage to human lymphoid tissue and support necroptosis, rather than apoptosis, as a major cell death pathway associated with infection. This ex vivo model reproduces relevant features of MPXV-induced tissue injury and provides a physiologically relevant platform for investigating viral cytotoxicity, host-pathogen interactions, and the molecular mechanisms underlying lymphoid tissue damage during MPXV infection and evaluating potential host-directed therapeutic strategies.

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PT.049

EPSTEIN–BARR VIRUS INFECTION AND IL10 RS1800872 GENETIC VARIANT IN HODGKIN AND NON-HODGKIN LYMPHOMAS: A CROSS-SECTIONAL STUDY

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The Epstein-Barr virus (EBV) is associated with the pathogenesis of different lymphoma subtypes and its persistence may be influenced by host immunogenetic factors. Interleukin-10 (IL-10) regulates antiviral immune responses and tumor immune evasion, while genetic variations in the *IL10* gene may affect its expression and influence EBV-host interactions. This study evaluated the association between EBV infection and rs1800872 (C/A) polymorphism of the *IL10* gene in patients with Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL). A cross-sectional study was conducted with lymphoma patients recruited between 2022 and 2026. EBV detection was performed by qPCR in saliva and genotyping of rs1800872 was carried out by PCR-RFLP using peripheral blood samples. Statistical analyses included the Mann–Whitney test and Kendall's Tau-b ($p<0.05$). A total of 98 patients were included, comprising 75 NHL and 23 HL cases. EBV positivity was observed in 38.8% of patients, with a higher frequency in NHL (31 cases), although no significant difference was found between lymphoma subtypes ($p=0.8297$). Mean viral load was higher in NHL (7.19×10^7 copies/mL) than in HL (3.58×10^5 copies/mL) and was increased among patients who progressed to death, suggesting a possible association with poorer prognosis. In HL, EBV positivity was associated with younger age (Tau=-0.477; $p=0.004$), whereas in NHL it was associated with female sex (Tau=0.332; $p=0.002$). No significant association was observed between rs1800872 genotypes and EBV positivity, viral load or clinical parameters. In conclusion, EBV was frequently detected in lymphoma patients, whereas the *IL10* rs1800872 polymorphism showed no apparent influence on EBV-related clinical features.

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PT.050

DEVELOPMENT OF AN *IN VITRO* SCREENING ASSAY FOR MUCIN-ADAPTED LYTIC PHAGES TARGETING *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is a pathogenic Gram-negative bacterium that affects immunosuppressed individuals with cystic fibrosis, carcinomas, or burns. Due to the limited effectiveness of antibiotics against multidrug-resistant strains, phage therapy has shown promising results in clinical trials against bacterial infections. Optimizing the isolation of phages with protective effects on mucous membranes enables the development of prophylactic, sanitizing, and therapeutic formulations. This study aimed to develop an in vitro screening methodology to isolate lytic bacteriophages against *P. aeruginosa* with differential replication in the presence of mucin. Two phages with distinct activity in mucin were used as controls: VAC1 (negative) and VAC3 (positive). The *P. aeruginosa* PA14 strain was incubated with different phages (MOI: 1) in LB broth supplemented with mucin concentrations of 0.1%, 0.5%, and 1%. Bacterial (CFU/mL) and viral (PFU/mL) counts were determined after 24 hours. Results showed that all phages infected PA14, even at different mucin concentrations. Additionally, bacteria exhibited phenotypic changes and increased CFU/mL in the presence of mucin. LB broth with 0.1% mucin did not influence phage replication (VAC1 and VAC3). However, at 0.5% mucin, VAC03 showed enhanced replication, with titers four logarithmic orders higher than untreated cultures, while VAC01 showed no significant changes. This methodology may serve as a screening tool for lytic phages with enhanced replication in mucin-rich environments. Further studies are needed to understand the role of mucin binding in phage-mediated protection of mucosal surfaces.

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PT.051

Structural characterization of the mpxv uracil-dna glycosylase as a potential target for antiviral drug development

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Mpox is a disease caused by the Monkeypox virus (mpxv), which has spread rapidly in several countries in 2022 and has become a major public health concern. Mpxv belongs to the genus Orthopoxvirus and has a double-stranded DNA genome. Among the enzymes involved in viral replication and gene expression, the virus encodes a DNA polymerase (DNApol) that integrates the replicative holoenzyme along with 2 other proteins, including uracil-DNA glycosylase (UNG). UNG contributes to genome maintenance by recognizing and removing uracil residues misincorporated into DNA and, because of its essential role in viral replication, represents a potential antiviral target. This study aimed to structurally characterize mpxv-UNG. The corresponding gene was cloned and expressed in *Escherichia coli*, and the recombinant protein was purified by affinity chromatography. Crystallization trials were performed by sitting-drop vapor diffusion using commercial screens. The crystals obtained, containing 0.09M halogens, 30% GOL_P4K, 0.1M buffer pH 6.5, were submitted to X-ray diffraction in the MANACÁ beam line of the Brazilian Synchrotron (SIRIUS). The final model was refined to 1.8 Å resolution in space group $P2_12_12$, with four molecules in the asymmetric unit, R_{work} and R_{free} values of 0.19 and 0.25, respectively, and 96% of residues in favored Ramachandran regions. The structure comprises 24 α-helices and 24 β-strands turing a highly conserved overall fold compared to a closely related counterpart. Additional electron densities were observed near the active site, together with cavities likely suitable for ligand binding. This structure provides a foundation for future ligand-screening studies and antiviral drug discovery.
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PT.052

MAGNETIC PARTICLE-BASED CAPTURE AS AN ALTERNATIVE TO CENTRIFUGATION FOR BACTERIOPHAGE RECOVERY FROM WASTEWATER: METHOD VALIDATION AND LYTIC ACTIVITY AGAINST MDR *Escherichia coli*

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The isolation of bacteriophages from environmental samples is constrained by the absence of standardized concentration protocols, and conventional centrifugation-based methods frequently result in low viral recovery from complex matrices such as wastewater. This study aimed to validate magnetic particle-based capture as an alternative concentration strategy for bacteriophage recovery using wastewater samples collected from a sewage network point within the campus of the Universidade Federal de Ouro Preto (UFOP), Minas Gerais, Brazil. MDR *Escherichia coli* strains used as phage hosts were isolated from the same sewage samples and had their antibiotic resistance profiles characterized prior to phage screening. Samples were processed in parallel by magnetic particle-based capture and standard centrifugation. The Phage on Tap (PoT) protocol served as a methodological benchmark, reporting recovery efficiencies of up to 85% and titers reaching 10^{10}–^{11} PFU·mL^{-1}. Centrifugation-based processing yielded 4 lytic phage isolates after purification, while the magnetic method recovered 6 lytic isolates, representing a 50% increase in phage diversity. Active phage fractions demonstrated lytic profiles against 5 of 8 tested MDR strains, including EC-01, EC-03, and EC-07. These results indicate that magnetic particle-based capture outperforms conventional centrifugation in recovering lytically active and diverse bacteriophages from wastewater, establishing a more sensitive and scalable approach for environmental phage prospecting with potential applications in phage therapy and AMR surveillance.

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PT.053

Comparative structural analysis of rodent transferrin receptor 1 identifies potential determinants of Sabiá virus host susceptibility

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New World mammarenavirus that causes severe hemorrhagic fever in humans, uses transferrin receptor 1 (TfR1) as its primary entry receptor. While the interaction between human TfR1 and arenavirus glycoproteins has been characterized, little is known about the diversity of TfR1 in wild rodents and its influence on host susceptibility. Here, we performed comparative sequence and structural analyses of TfR1 orthologs from multiple rodent species to identify molecular features potentially associated with SABV entry. The receptor-binding interface of the recently solved SABV GP1 structure was compared with the Machupo virus GP1–human TfR1 complex, revealing conserved and variable interaction loops. TfR1 sequences from representative rodent species were analyzed for the conservation of key residues involved in arenavirus recognition, particularly positions equivalent to human TfR1 N204, Y211, and N348. Based on these signatures, receptors were grouped according to their predicted compatibility with SABV GP1 binding. Structural modeling and molecular docking further suggest that specific amino acid combinations may modulate receptor accessibility and virus attachment. These findings establish a structural framework for prioritizing Brazilian wild rodent species for experimental characterization of TfR1 through gene cloning, receptor-binding assays, and structural studies. Understanding the diversity of TfR1 among potential reservoir hosts will provide valuable insights into the molecular determinants of Sabiá virus host range, reservoir ecology, and zoonotic emergence. This work was supported by the Brazilian Ministry of Science, Technology and Innovation (MCTI).

PT.054

Influenza A persistence in experimentally infected mice is independent of IL-10.

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The Influenza A virus (IAV) is of great public health relevance, causing 1 billion seasonal infections and 290,000 to 646,000 deaths annually worldwide. In addition, emerging IAVs can cause pandemics. We have previously shown that IAV can cause prolonged asymptomatic infection in lymphomonocytic cells from children's tonsils; however, the mechanisms resulting in this non-lytic infection of lymphocytes remain unknown. Interleukin-10 (IL-10) is a cytokine strongly related to the persistence of viral infections. We investigated the infection of lymphomonocytic cells and the persistence of IAV infection in an experimental mouse model. We infected wild type (WT) and IL-10 knockout (IL-10 KO) C57Bl/6 mice intranasally with IAV H1N1 and detected IAV in tissue samples collected over time until 120 days postinfection (dpi). Here we report the persistence of IAV RNA or antigens in various tissues up to 120 days post-infection in both WT and IL-10 KO mice, suggesting that IAV causes prolonged infection independently of IL-10 in this murine model. This model of IAV persistence in vivo is a valuable tool to investigate mechanisms of IAV persistence and possible preemptive experimental interventions that may block it.

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PT.055

FUNCTIONAL STUDY OF A MUTATED VERSION OF THE NSP3 PROTEIN FROM A CHIKV ISOLATE IN VIRAL REPLICATION SYSTEMS IN MAMMALIAN CELL LINES

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Chikungunya virus (CHIKV) is an emerging arbovirus transmitted by *Aedes aegypti*, causing debilitating musculoskeletal disease. Its single-stranded RNA genome encodes four non-structural proteins (nsP1–nsP4), with nsP3 being essential for replication. A natural mutant (CHIKVAA), carrying a 45-amino acid deletion in the hypervariable domain (HVD) of nsP3, maintains infectivity in insects but has lost the ability to infect mammalian cells. nsP3 recruits host G3BP1 via HVD, inhibiting stress granules. To investigate this loss, Vero cells were infected with wild-type CHIKV and CHIKVAA. Viral replication was analyzed by RT-qPCR at 6hpi, 16hpi, and 24hpi, along with nsP3 quantification. At 6hpi, both viruses showed similar levels, indicating that cell entry was not affected by the mutation. However, at 16hpi and 24hpi, CHIKVAA displayed a decrease in viral RNA and nsP3 levels, while wild-type CHIKV showed progressive replication. Gene expression analysis of IL4, IL6, IL1B, and IL10 at 16hpi revealed a similar immune response between both viruses. As next steps, other immune-related genes and G3BP1 will be analyzed, along with nsP3-G3BP1 colocalization by confocal microscopy. The data indicate that the HVD deletion does not compromise cell entry but prevents maintenance of viral replication in mammals. Although cytokine expression was similar at 16hpi, the failure in nsP3-G3BP foci formation in the mutant likely determines the infection outcome.This study reinforces nsP3 as a determinant of tropism and pathogenicity, highlighting how genomic alterations can impact the emergence of variants with different zoonotic potentials.

Financial support: CAPES, CNPq, FAPDF

PT.056

Role of Arginine-Rich Protein Domain in Genome Packaging and Capsid Assembly of Geminiviruses

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The Geminiviridae are a family of plant-infecting ssDNA viruses that cause significant economic losses in important crops. This family is characterized by a unique geminate capsid architecture: two incomplete T=1 capsids joined at a missing pentameric vertex, comprising 110 capsid protein (CP) subunits. However, the molecular determinants of geminivirus particle assembly remain unclear. Previous studies showed that expanding the genome of *Ageratum yellow vein virus* (AYVV) from 2.7 to 3.7 kb induced triple-geminate particle formation. Furthermore, comparative analysis across species revealed a positive correlation between genome size and the net positive charge of the CP arginine-rich N-terminal domain (R-arm). We hypothesize that increased R-arm charge enhances encapsidation of genomes > 3.0 kb, thus enabling geminate particle assembly. To test this hypothesis, we utilized the 3.7 kb AYVV replicative vector that generates triple particles. This vector encodes a CP with an R-arm net charge of +14. We engineered two variants with additional arginine and lysine residues to increase the R-arm net charge to +21 and +25. The three vectors were agroinfiltrated into Nicotiana benthamiana, leading to symptomatic leaves. To improve particle recovery, purification conditions were refined by increasing the amount of plant material and optimizing buffer pH. SDS-PAGE analysis of the purified material revealed a band at ~25–35 kDa, consistent with the expected CP size. Particle assembly, morphology and homogeneity will be further evaluated by negative-staining electron microscopy and dynamic light scattering (DLS). Through these experiments, we aim to elucidate and provide insights into geminivirus capsid assembly.

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PT.057

Human Cytomegalovirus vIL-10 Transcripts Detected for the First Time in Glioblastoma Tissues and Their Role in Modulating Tumor Progression

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Human cytomegalovirus (HCMV) is implicated in glioblastoma multiforme GBM initiation and progression via viral proteins with immunomodulatory and oncomodulatory activities. The HCMV UL111A gene encodes multiple viral interleukin-10 (vIL-10) isoforms A-H through alternative splicing. However, only isoforms A (cmvIL-10) and B (LAcmvIL-10) have been extensively characterized. This study first investigated UL111A transcript expression in human GBM tissues and evaluated cmvIL-10 isoform A effects on proliferation and migration in U138 GBM cells. Also, UL111A isoforms were tested for oncogenic transformation of hMSCs, with no effect result. Viral DNA was detected by qPCR, while isoform-specific RT-qPCR assays were developed for the transcripts. Amplicons were confirmed by DNA sequencing. Lentiviral vectors encoding UL111A isoforms A-F were constructed, and a doxycycline-inducible U138 cell line expressing cmvIL-10 was generated. Protein expression was confirmed by immunofluorescence and western blotting. Cell migration was evaluated using wound healing assays and cellular proliferation with viability cell kit and manual counting. UL111A transcripts A, B, E and F were detected in GBM tissues with different patterns of expression, representing the first demonstration of these viral transcripts in human glioblastoma. However, functional assays in U138 cells demonstrated that cmvIL-10 expression reduces cell proliferation, while it increases cell migration. These findings expand the spectrum of HCMV transcripts identified in GBM and provide the first functional evidence that cmvIL-10 promotes a more migratory phenotype in glioblastoma cells, supporting a role for vIL-10 in HCMV-associated tumor progression rather than oncogenic transformation. This work was funding by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

PT.058

***In vitro* assessment of the genetic diversity and evolutionary dynamics of DENV-3 modulated by pre-existing immunity to DENV-1.**

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Dengue virus (DENV) has four serotypes that are genetically different but immunogenically related, where the phenomenon of cross-neutralization may act as a genetic selection bottleneck. This study investigates how the genetic diversity of DENV-3 is modulated under selective pressure imposed by a DENV-1-specific cross-reactive serological response. A total of 59 samples were selected by FRNT that were serologically monotypic for DENV-1 (FRNT50 mean of 166.42). To evaluate the selective bottleneck caused by cross-neutralization, two DENV-3 lineages, isolated in 2020 and 2025, respectively, were independently incubated (MOI 0.1) for 1 h at 37 °C with each one of the 59 DENV-1-monotypic samples, under sub-neutralizing conditions (FRNT50 concentration). After incubation, Vero cells were infected and, for all infections (for both DENV-3 lineages and all samples), a cytopathic effect was observed between days 4 and 7, indicating successful infection. Supernatants were collected and analyzed by RT-qPCR, showing variation of CT values for all infections (from 10.88 to 28.54).Supernatants (59 samples tested in duplicate for each DENV-3 variant), were sequenced by NGS and are now under bioinformatic analysis to investigate mutation selection and to understand how pre-existing humoral immune response against DENV-1 may affect the evolutionary dynamics of different DENV-3 lineages. In addition, it will be possible to observe if fixed mutations tend to appear at the population level (arising from pressures exerted by different samples), or if each sample, with its specific serological response to DENV-1, modulates DENV-3 variants independently.

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PT.059

Human cytomegalovirus IL-10 isoforms cmvIL-10 and vIL-10F modulate the PI3K/AKT pathway and reduce THP-1 cell viability.

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Human cytomegalovirus (HCMV) is a prevalent agent worldwide. The infection causes different clinical presentations depending on the host's immune system and can be acute, persistent, or latent. HCMV is a highly successful virus due to its ability to subvert the host immune system through the action of various proteins; one of these is the viral interleukin-10 (vIL-10), a homolog of human interleukin-10 (hIL-10). The vIL-10 is encoded by the viral gene UL111A, which generates multiple transcripts and protein isoforms through alternative splicing (A-H). While some biological properties of cmvIL-10 (A) and LAcmvIL-10 (B) are known, the signaling pathways modulated by these proteins remain underexplored. It is known that cmvIL-10, best studied, activates the JAK/STAT and the PI3K/AKT pathways. In this study we investigated the ability of cmvIL-10, LAcmvIL-10, vIL-10E and vIL-10F to mediate PI3K/AKT signaling pathway. THP-1 cells were treated with conditioned media containing the viral proteins and modulation of the PI3K/AKT pathway was analyzed by Western blotting. Our results demonstrate that cmvIL-10 induces AKT activation (S473/T308), whereas inhibit p70S6K (T389) phosphorylation and reduces cell viability. The vIL-10F also reduces cell viability and induces AKT T308 phosphorylation. Additionally, we demonstrate that vIL-10F induced is dependent on the hIL-10 receptor and PI3K. The results further validate our previous in silico analysis indicating that isoform F is very likely to bind the hIL-10 receptor and induce intracellular signaling. Future studies investigating the cellular effects of PI3K/AKT pathway modulation by vIL-10F should be conducted to understand its functions during viral infection. This work was funded by the São Paulo Research Foundation (FAPESP).

PT.060

CLONING AND HETEROLOGOUS EXPRESSION OF OROPOUCHE VIRUS NSM, ENDONUCLEASE, RDRP, AND FULL-LENGTH L PROTEIN IN PROKARYOTIC AND EUKARYOTIC SYSTEMS

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Oropouche virus (OROV) is an emerging arbovirus with a negative-sense, single-stranded RNA genome divided into three segments, designated L, M, and S. The L segment encodes the L protein, which contains endonuclease and RNA-dependent RNA polymerase (RdRp) domains, whereas the M segment encodes a polyprotein precursor comprising the Gn and Gc glycoproteins and the nonstructural protein NSm. Despite the increasing epidemiological relevance of OROV, its proteins remain poorly characterized, highlighting the need to obtain recombinant proteins for structural and functional studies. This study established cloning strategies and evaluated the heterologous expression of NSm, the endonuclease and RdRp domains, and the full-length L protein. The NSm and endonuclease coding regions were amplified by PCR, generating fragments of approximately 523 and 498 bp, respectively, and cloned into the pET-M11 expression vector using ligation-independent cloning for expression in *Escherichia coli*. The full-length L construct was obtained in a pFastBac vector, and the approximately 2,240 bp RdRp domain was amplified and cloned into pFB-BSE. Both constructs were used in the Bac-to-Bac expression system. Bacterial expression tests were performed in *E. coli* BL21(DE3) and Rosetta 2(DE3) using different culture media and IPTG concentrations. In insect cells, recombinant baculovirus propagation passages were evaluated for protein expression. SDS–PAGE analysis revealed soluble bands of approximately 261 kDa and 20 kDa, corresponding to the full-length L protein and NSm, respectively. No clear overexpression was detected for the endonuclease or RdRp domains. These findings support further optimization of expression and purification conditions for subsequent structural and functional characterization.

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PT.061

DISCOVERY OF A POTENT CHLOROQUINOLINE-TRIAZOLE DERIVATIVE ACTIVE AGAINST EMERGING ARBOVIRUSES

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Arboviruses are responsible for an increasing number of infectious disease outbreaks worldwide. Among the most clinically relevant are members of the *Flaviviridae* and *Togaviridae* families, including *Alphavirus chikungunya* (CHIKV), *Orthoflavivirus zikaense* (ZIKV), and *Alphavirus mayaro* (MAYV). To date, no specific and effective antiviral drugs have been approved for the treatment of these infections. Consequently, drug discovery has become a critical area of research, particularly focusing on compounds with broad-spectrum antiviral activity, including those containing heterocyclic scaffolds such as quinoline and triazole. This study aimed to evaluate the in vitro antiviral activity of a novel chloroquinoline–triazole derivative against CHIKV, MAYV, and ZIKV. Cytotoxicity was assessed using the MTT assay and expressed as the median cytotoxic concentration (CC_{50}). Antiviral activity was determined by the MTT method and quantified through the median effective concentration (EC_{50}) and selectivity index (SI). Antiviral effects were further confirmed by cytopathic effect (CPE) inhibition assays and RT-PCR quantification, and virucidal activity was also evaluated. The tested compound exhibited potent antiviral activity against all three arboviruses, with EC_{50} values ranging from 49.79 to 71.60 µM and SI values greater than 4.1, surpassing the efficacy of the reference drugs. At 100 µM, the compound significantly inhibited virus-induced cytopathic effects and reduced viral replication, as noted by titration and RT-PCR. Notably, no virucidal activity was observed. These findings highlight this compound as a promising lead for the development of novel antiviral agents targeting emerging and re-emerging arboviral infections. Financial support: FAPEMIG, CNPq, UFOP, Rede Mineira de Antivirais.

PT.062

IN VITRO ANTIVIRAL ACTIVITY OF EUGENOL-DERIVED 1,2,3-TRIAZOLE COMPOUNDS AGAINST ORTHOPOXVIRUS

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Orthopoxvirus infections remain a significant public health concern due to the re-emergence of zoonotic outbreaks and the circulation of viruses related to variola virus, including Monkeypox, Cowpox, and Vaccinia viruses (VACV). In Brazil, VACV circulates in zoonotic cycles, affecting cattle and rural workers. Although some antiviral strategies exist, therapeutic options are still limited by toxicity, cost, and the risk of viral resistance. The identification of new anti-poxvirus agents is therefore essential. Heterocyclic compounds, particularly 1,2,3-triazole derivatives, have shown promising antiviral properties. In addition, eugenol-derived compounds have been widely investigated for their pharmacological activities. The combination of these scaffolds may generate hybrid molecules with enhanced biological potential. This study evaluated the in vitro antiviral activity of four eugenol-derived 1,2,3-triazole compounds against VACV-WR in BSC-40 cells. Cytotoxicity (CC_{50}) was determined by the MTT assay and neutral red (NR), and antiviral activity was assessed through cytopathic effect (CPE) inhibition and densitometric analysis. The most active compound was further evaluated using the NR uptake assay to determine EC_{50} and selectivity index (SI). All compounds showed no cytotoxicity at 200 µg/mL. Only one derivative exhibited detectable antiviral activity using densitometric analysis. Further, it was evaluated with NR method exhibiting an EC_{50} of 114.30 ± 2.28 µg/mL and an SI of 1.75. Despite its limited selectivity, this compound was able to inhibit VACV-WR replication. These findings highlight eugenol–triazole hybrids as promising prototypes for the development of novel anti-poxvirus agents. Financial support: FAPEMIG, CNPq, UFOP, Rede Mineira de Antivirais.

PT.063

FLAVONOID-RICH EXTRACTS FROM Luxemburgia poliandra AND Luxemburgia octandra EXHIBIT IN VITRO ANTI-ARBOVIRAL ACTIVITY

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Arboviruses represent a major public health concern, particularly in tropical regions, where effective antiviral therapies remain limited. The Ochnaceae family is a rich source of bioactive compounds, especially biflavonoids, with reported pharmacological potential. In this study, we evaluated the in vitro antiviral activity of ethanolic extracts from stems, leaves, and flowers of *Luxemburgia poliandra* and *Luxemburgia octandra* against Zika virus (ZIKV), Chikungunya virus (CHIKV), Mayaro virus (MAYV), and Oropouche virus (OROV). Cytotoxicity and antiviral activity were assessed using the MTT assay, and effective concentration (EC_{50}) values were determined. The most active extract (*L. poliandra*) was further fractionated by liquid–liquid partitioning, and its chemical profile was characterized by thin-layer chromatography (TLC) and UHPLC-HRMS. Extracts from stems and leaves of *L. poliandra* showed antiviral activity against ZIKV (EC_{50} = 71.2 µg/mL) and MAYV (EC_{50} = 48.9 µg/mL). The chloroform fraction exhibited enhanced activity against CHIKV, MAYV, and ZIKV, with EC_{50} values of 12.8, 47.07, and 25.3 µg/mL, respectively. Extracts from *L. octandra* displayed activity against OROV, with EC_{50} values ranging from 68.52 to 195.80 µg/mL and selectivity indices between 1.42 and 2.95. Phytochemical analyses revealed the presence of flavonoids, and UHPLC-HRMS allowed the tentative identification of multiple flavonoid derivatives. These findings suggest that flavonoids may contribute to the observed antiviral activity and highlight *Luxemburgia* species as promising sources of anti-arboviral compounds. **Financial support:** FAPMIG, CNPq, CAPES, UFOP.

PT.064

A SYNTHETIC CHLOROQUINE DERIVATIVE INHIBITS CHIKUNGUNYA VIRUS REPLICATION IN VITRO AND EMERGES AS A PROMISING ANTIVIRAL CANDIDATE

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Arboviruses pose a major and growing public health challenge, particularly in tropical regions, where effective antiviral therapies remain unavailable. Drug repurposing and synthetic optimization of known scaffolds represent a strategic approach for rapid antiviral discovery. In this context, we evaluated the in vitro antiviral activity of a synthetic chloroquine derivative against Zika virus (ZIKV), Chikungunya virus (CHIKV), and Mayaro virus (MAYV). Cytotoxicity was determined by the MTT assay (CC_{50}), and antiviral activity was assessed by EC_{50} and selectivity index (SI). Antiviral effects were further validated by cytopathic effect (CPE) inhibition and virucidal assays. The compound exhibited low cytotoxicity (CC_{50} = 31.51 ± 1.35 µM) and selective antiviral activity against CHIKV (EC_{50} = 10.25 ± 2.07 µM; SI = 3.07), which was confirmed by CPE inhibition assays. No virucidal activity was observed, suggesting a mechanism related to inhibition of viral replication rather than direct viral inactivation. Notably, the observed antiviral profile highlights the potential of chloroquine-based derivatives as viable scaffolds for anti-CHIKV drug development. These findings provide a basis for further mechanistic studies and structural optimization aimed at improving antiviral potency and selectivity. **Financial support:** FAPEMIG, CNPq, CAPES, UFOP, Rede Mineira de Antivirais.

PT.065

Hantavirus-Positive Human Sera Do Not Exhibit Serological Cross-Reactivity with Recombinant Oropouche Virus Gn and Gc Proteins

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Oropouche virus (OROV), an emerging orthobunyavirus widely distributed in Central and South America, has recently expanded into new geographic regions, increasing concerns regarding surveillance, diagnosis, and epidemic preparedness. Because OROV co-circulates with other arboviruses and emerging RNA viruses, the development of highly specific serological assays is essential. Although previous studies suggest limited serological cross-reactivity between OROV and hantaviruses, antigenic overlap among members of the order *Bunyavirales* highlights the need to evaluate assay specificity.
Here, we describe the development and preliminary antigenic evaluation of a recombinant OROV glycoprotein-derived construct, GnGcF, comprising the Gn ectodomain fused to a conserved region of the Gc glycoprotein. The recombinant protein was expressed in a heterologous system and purified by AKTA chromatography, yielding a final concentration of 1.945 mg/mL. SDS-PAGE under reducing and non-reducing conditions confirmed the expected molecular profile (~75 kDa).
To investigate potential serological cross-reactivity, ELISA assays were performed using sera from 10 individuals previously exposed to Araraquara hantavirus. Sequence and structural analyses identified limited conserved regions between the Gc proteins of OROV and hantavirus, whereas no relevant similarity was observed for Gn or nucleoproteins. Two predicted B-cell epitopes in OROV Gc and one in hantavirus Gc were located within or adjacent to the conserved region and were predicted to be surface exposed. Despite this limited conservation, ELISA detected no significant serological cross-reactivity between hantavirus-positive sera and recombinant GnGcF.
These findings support the antigenic specificity of the recombinant GnGcF protein and its potential application as a specific antigen for OROV serological assays
Financial support: FAPESP (São Paulo Research Foundation)

PT.066

INHIBITION OF MAYARO VIRUS BY A G-QUADRUPLEX-BINDING LIGAND

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Mayaro virus (MAYV) causes Mayaro fever, a disease lacking specific vaccines or treatments. G-quadruplexes (G4s) are non-canonical secondary structures that represent promising antiviral targets. Here, we evaluated the antiviral potential of a G4-binding compound (CompG4) against MAYV. Bioinformatic analysis of NC_003417.1 and MK070492 sequences (L and D genotypes) using three predictive algorithms identified several conserved Putative Quadruplex Sequences (PQSs). One PQS on the positive strand and three on the complementary strand showed high scores across all algorithms, indicating high probability of G4 formation. Dose-response assay using Vero E6 cells was performed with CompG4, ranging from 1.5-200 µM in the presence or absence of MAYV-*nanoluc* at a MOI of 0.1. MTT and *Renilla*-Luciferase assays were performed to measure cytotoxicity and viral replication levels, respectively, 24 hpi. The mechanism of action of CompG4 (50 µM) against MAYV-nanoluc was evaluated through a time-of-drug-addition (ToDA) assay (0-7 hpi). The inhibitory effect was analyzed via bioluminescence expression and viral titration of both intracellular and extracellular fractions. Dose-response assays revealed an EC_{50} of 16.2 µM and a CC_{50} >200 µM, resulting in a selectivity index (SI) >12.3. ToDA assays demonstrated that CompG4 treatment significantly reduces MAYV replication at 6 and 7 hpi, evidenced by decreased bioluminescence and substantial reduction in viral titers (up to 4 logs intracellularly and 3 logs extracellularly). Our findings demonstrate that MAYV genome contains conserved PQS and CompG4 exerts potent inhibitory effects. These results validate G4 as viable therapeutic targets and position G4-binding ligands as promising candidates for antiviral development against MAYV.
Financial Support: FAPEMIG, CNPq, CAPES.

PT.067

Whole-inactivated SARS-CoV-2-based equine F(ab′)₂ serum reduces viral RNA and protects K18-hACE2 transgenic mice from severe disease

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Equine hyperimmune F(ab')$_2$ preparations represent a scalable strategy for treating emerging viral infections. Previous work established the production and promising *in vitro* activity of an anti-SARS-CoV-2 serum developed at Fundação Ezequiel Dias (FUNED) using whole inactivated virus as the immunogen. Here, we evaluated its efficacy in K18-hACE2 transgenic mice, which express human ACE2 and are highly susceptible to severe SARS-CoV-2 infection. Animals were intranasally infected with 2.0 × 10^2 PFU of the SARS-CoV-2 Gamma variant (P.1) and received either placebo or a single 100 mg/kg dose of hyperimmune serum administered intraperitoneally. Oral swabs were collected at 1, 6, and 12 days post-infection (dpi), and viral RNA was assessed by RT-qPCR. The placebo and serum-treated groups included 10 and 12 animals, respectively. Clinical monitoring demonstrated marked protection in treated animals, with 100% survival and no severe disease, while 90% of placebo-treated mice developed severe clinical signs and 9/10 required early euthanasia. At 6 dpi, viral RNA was undetectable in 8 of 12 serum-treated mice, increasing to 10 of 12 by 12 dpi; the remaining two animals showed only low viral RNA levels. In contrast, only one placebo-treated animal survived until 12 dpi. These findings demonstrate that the FUNED equine F(ab')$_2$ preparation reduces viral RNA and prevents severe SARS-CoV-2 disease *in vivo*, supporting the K18-hACE2 model for preclinical evaluation of antibody-based therapies and reinforcing the potential of whole-inactivated-virus immunization for the scalable development of hyperimmune sera against emerging viral threats.

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PT.068

Identification of Antiviral Peptides Targeting nsP2 Proteases of Chikungunya and Mayaro Viruses by Phage Display

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Chikungunya and Mayaro viruses are emerging mosquito-borne arboviruses responsible for recurrent outbreaks in Brazil. Although vaccines and treatments are available for some arboviruses, the increase in viral variants and antiviral resistance highlights the urgent need for more effective therapeutic strategies. Phage display technology is a promising strategy for identifying peptides with high specificity for viral proteins. The cysteine protease domain in the C-terminal region of the nsP2 protein ($nsP2^{PRO}$) of CHIKV and MAYV is essential for viral replication and virulence, making it an attractive antiviral target. Therefore, phage display assays were performed to identify antiviral peptides targeting these proteases and to computationally analyze their interaction interfaces. Recombinant $nsP2^{PRO}$ proteins were successfully expressed in high yields under soluble conditions using Terrific Broth medium, with IPTG induction (0.4–0.5 mM). Proteins were purified by affinity and size-exclusion chromatography and validated by 15% SDS-PAGE and Western blotting. Biopanning experiments with the Ph.D.-12 phage display library (NEB Biolabs), leading to the selection of phages displaying peptides with strong affinity for the target proteins. DNA from selected phages was purified and analyzed by next-generation sequencing. The most enriched peptide sequences were synthesized and are currently being evaluated through enzymatic and cell-culture assays. Cytotoxicity assays performed in BHK-21 cells demonstrated that the peptides were non-toxic at concentrations up to 160 µM. This study proposes an innovative peptide-based strategy targeting essential viral proteases of CHIKV and MAYV, contributing to the development of novel antiviral agents and understanding of host–virus interactions. Financial Support: CAPES

PT.069

PLATINUM(II) ORGANOMETALLIC COMPLEXES WITH DISTINCT PHOSPHINE LIGANDS: POTENTIAL ANTIVIRAL AGENTS AGAINST ALPHAVIRUS

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Alphaviruses, a genus of viruses belonging to the *Togaviridae* family, are transmitted by the bite of arthropods. Chikungunya virus (CHIKV) and Mayaro virus (MAYV) are the etiologic agents of chikungunya and Mayaro fevers, respectively, diseases that cause hyperthermia, muscle pain, and skin rashes. Currently, there are no licenced antiviral drugs against CHIKV and MAYV infection. In this regard, metallic complexes with platinum(II) [Pt(II)] have demonstrated antiviral activity against several virus including CHIKV and SARS-COV-2. Here we assessed the potential of new compounds in which phenylpyridine C^N cyclometallated platinum(II) was coordinated to different phosphines and chloride, with general formula [Pt(II)(phpy)Cl(L)], where L is PPh_3, PPh_2(Ph-COOH), and DMSO, against CHIKV and MAYV infection. Dose-response assays were performed with PTL04PPh_3, PPh_2(Ph-COOH) and PrePTL04 in presence or absence of CHIKV or MAYV expressing the nanoluciferase gene (CHIKV-*nanoluc* and MAYV-*nanoluc*) for 24h. Additionally, time-of-drug-addition assays were performed to investigate the effect of compounds on different stages of virus replicative cycle. Renilla-luciferase assay was performed to measure viral replication levels 24h post-infection and MTT assay for cytotoxicity. Pt(II) compounds showed selective indexes (SI) for CHIKV and MAYV, respectively: PTL04PPh_3= 6.2 and 6.5; PTL04PPh_2(Ph-COOH) >11.8 and 20; PrePTL04 14.7 and 18.2. In time-of-drug-addition assay, strong antiviral activities were observed on entry steps, when all compounds inhibited > 60% of CHIKV infection, and > 50% for MAYV. In this context, Pt(II) coordinated compounds demonstrated an effective anti-CHIKV and MAYV activity *in vitro*, presenting potential as source of molecules for the development of novel antivirals against alphavirus.

Financial Support: FAPEMIG, CAPES, CNPq, FAPESP

PT.070

ANTIVIRAL EFFECTS OF APIGENIN AND RUTIN AGAINST HUMAN RESPIRATORY SYNCYTUAL VIRUS (HRSV) IN CEL CULTURE

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Acute respiratory infections (ARI) are a major global public health challenge, and human Respiratory Syncytial Virus (hRSV) is one of the most prevalent pathogens affecting children, the elderly, and immunocompromised individuals. Due to limited therapeutic options and the absence of vaccines for pediatric populations, the search for novel antiviral agents remains essential. This study investigated the antiviral potential of the flavonoids apigenin and rutin against hRSV in A549 cells. The cytotoxicity was evaluated by MTT assay, and antiviral activity was assessed through virucidal and post-infection treatment assays using multiplicities of infection (MOI) of 0.01 and 0.1. Apigenin showed higher cytotoxicity than rutin, with CC_{50} values of 550 µM and 611 µM at 24 and 48 hours, respectively, while rutin presented CC_{50} values of 2400 µM and 1000 µM. In viral entry inhibition assays, apigenin exhibited strong antiviral activity, with IC_{50} values of 0.41 µM and 7.2 µM at MOI 0.01, and 4.7 µM and 7.7 µM at MOI 0.1 (24 and 48 hours, respectively). Rutin showed moderate activity, with IC_{50} values of 97.6 µM and 408 µM at MOI 0.01, and 113.2 µM and 186 µM at MOI 0.1. Apigenin displayed higher selectivity indices (1341–79) than rutin (25.5–2.4). Post-infection assays showed reduced effect for both compounds, suggesting their primary mechanism involves direct viral neutralization before cell adsorption. The results showed that apigenin and rutin are promising antiviral candidates against hRSV.
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PT.071

MPOX INFECTION DRIVES CELL ACTIVATION, PROLIFERATION, AND IMMUNE SIGNALLING AT THE SINGLE B CELL RESOLUTION

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The global spread of mpox has highlighted the need to better understand the cellular mechanisms underlying protective immunity against Mpox virus (MPXV). Although both natural infection and vaccination induce MPXV-specific antibody responses, the transcriptional programs associated with antigen-specific memory B cells remain poorly characterized. Here, we performed paired single-cell RNA sequencing and B-cell receptor (BCR) repertoire analysis of MPXV-specific B cells isolated from individuals following natural infection or vaccination with JYNNEOS. Antigen-specific B cells recognizing the viral antigens A35, H3, A29 and E8L were sorted and analysed to investigate their transcriptional landscape and antibody repertoire.
Analysis of 270 high-quality MPXV-specific B cells revealed broadly comparable immunoglobulin gene usage between groups, indicating similar patterns of antigen-driven B-cell selection. In contrast, transcriptomic profiling identified distinct cellular states associated with natural infection, including increased expression of genes involved in B-cell activation, antigen receptor signalling, immune regulation and differentiation. These transcriptional signatures are consistent with a more functionally activated memory B-cell compartment following MPXV infection compared with vaccination.Our findings provide a high-resolution view of the human B-cell response to MPXV and identify molecular programs associated with infection- and vaccine-induced immunity. These results advance our understanding of the host immune response to MPXV and provide insights that may support the development and optimization of next-generation orthopoxvirus vaccines and antibody-based interventions.

Financial Support: FAPESP and NIH

PT.072

SNAKE VENOM-DERIVED TOXIN: A PROMISING ANTIVIRAL APPROACH AGAINST MAYARO AND CHIKUNGUNYA VIRUSES

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Mayaro virus (MAYV) and chikungunya virus (CHIKV) are arboviruses of the *Alphavirus* genus associated with febrile illness and arthralgia, representing apublic health concern. Although vaccines have been recently developed for CHIKV, therapeutic options remain limited against MAYV and CHIKV. In this context, snake venom-derived compounds have shown previously promising antiviral activity against arboviruses. Toxin-1, a heterodimeric compound, derived from the venom of *Crotalus durissus terrificus*, demonstrated activity against flaviviruses, supporting its investigation as an antiviral compound. Here, we aim to evaluate the antiviral activity of toxin-1 against MAYV and CHIKV. Cytotoxicity was assessed in Vero E6 cells treated with toxin-1 in concentrations ranging from 500 µg/mL to 0.0005 ng/mL for 24 h using MTT assay. Cells were infected with MAYV-*nanoluc* or CHIKV-*nanoluc* at a MOI of 0.1 in the presence of the toxin-1 following the same range of concentrations. Viral replication was quantified by luminescence after 24 h. Values of CC_{50}, EC_{50}, and the selectivity index (SI) were determined. As a result, toxin-1 demonstrated no cytotoxicity at all concentrations tested, with a CC_{50} of > 500 µg/mL. Against MAYV, the EC_{50} was 0.01 µg/mL, resulting in an SI $> 5 \times 10^4$, while against CHIKV, the EC_{50} was 0.004 µg/mL, resulting in an SI $> 1.25 \times 10^5$. In conclusion, toxin-1 exhibits potent anti-alphavirus activity with high selectivity and no cytotoxicity at the tested concentrations. These findings highlight its potential for antiviral drug development. Further studies are in progress to investigate its mechanism of action.

Keywords: *Alphavirus*, *Crotalus durissus terrificus.* Antiviral, toxin

Financial Support: FAPEMIG, CAPES, CNPq, FAPESP.

PT.073

Nanoparticles Containing VAC1 Bacteriophages Modulate Resistance and Phenotypic Alterations in Pseudomonas aeruginosa

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Pseudomonas aeruginosa is an aerobic Gram-negative bacillus and an opportunistic pathogen of high clinical relevance, representing one of the primary agents of nosocomial infections. Its pathogenicity is heightened in immunocompromised individuals or those with comorbidities, such as cystic fibrosis and chronic obstructive pulmonary disease. The ability of this species to form biofilms and its rapid genetic adaptation confer a multidrug-resistance profile, motivating the search for therapeutic alternatives such as phage therapy. Bacteriophages are viruses that specifically infect bacteria, with lytic types being ideal for treatment due to their ability to induce host cell lysis. Previous results demonstrated that encapsulation of the VAC1 phage in albumin nanoparticles shows therapeutic superiority over the free phage against the P. aeruginosa PA14 isolate. This study aimed to verify whether this treatment reduces the emergence of resistant isolates and alters their phenotypic growth and virulence profiles. To this end, planktonic cultures of PA14 were inoculated into LB broth and subjected to four treatments: empty nanoparticles, phage-loaded nanoparticles, free phages, and a control group. After 24 hours, samples were plated on 1.5% agar for colony isolation; five colonies per group were selected for morphological analysis in swimming medium. Results indicated significant changes in border, coloration, and area in the phage-loaded nanoparticle group, suggesting bacterial stress. Optical density assays in 96-well plates, conducted using three colonies per group, indicated that although treatment with phage-loaded nanoparticles was the most effective in reducing bacterial growth, resistance to the VAC1 bacteriophage was detected in the remaining isolates.

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PT.074

Antiviral mechanisms of justicidin B and tuberculatin from *Phyllanthus brasiliensis* against Oropouche virus infection

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Oropouche virus (OROV) has emerged as a growing public health concern in Latin America, with an unprecedented surge in Oropouche fever cases since 2023. Previously considered a sporadic and neglected arboviral infection, Oropouche fever now represents one of the most rapidly expanding arboviral threats in the region. Despite its increasing incidence, no approved vaccines or antiviral therapies are available, highlighting the urgent need for effective interventions. Natural compounds, particularly those derived from *Phyllanthus brasiliensis*, have shown promising antiviral properties against several RNA viruses. This study investigated the anti-OROV activity and mechanism of action of two lignans isolated from *P. brasiliensis*, justicidin B and tuberculatin. Cytotoxicity was evaluated by MTT assay, and antiviral efficacy was quantified by plaque-forming assays. Mechanistic analyses included virucidal assays and evaluations at distinct stages of the viral replication cycle. Both lignans exhibited potent anti-OROV activity, with selectivity indices of 2,307 (justicidin B) and 1,153 (tuberculatin). Chloroquine was used as a positive control and was found to be 47.7 and 30.1 times less potent than justicidin B and tuberculatin, respectively. Treatments initiated within 6 hpi reduced viral titers by >99.99% (>4$\log_{10}$), and by ~2$\log_{10}$ when added at 12 hpi. These results demonstrate that justicidin B and tuberculatin act mainly in intracellular processes occurring after viral entry. Molecular coupling analysis corroborated these findings, as both demonstrated a propensity to interact with residues of the OROV Gc glycoprotein, highlighting their strong therapeutic potential as lead candidates for antiviral development against Oropouche fever.

Keywords: justicidin B, tuberculatin, Oropouche virus, antiviral.
Financial support: FAPEMIG, CNPq, Capes, UFOP.

PT.075

Baculovirus-Based Production of West Nile Virus-Like Particles as a Vaccine Candidate Platform

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West Nile vírus (WNV) is an arthropod-borne arbovirus, primarily transmitted by mosquitoes of the *Culex* genus. Infection can be asymptomatic or cause clinical symptoms ranging from mild illness to severe neurological complications. WNV is considered a potential epidemic pathogen in Brazil, highlighting the need for preventive strategies beyond vector control.Virus-like particles (VLPs) have emerged as promising vaccine candidates because they mimic viral morphology and induce immune responses without the risk of replication.This study aimed to produce recombinant baculovirus stocks to expression WNV-VLPs in *Spodoptera frugiperda* (Sf9) insect cells. First, the recombinant bacmid, containing WNV structural proteins, was generated. Following extraction and PCR confirmation, the recombinant bacmid was transfected into Sf9WT cells to produce the initial baculovirus stock (L1). Viral amplification was subsequently performed to generate L2 and L3 stocks, all viralstocks were titrated using Sf9ET cells. Envelope protein expression was evaluated by dot and western blot, while VLP morphological was analyzed by transmission electron microscopy. The L1 stock was generated using two different bacmid amounts, 5µg and 10µg. Although the 5µg condition produced a more pronounced cytopathic effect in cell culture, both conditions yielded similar titers, 1.24×10^4 PFU/mL and 1.45×10^4 PFU/mL, respectively. The L3 stock reached a titer of 3.55×10^6 PFU/mL and it was used for VLP production. Envelope protein expression was confirmed by the transmission electron microscopy revelead particles measuring 45-55nm. The system successfully expressed WNV envelope proteins and produced correctly sized VLPs, demonstrating its potential as a vaccine platform.

PT.076

DEFINITION OF CRITICAL PROCESS PARAMETERS FOR RABIES VIRUS-LIKE PARTICLE PRODUCTION USING THE BACULOVIRUS/INSECT CELL PLATFORM

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Rabies remains responsible for more than 59,000 deaths annually worldwide, highlighting the need for scalable and cost-effective vaccine platforms. Virus-like particles (VLPs) produced using the baculovirus/insect cell expression system represent a promising alternative; however, process optimization is required to maximize productivity. This study applied a Quality by Design (QbD) approach to identify critical process parameters affecting rabies VLP production. A full three-level factorial design was performed to evaluate the effects of host cell line, Time of Infection (TOI), and Harvest Time (HT) on the expression of rabies glycoprotein (RVGP) and matrix protein (RVM), proteins required for VLP assembly. *Spodoptera frugiperda* (Sf9) cells cultivated in EX-CELL® CD medium and High Five™ cells cultivated in Express Five™ SFM medium were co-infected with recombinant baculoviruses expressing RVGP and RVM. TOI values of 24, 48, and 72 h and HT values of 48, 72, and 96 h post-infection were evaluated. Protein expression was analyzed by Dot Blot and Western Blot assays. Distinct expression profiles were observed between the cell lines. Sf9 cells achieved the highest RVGP expression (401.6 ng/mL), whereas High Five™ cells showed superior RVM production (1294.5 ng/mL).
Statistical modeling identified Sf9 cells infected at TOI 72 h and HT 96 h post-infection as the optimal condition for simultaneous expression of both structural proteins. Transmission electron microscopy confirmed the assembly and formation of rabies VLPs. These findings demonstrate the usefulness of the QbD framework for defining critical process parameters and establishing a design space for rabies VLP production using the baculovirus/insect cell platform.

PT.077

DEVELOPMENT AND VALIDATION OF AN IN-HOUSE PLATFORM FOR PRODUCING OF RECOMBINANT ENZYMES FOR SARS-CoV-2 MOLECULAR DIAGNOSIS BY RT-qPCR: A STRATEGY TO STRENGTHEN BIOTECHNOLOGICAL AUTONOMY IN COLOMBIA

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The availability of commercial enzymes for molecular diagnostics is a critical factor that can limit the response to public health emergencies, particularly in countries that depend on imported supplies. This study aimed to develop and validate an *in-house* platform for the production of RT-MMLV and Taq DNA polymerase for the molecular diagnosis of SARS-CoV-2 by RT-qPCR. This was an experimental laboratory study. Both enzymes were expressed in *Escherichia coli* BL21 (DE3), purified by Ni-NTA affinity chromatography, desalted, concentrated, and evaluated for purity and enzymatic activity by SDS-PAGE, complementary DNA synthesis, conventional PCR, and one-step RT-qPCR. Diagnostic performance was assessed using clinical nasopharyngeal swab samples and compared with a commercial RT-qPCR kit and digital PCR (dPCR). The recombinant enzymes exhibited high purity and enzymatic activity comparable to their commercial counterparts, with no significant differences in cDNA synthesis or amplification of the SARS-CoV-2 *E* gene. The *in-house* RT-qPCR system achieved 90.0% sensitivity, 85.7% specificity, and a Cohen's kappa coefficient of 0.756 compared with the commercial assay. It also showed an amplification efficiency of 94.3%, a coefficient of determination (R^2) of 0.9946, and a limit of detection of 100 copies/µL. Compared with dPCR, the assay showed 78.6% sensitivity, 81.8% specificity, and an area under the ROC curve of 0.802. These findings demonstrate the technical feasibility of locally producing recombinant enzymes for SARS-CoVV-2 molecular diagnosis and highlight the importance of strengthening national biotechnological capabilities to improve preparedness for future public health emergencies in Colombia. **Financial Support:** This work was funded by MINCIENCIAS (Colombia) (project **84730 – CT631–2021)**, with additional support from ANNAR Diagnostics.

PT.078

Phytochemistry and Anti-Arboviral Activity of Plant Species Occurring in Campos Rupestres

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Arboviruses pose recurrent threats in tropical regions, driven by climate change, deforestation, poor sanitation, unplanned urbanization, and socioeconomic challenges. In Brazil, Dengue, Chikungunya, and Zika viruses significantly impact public health, with clinical management limited to supportive care due to the lack of approved specific antivirals. Thus, prospecting natural and synthetic molecules for new antivirals is crucial. Campos rupestres, with high local/regional floristic richness, relicts, and endemism, are promising sources of anti-arboviral compounds. This study investigated the phytochemistry and in vitro antiviral activity against Mayaro, Chikungunya, and Zika viruses of ethanolic extracts from four native Serra do Cipó plant species. Preliminary chemical characterization used LC-DAD-ESI-HRMS/MS. For antiviral activity screening, the application of in vitro assays using tetrazolium salt-based colorimetric methods (MTT assay) enabled the efficient simultaneous evaluation of antiviral activity and cytotoxicity across the extracts.. Preliminary results highlight their potential: the ethanolic leaf extract of *Calliandra fasciculata* Benth showed activity against all three viruses; the *Microstachys daphnoides* stem extract exhibited selectivity indices (SI) of 29.4 (Zika) and 17.4 (Mayaro), while its leaf extract had SI 15.6 (Zika); finally, *Marcetia taxifolia* leaf extracts demonstrated activity against all three viruses. These findings underscore the species' potential as sources of bioactive anti-arboviral compounds.

Financial Support: CAPES, UFOP, FAPEMIG.

PT.079

BIOPROSPECTING OF SULFATED PECTIN FROM *PASSIFLORA EDULIS* WITH ANTIVIRAL POTENTIAL AGAINST SARS-COV-2 AND RESPIRATORY SYNCYTIAL VIRUS

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Viral respiratory infections, including COVID-19 and bronchiolitis caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and Respiratory Syncytial Virus (RSV), remain major public health challenges due to their high morbidity and limited therapeutic options. In this context, naturally derived sulfated polysaccharides have attracted increasing biotechnological interest as potential broad-spectrum antiviral agents. This study evaluated the cytotoxicity, antiviral activity, and mechanism of action of sulfated pectin (PecMS2.4) extracted from the peel of *Passiflora edulis* f. *flavicarpa* against SARS-CoV-2 and RSV *in vitro*. Cytotoxicity and antiviral activity were assessed in Vero and HEp-2 cells using CellTiter-Glo® and MTT assays to determine the 50% cytotoxic concentration (CC50), 50% inhibitory concentration (IC50), and selectivity index (SI). The mechanism of action was investigated through simultaneous treatment, virucidal, and viral adsorption inhibition assays, with viral quantification by TCID50 and RT-qPCR. PecMS2.4 showed no cytotoxicity at the highest concentration tested (CC50 > 250 µg/mL). The compound exhibited potent antiviral activity against RSV (IC50 < 1.9 µg/mL; SI > 131.6) and antiviral activity against SARS-CoV-2 (IC50 = 40.7 µg/mL; SI > 6.14). PecMS2.4 interfered with early stages of viral infection, exhibiting simultaneous, virucidal, and viral adsorption inhibition effects against SARS-CoV-2, and simultaneous and viral adsorption inhibition effects against RSV. These findings demonstrate the biotechnological potential of PecMS2.4 as a promising broad-spectrum antiviral candidate. Furthermore, its production from *Passiflora edulis* peel adds value to agro-industrial waste, promoting the sustainable use of plant biomass as a renewable source for innovative biopharmaceutical development.

PT.080

A RHAMNOLIPID-BASED AFTERSHAVE ACHIEVES COMPLETE INHIBITION OF HSV-1: FROM AGRO-RESIDUAL BIOMASS TO AN ANTIVIRAL COSMECEUTICAL

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The circular bioeconomy encourages the development of sustainable biomolecules, driving technological innovation, waste valorization, and the reduction of reliance on petrochemical inputs.This study developed na innovative antiviral aftershave formulation containing a rhamnolipid biosurfactant produced by *Pseudomonas aeruginosa* using residual andiroba (*Carapa guianensis* Aubl.) biomass, and to evaluate the antiherpetic (HSV-1) potential of both the isolated active compound and the final formulation. InitiallyCytotoxicity assays demonstrated a CC50of 56.41 $\mu g \cdot mL^{-1}$ to the isolated rhamnolipid. Antiviral activity was investigated throughout the HSV-1 infection cycle, at different assays: pre-treatment (-3 h), simultaneous (0 h), post-infection (+1 h, +3 h, and +6 h), adsorption, and penetration, at concentrations ranging from 25.0, to 3.12 $\mu g \cdot mL^{-1}$. A moderate inhibition occurred during simultaneous treatment with IC50 of 22.6 $\mu g \cdot mL^{-1}$ and selectivity index of 2.54). The strongest antiviral activity was detected during viral adsorption and penetration, indicating that the the rhamnolipid primarily acts by preventing viral entry into host cells. Based on these findings, two aftershave formulations were developed, including a rhamnolipid-enriched prototype. Both formulations exhibited excellent stability, and appropriate organoleptic and physicochemical characteristics. In efficacy tests, the rhamnolipid-containing aftershave achieved complete (100%) inhibition of HSV-1 replication, whereas the control formulation showed no antiviral activity, with results confirmed by real-time PCR. These results demonstrate that rhamnolipid-type biosurfactants, obtained through circular biotechnology, are promising bioactive agents for the development of multifunctional cosmetics with preventive antiviral properties.

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PT.081

PLATINUM COORDINATED COMPOUNDS STRONGLY INHIBIT ZIKA VIRUS INFECTION *IN VITRO*

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Introduction: Zika virus (ZIKV), an arbovirus from the *Flaviviridae* family, is the etiological agent of Zika fever, a disease characterized by dengue-like symptoms. It has been previously associated with cases of microcephaly and Guillain-Barré syndrome, raising major international public health concerns. Nonetheless, there are no specific antiviral drugs or vaccines available against ZIKV, being the clinical management based on controlling symptoms. In that regard, metal complexes emerge as an approach in drug development due to their therapeutic potential against different viruses. Therefore, this study aimed to evaluate the antiviral activity of the metal platinum (Pt) coordinated to phosphines (tpp, dppe and dcpe) against ZIKV. **Methods:** Dose-response assays were performed for Pt(tpp), Pt(dppe) and Pt(dcpe) in Vero E6 cells, at concentrations ranging from 2.34 to 300 µM, in the presence or absence of $ZIKV_{PE243}$ for 24 h. Cell viability and viral infectivity were evaluated through MTT and immunofluorescence assays, respectively. **Results:** In dose-response assays with $ZIKV_{PE243}$, Pt(tpp), Pt(dppe) and Pt(dcpe) had an EC_{50} of 15.3 µM, <2.3 µM and 2.4 µM, resulting in selectivity indexes (SI) of >19.6, >128 and 78.8, respectively. **Conclusion:** Data suggests that Pt metal coordination to tpp, dppe and dcpe may provide a promising source for the development of future antivirals drugs against Zika virus.

Financial support: FAPEMIG, CNPq, CAPES, FAPESP.

PT.082

SYNTHESIS OF NEW PEPTIDES AND BIOCONJUGATES WITH BENZOIC DERIVATIVES AND IN VITRO ANTIVIRAL EVALUATION AGAINST CHIKUNGUNYA VIRUS (CHIKV)

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Chikungunya is an arboviral disease endemic to Brazil, caused by the Chikungunya virus (CHIKV) and transmitted by mosquitoes of the *Aedes* genus. Clinical manifestations include edema, myalgia, joint pain, and fever; the disease can progress to chronic forms and, in severe cases, lead to death. Currently, treatment is limited to symptom management, as there are no approved specific antivirals for the infection. In this context, there is a need to search for new therapeutic alternatives. With advances in peptide synthesis and drug development, these compounds have established themselves as a promising class of therapeutic agents of interest to the pharmaceutical industry. This study proposed the synthesis of new peptides incorporating substituted benzoic acids and structural modifications to peptides previously studied in the laboratory. The compounds were obtained via solid-phase peptide synthesis (SPPS), purified by high-performance liquid chromatography (HPLC), and characterized by mass spectrometry. Subsequently, the cytotoxicity of the compounds was evaluated in Vero cells using the MTT cell viability assay; this allowed for the determination of the highest non-cytotoxic concentration—defined as the concentration maintaining cell viability above 80%—which was then used in antiviral assays.

The synthesized peptides were evaluated for their ability to inhibit the progression of CHIKV infection in vitro, analyzed by relative RNA levels after 72 hpi using RT-qPCR. Among the compounds analyzed, peptide BO2505 demonstrated promising antiviral activity at 6.25 µM, standing out as a potential candidate for the development of new therapeutic strategies against Chikungunya.

PT.083

BretVIR® and BretMUNE® as Antiviral Candidates for Influenza A Infection

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The Influenza A (H1N1) virus has been responsible for major pandemics throughout history, including the 2009 pandemic, which affected approximately 214 countries and resulted in more than 18,000 deaths. Since then, seasonal strains have continued to circulate globally, accounting for the leading causes of acute respiratory infections. The high mutation rate of this virus compromises the efficacy of specific antivirals and favors the emergence of resistant variants. Furthermore, the high cost of available therapies limits access to treatment, reinforcing the need to develop effective and affordable therapeutic alternatives. In this context, the present study aimed to evaluate the antiviral potential of two plant extracts, BretVIR® and BretMUNE®. For that, cytotoxicity assays and analyses of antiviral mechanisms of action were conducted at different stages of the virus's replicative cycle. The results demonstrated low cytotoxicity in cell cultures for both extracts. Furthermore, viral titration showed a reduction of up to 6 logs in viral load at the highest concentration tested, maintaining significant activity even after dilution by up to 10-fold. These findings highlight the promising potential of these compounds as candidates for new therapeutic strategies against the Influenza A virus. Additional studies are underway to elucidate the mechanisms of action involved and validate their potential as candidates for anti-influenza agents.

Financial Support: FAMPEMIG; Capes, CNPq, Novandina, Embrapii

PT.084

EVALUATION OF THE ANTIVIRAL ACTIVITY OF EXTRACTS FROM BRAZILIAN AMAZON AGAINST WEST NILE VIRUS

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West Nile Virus (WNV) has been spreading widely throughout the Americas, affecting both animals and humans. At the moment, there is no approved treatment or vaccine for humans. Therefore, Brazil´s biodiversity is a valuable source of phytocompounds with pharmacological potential that may treat and/or prevent the disease. In this context, developing antiviral strategies enables improved prevention and response regarding future emerging arboviral cases. This study utilized five natural extracts collected in Belém (Pará) from the leaf, stem, bark, sapwood of *Byrsonima sp.* plant, along with a stem extract from *Apuleia leiocarpa*, to evaluate their antiviral activity against WNV using a cellular infection model (Vero CCL-81 cells). The CellTiter-Glo luminescent viability assay-which quantifies ATP-was used to estimate cellular metabolic viability and also quantification by RT-qPCR to evaluate viral load under different experimental conditions. A previous study showed no cytotoxic effect at concentrations of 100, 50, 25, 12.5 and 6.25ug/ml that were employed in three strategies: pre-treatment, post-treatment and virucidal, all under BSL3 conditions. Neither the pre-treatment nor the post-treatment showed inhibitory activity against WNV infection after 72h. In contrast, the virucidal strategy yielded positive results at the highest concentration for: *Byrsonima sp.* leaf and bark after 72h that exhibit a concentration-dependent virucidal activity. It suggests that their active principles act by inactivating the viral particle extracellularly prior to cellular adsorption, positioning them as promising candidates for preventive formulations or disinfectants against WNV.

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Keywords: West Nile Virus, antiviral activity, natural extracts, viability, quantification

PT.085

EVALUATION OF THE ANTIVIRAL ACTIVITY OF *Ipomoea setifera* Poir

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Herpes simplex virus type 2 (HSV-2) is a highly prevalent human pathogen associated with painful, recurrent genital lesions and an increased risk of human immunodeficiency virus (HIV) acquisition, further emphasizing its significance as a public health concern. Moreover, the emergence of viral strains resistant to currently available antiviral drugs has intensified the search for novel antiviral agents, particularly those derived from natural sources. In this context, this study aimed to evaluate the anti-HSV-2 activity of ethanolic extracts obtained from the leaves (EEF) and branches (EER) of *Ipomoea setifera* Poir. Cytotoxicity was assessed using the Sulforhodamine B colorimetric assay, and antiviral activity against HSV-2 strain 333 was evaluated by plaque reduction assays under post-treatment, pre-treatment, virucidal, and adsorption conditions. In the cytotoxicity assay, the leaf extract (EEF) of *I. setifera* exhibited a $CC_{50} > 500$ µg mL^{-1}, whereas the branch extract (EER) showed a CC_{50} of 485.47 ± 29.07 µg mL^{-1}. The EEF exhibited selectivity index ($SI=CC_{50}/EC_{50}$) values of 14 and 16 in the post-treatment and virucidal assays, respectively, whereas the EER showed SI values of 8 and 3 under the same experimental conditions. Because the EEF exhibited higher SI values, adsorption and pre-treatment assays were subsequently performed, yielding SI values > 32 for both conditions. Overall, both extracts demonstrated anti-HSV-2 activity. However, the leaf extract exhibited greater antiviral potential, and the findings suggest that it may interfere with multiple stages of the viral replication cycle, particularly the early stages. Further studies to elucidate its mechanism of action are underway."Financial Support" CNPq e UFMT.

PT.086

Inhibition of Herpes Simplex Virus Type 2 (HSV-2) *In Vitro* by an Extract of *Byrsonima*, a Native Amazonian Plant

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Although nucleoside analogues are the standard treatment for herpes simplex virus (HSV) infections, their use is limited by drug resistance, toxicity, recurrent disease, and viral latency. Therefore, Amazonian plants have emerged as promising sources of novel antiviral compounds. This study investigated the *in vitro* anti-HSV-2 activity of the leaf extract from a *Byrsonima* species (B.a-0110). Cell viability was assessed using AlamarBlue, and antiviral activity was evaluated in Vero CCL-81 cells by plaque reduction assays, including simultaneous treatment, direct virucidal, pre-treatment, post-infection, and combination with acyclovir. Data were analyzed using ordinary one-way ANOVA followed by Tukey's multiple comparisons test. The extract was non-cytotoxic at 1–100 µg/mL, maintaining cell viability near 100%. B.a-0110 showed dose-dependent antiviral activity in the simultaneous treatment assay, reaching 48% (±11.26) inhibition at 5 µg/mL and ≥96% from 12.5–100 µg/mL. Direct virucidal activity exceeded 97% at all concentrations (5–100 µg/mL). Pre-treatment for 2 h before infection resulted in concentration-dependent antiviral activity, exceeding 97% inhibition at 75 and 100 µg/mL. In contrast, post-infection treatment did not reduce viral replication, suggesting activity during the early stages of the HSV-2 replication cycle. Combining 2.5% of the standard acyclovir concentration with B.a-0110 (1–5 µg/mL) achieved nearly 100% inhibition at all concentrations, markedly exceeding the extract alone. These findings demonstrate potent *in vitro* anti-HSV-2 activity of the leaf extract from a *Byrsonima* species, supporting its potential as a source of novel antiviral compounds. This study was financial supported by CAPES, INCT-PROBIAM, CNPq, and FAPERGS.

PT.087

DIRECT RNA SEQUENCING REVEALS M6A EPITRANSCRIPTOMIC REMODELING AFTER CHADOX1 NCOV-19 (ASTRAZENECA ®) VACCINATION IN PAIRED HUMAN BLOOD SAMPLES

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Introduction: The epitranscriptomic response to COVID-19 vaccination remains poorly understood. **Objective:** To investigate blood RNA m⁶A methylation following ChAdOx1 nCoV-19 immunization using direct RNA sequencing. **Methods:** Fifty-eight participants were included: 29 received ChAdOx1 nCoV-19 and 29 meningococcal ACWY vaccine as controls. Paired day-0/day-28 samples were assessed by ELISA against SARS-CoV-2 S and N proteins and viral neutralization testing. Peripheral-blood RNA was sequenced by Oxford Nanopore/MinION, basecalled with DORADO, quality-assessed with ToulligQC, aligned to GRCh38 with Minimap2, and analyzed with m6Anet for m⁶A detection at DRACH motifs. **Results:** Analysis focused on the 29 ChAdOx1-vaccinated participants. Twelve were excluded due to N-protein reactivity indicating previous SARS-CoV-2 infection. Among the 17 N-negative participants, nine were S-reactive at day 28 and selected for sequencing, totaling 18 libraries. To date, five participants have been sequenced and analyzed, corresponding to 10 libraries. None of the nine participants had neutralizing antibodies at either time point. Among the five analyzed participants, 75 candidate m⁶A sites showed modification probability ≥60%. Relative to day 0, five sites crossed the ≥60% threshold after vaccination and one fell below it. MT-RNR2 showed the most recurrent signals: position 692 reached the threshold in all five participants, while positions 580 and 1209 did so in four. Overall, 38 sites (50.7%) mapped to MT-RNR1/MT-RNR2, with the remainder distributed across respiratory-chain complexes I, III, IV, and V. **Conclusion:** ChAdOx1 vaccination was associated with a heterogeneous mitochondrial m⁶A profile, suggesting a role for mitochondrial RNA regulation in oxidative phosphorylation and post-vaccination immunometabolic responses. **Financial support:** FAPESP.

PT.088

DEVELOPMENT OF A SYNTHETIC POSITIVE CONTROL FOR THE MOLECULAR DIAGNOSIS OF HEPATITIS E VIRUS GENOTYPE 3 IN BRAZIL

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Hepatitis E virus (HEV) is an emerging zoonotic pathogen that causes acute and chronic hepatitis. Despite its global distribution, HEV infection remains underdiagnosed due to limited access to molecular diagnostic methods. Detection of HEV RNA by RT-qPCR is considered the gold standard for laboratory diagnosis. However, the lack of standardized positive controls hampers assay implementation and quality assurance. This study aimed to develop a synthetic positive control for molecular detection of HEV genotype 3 using molecular cloning and PCR standardization. A synthetic HEV ORF1 sequence was cloned into the pGEM-T Easy vector and transformed into competent *Escherichia coli* cells. Recombinant plasmids were confirmed and used as positive controls during assay optimization. A semi-nested PCR targeting the HEV RdRp region was optimized using a touchdown protocol with annealing temperatures decreasing from 60°C to 50°C, . over 10 cycles. Amplification was performed in two rounds, generating expected 371 bp and 330 bp amplicons. PCR products were analyzed by 1.5% agarose gel electrophoresis, and two HEV-positive human samples were included for preliminary validation. The recombinant plasmid produced the expected amplification profile, while human samples were detected after the second amplification, confirming the sensitivity of the semi-nested approach. These findings support the recombinant plasmid as a reliable positive control for HEV molecular detection. Further studies will evaluate the analytical reproducibility of the assay to support its application in routine diagnosis of HEV-3 in Brazil. This project has financial support from Fiotec - Fiocruz, Pernambuco Research Support Foundation (FACEPE) and CNPq (Processo n° 420953/2023-0).

PT.089

A NEW ALTERNATIVE METHOD FOR EVALUATING IMMUNOBIOLOGICALS DEVELOPED TO COMBAT THE H5 AVIAN INFLUENZA VIRUS—DETERMINATION OF THE POTENCY OF ANTI-H5 SERUMS

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The use of animals for drug testing, although routine, has disadvantages such as high cost, complex maintenance, and limitations on the number of animals. Chicken embryo eggs (CEE) are currently considered an alternative to the use of animals. From this perspective, the objective of this study was to develop a CEE-based platform to evaluate the 50% effective dose (ED_{50}) and potency of equine anti-H5 therapeutic serum as an alternative to the use of ferrets. Initially, the 50% lethal dose (LD_{50}) of IA/H5 (strain A/Astrakan/3212/2020-IDCDC-RG71A) was determined by inoculating 5 groups of 10 eggs each with different concentrations of the virus in three independent experiments. A group of uninoculated eggs (CC) and a group inoculated with vehicle (CI) served as controls. After inoculation, the eggs were incubated and subjected to daily candling. The LD_{50} was calculated by Trimmed-Spearman-Karber method and corresponded to 0.5 EID_{50}/mL. Anti-H5 equine sera were serially diluted and incubated with 60xLD_{50} (an amount sufficient to achieve 100% embryo mortality). The mixtures were then innoculated into groups of 10 eggs for each dilution in three independent experiments. Negative serum and CC and CI groups were used as controls. After incubation, the ED_{50} was calculated by Trimmed-Spearman-Karber method. The three sera evaluated demonstrated protection against embryo death, with ED_{50} values of 1.3, 1.9 and 18 µg/mL and potency of 7,776; 12,164 and 819 EID_{50}/mg (depending on the IHA titer of the antiserum). The method is novel, easy to perform, and a good alternative for screening new Influenza vaccine prototypes.

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PT.090

EXTRACTS OF *BANISTERIOPSIS SPP.* INHIBIT THE REPLICATION STAGE OF CHIKUNGUNYA VIRUS REPLICATION CYCLE

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Introduction: Chikungunya virus (CHIKV) is the etiological agent of chikungunya fever, a condition characterized by dengue-like symptoms, including fever and severe arthralgia, which can persist and become chronic and debilitating in some cases. Currently, there are no specific antivirals available for CHIKV, highlighting the urgent need to develop new therapeutic strategies. Within this context, extracts of species of the genus *Banisteriopsis*, native to the Brazilian biomes Cerrado, Atlantic Forest and Amazon Rainforest, are potential candidates for new treatments, due to their known medicinal properties, including anti-inflammatory and antioxidant activities. With this, our study aimed to investigate the antiviral potential of sixteen extracts from native Brazilian plants on the replicative stage of CHIKV cycle. **Material and Methods**: Assays were performed with each extract using a subgenomic replicon system in BHK-21 cells expressing CHIKV non-structural proteins and a *Renilla luciferase* reporter gene. Cytotoxicity was evaluated by MTT method to determine the maximum non-cytotoxic concentration. Antiviral activity was evaluated with the highest non-cytotoxic concentration of each extract. Viral replication levels were measured 24 h post-treatment through luminescence assay. **Results:** Among the sixteen tested compounds, the extracts from *B. stellaris* (stem)*, B. anisandra* (stem) and *B. argyrophylla* (stem) reduced CHIKV replication by 34%, 41% and 64%, respectively ($p < 0.0001$). **Conclusion:** These findings suggest that *B. stellaris, B. anisandra*, and *B. argyrophylla* possess significant antiviral activity on CHIKV replicative stage, highlighting the importance of further studies to elucidate their mechanisms of action and support the development of innovative antiviral therapies.

Keywords: Antivirals; Natural products; Alphaviruses.
Financial support: CNPq; FAPEMIG; CAPES.

PT.091

MOL22 EXHIBITS POTENT BROAD-SPECTRUM ANTIVIRAL ACTIVITY AGAINST ORTHOPOXVIRUSES

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Introduction: Orthopoxviruses represent a re-emerging public health threat, as highlighted by the recent mpox outbreak. The identification of broad-spectrum antivirals with high selectivity is urgently needed. In this study, we evaluated the antiviral potential of the synthetic compound Mol22 against Vaccinia virus (VACV-WR), Cowpox virus (CPXV), and Monkeypox virus (MPXV). Methods: Cytotoxicity (CC_{50}) was determined by MTT assay in Vero CCL-81 and BSC-40 cells. Antiviral efficacy (EC_{50}) was assessed by densitometry and confirmed by viral titration. Mechanism-of-action studies included adsorption, internalization, virucidal activity, pre-treatment, co-treatment, and post-infection kinetics assays. Guanosine reversal assays were performed to investigate potential interference with nucleotide biosynthesis. Results: Mol22 exhibited potent antiviral activity against all three Orthopoxviruses, with a consistent and robust profile. High selectivity indices were observed: VACV (SI ≈ 44.7), CPXV (SI ≈ 40.4), and MPXV (SI >65). Mol22 showed no virucidal activity but strongly inhibited intracellular viral replication, with the most pronounced effects observed under co-treatment and early post-infection conditions (up to 6 h), indicating action at post-entry stages of the viral life cycle. Antiviral activity was further supported by fluorescence microscopy, showing a dose-dependent reduction in GFP expression, with near-complete signal suppression at higher concentrations. Guanosine supplementation did not reverse antiviral activity. Transmission electron microscopy revealed the absence of viral factories and preservation of cellular structure in treated cells. Conclusion: Mol22 demonstrates potent broad-spectrum antiviral activity against orthopoxviruses highlighting its potential as a promising candidate for antiviral drug development.

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PT.092

MOLECULAR DOCKING AS A STRATEGY TO IDENTIFY GLYCOSIDE DERIVATIVES FROM BRACOLI, THE NATIONAL LIBRARY OF COMPOUNDS, AS INHIBITORS OF HTLV-1 REVERSE TRANSCRIPTASE

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Over 10-20 million people worldwide are infected with the HTLV-1 retrovirus and in Brazil, more than 800,000 people are infected, representing a significant neglected public health problem. To date, there are no recommendations for effective medications or treatments, and the lack of specific inhibitors is a limiting factor in the discovery of antivirals. This work aims, in an unprecedented way, the search for new glycoside compounds from BraCoLi, a national library of synthetic compounds, as specific inhibitors for HTLV reverse transcriptase and potential candidates for specific drugs for viral control and treatment of patients infected with HTLV-1. Initially, the polymerase domain of RT transcriptase was predicted by Alphafold 3. A consensus molecular shell approach was performed with 61 compounds, with 20 poses, 10Å box, using Dideoxycytidine 5'-triphosphate as a control. These compounds will be tested in MT-2 cells to determine cytotoxicity and viral load reduction by real-time PCR and ELISA. Subsequently, recombinant protein production will be carried out, where crystallization conditions will be determined to obtain its structure bonded with inhibitors. Visual inspection showed 12 potential inhibitors of the polymerase domain and two predicted structures with larger surface areas compared to the control, with more polar interactions with the active site. These data demonstrate that computational strategies can be important tools for the search for specific polymerase inhibitors and that BraCoLi is an important national library with secrets and potential compounds with antiviral activity against HTLV.

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PT.093

EVALUATION OF THE IMPACT OF NEUROVIRULENCE-ASSOCIATED MUTATIONS IN THE ZIKA VIRUS ON VIRAL FITNESS IN GLIOBLASTOMA CELL LINES.

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Glioblastoma is the primary malignant tumor of the central nervous system with the highest incidence and aggressiveness, characterized by marked cellular heterogeneity and resistance to currently available therapies, highlighting the need to develop alternative therapeutic approaches. In this context, oncolytic virotherapies stand out; composed of viruses that selectively infect and destroy tumor cells while sparing healthy cells. Among candidate viruses for an oncolytic virotherapy against glioblastoma is Zika virus, because of its tropism for glioblastoma stem cells. This study aims to evaluate the impact of two mutations, previously described as positive modulators of neurovirulence, on ZIKV's fitness and virulence in differentiated (U-87 MG) and stem-like (TG1) GBM cell lines. An infectious clone of ZIKV PE/243 was developed by in vitro assembly of genomic fragments, while also introducing the K101R (Capsid) and E21K (prM) mutations individually and combined, allowing the recovery of rZIKV wild-type, rZIKV K101R, rZIKV E21K, and rZIKV K101R/E21K. Fitness assessment in U-87 MG cells revealed that the mutations reduced viral titer, but did not alter ZIKV’s capacity to induce cell death in these cells. Conversely, in TG1, fitness evaluation of the mutant viruses showed no change in viral titer nor load, yet the mutations caused a marked increase in virulence, especially E21K. These results demonstrate that the evaluated mutations enhanced the oncolytic potential of ZIKV in GBM stem-cell models, precisely the targets most resistant to conventional GBM therapy, making them candidate tools to compose ZIKV-based oncolytic virotherapies against GBM.

PT.094

EVALUATION OF CRITICAL QUALITY ATTRIBUTES TO SELECT CANDIDATE STABILIZER FORMULATIONS FOR A COMBINED MEASLES, RUBELLA AND YELLOW FEVER (MR-YF) VACCINE

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Live attenuated combination vaccines can improve vaccination coverage and simplify immunization programs, but formulation compatibility and viral stability remain major challenges. This study compared two candidate stabilizer formulations (TmE1 and TmE2) for a combined measles-rubella-yellow fever (MR-YF) vaccine by evaluating critical quality attributes (CQAs), established for commercial vaccines by the Brazilian Pharmacopoeia and ANVISA. Six batches of each formulation were produced and assessed for osmolality, pH, residual moisture content, viral potency and thermostability, after lyophilization. Accelerated stability was evaluated in four batches (two/formulation) stored at 30°C for 180 days. Viral potency and thermostability were determined for yellow fever virus (YFV; FFU/dose), measles virus (MeV; FFU/dose), and rubella virus (RuV; $CCID_{50}$/dose). Mean values for osmolality, pH and residual moisture content remained within specification limits. Viral potency remained within established specifications after lyophilization, with batch-to-batch variations below 1.0 log. During accelerated stability testing, potency losses remained below 0.5 log for YFV, MeV and RuV at all time points compared with samples maintained at 2–8°C, the shelf temperature. However, statistical analysis of the stability kinetic revealed formulation-dependent differences in the stability profiles of YFV and MeV, whereas RuV remained highly stable regardless of formulation. Although both candidate formulations exhibited physicochemical characteristics consistent with the regulatory specifications adopted for licensed vaccines, TmE2 showed superior stability performance for YFV and MeV, supporting its selection as the preferred stabilizer formulation for the MR-YF vaccine. These findings demonstrate the technological feasibility of the MR-YF formulation strategy and support the rational selection of the stabilizer formulation.

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PT.095

BEYOND ANTIVIRAL ACTIVITY: AMINOADAMANTANE DERIVATIVE MODULATES SARS-COV-2–INDUCED INFLAMMATION IN VITRO

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There is a vast literature on how patients with COVID-19 may experience a severe systemic inflammation associated with cytokine storm caused by an imbalanced immune cells recruitment and activation during SARS-CoV-2 infection. In this context, immunomodulatory treatments are promising as they attenuate the patient's abnormal inflammatory response without hindering the viral clearance. In this regard, a synthetic derivative of the approved antiviral Amantadine, named 3F4, presented in vitro and in vivo antiviral effect against SARS-CoV-2 and was now evaluated in the context of the coronavirus-specific inflammatory response. Blood samples from healthy donors were employed in the study of soluble mediators by Luminex magnetic bead assay after short-term culture with inactivated virus (iSARS-CoV-2) in the presence and absence of 3F4. The aminoadamantane derivative presented a robust modulatory activity on the soluble mediators, reducing the levels of chemokines and proinflammatory cytokines, such as CCL3, CCL4, IL-1b, IL-6, IFN-g and IL-17, following the short-term stimulation. Furthermore, reduced levels of regulatory cytokines (IL-4, IL-5), and growth factors (FGF-basic, IL-2) were also observed following 3F4 treatment. Aiming to obtain an in-depth assessment of the immunomodulatory effects of 3F4 at a cellular level, immunophenotyping assays are currently being performed to evaluate lymphocytes and phagocytes regarding their activation following short-term culture with iSARS-CoV-2. However, there is still the need to understand the drug's mechanisms for its anti-inflammatory effect and proceed with the in vivo tests. Nevertheless, these preliminary results associated with the robust antiviral effects of 3F4, indicate its potential as a therapeutic strategy for COVID-19.

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PT.096

Self-adjuvanting vaccines: a novel strategy to enhance innate immune activation

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Modified Vaccinia virus Ankara (MVA) is a widely used vaccine vector with an excellent safety profile but limited intrinsic immunogenicity. To enhance its adjuvant properties, we investigated a self-adjuvanting MVA (saMVA) engineered to express human cyclic GMP–AMP synthase (cGAS), aiming to potentiate cytosolic DNA sensing through the cGAS–STING pathway. saMVA induced markedly increased cGAMP production and enhanced downstream signaling, including phosphorylation of STING and IRF3, compared to control MVA in PMA-differentiated THP-1 macrophage-like cells and human fibroblasts.Transcriptomic analysis of infected human macrophages further revealed a strong upregulation of innate immune pathways in response to saMVA, with increased expression of type I interferons, interferon-stimulated genes, and inflammatory mediators compared to control MVA. Differential gene expression analysis confirmed a more robust antiviral signature associated with enhanced cGAS–STING activation. In contrast, murine bone marrow–derived macrophages showed comparable activation profiles following infection with both vectors. Immunization of C57BL/6 mice resulted in similar humoral and cellular immune responses, including equivalent antibody titres and antigen-specific T cell responses. These findings demonstrate that saMVA enhances innate immune activation in human systems while revealing species-specific limitations of murine models to capture cGAS–STING-driven effects. While animal models remain indispensable for assessing safety, they may underestimate to capture enhancements in innate immune activation that are specific to humans. This is especially pertinent for DNA virus–based vectors such as MVA, where cGAS-dependent sensing plays a dominant role.

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PT.097

DUAL-ACTION DRUGS: HYDRAZONE AND HYDRAZIDE-TRIAZOLE DERIVATIVES EXHIBIT ANTIVIRAL AND IMMUNOMODULATORY ACTIVITY AGAINST ORTHOPOXVIRUSES

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Orthopoxvirus infections have re-emerged as a major public health concern, evidenced by global outbreaks of mpox (MPV) in recent years and recurrent outbreaks of vaccinia virus (VACV) in Brazil. Given the limited therapeutic options available, this study investigated the antiviral and immunomodulatory potential of four compounds: two cannabidiol-analogous hydrazones (HAC1 and HAC2) and two hydrazide-triazole hybrids (HIT1 and HIT2), against orthopoxviruses. The compounds were evaluated for their cytotoxicity, antiviral activity, mechanisms of action, and immunomodulatory effects. VACV was used as a model for antiviral evaluation, while MPV was used for immunological assays. All compounds showed absent or low cytotoxicity in BSC-40 cells, corroborating detailed selectivity profiles. At a concentration of 100 μM, all four compounds exhibited antiviral activity in vitro, with HAC1 and HIT1 showing selectivity indices > 37,000. Mechanistic assays revealed that HIT1 exhibited virucidal activity, while HIT2 interfered with viral discovery. Overall, the compounds primarily interfere with the initial events of the viral multiplication cycle. Transmission electron microscopy revealed a significant decrease in the number of mature VACV viral particles, suggesting that the compounds interfere with viral particle assembly. In immunomodulatory assays, treatment with HAC1 or HIT1 in MPV-infected PBMCs restores the profile of chemokines (CCL3, CCL4, CXCL10, CXCL12), pro-inflammatory cytokines (IL-1α, IL-12p40, IL-15), regulatory cytokines (IL-4, IL-13), and growth factors (PDGF, NGF-β, VEGF), in addition to re-establishing the activation of key immune response populations (NK cells, NKT cells, and T lymphocytes). Taken together, these results indicate promising candidates for dual-action drugs against orthopoxvirus infection.

Financial Support: FAPEMIG, CNPq, CAPES, INCT-POX

PT.098

EUGENOL DERIVED PIPERAZINE AS PROMISING ANTIVIRAL AGENTS AGAINST *ALPHAVIRUS MAYARO*

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Arboviruses represent an ongoing global health challenge, particularly in tropical regions where diseases caused by Chikungunya (CHIKV) and Mayaro (MAYV) viruses continue to emerge and spread. Given the absence of licensed antiviral therapies, the identification of effective small molecules remains a critical priority. In this work, sixteen eugenol and dihydroeugenol derived piperazine Mannich bases were evaluated for antiviral activity against CHIKV, MAYV, and Zika virus (ZIKV) in Vero cells. Cytotoxicity was assessed using the MTT assay and expressed as median cytotoxicity concentration (CC_{50}), and antiviral activity was determined by MTT method and quantified through median effective activity (EC_{50}) and selectivity index (SI) values. Three compounds (D3, D7, and E7) exhibited selective antiviral activity, with D7 demonstrating potent inhibition of MAYV (CC_{50} > 239.12 μM; EC_{50} = 14.43 μM; SI > 16.6), surpassing ribavirin by more than seventeen-fold in relative potency. Time-dependent cytopathic effect inhibition and plaque reduction assays revealed progressive viral titer reductions of up to 3 log10 $PFU.mL^{-1}$ over 48 h. Virucidal assays confirmed that D7 does not directly inactivate viral particles, supporting a post-entry mechanism of action. These findings identify D7 as a promising antiviral hit compound and provide a foundation for further mechanistic, optimization, and *in vivo* studies aimed at developing anti-alphavirus therapeutics. Financial Support: FAPEMIG, CNPq, UFOP, Rede Mineira de Antivirais.

PT.099

SILYMARIN INHIBITS MAYARO VIRUS INFECTION AT POST-INFECTION STAGES

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Mayaro virus (MAYV), an arbovirus from the Togaviridae family, causes Mayaro fever, a neglected disease characterized by acute febrile illness and persistent arthralgia. Despite the increasing incidence of cases, there are no specific antiviral therapies available. Our group previously demonstrated that silymarin, a natural compound derived from Silybum marianum with antioxidant, anti-inflammatory, and hepatoprotective properties, exhibits antiviral activity against MAYV both in vitro and in vivo. However, its mechanism of action remains unclear. This study aimed to characterize the antiviral mechanism of silymarin against MAYV. When administered at the time of infection, silymarin completely inhibited viral replication (6-log reduction) at 150 µg/mL. In contrast, virucidal activity and interference during viral adsorption and penetration resulted in approximately 50% reduction in viral load. Notably, post-infection treatment demonstrated robust antiviral effects across all evaluated time points: complete inhibition at −2 h and 0 h, with sustained, albeit reduced, activity up to 24 hours post-infection. Transmission electron microscopy performed 6 hours after treatment revealed a marked decrease in viral particles compared to untreated controls, which displayed abundant virions. Ongoing analyses, including RT-PCR and immunofluorescence assays, aim to further elucidate the molecular mechanisms underlying this inhibitory effect. These findings highlight silymarin as a promising candidate for antiviral therapy against MAYV.
Keywords: Alphavirus, Silymarin, Antiviral.
Financial Support: FAPEMIG, CNPq, CAPES and UFOP

PT.100

IN SILICO CONSTRUCTION OF A CHIMERIC ANTIGEN FOR THE SERODIAGNOSIS OF CHIKUNGUNYA FEVER

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Chikungunya fever is a widespread arboviral disease in Brazil that co-circulates with other infections presenting similar clinical manifestations, making laboratory diagnosis essential for appropriate patient management and epidemiological surveillance. Although RT-qPCR is the gold-standard method during the acute phase of infection, its high cost and dependence on specialized laboratory infrastructure limit its routine use. Conversely, serological assays are more accessible but may exhibit cross-reactivity with Mayaro virus and other antigenically related arboviruses, reducing diagnostic specificity. Therefore, the development of highly specific recombinant antigens represents a promising strategy to improve serological diagnosis. This study aimed to design, *in silico*, a recombinant chimeric antigen containing B-cell epitopes of Chikungunya virus for future application in serodiagnostic assays. B-cell epitopes were selected from the Immune Epitope Database based on bioinformatic predictions and experimental validation and subsequently assembled into a chimeric gene. The encoded recombinant protein was analyzed using ExPASy ProtParam to predict its molecular weight, isoelectric point, hydrophobicity, and extinction coefficient. The predicted protein exhibited favorable physicochemical properties, including an estimated molecular weight of 58.4 kDa, an isoelectric point of 5.78, an extinction coefficient of 60,780, and a grand average of hydropathicity of −0.643. These characteristics indicate a predominantly hydrophilic profile and suggest favorable properties for heterologous expression and purification. The proposed construct represents a promising candidate for recombinant production and subsequent development of more specific serological assays for chikungunya, with the potential to reduce cross-reactivity and strengthen laboratory diagnosis and epidemiological surveillance.

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PT.101

I DENTIFICATION OF A NOVEL CHLOROQUINOLINE–TRIAZOLE DERIVATIVE TARGETING *A lphavirus C hikungunya* (CHIKV)

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Arboviruses are increasingly associated with infectious disease outbreaks across the globe. Among the most clinically significant are chikungunya virus (CHIKV), Zika virus (ZIKV), and Mayaro virus (MAYV). Despite their impact, there are currently no approved antiviral therapies specifically indicated for that infection. This scenario underscores the importance of drug discovery efforts, including molecules built on heterocyclic frameworks like quinoline and triazole. In this context, the present study investigated the in vitro antiviral potential of a new chloroquinoline–triazole derivative (AM1009B - 6.3 uM) against CHIKV. Cytotoxicity and antiviral activity was evaluated using the MTT assay. Additional confirmation of antiviral effects was obtained through cytopathic effect (CPE) inhibition assays and RT-PCR analysis. The compound was also tested for virucidal activity. The tested compound exhibited antiviral activity against CHIKV. Plaque reduction assays demonstrated that the compound significantly decreased CHIKV viral titers, reaching reductions of up to 1 $\log_{10}$ PFU·mL^{-1} at 48 hours post-infection. Consistently, RT-qPCR results showed a marked inhibition of CHIKV replication at 24, 36, and 48 hours. No direct virucidal effect was detected. Overall, these findings suggest that this compound is a promising candidate for further investigation as a potential antiviral agent against CHIKV. Financial support: FAPEMIG, CNPq, UFOP, Rede Mineira de Antivirais.

PT.102

IN VITRO ANTIVIRAL ACTIVITY OF FLAVONOIDS AGAINST CHIKUNGUNYA VIRUS: EVALUATION OF APIGENIN, GENISTEIN AND LUTEOLIN

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Chikungunya virus infection remains a public health challenge due to the lack of licensed antiviral therapies and its association with intense persistent arthralgia. Natural compounds have been widely investigated due to their diverse biological activities, and flavonoids have emerged as promising antiviral candidates. This study aimed to evaluate the antiviral activity of selected flavonoids against *Alphavirus chikungunya* (CHIKV) *in vitro*. Cytotoxicity assays were performed using apigenin, genistein and luteolin at concentrations ranging from 25 to 400 µM in Vero E6 cells, which were incubated for 48h at 37 ºC and 5% CO_2. Cell viability was assessed by the MTT assay. All compounds showed a maximum non-toxic concentration of 100 µM, with CC_{50} values > 400 µM. Subsequently, an antiviral assay was performed by infecting cells with CHIKV (MOI 0.01) for 2h adsorption, followed by treatment with compounds at concentrations ranging from 12,5 to 100 µM for 48h. Viral inhibition was evaluated by the MTT assay. All tested compounds demonstrated dose-response antiviral activity. Apigenin, genistein, and luteolin showed inhibition rates of 92.20 ± 7.38%, 83.87 ± 2.93% and 85,64 ± 3,88%, respectively, with promising selectivity indexes (>18.89, >11.02 and >15.37, respectively). These findings demonstrate the antiviral potential of apigenin, genistein, and luteolin against CHIKV and support further investigation on their mechanisms of action as potential candidates for antiviral development.
Financial support: FAPEAL; CNPq; CAPES.

PT.103

Evaluation of the Mechanism of Action and Therapeutic Efficacy of the Algal Fraction from *Dictyota menstrualis* against Mayaro Virus

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Mayaro virus (MAYV), from the Togaviridae family, is an arbovirus mainly transmitted by the bite of mosquitoes of the genus *Haemagogus*. Among the main symptoms caused by this infection are fever and joint pain, and currently there are no vaccines or antivirals available for the prevention and treatment of MAYV infection. Therefore, the search for new antiviral therapies with anti-MAYV activity is important. Previous studies have shown that the FAc-1 fraction of the alga *Dictyota menstrualis* exhibited high anti-MAYV activity. Furthermore, *in vivo* studies demonstrated that the fraction was able to reduce virus-induced paw edema and modulate the immune response in infected mice. This study aimed to determine, *in vitro*, the mechanism of action of the FAc-1 fraction of *D. menstrualis* against MAYV, as well as to evaluate its therapeutic efficacy (*in vivo*) through histopathological analysis of the paw muscle of infected animals, treated or untreated. Our studies showed that, in the virucidal assay, there was 28% inhibition of MAYV infectivity at the concentration of 20 µg/mL, while in the adsorption assay, inhibition reached 81%, indicating that the main mechanism of action of the fraction is interference with viral adsorption. Histopathological analyses demonstrated that treatment with the fraction reduced paw muscle damage compared to infected control groups, which presented edema, inflammatory infiltrates, muscle disorganization, and necrosis. Based on these results, the FAc-1 fraction appears promising for the development of natural-product-derived antivirals against MAYV.

PT.104

DIAGNOSTIC GAPS IN EQUINE VIRAL ENCEPHALITIS: THE IMPACT OF SAMPLE TYPE ON VIRAL DETECTION AND SURVEILLANCE

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Equine neurological disease presents a major diagnostic challenge because multiple neurotropic viruses may produce similar clinical signs and optimal biological samples are frequently unavailable under field conditions. This study evaluated viral detection in different biological matrices from 76 horses presenting neurological disease submitted for routine laboratory investigation in Brazil. Molecular assays were performed for rabies virus (RABV), equine herpesvirus type 1 (EHV-1), Madariaga virus (MADV), flaviviruses and equine gammaherpesviruses. At least one encephalitis-associated virus was detected in 43.4% of animals. EHV-1 was the most frequently identified virus (25.0%), followed by RABV (15.8%) and MADV (9.2%). Viral detection differed markedly according to sample type. Central nervous system (CNS) samples showed the highest diagnostic yield and significantly greater odds of viral detection than other biological matrices. Rabies virus was detected almost exclusively in CNS tissues, confirming their essential role for definitive diagnosis. Among ante-mortem samples, nasal swabs consistently showed the highest diagnostic performance, allowing detection of EHV-1 and MADV more frequently than blood or cerebrospinal fluid. These findings demonstrate that inadequate submission of CNS samples remains a major limitation for etiological diagnosis of equine neurological disease in Brazil. Although alternative biological matrices cannot replace CNS tissues, nasal swabs represent a valuable complementary sample for molecular diagnosis when post-mortem examination is not possible. Strengthening adherence to official diagnostic protocols while incorporating optimized ante-mortem sampling strategies may substantially improve surveillance of equine neurotropic viruses.

PT.105

A SIMPLE ERYTHROCYTE-BASED CONCENTRATION METHOD IMPROVES INFLUENZA A VIRUS GENOME RECOVERY FOR MOLECULAR SURVEILLANCE

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Detection of Influenza A virus (IAV) in clinical samples with low viral load remains challenging because viral RNA is frequently present in low concentrations and mixed with PCR inhibitors, particularly in fecal matrices. This study evaluated a turkey red blood cell (RBC)-based adsorption–desorption method to improve viral concentration and molecular detection. Rectal swabs experimentally spiked with IAV were used to optimize the protocol, while clinical nasal swabs with Ct values between 30 and 37 were used for field validation. Two RBC concentrations (1.5% and 3%) and different desorption times were evaluated by RT-qPCR and multisegment RT-PCR. Statistical analysis demonstrated that viral recovery depended on the interaction between RBC concentration and desorption time. The 1.5% RBC condition with 30 min of desorption consistently produced the lowest Ct values and significantly improved viral recovery compared with untreated samples. Multisegment RT-PCR demonstrated superior amplification of HA and NA genes following RBC treatment, indicating improved recovery of intact viral RNA. In low viral load field samples, RBC processing enabled amplification of viral genomic segments that were undetectable in untreated samples, substantially improving molecular characterization. The method increased analytical sensitivity without requiring specialized equipment and improved the quality of viral RNA for downstream applications, including subtyping, sequencing, and genomic surveillance. These findings demonstrate that turkey RBC adsorption is a simple, inexpensive, and scalable strategy to enhance Influenza A virus detection in challenging clinical samples and may improve surveillance of influenza viruses in both veterinary and human health programs.

PT.106

DEVELOPMENT OF AN ALTERNATIVE SEROLOGICAL TEST FOR THE DETECTION OF RABIES VIRUS NEUTRALIZING ANTIBODIES IN SERUM ELUTED FROM FILTER PAPER

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Rabies is a disease caused by *Lyssavirus rabies* (RABV), and the Rapid Fluorescent Focus Inhibition Test (RFFIT) is the most widely used test for evaluating anti-RABV neutralizing antibodies. The objectives of this study were to develop and standardize an alternative serological method for detecting anti-RABV neutralizing antibodies using filter paper. For this, 12 bovine serum samples previously analyzed by the RFFIT (4 positive, 4 negative, and 4 weakly positive) were used to standardize a method for antibody detection using samples impregnated on filter paper. Different experimental variables were evaluated, including type of eluent, incubation time, eluent volume, and number of discs used. The method correctly identified all positive and negative samples, whereas weakly positive samples were not detected. Subsequently, the optimized protocol was applied to whole blood samples impregnated on filter paper, obtained from dogs, cats and bats. From these samples, neutralizing antibodies were detected only in dog and cat samples (6/14, 42.86%). When serum was impregnated onto filter paper, neutralizing antibodies were detected in 10 out of 14 animals evaluated (71.43%). To evaluate sample stability during storage at room temperature, analyses were repeated after 60 days. Similar results were obtained, with antibodies remaining detectable in samples with higher titers, although a reduction in assay sensitivity was observed over time. These findings indicate the potential use of filter paper as an alternative for the collection, transport, and storage of blood/serum samples for serological testing to detect anti-RABV antibodies.

Keywords: rabies; antibody; RFFIT; filter paper
Financial support: Instituto Pasteur de São Paulo; CAPES

PT.107

EVALUATION OF ALTERNATIVE READOUT METHODS FOR ACCURATE PORCINE PARVOVIRUS TITRATION VIA $TCID_{50}$.

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Porcine Parvovirus (PPV) is one of the primary causative agents of reproductive failure in breeding sows worldwide. Accurate laboratory diagnosis of PPV is crucial for mitigating reproductive losses and differentiating PPV from other pathologies. Because the virus causes no apparent clinical symptoms in adult sows, laboratory tests are the sole means to confirm parvovirus infection. To identify and quantify infectious particles, the gold standard method is the 50% Tissue Culture Infectious Dose ($TCID_{50}$) assay. However, because PPV often causes a subtle, delayed, or absent cytopathic effect (CPE) in common cell lines, direct visual reading may not be accurate. This study aimed to compare three readout methods for PPV titration using $TCID_{50}$. Pig kidney cells (PK-15) were seeded into 96-well plates and inoculated with serial dilutions of the viral sample in serum-free DMEM. Following the total incubation period, three methods were performed to score the presence (+) or absence (0) of CPE: an inverted microscope examination of the cells (both fresh and stained with naphthol blue-black) and a hemagglutination assay using the supernatant from the infected plate and guinea pig red blood cell solution. Viral titers were calculated by Reed & Muench method. Different assays were conducted to standardize the optimal initial cell concentration, incubation time, and the recovery of infectious titers starting from a known titer. When evaluating the different readout methods, it was verified that the results showed variations ≤20% and recovery between 90-110%, both parameters within the acceptance criteria, demonstrating that all methods are applicable for $TCID_{50}$ titration.

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PT.108

Antiviral activity of natural compounds against Mayaro virus *in vitro*

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Mayaro virus (MAYV) belongs to the genus *Alphavirus* and the family *Togaviridae*. It has become a growing public health concern due to its geographic expansion, potential adaptation to urban environments, and clinical similarity to other arboviral infections, which complicates diagnosis and surveillance. Cases have been reported in several Latin American countries, with Brazil presenting the highest number, particularly in the Amazon region. Transmission occurs mainly through *Haemagogus* mosquitoes in wild areas; however, evidence suggests that urban vectors, especially *Aedes aegypti*, may also contribute to viral dissemination. Currently, there are no specific vaccines or antiviral therapies available for MAYV infection. Therefore, this project aims to evaluate the antiviral activity and mechanisms of action of natural compounds against MAYV using *in vitro* and *in vivo* approaches. *In vitro* assays include cytotoxicity, initial screening, dose-response, time to addition, and virucidal activity, while *in vivo* analyses will be performed using C57BL/6 mice. A non-toxic concentration of 80 μM was established for hesperetin and thymoquinone after 24 hours in BHK-21 and Huh-7 cells. Viral replication kinetics using the MAYV-NLuc strain identified 24 hours as the ideal endpoint for antiviral assays. Hesperetin inhibited viral replication by 51%, while thymoquinone achieved 99% inhibition in BHK-21 cells. These findings identify both compounds as promising antiviral candidates against MAYV, supporting further *in vivo* investigations and contributing to the development of effective therapies for emerging arboviral diseases.

Financial Support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

PT.109

DEVELOPMENT OF FORMULATIONS FOR BACTERIOPHAGE STABILIZATION AIMING AT APPLICATIONS IN PHAGE THERAPY

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Antibiotic resistance is considered one of the greatest threats to global public health by the World Health Organization. *Pseudomonas aeruginosa* stands out as a priority pathogen due to its frequent association with difficult-to-treat hospital infections and its high capacity for antimicrobial resistance. As a promising alternative therapy, lytic bacteriophages have emerged. These viruses specifically infect and lyse bacterial cells. Since bacterial infections may involve multiple strains and species, phage cocktail formulations represent an important strategy to broaden the spectrum of activity and improve therapeutic efficacy. The aim of this study was to develop a phage cocktail against *P. aeruginosa*, formulate it in a veterinary spray solution or carbopol hydrogel, and evaluate its stability and activity *in vitro* and *in vivo*. Strictly lytic phages, selected from the group's collection based on their host range, were combined at a concentration of ~10^8 PFU/mL. Stability and activity were periodically assessed by drop tests, paper filter diffusion assays, and bacterial growth curves. *In vivo* assays using *Galleria mellonella* larvae were performed only with the liquid formulation. The results demonstrated that the phages remained viable in the spray formulation for 5 months and completely inhibited bacterial growth at an MOI of 0.1. Treatment prolonged the survival of infected larvae by 24 h. The hydrogel containing the phage cocktail produced lytic halos and inhibited bacterial growth in liquid medium. We conclude that both formulations preserve the stability and lytic activity of the phages against different virulent clinical strains of *P. aeruginosa*. Financial support: FAPESP, CNPq, PUB-USP.

PT.110

IDENTIFICATION OF A SYNTHETIC CHLOROQUINOLINE DERIVATIVE AS A PROMISING ANTIVIRAL LEAD AGAINST MAYARO VIRUS

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Arboviral diseases are viral infections transmitted by arthropods and, in Brazil, Chikungunya virus, Zika virus, and Mayaro virus are among the main emerging pathogens, with no specific antiviral therapy currently available. In this context, the discovery of natural products rich in bioactive compounds is essential for the development of synthetic drugs, such as chloroquinoline derivatives. This study investigated the in vitro antiviral activity of a chloroquinoline derivative against CHIKV, MAYV, and ZIKV. Cytotoxicity was assessed using the MTT assay (CC_{50}), and antiviral activity was determined by the median effective concentration (EC_{50}) and selectivity index (SI). Antiviral activity was evaluated through cytopathic effect (CPE) inhibition assays, viral load quantification by RT-qPCR, and virucidal activity testing. The tested compound exhibited potent antiviral activity against CHIKV and MAYV, with relative potency values of 42.24 and 88.71, EC_{50} values of 9.92 and 4.97, and SI values greater than 4.1, demonstrating superior efficacy compared to the control drug, respectively. At a concentration of 14.7 µM, the compound inhibited the cytopathic effect, resulting in significant reductions in viral titers, reaching 3 $\log_{10}$ PFU·mL^{-1} for CHIKV and 5 $\log_{10}$ PFU·mL^{-1} for MAYV at 48 hours post-infection (hpi). These findings were confirmed by RT-qPCR, which showed a significant reduction in CHIKV replication at 24, 36, and 48 hpi, as well as in MAYV replication at 36 and 48 hpi. Notably, no virucidal activity was observed. These results highlight the promising potential of this compound as a candidate for the development of new antiviral agents targeting emerging arboviral infections. Funding: FAPEMIG, CNPq, UFOP, Rede Mineira de Antivirais.

PT.111

FULLEROL ATTENUATES OXIDATIVE STRESS AND INFLAMMATORY SIGNALING IN ZIKA VIRUS-INFECTED MACROPHAGES

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Zika virus (ZIKV) infection triggers oxidative stress and inflammatory responses in host cells, yet no approved antiviral therapy currently exists. This study investigated the antioxidant and cytoprotective effects of fullerol (C60(OH)22-24) in ZIKV-infected macrophages, focusing on redox imbalance and associated inflammatory pathways. J774A.1 murine macrophages and MRC-5 human fibroblasts were infected with ZIKV (MOI 5) and treated with fullerol at 3.125 nM. Intracellular ROS were quantified by Carboxy-H2DCFDA fluorescence; NRF-2 and NF-κB/AP-1 transcriptional activities were assessed by reporter assays; SOD and CAT activities were determined by enzymatic methods; and oxidative damage was evaluated by TBARS and protein carbonyl assays. ZIKV infection increased intracellular ROS by approximately 13%, reduced NRF-2, SOD and CAT activity, elevated lipid peroxidation and protein carbonylation, and activated NF-κB and AP-1 pathways, leading to IL-6 upregulation — a dual inflammatory activation not observed with DENV2 or YFV. Fullerol at 3.125 nM reduced ROS levels by approximately 18% in both J774A.1 and MRC-5 cells, restored NRF-2/ARE activity, increased SOD activity, reduced TBARS and carbonylated protein content to near-basal levels, and demonstrated direct antiviral activity against ZIKV. No cytotoxicity was observed across all tested concentrations. These results establish fullerol as a promising antioxidant and antiviral candidate against ZIKV, acting simultaneously on redox imbalance and NF-κB/AP-1-mediated inflammatory pathways.
Financial support: FAPEMIG, CAPES, CNPQ.

PT.112

Validation protocol for RT–qPCR primers and probes for persistent variants of SARS-CoV-2

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The demand for the molecular diagnosis of pathogens has increased dramatically since the onset of the COVID-19 pandemic. The emergence of new variants of SARS-CoV-2 raises questions about whether the molecular tests available for diagnosis are still effective in detecting the virus. This study analyzed the validity of primers and probes designed for RT–qPCR based on the Wuhan strain of SARS-CoV-2 against new variants and subvariants of the virus. For this purpose, we used bioinformatics tools to analyze SARS-CoV-2 genomic data of different variants and subvariants deposited in genomic databases to assess the accuracy of the molecular tests available for the diagnosis of COVID-19. Mutation frequency analysis revealed high SNP rates in the nucleocapsid phosphoprotein (NP), envelope protein (E) and RNA-dependent RNA polymerase (RdRp) genes and targets, although the target regions were conserved.Only nine SNPs were recurrent in the sequences of the variants identified on different continents and all in different targets (N, E and RdRp). The registered mutations are not consistent and do not appear frequently in isolates of the same variant in all regions worldwide. We also tested two RT-qPCR protocols using designed long oligonucleotides containing SNPs found in variants, and both were able to detect them. Our data suggest that the molecular targets designed for reference to the SARS-CoV-2 genome remain valid for the identification of new virus variants and subvariants despite the large number of identified haplotypes.

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PT.113

COMPUTATIONAL PIPELINE FOR STRUCTURAL MODELING AND VALIDATION OF OROPOUCHE VIRUS GLYCOPROTEINS

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The Oropouche virus, which belongs to the genus *Orthobunyavirus* and the family *Peribunyaviridae*, is an emerging arbovirus associated with febrile illness and neurological symptoms. This study established a pipeline for the structural modeling and validation of the Gn and Gc glycoproteins based on viral sequences circulating in Alagoas. We analyzed 37 amino acid sequences of the M-segment polyprotein, derived from genomes obtained from human sera positive for the Oropouche virus and sequenced by our research group. The sequences were aligned using MAFFT v.7.526; the consensus sequence was generated using cons from the EMBOSS package and inspected in Jalview. The signal peptide was predicted using SignalP-6.0, and the topology of the Gn, NSm, Gc, and transmembrane regions was defined based on structural data. The Gc head, the Gc head-stem region, and a complex comprising Gn and Gc were modeled. For each construction, five models were generated by AlphaFold2 on the COSMIC² platform, and the model with the highest global pLDDT was selected. The models were relaxed using AMBER and refined in ISOLDE integrated with ChimeraX. For the three structures, respectively, the pTM values were 0.89, 0.86, and 0.64, and the ERRAT global quality factors were 99.53%, 97.51%, and 92.20%. MolProbity scores ranged from 1.53 to 1.59, Clashscores ranged from 3.14 to 7.18, and 96.16% to 97.31% of the residues were located in favored regions of the Ramachandran diagram. The models showed stereochemical consistency and provided a structural basis for future analyses of conservation, surface accessibility, antibody recognition potential, and antiviral activity.

Keywords: Bioinformatics; Oropouche; Alagoas

Financial Support: FAPEAL, CAPES, CNPq e FINEP.

PT.114

Genomic and phylogenetic characterization of a *Ortoflavivirus denguei* serotype 2 isolate associated with a neurotropic case in Northern Brazil

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Neurotropic cases associated with *Orthoflavivirus denguei* (DENV) infection are clinically significant events, yet the viral determinants associated with these manifestations remain poorly understood. In this study, we performed the genomic and phylogenetic characterization of a DENV-2 isolate obtained from a neurotropic case that occurred in 2000 in Belém, Pará, Brazil. The complete viral genome was recovered through next-generation sequencing on the Illumina NextSeq 500 platform. Sequencing reads underwent quality control and filtering using Fastp, removal of ribosomal sequences using SortMeRNA, and de novo assembly using MEGAHIT. Viral sequence identification and annotation were performed through comparison with protein databases using DIAMOND. Genotype assignment was confirmed by Maximum Likelihood phylogenetic analysis using IQ-TREE and a dataset comprising 60 complete DENV-2 coding sequences available in GenBank. The obtained sequence was assigned to genotype 2.III (Asian-American), lineage E, clustering with isolates collected between 1997 and 2006 in Brazil, the United States, and French Guiana. Nucleotide and amino acid identities of 99.4% and 99.7%, respectively, were observed relative to contemporary Brazilian sequences collected between 2000 and 2001. Eight unique nonsynonymous mutations were identified in the E, NS1, NS2A, NS4B, and NS5 genes, including the A249T substitution in NS5, corresponding to nucleotide position 8.221 and resulting in a change in amino acid polarity. This study provides novel genomic data on a historical DENV-2 isolate associated with a neurotropic manifestation in Northern Brazil and contributes to the understanding of the molecular diversity of Asian-American lineages circulating during this period.

Financial Support: CNPQ, CAPES, SVSA/MS.

PT.115

Computational platform for the rational design of antigens and antibodies for the diagnosis and treatment of viral diseases

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Despite advances in antibody engineering, the development of highly specific ligands for viral diagnosis and therapy still relies on fragmented workflows, in which computational prediction and experimental validation are conducted independently, limiting efficiency and translational applicability. To address this gap, we developed a fully integrated platform that connects epitope selection, antibody retrieval from curated databases, reformatting into scFv and VHH single-domain formats, 3D antigen-antibody modeling, molecular docking simulations, identification of key binding residues, and targeted mutagenesis into a single cohesive pipeline. As a proof of concept, we applied the platform to *Orthoflavivirus* NS1 and E antigens, targets for which cross-reactivity responses between serotypes remain a central diagnostic challenge. Epitope mapping identified 3 candidate regions with high predicted specificity (at least 70% distinct between species), and the corresponding antibodies showed distinct binding profiles, with the top-ranked candidate exhibiting a HADOOCK score of 94.1. An anti-E scFv targeting DENV2 was cloned and is in the expression phase in eukaryotic cells. These results demonstrate that end-to-end integration of computational and experimental steps accelerates the rational engineering of antibodies with optimized binding profiles, offering a replicable framework applicable to emerging and neglected viral pathogens.

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PT.116

SMALL RNAS, LARGE CONSEQUENCES: UNCOVERING VIROPHAGE MICRORNA-MEDIATED REGULATION

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Giant viruses, such as mimiviruses, are parasitized by virophages, small dsDNA satellite viruses that use giant viruses factories for replication. Mimiviruses parasitized by the Sputnik virophage produce abnormal capsids, reducing viral fitness. MicroRNAs are small non-coding RNA molecules involved in post-transcriptional regulation, but it is unknown whether virophages employ miRNA mediated interference to harm giant viruses' replication. In the present study, we aimed to computationally predict microRNAs encoded by Sputnik and to identify candidate target sites within the genomes of their giant virus hosts. We retrieved Sputnik and mimivirus genomes, identified hairpin-like regions via sliding-window secondary-structure prediction (using RNAfold), and performed target prediction with RNAhybrid, retaining only miRNA–target pairs with minimum free energy ≤ −25 kcal/mol. A limited number of mimivirus genes accumulate a disproportionately high number of predicted miRNA interactions, supported by high interaction counts, recurrent targeting, and strong binding energies. Functional enrichment of top targeted genes revealed structural functions, RNA and DNA processing, and protein–protein interactions. This implies that Sputnik miRNAs are capable of affecting viral transcript stability and translation efficiency and affect virion architecture. This strategy is biologically coherent: Sputnik depends on Mimivirus replication machinery. Together, our results support that Sputnik microRNAs exert a phase-dependent regulatory effect on mimivirus gene expression. Such targeted disruption provides a mechanistic explanation for the defective capsid phenotype observed and supports the existence of a coevolutionary regulatory coupling, in which Sputnik exploits the mimivirus transcriptional machinery and actively reshapes its expression landscape through microRNA-mediated regulation to improve its own propagation.

Financial support: FAPEMIG, CNPq, CAPES, PRPq-UFMG, PRPg-UFMG.

PT.117

LINE-1 REACTIVATION AT HPV INTEGRATION HOTSPOTS IN CERVICAL CANCER

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Cervical cancer is caused by persistent infection with high-risk human papillomavirus (HPV). While the genetic and epigenetic mechanisms underlying this cancer are not fully understood, the reactivation and dysregulated expression of transposable elements have been linked to genomic instability and oncogene activation across tumor types. Thus, this study characterized long interspersed nuclear element-1 (LINE-1) expression using RNA-seq data (GEO: GSE147009) from HPV16-associated normal cervical epithelium (NC), HPV-positive high-grade squamous intraepithelial lesions (HSIL), and squamous cell carcinoma (SCC), focusing on the HPV integration hotspots: 3q28, 13q22.1, and 21p11.2. Reads were aligned to the human reference genome (hg38) using HISAT2, and host gene expression was quantified using HTSeq-count. LINE-1 expression was quantified using atena (TEtranscripts method) in R. Differential expression was analyzed with DESeq2 based on two criteria: count sum > 50 and padj < 0.05, focusing on upregulated elements. RepeatMasker coordinates were intersected with GENCODE-annotated host genes within a 10 kb window. In NC vs. SCC comparison, upregulated elements included L1MD3 near *PIBF1* (13q22.1) associated with progesterone-induced blocking, L1MC5 near *LPP* (3q28) related to cell adhesion and motility and L1MC near *BAGE2* (21p11.2), a cancer/testis antigen. No functional genes were identified in the NC vs. HSIL comparison. In HSIL vs. SCC comparison, L1PA7 neighbored L1PA7 (13q22.1), and L1MB2 neighbored *TPRG1* (3q28), associated with the regulation of the p63 tumor protein. Therefore, LINE-1 activity may contribute to malignant transformation, as its reactivation was observed near cancer-related genes at HPV integration hotspots.

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PT.118

SAMPLING-ADJUSTED PHYLOGEOGRAPHY OF DENV-1 IN BRAZIL REVEALS A SOUTHEASTERN ORIGIN AND RECENT SOUTHWARD SPREAD

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Spatially uneven genomic sampling can distort dengue virus phylogeographic reconstructions by inflating the importance of highly sampled locations. We investigated the origin, persistence, and dissemination of a dengue virus type 1 (DENV-1) lineage using 264 sequences from five macroregions and 19 states in Brazil. Sampling-adjusted Bayes factors (BFadj) were estimated through tip-state randomization, and Markov jumps and rewards were reconstructed using BEASTXv10.5.0. The Southeast region was strongly supported as the ancestral macroregion (pp = 0.984; BFadj = 42.97), with Rio de Janeiro identified as the most probable ancestral state (pp = 0.705; BFadj = 12.08). Although São Paulo had the second-highest unadjusted root probability, its support disappeared after adjustment (BFadj = 0.64), indicating a strong influence of sampling structure. Nevertheless, São Paulo accounted for 47.6% of the total lineage-time, identifying it as the principal center of persistence rather than the ancestral source. Extensive circulation was reconstructed between the Southeast and Central-West, with Central-West-to-Southeast representing the most strongly supported regional pathway (BFadj = 147.83). The clearest recent expansion, however, occurred towards southern Brazil after 2020. The Southeast-to-South transition was recovered in all posterior histories (BFadj = 23.27), while Paraná-to-Rio Grande do Sul showed the strongest state-level support overall (BFadj = 299.39; median first-jump date, 2022.5). Additional support for Bahia-to-Paraná suggested contributions from multiple sources. These findings support a southeastern origin of the DENV-1 lineage, prolonged persistence in São Paulo, and recent multi-source expansion towards southern Brazil. They also highlight the importance of prioritizing genomic surveillance beyond the most heavily sampled locations.

PT.119

THE EPITRANSCRIPTOMIC LANDSCAPE OF SARS-COV-2: A MUTUAL INFORMATION APPROACH TO m6A MODIFICATIONS

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Introduction: SARS-CoV-2 is a positive-sense single-stranded RNA virus whose replication and gene expression are regulated by post-transcriptional modifications, particularly N^6-methyladenosine (m^6A). This study investigated whether viral transcripts exhibit a coordinated pattern of modification co-occurrence using Oxford Nanopore direct RNA sequencing (dRNA-seq) data. **Methods:** The public dRNA-seq data from the Wuhan-Hu-1 and South Korean strains were reprocessed under two conditions: in vitro transcribed (IVT) RNA as a negative control, and infected VeroE6 cells as a positive control. The raw electrical signal files were standardized from ".fast5*"* into ".pod5" and ".blow5" formats. The basecalling step was executed using the DORADO software (v0.9.6), supporting the SQK-RNA002 kit with GPU acceleration, while sequence alignment was conducted using Minimap2 (v2.30). Methylation prediction was performed by the m6Anet software (v2.1), selecting high-confidence sites with a probability greater than ≥60%. Finally, 15-mer motif analyses and quality control were carried out using ggseqlogo and ToulligQC. **Results:** The results demonstrate that reprocessing the data with deep neural network models increased. Moreover, m6Anet identified high-confidence sites, confirming a bias toward the DRACH motif. A striking overrepresentation of methylation was observed (19 sites IVT versus 27 sites Vero with ProbM > 60%), concentrated within the structural and accessory genes *S*, *ORF3a*, *ORF7b*, and *N*. The application of mutual information measures based on Shannon entropy revealed statistical dependencies and non-random co-occurrence patterns among the modified sites. **Conclusion:** Ultimately, the successful application of mutual information metrics confirmed the existence of strong statistical dependencies between these modified sites. **Financial support:** CAPES and FAPESP.

PT.120

DECIPHERING THE EPITRANSCRIPTOMIC IMPACT OF SARS-COV-2 RECOMBINATION: A MACHINE LEARNING AND RDP5-DRIVEN FRAMEWORK

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Introduction: In SARS-CoV-2, N6-methyladenosine (m6A) modifications modulate viral replication, immune evasion, and infectivity. However, how recombination reshapes the epitranscriptomic landscape of SARS-CoV-2 Omicron subvariants remains poorly understood. **Methods:** We expanded our bioinformatics workflow to analyze a comprehensive dataset of more than 9,000 complete, high-coverage SARS-CoV-2 genomic sequences retrieved from the GISAID EpiCoV database. To systematically identify mosaic structures and evolutionary outliers, a Principal Component Analysis (PCA) was implemented using PLINK (v1.9) to detect intermediary sequences located at the boundaries between distinct subvariant clusters. Genomic mosaic structures and breakpoints were validated using the Recombination Detection Program methods. To assess functional impacts, sequence based m^6A modification profiles were predicted using the SRAMP software across mature mRNA and full-transcript models. **Results:** The macro-scale PCA efficiently stratified major lineages and identified unique Omicron hybrid candidates. RDP5 analysis confirmed statistically significant breakpoints ($p<0.05$), showing a high predisposition for genomic exchanges spanning from the Spike (*S*) gene toward the 3' terminus. SRAMP profiling revealed distinct epitranscriptomic differences, showing that recombinant lineages exhibit a highly confident hypermethylation (23 sites with high confidence by SRAMP) profile compared to non-recombinant strains. Conserved modified sites were significantly enriched within the canonical DRACH motif, specifically in previously unexplored regions of the *ORF1ab* and *S* genes. **Conclusion:** Our pipeline demonstrates that SARS-CoV-2 recombination events significantly alter the viral m^6A landscape. The heightened methylation observed via SRAMP in these novel recombinant strains suggests a potential mechanism for enhanced viral fitness, emphasizing the need to monitor multi-layered evolutionary shifts. **Financial support:** CAPES and FAPESP.

PT.121

Genomic and Phylogenetic Characterization of Anelloviruses Detected in Rodents from French Guiana

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Anelloviruses are circular single-stranded DNA viruses widely distributed among mammals; however, information regarding their genetic diversity and distribution in rodents from South America remains limited. This study aimed to genomically characterize anelloviruses in rodents from French Guiana through the reanalysis of publicly available metagenomic data. A descriptive and retrospective study was conducted using five rodent metagenomic datasets deposited in the Sequence Read Archive under accession numbers SRR12203285, SRR12203289, SRR12203296, SRR12203318, and SRR12203319. The datasets were analyzed using Genome Detective for viral identification, Geneious for genomic characterization, BLAST for sequence comparison, and IQ-TREE for phylogenetic analysis. Five rodent-associated anellovirus sequences, provisionally designated R-TTV1, R-TTV2, R-TTV3, R-TTV4, and R-TTV5, were identified. Their genome lengths ranged from 2 517 to 2 536 base pairs. BLASTn analysis identified Rodent Torque teno virus 6 isolate 2404 (NC_040618.1) as the closest match, with nucleotide identities ranging from 75.47% to 76.43% and query coverage of 90%–91%. Genomic organization revealed three open reading frames, ORF1, ORF2, and ORF3, consistent with members of the family Anelloviridae. Phylogenetic analysis placed all five sequences within the genus Wawtorquevirus, where they formed a well-supported rodent-associated cluster. These findings demonstrate the usefulness of publicly available metagenomic data for identifying and characterizing anelloviruses and expand current knowledge of their presence, genetic diversity, and phylogenetic relationships in rodents.
Financial Support: San Juan Bautista Private University, Ica, Peru.

PT.122

THERAEPIFLOW: AN INTEGRATED LOCAL PIPELINE FOR RATIONAL SELECTION OF MHC CLASS I EPITOPES IN VACCINE DESIGN

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Multi-epitope therapeutic vaccines have gained growing relevance in personalized cancer treatment and infectious disease control. Rational epitope design depends on immunoinformatics tools capable of predicting MHC binding, immunogenicity, and conservation; however, available tools operate in isolated stages, require weeks of manual processing, and rely on external servers, raising data security concerns. This study developed and validated a fully local, open-source computational tool that integrates, within a single automated workflow, all steps required to identify MHC class I-restricted CD8 T-cell epitopes, concluding with linker and adjuvant selection and generation of a FASTA file containing the synthetic antigen sequence. The methodology comprised a pipeline that retrieves target protein sequences via the UniProt API, predicts peptide-HLA binding through consensus between two independent methods, evaluates immunogenicity, screens toxicity, clusters similar epitopes selecting the most promising representative through a normalized priority score, and analyzes conservation, human population coverage, and murine cross-reactivity, culminating in an interactive HTML interface for construct assembly. Functional validation used the HPV16 E7 protein and the SARS-CoV-2 nucleocapsid protein, each processed across ten independent runs. Results showed the epitope set reduced from 27 to 7 final candidates for E7 and from 105 to 30 for the nucleocapsid, of which six and twenty, respectively, had documented experimental support, with average execution times of 72–75 seconds per protein and 4.3% variation between proteins of different sizes. The platform represents an original, reproducible, and auditable approach for rational vaccine antigen screening, reducing synthetic antigen construction time from weeks to minutes.

Financial Support: CNPq and CAPES.

PT.123

EVOLUTIONARY ANALYSIS OF MAYARO VIRUS COMPARED WITH CHIKUNGUNYA VIRUS REVEALS GENOTYPE-SPECIFIC CODON USAGE PATTERNS

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Alphavirus genus belongs to the Togaviridae family, comprising viral species epidemiologically relevant to public health, among which Chikungunya virus (CHIKV) and Mayaro virus (MAYV). CHIKV and MAYV can cause acute disease in humans, with overlapped clinical manifestations. While MAYV is restricted to the Americas, CHIKV has caused several epidemics throughout subtropical and tropical regions worldwide. Two of the three CHIKV genotypes sustained urban transmission cycles, whereas MAYV genotypes circulate in a sylvatic environment, with the Dispersed (D) genotype currently circulating as an emerging genotype with potential to establish urban transmission. To investigate genotype D of MAYV, this study aimed to compare MAYV and CHIKV by considering the evolutionary and ecological aspects associated with each of their genotypes. Here, we used Relative Synonymous Codon Usage (RSCU), and Codon Adaptation Index (CAI) analysis. Our results indicate that MAYV and CHIKV exhibit distinct codon usage patterns, but for each virus, there is clustering according to genotypes. Considering all MAYV genotypes, it was possible to observe a similarity in codon usage patterns between MAYV genotype D and urban genotypes of CHIKV. Interestingly, CAI values are higher (for both humans and Aedes aegypti) for MAYV compared to CHIKV. In conclusion, our results indicate distinct codon usage patterns associated with viral species and even genotypes. Furthermore, the adaptation to urban environments can not be merely explained by the association of high CAI values, but rather by a balance between codon usage optimization and/or de-optimization involving the host and the vector.

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PT.124

First Evidence of Highly Pathogenic Avian Influenza (H5N1) Clade 2.3.4.4b in Weddell Seal (*Leptonychotes weddellii*) Feces from Antarctica

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Highly pathogenic avian influenza virus (HPAIV) H5N1 has expanded globally, causing unprecedented mortality in wild birds and increasing spillover events into marine mammals. Although the virus has recently been detected in Antarctic wildlife, information on its occurrence in Weddell seals (*Leptonychotes weddellii*), one of the continent's most abundant pinniped species, remains scarce. Here, we investigate the presence of influenza A virus (IAV) in fecal samples collected from Weddell seals in Antarctica. During November and December 2024, 14 fecal samples were collected on Nelson Island with logistical support from the Brazilian Navy, the Brazilian Antarctic Program (PROANTAR), and the Chilean Antarctic Institute (INACH). Viral RNA was extracted using the MagMAX™ CORE kit on the KingFisher™ Flex platform and screened for IAV by RT-qPCR targeting the matrix gene. Five samples were positive for IAV and one sample underwent whole-genome amplification and high-throughput sequencing on the Ion Torrent GeneStudio S5 Plus platform, with genome assembly performed using the IRMA pipeline. Partial H5 and complete N1 gene sequences confirmed HPAIV H5N1 clade 2.3.4.4b. Phylogenetic analyses placed the virus within the South American lineage currently circulating among wild birds and marine mammals. To our knowledge, this is the first evidence of HPAIV H5N1 clade 2.3.4.4b in Weddell seals in Antarctica. These findings provide the first molecular evidence of H5N1 in Weddell seal feces and demonstrate the value of non-invasive fecal sampling for pathogen surveillance.

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Keywords: Marine mammals, Highly pathogenic avian influenza virus, Environmental surveillance

PT.125

Surveillance of influenza A virus in marine mammals along the coast of Rio Grande do Sul, Brazil

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Influenza A viruses (IAVs), members of the family *Orthomyxoviridae*, infect a wide range of hosts, including domestic and wild birds, humans, and marine mammals. In marine mammals, IAV infections have been reported predominantly in pinnipeds, where they are frequently associated with outbreaks and high mortality, whereas infections in cetaceans remain uncommon. Following the recent spread of highly pathogenic avian influenza (HPAI) H5N1 across South America, this study investigated the occurrence of IAV in stranded marine mammals along the coast of Rio Grande do Sul, southern Brazil. Between January and July 2026, a 485-km stretch of coastline was monitored, and samples were collected from stranded animals. Swab samples were preserved in sterile transport medium, and total nucleic acids were extracted using the MagMAX™ CORE Nucleic Acid Purification Kit on the KingFisher™ Flex automated platform. Influenza A virus detection was performed by RT-qPCR targeting the matrix (M) gene, followed by H5 subtype screening. A total of 99 marine mammals were analyzed: 27 cetaceans and 72 pinnipeds. Six animals tested positive for both the M gene and the H5 subtype: one southern elephant seal (*Mirounga leonina*), four South American fur seals (*Arctocephalus australis*), and one franciscana dolphin (*Pontoporia blainvillei*). These findings demonstrate the circulation of HPAI H5 viruses among marine mammals along the southern Brazilian coast and highlight the importance of stranded animal surveillance for the early detection of relevant pathogens. Continued monitoring is essential to better understand viral transmission, host susceptibility, and the ecological role of marine mammals in IAV dynamics.

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PT.126

MOLECULAR DETECTION OF DIVERGENT POXVIRUSES IN NON-VOLANT SMALL MAMMALS FROM MINAS GERAIS, BRAZIL

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Poxviruses have a significant history of impacting public health. With the current Mpox outbreak, concerns regarding the zoonotic and epidemic potential of other poxviruses have increased. Therefore, this study screened for poxvirus DNA in rodents and marsupials captured between 2018 and 2020 (SISBIO 68066-1; CEUA 041/2019) across five Cerrado forest fragments in the Triângulo Mineiro region, Minas Gerais, Brazil. DNA samples from ear fragments (n=236), liver (n=20), spleen (n=20), and skin lesions (n=8) were subjected to a universal PCR detecting low- and high-GC content poxviruses. A total of 568 reactions were conducted on 174 rodent and 70 marsupial individuals. Overall, 11% (27/236) of ear fragments, a spleen from *Rhipidomys macrurus*, and skin lesions from *R. macrurus*, *Calomys* sp., and *Hylaeamys megacephalus* individuals tested positive only in the low-GC PCR. No samples were positive in the high-GC content PCR. By species, positivity rates were 100% (1/1) for *Calomys* spp., 19% (9/48) for *Gracilinanus agilis*, 16% (18/111) for *R. macrurus*, 5% (1/22) for *Didelphis albiventris*, 2% (1/46) for *H. megacephalus*, and 0% for *Oecomys cleberi* (0/15) and *Rattus rattus* (0/1). Bidirectional Sanger sequencing generated consensus sequences sharing 75–80% nucleotide identity with NY_014 poxvirus. Subsequently, all positive samples were subjected to a PCR targeting Orthopoxvirus hemagglutinin, but no amplification occurred. These findings suggest the presence of a divergent poxvirus in these non-volant small mammals; however, further molecular characterization is required to determine its precise taxonomic identity.

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PT.127

MOLECULAR EVIDENCE OF A DIVERGENT GAMMAHERPESVIRUS IN PECCARIES FROM THE BRAZILIAN AMAZON

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Gammaherpesviruses infect diverse mammals, but their diversity in Neotropical wildlife remains poorly characterized. We investigated herpesvirus DNA in 13 peccaries from the Brazilian Amazon sampled from February 2009 to June 2012: three collared peccaries (*Pecari tajacu*; Mato Grosso, n=1; Pará, n=2) and ten white-lipped peccaries (*Tayassu pecari*; Mato Grosso, n=2; Pará, n=8). Samples from Mato Grosso originated from animals hunted by Indigenous communities, whereas those from Pará originated from roadkill. DNA extracted from spleen was screened by broad-range nested polymerase chain reaction targeting a conserved region of the herpesvirus DNA polymerase gene, with expected products of 453–709 base pairs in the primary reaction and 208–315 base pairs in the nested reaction. Four animals (30.8%) were positive: three *T. pecari* and one *P. tajacu*. The *P. tajacu* sequence was insufficient for reliable characterization. In contrast, the three *T. pecari* sequences were identical and yielded a 170-nucleotide consensus. BLASTn showed no close match; the highest identity was 83.8% over 46% query coverage, while alignments covering up to 71% of the query showed approximately 73%–74% identity to gammaherpesvirus sequences. These findings support detection of a divergent gammaherpesvirus sequence in *T. pecari*, although the short fragment precludes genus- or species-level classification. The study expands knowledge of herpesvirus diversity in Neotropical wildlife and highlights the need for longer genomic regions and additional loci for robust taxonomic and evolutionary characterization.

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PT.128

DEVELOPMENT OF A REAL-TIME FLUORESCENT RT-LAMP ASSAY FOR RAPID DETECTION OF RESPIRATORY VIRUSES USING PORTABLE DEVICES

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Respiratory Syncytial Virus (RSV) is a leading cause of lower respiratory tract infections in children and is associated with high morbidity and hospitalization rates worldwide. Early detection is essential for clinical management and surveillance. However, the gold-standard method, RT-qPCR, has limitations such as high cost, long processing time, and need for specialized laboratory infrastructure, restricting its use in rapid diagnostic settings. In this context, reverse transcription loop-mediated isothermal amplification in a fluorescent format (fluorescent RT-LAMP) is a promising rapid and sensitive alternative for Point-of-Care (PoC) applications. In this study, a fluorescent RT-LAMP assay for RSV detection was developed and evaluated using commercial kits (WarmStart® Fluorescent and LyoPrime WarmStart™ Fluorescent LAMP/RT-LAMP). Reactions were performed on portable ISO-T8 and ISO-T16 devices (Axxin). Different primer sets and concentrations were tested, and optimal conditions were established to ensure amplification efficiency and specificity. On portable platforms, parameters such as fluorescence threshold and baseline adjustment were optimized to distinguish true signals from background noise. The assay showed high analytical sensitivity, with detection in less than 10 minutes for concentrated samples and increased time with serial dilutions. No amplification was observed in negative controls. These findings indicate that fluorescent RT-LAMP is a rapid and robust method for RSV detection using portable platforms. Further validation with clinical samples is required.

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PT.129

CLINICAL AND EPIDEMIOLOGICAL CHARACTERISTICS OF AIDS CASES IN THE BRAZILIAN AMAZON: A POPULATION-BASED STUDY

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Background: Despite advances in HIV prevention and treatment, AIDS remains a major public health challenge in the Brazilian Amazon, where social inequalities and barriers to healthcare may influence disease occurrence and outcomes. **Methods:** A population-based cross-sectional study was conducted using secondary surveillance data on reported AIDS cases in Pará, Brazil, from 2022 to 2025. Sociodemographic, clinical, laboratory and exposure variables were analyzed using descriptive statistics. **Results:** A total of 3,974 AIDS cases were identified. Most cases occurred in men (70.2%), individuals aged 25–34 years (29.8%), and people of mixed race/ethnicity (79.1%). Urban residents accounted for 89.1% of notifications. Heterosexual exposure was the predominant transmission route (60.8%), followed by homosexual exposure (23.1%). The mean age was 38.3 years (95% CI: 37.86–38.65), with a non-normal age distribution. Cachexia (51.2%), asthenia (48.0%), fever (39.2%) and diarrhea (33.0%) were the most frequent clinical manifestations, indicating advanced clinical impairment in a substantial proportion of patients. Laboratory confirmation showed high positivity rates, although approximately one-third of screening and confirmatory tests were not performed. Most patients were alive at the time of notification (85.1%); however, AIDS-related mortality reached 12.8%, highlighting the persistent burden of severe disease. **Conclusions:** AIDS in Pará predominantly affects young adult men living in urban areas and remains associated with advanced clinical manifestations and considerable mortality. These findings reinforce the need to strengthen early diagnosis, expand access to timely treatment and improve the completeness and quality of surveillance data to support more effective public health interventions in the Brazilian Amazon.

Financial Support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)

PT.130

COMPARATIVE EPIDEMIOLOGICAL PROFILE OF HIV, AIDS AND VIRAL HEPATITIS IN THE BRAZILIAN AMAZON

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HIV infection, AIDS and viral hepatitis share transmission routes and social determinants but differ in their epidemiological dynamics. Understanding these differences is essential for developing targeted public health strategies in the Brazilian Amazon. A population-based cross-sectional study was conducted using surveillance data from reported HIV, AIDS and viral hepatitis cases in Pará, Brazil, between 2022 and 2025. Sociodemographic characteristics were compared using descriptive statistics and Pearson's chi-square test. The epidemiological profiles differed significantly among the three conditions. HIV and AIDS predominantly affected males (71.3% and 70.2%, respectively), whereas viral hepatitis showed a more balanced sex distribution (53.8% male). HIV and AIDS cases were concentrated among young adults aged 25–44 years, while viral hepatitis occurred mainly in individuals aged 45 years or older, suggesting a predominance of chronic infection and later diagnosis. Individuals of mixed race/ethnicity represented the majority of cases across all diseases (76.2%–79.1%). Urban residence predominated in all groups but was less frequent among viral hepatitis cases (80.9%) than among HIV and AIDS cases (86.4% and 89.1%, respectively). Differences in sex, age group, race/ethnicity and area of residence were statistically significant ($p<0.05$), demonstrating distinct epidemiological patterns among the diseases. Although HIV, AIDS and viral hepatitis share common social determinants, they present different demographic and geographic profiles. These findings support the implementation of disease-specific surveillance, prevention and healthcare strategies, considering the epidemiological particularities of each condition to improve health outcomes in the Brazilian Amazon.

Financial Support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)

PT.131

Imunochromatographic and molecular detection of Bovine Viral Diarrhea (BVD) on a beef cattle farm

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Bovine Viral Diarrhea (BVD) is caused by a *Pestivirus* and is associated with respiratory, gastrointestinal, hemorrhagic, and cutaneous diseases, immunosuppression, and reproductive disorders. Due to the clinicopathological consequences of infection during pregnancy, Bovine Viral Diarrhea Virus (BVDV) is considered one of the most important pathogens affecting cattle reproduction. The study began in May 2026 after an abortion at the fifth month of gestation in a cow from 120 pregnant cows in a herd of 600 animals. Ear notch and eyelid tissues from the aborted fetus tested positive by the immunochromatographic assay (IDEXX BVDV Ag Point-of-Care®). Based on this finding, the herd was screened for persistently infected (PI) animals. Whole blood samples collected in EDTA tubes obtained from calves (n = 71) born between January and June 2026 were tested for BVDV using immunochromatographic assay and RT-qPCR. RNA was obtained with the QIAamp® Viral RNA Mini Kit and SYBR RT-qPCR results were interpreted based on quantification cycle (Cq) and melting temperature (Tm) values. Eight calves (11.26%) tested positive on both diagnostic methods, suggesting persistent BVDV infection. As PI animals are the primary source of BVDV transmission, the positive calves may have been responsible for infecting the pregnant cow that subsequently aborted. Identifying PI is one of the main measures for disease control in herds. Hence, early BVD detection and control may prevent reproductive losses, contributing to the sustainability of cattle production.

Keywords: *Pestivirus tauri*, RT-qPCR, calves, diagnostic.

Financial Support: CAPES - Finance Code 001, INCT-One, PREPROMV, Ministério da Saúde e Fundect.

PT.132

Detection of Bovine Viral Diarrhea Virus (BVDV) in bovine fetal tissues by RT-qPCR using magnetic bead-based RNA extraction

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Bovine Viral Diarrhea (BVD) is an infectious disease caused by the *Pestivirus tauri* (Bovine Viral Diarrhea Virus; BVDV), Pestivirus genus, *Flaviviridae* family, and is responsible for significant respiratory, reproductive, and economic losses in cattle production. This study aimed to report the molecular detection of the BVDV in tissues collected from an aborted bovine fetus. The samples were obtained from a cow that aborted during the fifth month of gestation, belonging to a group of 120 pregnant cows from a herd comprising approximately 600 cattle. Following tissue maceration, viral RNA was extracted using a magnetic bead-based commercial kit. BVDV was detected by RT-qPCR using the QuantStudio™ system. The results were interpreted based on quantification cycle (Cq) values and melting temperature (Tm), and Cq 34 was used as cut-off. Heart (Cq 33.15), purulent content (Cq 30.88), umbilical cord (Cq 30.23), placental fluid (Cq 32.33), small intestine (Cq 32.50), spleen (Cq 30.70), brain (Cq 31.44), large intestine (Cq 32.72), and abomasum (Cq 28.65) tested positive for BVDV, while kidney, lung, liver, and placenta samples were negative. These findings demonstrate the presence of the viral genome in multiple fetal tissues and reinforce the importance of the molecular detection for early detection of BVDV in cases of bovine abortion.

Keywords: BVDV; RT-qPCR; bovine abortion; *Pestivirus*; molecular diagnosis.

Financial Support: CAPES - Finance Code 001, INCT-One, PREPROMV, Ministério da Saúde e Fundect.

PT.133

SILENT CIRCULATION OF ENTEROVIRUS D68 DETECTED THROUGH ENVIRONMENTAL SURVEILLANCE IN DIAMANTINA, MINAS GERAIS, BRAZIL

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Enterovirus D68 (EV-D68) is associated with outbreaks of acute respiratory disease in children and, in severe cases, the infection may progress to the central nervous system, causing symptoms such as encephalitis and acute flaccid paralysis. In Brazil, systematic surveillance of this virus has not yet been established, meaning that detected cases likely underestimate its true extent and prevalence in the country. In this context, the present study investigated the silent circulation of EV-D68 in Diamantina, Minas Gerais, through molecular analyses of wastewater samples. From January 2023 to March 2025, a total of 351 samples of 100 mL each were collected from seven strategic points at wastewater treatment plants. After collection, the samples were transported on ice, stored at –80 °C, and concentrated using PEG 8000/NaCl precipitation. RNA extraction was performed using a commercial Invitrogen Thermo Fisher kit, followed by RT-qPCR using primers specific for EV-D68. A total of 25 samples showed amplification for EV-D68, with Ct values below 40. Of these, 8 samples were sequenced. The results demonstrate the circulation of EV-D68 in the municipality’s sewage system, suggesting underdiagnosed community transmission and reinforcing the importance of environmental surveillance as a complementary tool for the early detection of respiratory viruses. Financial Support: CAPES, FAPEMIG PCE-00181-26 and APQ-04955-23, SES-MG Agreement no. 007/2022.

PT.134

INFLUENZA A VIRUS SCREENING IN WILD BIRDS IN SAO PAULO CITY

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Wild aquatic birds are the recognized reservoir for Influenza A viruses and migratory species contribute to virus dispersal. Some species, such as *Dendrocygna* species (whistling ducks), were identified with HPAI Influenza H5N1 in Brazil and despite no typical migratory behavior these species present regional displacements and occurrence in areas with migratory groups. Between September 2025 and April 2026, 145 Oral and cloacal swabs samples were collected from wild birds in São Paulo City, close to Guarapiranga Dam (23°44'25.71"S, 46°43'5.06"W 750 m) and immediately stored in liquid nitrogen. Ground traps were used to capture aquatic species and mist-nets for other species. Bird rings (CEMAVE-ICMBio) were used to identify individuals captured for the first time. Total RNA was extracted and screened for Influenza A virus using broad conventional PCR assay targeting the PB1 gene. A total of 26 distinct bird species including 55 *Dendrocygna viduata* (37,9%) and 66 *D. autumnalis* (45,5%) were analyzed. All collected samples were negative for Influenza virus. This result indicates the absence of influenza virus circulation in this specific area and during the analyzed period and contributes to Influenza virus monitoring and surveillance in São Paulo City. Continuous surveillance for Influenza A viruses in migratory and non-migratory birds is essential for the early detection of viral introduction into Brazil, enabling the rapid implementation of control measures to minimize impacts on native and wild bird populations, and viral introduction into commercial poultry, reducing the risk of zoonotic spillover to humans.

Financial Support: FAPESP

PT.135

EPIDEMIOLOGICAL AND GENOMIC CHARACTERIZATION OF SARS-COV-2 IN SOUTHERN MINAS GERAIS, BRAZIL (2024–2026).

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SARS-CoV-2 genomic surveillance remains essential to monitor viral evolution and transmission dynamics, particularly in non-metropolitan regions. This study aimed to characterize the epidemiological, molecular, and genomic profile of SARS-CoV-2-positive samples from southern Minas Gerais, Brazil. A total of 120 clinical samples collected between October 2024 and April 2026 from multiple municipalities were analyzed. Viral detection was performed by RT-qPCR, and cycle threshold (Ct) values were recorded. A subset of samples underwent whole-genome sequencing and phylogenetic analysis. Ct values ranged from 12.0 to 29.4, with a median of approximately 22.0 and an interquartile range of 19.5–25.5, indicating heterogeneous viral loads. A predominance of female individuals (65%) was observed, with higher frequency among elderly patients (≥60 years), although cases occurred across all age groups. All samples tested negative for flaviviruses. Phylogenetic analysis of 28 genomes revealed substantial genetic diversity and the co-circulation of multiple Omicron-derived recombinant lineages, with predominance of XFG-related variants and detection of XEC, KP, MC, and QF lineages. The presence of closely related sequences within major clades supports local transmission clusters, while lineage diversity indicates multiple independent viral introductions over time. The results demonstrate sustained SARS-CoV-2 circulation across different epidemiological periods, with genetic diversification and heterogeneous demographic patterns. These findings demonstrate sustained viral circulation, genetic diversification, and reinforce the importance of decentralized genomic surveillance for public health decision-making.

PT.136

SEROLOGICAL EVIDENCE OF HUMAN EXPOSURE TO *ORTHOPOXVIRUS* IN INDIVIDUALS FROM AN URBAN AREA IN NORTHEASTERN BRAZIL.

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The interruption of smallpox vaccination contributed to the formation of a growing contingent of individuals without vaccine protection against *Orthopoxviruses* (OPV), increasing concern about infections caused by these viruses. This study evaluated the presence of neutralizing antibodies and exposure factors associated with OPV in individuals from Parnaíba-PI/Brazil. Serum samples and epidemiological information were obtained from 104 volunteers seen at a clinical laboratory. Based on age, participants were classified as presumably vaccinated against smallpox (≥45 years) or unvaccinated (<45 years). Antibodies were detected by the plaque reduction neutralization test with *Orthopoxvirus vaccinia*, Western Reserve strain. Samples with a minimum 50% reduction in plaques were considered positive. Associations were evaluated by univariate analysis and logistic regression, adopting $p < 0.05$. Seventeen participants presented antibodies, corresponding to 16.3% (95% CI: 9.8–24.9), with titers between 50 and 100 UN/mL. The frequency of positives was higher among individuals ≥45 years (33.3%) than among those <45 years (7.4%). In logistic regression, participants <45 years had a lower chance of a positive result (odds ratio: 0.159; 95% CI: 0.0505–0.498; $p=0.002$). Although 90.4% reported contact with animals, no exposure variable showed a significant association. Five positive individuals were <45 years old, suggesting previous exposure. These findings represent the first serological evidence of human exposure to OPV in Piauí, demonstrate antibodies in a predominantly urban sample, and reinforce the integrated surveillance of human, animal, and environmental health. Financial support: Coordination for the Improvement of Higher Education Personnel (CAPES) and the National Institute of Science and Technology in Poxviruses (INCT-POX).

PT.137

Molecular investigation of astroviruses in rectal swabs from non-human primates in Minas Gerais, Brazil

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Astroviruses (family Astroviridae) are enteric viruses commonly associated with gastroenteritis. Viral enteropathogens are important etiological agents of acute gastroenteritis and may be involved in diarrheal outbreaks. Transmission occurs mainly through the fecal–oral route, including ingestion of contaminated water or food. Non-human primates (NHPs) can be infected by viruses relevant to human health and may contribute to the maintenance, circulation, or emergence of enteric viruses in different ecological contexts. This study aimed to investigate the presence of astroviruses in rectal swab samples from NHPs collected in Minas Gerais, Brazil. Samples were obtained from NHPs in urban parks in Belo Horizonte or from animals referred to the CETAS. A total of 103 rectal swab samples underwent total RNA extraction, followed by semi-nested PCR and nested PCR targeting conserved regions of pan-astrovirus genes. To assess amplicons of the expected size, 40 PCR products were analyzed by polyacrylamide gel electrophoresis. Nineteen samples with compatible amplification profiles were selected for sequencing to investigate astrovirus presence and enable molecular characterization. Besides the expected band, other amplification products were observed. Therefore, the corresponding band was excised, purified, and submitted for sequencing. Sequencing results are pending. Given the nonspecific amplification pattern, viral detection cannot yet be confirmed and depends on sequence analysis. The remaining samples from 184 NHP will be analyzed. This ongoing molecular investigation contributes to surveillance of enteric viruses in free-ranging and rescued NHPs from MG, and may expand knowledge on astrovirus diversity, host range, and circulation in wildlife, especially in urban and peri-urban environments.

Financial Support: FAPEMIG, CAPES, and CNPq.

PT.138

HPV32 AND VIRAL COINFECTION ARE ASSOCIATED WITH ORAL MUCOSAL LESIONS IN A BRAZILIAN METROPOLITAN POPULATION

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Oral HPV infection encompasses a broader spectrum of genotypes and epidemiological studies often ignore low-risk oral HPV genotypes and coinfections. Data on oral HPV diversity in heterogeneous clinical populations remain scarce, particularly in Brazil. This study investigated HPV prevalence and genotypes in individuals with and without oral lesions, analyzing associations with clinical, cytopathological, and behavioral traits. A retrospective cross-sectional study was conducted with 281 individuals attending an oral diagnosis outpatient clinic in a metropolitan region of Rio de Janeiro, Brazil. Sociodemographic, clinical, and behavioral data were collected from medical records. Oral mucosal scraping samples were analyzed by nested PCR for HPV detection and by type-specific touchdown PCR for genotyping of HPV6/11, HPV16, HPV18, and HPV32. Overall HPV prevalence was 29.9% (84/281). HPV16 was the most frequent genotype (41.7%). Low-risk genotypes were detected in 39.3% of positive individuals. HPV32 showed the strongest association with clinical oral lesions (OR = 7.08; 95% CI: 2.13–23.54; p = 0.001), while coinfection with multiple HPV types was also associated with clinical lesions (OR = 3.53; p = 0.034). Immunocompromise was independently associated with cytopathological alterations (aOR = 3.10; 95% CI: 1.15–8.34; p = 0.025). No HPV was detected in cancer cases. A high HPV prevalence was observed in this Brazilian population. Although HPV16 was most prevalent, the strongest clinical associations involved HPV32 and HPV coinfection. These findings highlight the relevance of non-classical genotypes in oral mucosal disease and support broader genotyping strategies in oral diagnostic settings.

PT.139

CHARACTERIZATION OF HUMAN VIRUSES IN HOSPITAL WASTE LEACHATE USING TARGETED METAGENOMIC SEQUENCING

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Healthcare waste management follows composition and risk. Brazilian law categorizes biological waste (Group A) and non-hazardous (Group D). Waste storage and transport decomposition produces leachate. To assess the viral community in a hospital waste leachate, in Rio de Janeiro city, Brazil, 24 samples of 500 mL (12 from Group A and 12 from Group D) were obtained monthly between September 2024 and February 2025. Samples were collected using sterile flasks, transported under refrigeration, concentrated by ultracentrifugation and combined into 12 pools (6 pools of 2 samples from each Group). Nucleic acid extraction was performed using the EXTRACTA® FAST – Viral DNA and RNA kit (Loccus, Brazil). The Illumina Viral Surveillance Panel v2 kit (Illumina, USA) was used to determine viral diversity. Libraries were sequenced on the NextSeq™ 2000 platform (Illumina) using the P3 sequencing kit (300 cycles), generating paired-end reads of 2×150 bp. Raw sequencing data were processed using the Illumina DRAGEN™ microbial enrichment pipeline. A total of 9,244,626 reads were obtained (444,362 from Group A leachate samples and 8,805,242 from Group D). Overall, 21 viruses from 8 species and 4 families were detected. The most predominant family was Polyomaviridae (71.4%), followed by Anelloviridae (14.3%), Herpesviridae (9.5%), and Papillomaviridae (4.8%). Predominant reads (90.5%) belonged to dsDNA viruses, with 33.3% achieving complete genome coverage (100%), predominantly from Group A leachate samples. Alphapolyomavirus quintihominis and Betapolyomavirus secuhominis were the most frequently detected species. This study provides the first characterization of the viral composition in healthcare waste leachate using targeted metagenomic sequencing.

Financial support: CNPq/PQ; CNPq PROEP/IOC; CNPq/PIBIC; FAPERJ/CNE; FAPERJ/JCNE; CAPES.

PT.140

High Prevalence of HTLV-1 Infection in Patients with Prostate Cancer

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Introduction: Cancer development is usually associated with multifactorial factors, including behavioral, environmental, and genetic components. Human oncovirus infections are among the associated factors, and human T-lymphotropic virus 1 (HTLV-1) was the first human retrovirus isolated from a patient presenting a rare type of leukemia. Material and Methods: In the present study, in collaboration with the National Institute of Science, Technology and Innovation in Genitourinary Cancer (INCT-UroGen), the presence of HTLV-1 infection was investigated in genomic DNA extracted from prostate tumor fragments preserved in paraffin blocks. A total of 200 samples were subjected to real-time polymerase chain reaction (qPCR) for the detection of HTLV-1 proviral DNA. Results: Molecular analysis detected proviral DNA in samples from 12 patients, a prevalence of 6%. Statistical analysis showed that the presence of the infection was not associated with clinical-laboratory parameters such as tumor staging (p=0.800), tumor extension (p=0.666), perineural invasion (p=0.357), lymphovascular invasion (p=1.00), pre-PSA (p=0.492), nadir-PSA (p=0.803), testosterone (p=0.936), and C-reactive protein (p=0.354). Although the presence of the infection was not associated with clinical-laboratory parameters, the high prevalence of this infection in tumor samples was six times higher than the national average for HTLV-1 infection in men. Conclusions: This is quite unusual and highlights the importance of more detailed investigations into this possible relationship between HTLV-1 and the development of prostate cancer, including the confirmation in prospective studies with larger cohorts.

Financial Support: National Institute of Science, Technology and Innovation in Genitourinary Cancer (INCT-UroGen/CNPq #408576/2024-3)

PT.141

Predominance of High-Risk HPV Genotypes in Cervical Lesions from Women in Northeastern Brazil

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Cervical cancer is the second most common type of cancer in the Northeast region of Brazil. This type of carcinoma is strongly associated with the presence of Human Papillomavirus (HPV), mainly due to high-risk genotypes. However, there is a genotypic variation from region to region in the epidemiology of HPV. Therefore, the objective of the study was to evaluate the prevalence of HPV genotypes in women with cervical lesions in the state of Sergipe, Brazil. For this, molecular diagnosis and HPV genotyping were obtained using the PCR technique and DNA sequencing. Samples from 146 patients were included, of which 80 (54.79%) were positive for HPV. Of these 80, nine different types of HPV were found, six of which were high-risk types (HRHPV), among which HPV16 (55.32%), the most prevalent, followed by HPV52 (10.64%), HPV31 (8.51%), HPV66 (6.38%), HPV45 (4.26%) and HPV58 (2.13%), which were associated with high-grade cervical lesions (HSIL) and neoplastic histopathologies,specifically CIN1 (23,08%), CIN2 (19,66%), CIN3 (17,09%) but none were related to carcinoma (CC). Although the highest prevalence is HPV16, the second most prevalent is HPV52 followed by other high-risk type, whose genotypes are classified for developing cervical cancer. Given that HPV16 is covered by the quadrivalent vaccine, it is worth considering the implementation of the nonavalent vaccine within the SUS, in light of the circulation of other high-risk types. Research of this nature has significant social impact by updating data on local viral circulation and enabling the assessment of vaccine efficacy.

PT.142

Epidemiological Profile and Molecular Diagnosis of Exanthematous Diseases in Five Hospitals in the State of São Paulo

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Exanthematous diseases constitute an important public health problem due to the diversity of etiological agents, the overlap of clinical manifestations, and the risk of re-emergence of vaccine-preventable viruses. The reduction in vaccination coverage and the increase in the international circulation of pathogens reinforce the need for laboratory surveillance and molecular diagnosis to support actions of the Sistema Único de Saúde (SUS). This study evaluated the circulation of viruses associated with exanthematous syndromes in patients treated at five public hospitals in the state of São Paulo between January 2025 and June 2026. A total of 493 clinical samples (serum, swab, and/or urine) were analyzed by RT-qPCR for a panel of nine viruses. 102 samples (20.5%) were positive for at least one viral agent. The highest detection frequencies occurred in adults (31%), middle childhood (29%), and teenagers (28%). Erythrovirus B19 was the most prevalent virus (7.0%), followed by Epstein-Barr virus (6.5%), human herpesvirus 6 (4.5%), cytomegalovirus (2.0%), HSV-1 (1.5%), HSV-2 (1.0%), and varicella-zoster and measles viruses (0.4% each), while rubella virus was not detected. Among positive cases, 30.4% presented comorbidities, 56% manifested exanthem, and 28.5% presented fever without a defined focus. Erythrovirus B19 and human herpesvirus 6 were identified in all age groups, while measles virus was detected only in teenagers and adults. The results demonstrate the relevance of molecular panels for differential diagnosis, strengthening epidemiological surveillance and early detection of re-emerging viruses.

Financial Support: FAPESP

PT.143

PREVALENCE OF OROPOUCHE VIRUS IN LOWLAND TAPIRS (*Tapirus terrestris*) IN BRAZIL

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Oropouche virus (OROV) is an enzootic arbovirus of public health importance across Latin America. Outbreaks in the Northern region and autochthonous cases in western Brazil suggest the silent circulation of this neglected virus. This study aimed to investigate viral exposure in tapirs (*Tapirus terrestris*) across three Brazilian biomes: the Amazon, Cerrado, and Pantanal. A serological survey was conducted to identify neutralizing antibodies against OROV using the plaque reduction neutralization test (PRNT) with strains BeAn 19991 and OROV 43. Of the 88 tapir samples analyzed, results revealed a seropositivity rate of 18.18% (16/88). Titers ranged from 20 to 320 in the PRNT50 assay with OROV BeAn 19991 (11.36%; 10/88) and from 20 to 80 in the PRNT50 assay with OROV 43 (13.64%; 12/88); four samples tested positive for both variants. The Cerrado biome showed the highest positivity rate for both variants: 6.82% (6/88) for OROV BeAn 19991 and 7.95% (7/88) for OROV 43. The identification of seropositive animals in urban areas of the Cerrado and Pantanal, combined with the co-circulation of the new OROV variant, highlights a complex epidemiological scenario of viral transition and adaptability at the wildlife-urban interface. These results reveal the importance of surveillance for emerging arboviruses in wildlife and demonstrate the circulation and dispersal of OROV among tapirs across Brazilian biomes between 2017 and 2025, confirming exposure to the pathogen and establishing the species as an important sentinel indicator for the surveillance of enzootic arboviruses.
Keywords: OROV, one health, wild mammals.
Financial Support: CAPES Finance Code 001, INCT-One.

PT.144

PREVALENCE OF MAYARO VIRUS IN FREE-LIVING LOWLAND TAPIRS (*Tapirus terrestris*) IN BRAZIL

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Mayaro virus (MAYV) is an enzootic arbovirus associated with the Amazon region and is considered a public health concern across Latin America, as it causes an acute febrile illness transmitted primarily by the bite of sylvatic mosquitoes (*Haemagogus*). Evidence from Brazil suggests the silent circulation of this neglected virus. Endemic to the Amazon and wild areas, the virus is driven to seek new hosts as deforestation and human encroachment reduce forest biodiversity. Accordingly, the aim of this study was to investigate MAYV exposure in lowland tapirs (*Tapirus terrestris*) in areas of Central-West Brazil, spanning the Amazon, Cerrado, and Pantanal biomes. A serological survey was conducted to identify antibodies against MAYV using the plaque reduction neutralization test (PRNT) on samples from 88 lowland tapirs. The results revealed a seropositivity rate of 2.27% (2/88). Both positive samples showed neutralizing titers of 80, while one female (sampled in 2016) originated from the Cerrado, and one male (sampled in 2023) from the Pantanal. These results raise concerns regarding MAYV circulation and geographic spread among tapirs across these three biomes between 2016 and 2025. The study illustrates Mayaro dispersal routes within the context of enzootic arbovirus surveillance in Brazil. The detection of neutralizing antibodies confirms the exposure of these large mammals to the pathogen, highlighting the importance of wild species as sentinel indicators for enzootic arbovirus surveillance.

Keywords: Mayaro, tapirs, PRNT, wild mammals.
Financial Support:CAPES - Finance Code 001. INCT-One.

PT.145

HUMAN ADENOVIRUS AND JC POLYOMAVIRUS TRACE ANTHROPOGENIC FECAL CONTAMINATION ACROSS SURFACE WATERS OF THE GUANABARA BAY WATERSHED

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This study investigated the occurrence of human adenovirus (HAdV) and JC polyomavirus (JCPyV) and their physicochemical drivers in surface waters of the Guanabara Bay watershed, southeastern Brazil. In November 2025, 2-L water samples were collected at 22 sites, including beaches, coastal lagoons, urban rivers, and canals. Physicochemical parameters (pH, temperature, dissolved oxygen, and salinity) were measured in situ using a Hanna HI4829 multiparameter water meter. Biochemical oxygen demand (BOD), phosphorus, and total, fixed, and volatile suspended solids (TSS, FSS, VSS) were measured in the laboratory. Beach and lagoon samples were concentrated by filtration/ultrafiltration, whereas river and canal samples by ultracentrifugation. Viral nucleic acids were extracted using the Maxwell® RSC PureWater Kit (Promega, Madison, WI, USA). Viral targets were detected and quantified by qPCR (ABI PRISM 7500 Real-Time System, TaqMan assays). Statistical analyses were performed in R Studio (v.4.3.1). HAdV and JCPyV were detected in 82% and 64% of samples, with mean viral loads of 5.72 and 4.55 log10 GC L^{-1}, respectively. Viral loads differed significantly among aquatic environments (ANOVA: HAdV, $p = 0.040$; JCPyV, $p = 0.032$). Redundancy analysis (RDA) explained 92.4% of the variance in viral loads (RDA1 = 68.2%; RDA2 = 31.8%). Both viruses were strongly associated with phosphorus, BOD, and suspended solids, corroborating significant positive Spearman correlations. Their complementary responses highlight their value as indicators of human fecal contamination in surface waters. Overall, the results confirm human fecal contamination and anthropogenic impacts on water quality. Financial Support: AGEVAP; CBH-BG.

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EPIDEMIOLOGICAL ANALYSIS OF HBOV INFECTION IN INFANTS BETWEEN JUNE 2024 AND JUNE 2026

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Human Bocavirus (hBoV) predominantly affects infants and young children aged 6 to 24 months. Because hBoV is frequently detected in co-infections with other respiratory viruses, its independent clinical significance remains challenging to establish. Continuous epidemiological surveillance is essential to elucidate viral dynamics, clinical profiles, and seasonal trends, thereby supporting the Brazilian National Health System (SUS) in optimizing pediatric healthcare resources. This study aims to investigate hBoV positivity, co-infection rates, and basic clinical patterns among hospitalized children from five public hospitals in São Paulo. A total of 11,224 naso-oropharyngeal samples collected between June 2024 and June 2026 were tested by RT-qPCR for 15 respiratory viruses. Of these, 697 samples were positive for hBoV, with 60.8% occurring in children aged 7 to 24 months. Co-infections were identified in 63.84% of hBoV-positive cases, most frequently with RSV (49.44%) and Rhinovirus (23.82%). Moderate to severe clinical manifestations were predominantly observed in children with underlying medical conditions or RSV co-infection. These findings underscore a high rate of hBoV co-infections in pediatric hospitalizations in São Paulo, highlighting the importance of targeted molecular surveillance to guide resource allocation and clinical management within the SUS.

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ENVIRONMENTAL SURVEILLANCE OF HEPATITIS A AND HEPATITIS E VIRUSES IN THE GUAÍBA WATER SYSTEM, SOUTHERN BRAZIL

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Hepatitis A virus (HAV) and Hepatitis E virus (HEV) are enteric viruses transmitted through the fecal–oral route that may persist in aquatic environments and serve as indicators of fecal contamination. Environmental surveillance of surface waters has emerged as an important tool for monitoring viral circulation and assessing potential public health risks. Located in a highly urbanized region in southern Brazil, the Guaíba water system (GWS) receives water from more than 200 municipalities and undergoes significant hydrological changes during seasonal flooding. This study aimed to investigate the occurrence of HAV and HEV in surface waters along the Guaíba system. Water samples were collected quarterly at 17 monitoring sites distributed along the Guaíba system shoreline. Samples were concentrated by ultracentrifugation, followed by RNA extraction using the Maxwell® automated system and RT-qPCR targeting the HAV 5′-UTR and HEV ORF3 regions. During the first sampling campaign (March 2026), HAV RNA was detected in 5/17 samples, whereas HEV RNA was detected in 1/17 samples. Analyses of the second campaign (May 2026) revealed the presence of HAV in 2/17 samples, while HEV was not detected. These findings demonstrate the circulation of enteric hepatitis viruses within the population, whose wastewater is fed into the Guaiba system, and highlight the value of environmental surveillance for assessing fecal contamination in urban freshwater systems. Continued monitoring will provide insights into seasonal trends and support complementary data to public health strategies.

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ENVIRONMENTAL SURVEILLANCE OF SARS-CoV-2 AND INFLUENZA A VIRUSES IN THE GUAÍBA WATER SYSTEM, SOUTHERN BRAZIL

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Environmental surveillance of surface and wastewater has emerged as a complementary strategy for monitoring viral circulation and supporting public health. The Guaíba watershed system (GWS) receives water from 251 municipalities of Rio Grande Sul state (southern Brazil). During its seasonal flooding, from April to July, GWS undergoes hydrological changes, reinforcing its importance for environmental monitoring. This study aimed to investigate SARS-CoV-2 and Influenza A virus (IAV) in samples from two collections in March and May 2026 at 17 sites along the GWS. Water samples from both collections were concentrated by ultracentrifugation at 110,000 × g for 1 hour, followed by glycine incubation (pH 9.5) and centrifugation at 3,000 × g for 20 minutes, with a second ultracentrifugation cycle performed under the same conditions. Viral RNA was extracted using the Maxwell® RSC Viral Total Nucleic Acid Purification Kit and SARS-CoV-2 and IAV were investigated by RT-qPCR targeting the nucleocapsid (N) and matrix (M) genes, respectively. During the first sampling campaigns (March 2026), SARS-CoV-2 was detected in 7/17 samples and IAV in 1/17 samples. In samples from the second timepoint (May 2026), both SARS-CoV-2 and IAV were detected only in 1/17 samples. The detection of respiratory viruses in urban surface waters demonstrates the potential of environmental surveillance as a complementary component of epidemiological surveillance by monitoring viral circulation within the contributing population. Continued monitoring will improve the understanding of viral dynamics, support the early detection of changes in viral circulation, and strengthen integrated public health surveillance.

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WASTEWATER MONITORING OF HUMAN VIRUSES AT THE BRAZILIAN ANTARCTIC STATION

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Wastewater-based epidemiology has become an important tool for public health surveillance, particularly following the COVID-19 pandemic. Monitoring human viruses in wastewater enables the early detection of pathogens shed by infected individuals and complements traditional epidemiological surveillance. However, information on wastewater viral surveillance in remote regions such as Antarctica remains scarce. This study aimed to investigate the presence of human viruses in wastewater from the treatment plant of the Brazilian Antarctic Station Comandante Ferraz (EACF) as part of the VIDEANTAR project. Three wastewater samples were collected during the Brazilian Antarctic Expedition (OPERANTAR XLIV) in November and December 2025. Samples were concentrated by ultracentrifugation, viral nucleic acids were extracted using the MagMAX CORE Nucleic Acid Purification Kit, and analyzed by real-time RT-qPCR for SARS-CoV-2, Influenza A virus, hepatitis A and E viruses, norovirus GI and GII, poliovirus, Oropouche virus, Zika virus, and flaviviruses. SARS-CoV-2 RNA was detected in one of the three samples, whereas all samples tested negative for the remaining viruses. To our knowledge, this is the first report of SARS-CoV-2 detection in wastewater from the EACF. Despite the strict health and biosafety measures implemented by the Brazilian Antarctic Program (PROANTAR), including medical screening and vaccination requirements, the detection of SARS-CoV-2 suggests that viral circulation may still occur, highlighting wastewater surveillance as a valuable complementary tool to strengthen epidemiological surveillance and biosafety in Antarctic research stations.

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EPIDEMIOLOGICAL CHARACTERISTICS AND SUBTYPE DISTRIBUTION OF RESPIRATORY SYNCYTIAL VIRUS IN ALAGOAS, BRAZIL, 2024-2026

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Respiratory Syncytial Virus (RSV) causes severe respiratory infections worldwide. This study analyzed laboratory-confirmed RSV cases from the Central Public Health Laboratory of Alagoas (LACEN-AL) between 2024 and the first semester of 2026. Samples were tested by RT-qPCR and subsequently genotyped. A total of 1,025 cases were identified: 165 in 2024 (16.1%), 559 in 2025 (54.5%), and 301 in early 2026 (29.4%). Genotyping revealed an alternating circulation pattern: RSV-A predominated in 2024 (73.3%), whereas RSV-B was predominant in 2025 (71.5%) and 2026 (55.8%). No gender differences were observed. Infants under one year accounted for most cases across all years and genotypes (n = 573; 55.9%). Maceió recorded the highest number of cases for both subtypes, followed by Arapiraca, União dos Palmares, and Marechal Deodoro in the interior. These findings highlight the importance of laboratory surveillance in Alagoas to monitor seasonal subtype dynamics and guide targeted prevention strategies.

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HUMAN ENTERIC VIRUSES, CRASSPHAGE, AND PMMOV IN NILE TILAPIA FROM A TROPICAL COASTAL LAGOON: A SENTINEL SPECIES FOR HUMAN FECAL POLLUTION

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This study investigated the occurrence of the human enteric viruses *Rotavirus alphagastroenteritidis* (RVA), human astrovirus (HAstV; species *Mamastrovirus hominis*), and human mastadenovirus (HAdV) in Nile tilapia (*Oreochromis niloticus*) and evaluated their correlation with the human fecal contamination markers as bacteriophage *Carjivirus communis* (crAssphage) and *Pepper mild mottle virus* (PMMoV). A total of 64 samples, including gills and digestive tracts from 32 fish collected from an impacted lagoon in Rio de Janeiro, Brazil, between October 2022 and July 2023, were analyzed. Viral particles were recovered using an enzymatic processing protocol adapted from ISO 15216-1:2017, followed by nucleic acid extraction and viral genome quantification using qPCR and RT-qPCR (TaqMan assays). Mengovirus, used as an internal process control, was detected in 98.4% of the samples, with a median recovery efficiency of 83.5%. RVA was prevalent (26.5%; 17/64), with a median concentration of 3.9×10^3 genomic copies (gc)/g, followed by HAstV (15.6%; 1.3×10^2 gc/g) and HAdV (4.7%; 2.2×10^1 gc/g). PMMoV and crAssphage were detected in 71.9% and 59.4% of the samples, respectively, with median viral loads of 5.1×10^4 and 7.2×10^3 gc/g. Significant positive correlations ($p < 0.05$) were observed between crAssphage and HAstV ($\rho = 0.49$), as well as between PMMoV and HAdV ($\rho = 0.41$) and RVA ($\rho = 0.37$). This study provides the first application of crAssphage and PMMoV as viral fecal contamination markers in fish and demonstrates that Nile tilapia can serve as a sentinel species for monitoring human fecal pollution in impacted aquatic ecosystems.

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REGIONAL AND TEMPORAL DISPARITIES IN VIROLOGICAL CONFIRMATION AMONG VIRAL MENINGITIS CASES IN BRAZIL, 2015-2025

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Introduction: The etiological confirmation of viral meningitis depends on specific laboratory methods, whose availability may vary across Brazilian regions and compromise the comparability of epidemiological indicators. **Objective**: To assess regional inequalities and temporal trends in the virological confirmation of viral meningitis in Brazil between 2015 and 2025. **Methodology**: Ecological time-series study including viral meningitis cases with a known region recorded in the Notifiable Diseases Information System. Virological confirmation was defined as a record of PCR or viral isolation. Proportions and 95% confidence intervals were calculated using the Wilson method. Regional differences were assessed using Pearson's chi-square test, and temporal trends using quasibinomial regression with a logit link, including year, region, and the year × region interaction. A sensitivity analysis was performed excluding provisional data from 2025. **Results**: Among 67,393 cases, 7,041 had virological confirmation. The national proportion increased from 5.31% in 2015 to 20.56% in 2025. The highest proportion occurred in the South (15.76%) and the lowest in the North (2.63%), with statistically significant regional heterogeneity ($p<0.001$). The annual odds of confirmation increased in the Northeast (OR=1.52; 95% CI:1.401.66), Southeast (OR=1.21; 95% CI:1.18-1.24), and Central-West (OR=1.48; 95% CI:1.24-1.75), with no significant trends in the North or South. The year × region interaction was significant ($F=32.01$; $p<0.001$) and remained significant after excluding 2025 ($F=19.81$; $p<0.001$). **Conclusion**: There was an increase in virological confirmation, although regionally heterogeneous, suggesting persistent inequalities in the availability, use, or reporting of laboratory methods and reinforcing the need to expand and standardize laboratory surveillance throughout the country.

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REDISTRIBUTION OF OROPOUCHE FEVER DETECTIONS IN BRAZIL BETWEEN 2024 AND 2025

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Introduction: Oropouche fever, historically concentrated in the Amazon region, expanded to extra-Amazonian areas during the 2024 outbreak, suggesting a change in the distribution of detections. **Objective:** To compare the regional distribution of Oropouche fever detections in Brazil between 2024 and 2025. **Methods:** Ecological study using data from the Ministry of Health's Oropouche Epidemiological Dashboard, aggregated by region according to the state of the probable place of infection. Population estimates were obtained from the Brazilian Institute of Geography and Statistics. Detection rates per 100,000 inhabitants, regional proportions, rate ratios, and 95% confidence intervals were calculated. The regional distribution was compared using Pearson's chi-square test, with standardized residuals and Cramér's V. **Results:** A total of 13,856 detections were recorded in 2024 and 11,988 in 2025, with a reduction in the national rate from 6.52 to 5.62 per 100,000 inhabitants. In the Southeast, the proportion increased from 45.7% to 86.2%, and the rate increased by 62.8%, from 7.15 to 11.64 per 100,000 inhabitants (rate ratio=1.628; 95%CI: 1.578-1.679). In contrast, the North decreased from 41.9% to 1.4% of detections and showed a 97.0% reduction in the rate (rate ratio=0.030; 95%CI: 0.025-0.034). The regional distribution differed between the years ($p<0.001$; Cramér's V=0.491), mainly due to changes in the North and Southeast. **Conclusion:** A marked regional redistribution of detections was observed, with concentration in the Southeast in 2025. The results should be interpreted considering differences in testing, epidemiological investigation, definition of the probable place of infection, and the potentially provisional nature of the 2025 data.

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MOLECULAR DETECTION OF INFLUENZA VIRUS IN BIRDS FROM THE ANTARCTIC PENINSULA DURING THE 2024/2025 AND 2025/2026 SUMMERS

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Influenza A virus (Alphainfluenzavirus influenzae, FLUAV) is a negative-sense, segmented single-stranded RNA virus. FLUAV infects wild and domestic birds, including poultry, posing a significant threat to animal health and production. Wild waterfowl are the primary natural reservoirs of FLUAV, and contribute to the long-distance dissemination of the virus. Recent outbreaks of highly pathogenic H5N1 strains led to mass mortality of seabirds reported across South America between 2022 and 2023, followed by cases in the Antarctic region by the end of 2024. Since then, multiple cases of H5N1 FLUAV infection have been confirmed on the Antarctic Peninsula. During the summer of 2024/2025 and 2025/2026, a total of 90 and 54, respectively, bird fecal samples were collected from Chabrier Rock, Hannah Point, Hennequin Point and Two Summit. Total nucleic acids were extracted using the Kingfisher™ automated extraction system with the MagMAX™ CORE Nucleic Acid Purification Kit. Samples were analyzed by reverse transcription followed by a qPCR targeting the matrix (M) gene. Eight samples tested positive for FLUAV across both sampling seasons. These findings demonstrated the continuous presence of FLUAV in birds that breed and forage in Antarctic region since the 2023 outbreak. While some of the sampled species are Antarctic residents, others undertake medium and long-distance migrations outside the breeding season, coming into contact with other bird and mammal populations, facilitating the geographic spread of FLUAV. Thus, the maintenance of FLUAV surveillance in Antarctic is fundamental to predict new FLUAV outbreaks and comprehend the new patterns of virus circulation worldwide.

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INFLUENZA A VIRUS SURVEILLANCE IN AMERICAN OYSTERCATCHERS (*HAEMATOPUS PALLIATUS*) IN THE COAST OF RIO GRANDE DO SUL, BRAZIL

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Wild birds are the primary natural reservoirs of Influenza A virus (IAV), and the infection has previously been reported in American Oystercatchers (*Haematopus palliatus*) in South America. The American Oystercatcher is a coastal shorebird widely distributed along the Brazilian coastline, where it shares habitats with migratory species and may be exposed to a diverse range of pathogens. This study investigated the presence of IAV in American Oystercatchers sampled along the southernmost coast of Brazil. A total of 44 oropharyngeal and/or cloacal swab samples were collected between November 2025 and February 2026 from the northern and central regions of the coast of Rio Grande do Sul state. Viral RNA was extracted using the automated Maxwell® system, and molecular detection was performed using the AgPath-ID™ One-Step RT-PCR Kit targeting the influenza A matrix gene, followed by investigation of the H5 subtype in positive samples. IAV RNA was detected in three samples (6.8%; 3/44), and two were positive for the H5 subtype (4.5%; 2/44). These findings demonstrate the circulation of IAV, including H5, in coastal American Oystercatcher populations, highlighting the importance of this species as a sentinel for pathogen circulation in marine and estuarine ecosystems and the potential role of resident coastal birds that share habitats with migratory species in IAV transmission in IAV ecology. These results emphasize the need for continuous surveillance programs to improve understanding of viral ecology and support early warning strategies for pathogens of public health relevance.

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PT.156

Comparison of Sample Preparation Strategies for Metagenomic Detection of Enteric Viruses in Milk

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Metagenomics has emerged as an important tool for the surveillance of foodborne viruses; however, its sensitivity depends on the efficiency of sample preparation and viral enrichment procedures. This study aimed to compare two sample preparation strategies to optimize the recovery of enteric viruses from milk samples for metagenomic sequencing. A milk sample was artificially inoculated with human adenovirus type 5 (HAdV-5), norovirus, rotavirus A, and mengovirus, subjected to viral concentration, and divided into two protocols. The first protocol consisted of filtration through a 0.45 µm syringe membrane filter, whereas the second included an additional centrifugation step using a 25% sucrose cushion after filtration. Sequencing was performed on an Illumina platform, and primary data analysis was conducted using CZ ID. Both protocols enabled the detection of all inoculated viruses. However, the filtration-only protocol yielded higher viral sequence recovery, with 3,456 reads for HAdV-5, 2,710 for mengovirus, 328 for norovirus, and 22 for rotavirus A. The protocol including the sucrose cushion generated substantially fewer reads for all viruses evaluated. These findings indicate that the addition of a 25% sucrose cushion did not improve viral recovery and may have reduced recovery efficiency under the conditions evaluated. The proposed workflow, based on viral concentration followed only by 0.45 µm membrane filtration, represents a simpler and more efficient approach for metagenomic detection of enteric viruses in dairy matrices, contributing to the standardization of sample preparation protocols.

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Evaluation of Viral Concentration Protocols for Environmental and Fecal Samples Using Next-Generation Sequencing

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Next-generation sequencing (NGS) and metagenomics have transformed virology by enabling the detection of viral genomes in complex samples without prior knowledge of target viruses, thereby expanding epidemiological surveillance. This study evaluated the efficiency of viral concentration protocols for environmental and fecal samples using NGS by assessing the recovery of the mengovirus genome. In September 2025, two water samples and four fecal samples were collected from two dairy farms in the Vale do Paranhana region, Rio Grande do Sul, Brazil. Samples were processed using matrix-specific concentration methods: centrifugation in minimum essential medium (MEM) for fecal samples and ammonium sulfate precipitation followed by ultracentrifugation for water samples. Fecal samples were pooled (F1), and both matrices were inoculated with 20 µL of mengovirus (5.52 log10 genome copies/µL) before filtration through 0.45 µm membranes. Nucleic acids were extracted using the MagMAX Core Nucleic Acid Purification Kit on the KingFisher Duo Prime system. Libraries were prepared with the Illumina DNA Prep kit and sequenced on the Illumina NextSeq platform. Sequence assembly using the CZ ID platform generated 361 contigs for A1, 75 for A2, and 584 for F1. Mengovirus was successfully recovered from all samples, achieving 100% genome coverage and 99.8%–99.9% nucleotide identity with reference genomes (DQ294633.1 and L22089.1). No additional exogenous viruses were detected. These results demonstrate the effectiveness of the concentration protocols and metagenomic workflow for viral genome recovery from environmental and fecal matrices, supporting their use in viral surveillance studies. **Financial support:** CAPES, CNPq, Universidade Feevale, and PPSUS/MS/FAPERGS No. 01/2025.

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Integrated entomological surveillance reveals the co-circulation of arboviruses and insect-specific viruses in urban and sylvatic mosquitoes from southeastern Brazil

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Arboviral diseases have risen in recent decades, especially in tropical and subtropical regions. Brazil is a hotspot for the emergence and re-emergence of arboviruses, with frequent outbreaks. To investigate the circulation of arboviruses and insect-specific viruses (ISVs), we conducted surveillance from October 2023 to October 2025 in urban and sylvatic areas of São José do Rio Preto, Brazil. Mosquitoes were screened by RT-qPCR, and positive samples underwent virus isolation and whole-genome sequencing. We collected 3,312 mosquitoes from eight genera. Among 171 arbovirus-positive samples, chikungunya virus (CHIKV) was predominant (85.3%), followed by dengue virus (DENV), comprising DENV-2 (10.5%), DENV-3 (2.3%), DENV-1 (1.2%), and DENV-2/DENV-3 coinfection (0.6%). Zika virus, Yellow Fever virus, Oropouche virus, and Mayaro virus were not detected. The highest detection rate was in 2024, coinciding with the first CHIKV epidemic and the introduction of new dengue genotypes in the area. Whole-genome sequencing generated 66 CHIKV, four DENV-2, and one DENV-3 genomes. Phylogenetic analyses classified DENV-2 as genotype II, DENV-3 as genotype III, and all CHIKV genomes as the East/Central/South-African lineage. ISV screening revealed widespread circulation of Phasi-Charoen-like virus (PCLV) (25.6%) and Humaita Tubiacanga virus (HTV) (4.5%), with frequent coinfections (21.8%) in both urban and sylvatic mosquitoes, including species of the genera *Limatus, Sabethes,* and *Psorophora.* Four high-coverage PCLV genomes clustered with Brazilian and Asian isolates. Frequent ISV–arbovirus coinfections suggest potential interactions affecting vector competence, and the concordance between mosquito infections and human cases underscores the importance of integrated entomological surveillance.

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CRASSPHAGE AND HUMAN MASTADENOVIRUS AS VIRAL INDICATORS OF FECAL CONTAMINATION IN AN ANTHROPOGENICALLY IMPACTED URBAN LAGOON: A QPCR AND VIROME APPROACH

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This study evaluated crAssphage and human mastadenovirus (HAdV) as molecular indicators of human fecal contamination and integrated targeted qPCR with virome sequencing to improve environmental viral surveillance. Forty-eight surface water samples were collected quarterly from an urban lagoon between August 2024 and June 2025 and concentrated by filtration/ultrafiltration. Viral nucleic acids were extracted using the Maxwell® RSC PureWater Kit (Promega, Madison, WI, USA). Viral targets were detected and quantified by qPCR (ABI PRISM 7500 Real-Time System, TaqMan assays). To complement targeted detection, positive samples pooled by site were characterized using the Illumina Surveillance Panel V2 on the NextSeq™ 2000 (Illumina®, USA) with a P3 sequencing kit (300 cycles; 2 × 150 bp). Raw reads were processed in Galaxy, and viral sequences were identified by BLASTn against the NCBI database. Statistical analyses were performed using Mann–Whitney and Kruskal–Wallis tests in GraphPad Prism v10.2.3. CrAssphage was detected in 47.9% of samples (median: 4.61 $\log_{10}$ GC L^{-1}) and HAdV in 25.0% (median: 1.93 $\log_{10}$ GC L^{-1}). CrAssphage concentrations were significantly higher than HAdV (p<0.0001), with no spatial differences among sampling sites. CrAssphage remained temporally stable, whereas HAdV showed seasonal variation (p = 0.025), peaking in winter and not detected in spring. Virome analysis revealed 1.33–1.86% viral scaffolds, mainly viruses infecting cyanobacteria and halophilic algae; however, neither qPCR target was recovered, suggesting low abundance or metagenomic limitations. Together, targeted qPCR and virome sequencing provide complementary information for monitoring human fecal contamination and assessing the sanitary quality of urban coastal waters.

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SEROLOGICAL SURVEILLANCE OF YELLOW FEVER VIRUS IN SMALL MAMMALS FROM SERRA DO CIPÓ, MINAS GERAIS, BRAZIL

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The Parque Natural Municipal Mata da Tapera, located in the Serra do Cipó region, is a preserved area near areas with heavy human traffic. The state of Minas Gerais (MG) has intense circulation of several arboviruses, including the Yellow Fever virus (YFV), in urban, peri-urban, and sylvatic areas. In addition, during 2017–2018, the most significant Yellow Fever outbreak in Brazil was recorded, with 480 confirmed cases and 152 deaths in MG. Therefore, monitoring YFV circulation among wild animals inhabiting regions such as Serra do Cipó is essential, given the high influx of people in the area. This study aimed to conduct a serological survey in wild small mammals to investigate YFV circulation in Mata da Tapera. Between March 2023 and September 2024, animals were captured using Tomahawk traps (n = 17). Serum samples were tested by plaque reduction neutralization test (PRNT50) using a wild-type YFV strain (HUBR2018). Captured animals were identified as *Didelphis albiventris* (n = 4), *Marmosops incanus* (n = 9), and *Nectomys squamipes*(n = 4). Four *Nectomys squamipes* (100%) individuals were seropositive for wild-type YFV, with neutralizing antibody titers of 1:320. The remaining 13 individuals were negative. These findings suggest YFV circulation in the study area and provide evidence of the possible role of small mammals as sentinels or potential reservoirs, supporting improved surveillance and public health decision-making in MG.

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PT.161

Dengue epidemiology in Irecê-BA: a restrospective study over the lady 19 years in the semiarid

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Dengue is a public health problem in Brazil, especially in low-income regions, as the semiarid. However, long-term epidemiological data from the Brazilian semiarid are scarce, despite being regions heavily affected by dengue. This study presents the first epidemiological analysis of dengue in Irecê, a city located in the semiarid of the Bahia State, using surveillance data collected over the last 19 years. A retrospective ecological study was conducted using dengue notifications recorded in the Brazilian Notifiable Diseases Information System (SINAN) from January 2005 to December 2023. After data cleaning, deduplication, and validation, 12,832 confirmed notifications were included and grouped into two historical series (2005–2013 and 2014–2023). Descriptive and statistical analyses were performed using Chi-square and Fisher's exact tests considering a $p<0.05$. The second series (2014-2023) showed a marked reduction in notifications (9,469 vs. 3,363 cases). Comparing gender of notification, female proportion decreased in the second series. A significant increase in notifications among mixed-race individuals was observed. Compared with males, females had lower odds of fever, rash, nausea, positive tourniquet test, and epistaxis, but higher odds of vomiting. Hospitalization was significantly less frequent in the second series, whereas mortality increased. These findings reveal important temporal changes in the epidemiological profile of dengue in Irecê and provide the first long-term evidence to support surveillance and prevention strategies in the semiarid of Bahia state, Brazil.

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INTRAFAMILIAL TRANSMISSION OF HTLV-1/2 AMONG INDIVIDUALS ATTENDING AT A REFERENCE CENTER IN PARÁ

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Introduction: Human T-lymphotropic virus (HTLV) is considered a significant public health issue in endemic regions, particularly due to its potential to cause diseases such as Adult T-cell Leukemia/Lymphoma (ATL) and HTLV-associated myelopathy (HAM). Mother-to-child transmission, especially via breastfeeding, and sexual transmission are the primary routes of HTLV spread, contributing to the persistence of intrafamilial infection. This study describes intrafamilial HTLV-1/2 transmission among individuals monitored at a reference center in Pará. Material and methods: Socio-epidemiological data and kinship information from 160 HTLV-infected individuals were evaluated to identify probable transmission events within family units. Results: Of the individuals analyzed, 42 (26.3%) formed 21 pairs suggestive of intrafamilial transmission. Of these pairs, 12 (57.1%) involved a mother-child relationship, and 9 (42.9%) involved a spouse-spouse relationship. The prevalence of intrafamily infection ranged from 40% to 100%. The occurrence of cases within the same family unit, evidenced by mother-child pairs, suggests the importance of vertical transmission in perpetuating the infection across generations. At the same time, the identification of infected spouses reinforces the role of sexual transmission in the virus's spread. Conclusion: These results highlight the importance of serological screening for the family members of HTLV-infected individuals and underscore the necessity of implementing preventive measures and counseling aimed at halting vertical and sexual transmission. Such strategies are essential for minimizing viral spread and the impact of HTLV-associated diseases, particularly in areas of high endemicity.

Keywords: HTLV, intrafamilial, sexual, transmission

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ASSESSING VIRAL RISK IN FRESHWATER ECOSYSTEMS: DEVELOPMENT OF A FRAMEWORK FOR ENVIRONMENTAL EPIDEMIOLOGICAL ASSESSMENT

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Enteric viruses are important indicators of environmental contamination and a significant public health concern given their involvement in waterborne and foodborne disease outbreaks. Their occurrence, persistence, and transport in aquatic ecosystems are influenced by a complex interplay of hydrological dynamics, physicochemical conditions, and anthropogenic contaminants, including microplastics (MPs), which can affect pathogen dissemination and fate in the environment. Monitoring these viruses alongside environmental parameters and contaminant distribution is therefore essential for understanding transmission pathways, identifying areas of increased exposure risk, and supporting evidence-based public health management. This study aimed to create a spatial risk assessment map for environmental viral contamination in the metropolitan region of Florianópolis, Southern Brazil, by integrating data on enteric viruses, environmental parameters, and anthropogenic contamination indicators to identify potential hotspots and guide public health surveillance. Data from 13 previously monitored sampling sites were compiled into a georeferenced database. Enteric virus occurrence information was integrated with water physicochemical parameters, urbanization index, and microplastic contamination data using GIS-based spatial analysis, resulting in the development of a Viral Environmental Surveillance Index (IVV). Results revealed marked spatial variability in contamination levels, with high-, moderate-, and low- risk zones reflecting a heterogeneous distribution across the study area and allowing the identification of hotspot zones for surveillance and environmental management. The integration of these indicators proved effective for spatial risk assessment, positioning the IVV as a valuable decision-support tool for environmental monitoring and public health planning.

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PT.164

HIDDEN FROM SURVEILLANCE: THE PUBLIC HEALTH BURDEN OF HUMAN ADENOVIRUS IN ALAGOAS

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Human adenoviruses (HAdVs) are viruses belonging to the *Adenoviridae* family and are major causative agents of respiratory, ocular and gastrointestinal infections. Although HAdV infections are generally self-limiting, they represent a significant public health concern due to their widespread global distribution, high transmissibility and potential to cause outbreaks. This study aimed to determine the detection frequency of HAdV in clinical samples from nasal swabs and to describe the epidemiological profile of positive cases in Alagoas. Clinical samples collected between 2024-2026 were analyzed and processed at the Central Public Health Laboratory of Alagoas (LACEN-AL). The samples underwent genetic material extraction, followed by molecular detection of HAdV via qPCR (Kit molecular VR1/VR2 Bio-Manguinhos). Statistical analyses were performed using R software, examining the distribution of cases by sex, age group, municipality and positivity rate. A total of 17.897 clinical samples were analyzed, of which 648 tested positive for HAdV (3.6%). Positive cases were more frequent among females (51.2%) and were concentrated in the 1-4 age group (32.1%). Geographic distribution revealed the presence of cases across 13 municipalities, with the highest frequency in Maceió (18.5% of cases). Among the HAdV-positive samples, 33.2% showed coinfections with other viruses such as HHRV, RSV, INFA, HMPV, INFB and SARS-Cov-2. Furthermore, over 50% of the positive cases lacked a notification record in the epidemiological surveillance system. These data contribute to the understanding of HAdV epidemiology in the state and reinforce the importance of molecular detection as a tool for monitoring viral circulation.
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PT.165

IMPLEMENTATION OF LONG-ACTING CABOTEGRAVIR AS HIV PRE-EXPOSURE PROPHYLAXIS IN THE BRAZILIAN UNIFIED HEALTH SYSTEM: CHALLENGES AND PERSPECTIVES

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The incorporation of long-acting cabotegravir (CAB-LA) is a milestone in HIV prevention, particularly for vulnerable populations facing low adherence to daily oral PrEP. This study analyzes the implementation perspectives and operational challenges of CAB-LA within the Brazilian Unified Health System (SUS), integrating public health guidelines with epidemiological indicators from DataSUS, the National PrEP Monitoring Panel, and Brazilian implementation cohorts (ImPrEP). Although national databases register a robust scale-up exceeding 100,000 users, the data reveals critical disparities and alarming rates of treatment discontinuation and loss to follow-up, specially among young, black, and transgender individuals who struggle with daily pill burdens. Analysis indicates that while long-acting injectables overcome oral retention barriers, nationwide rollout faces structural bottlenecks. Qualitative and quantitative data highlight that Specialized Care Services (SAEs) concentrate infrastructure but impose access barriers due to HIV-related stigma, whereas Primary Care lacks human resources to absorb preventative demands. The transition to an injectable model introduces new operational complexities: the necessity of decentralized services to reach peripheral communities, and strict diagnostic protocols to prevent delayed seroconversion and viral resistance during the pharmacologic "tail effect". In conclusion, CAB-LA has the virological potential to alter the HIV epidemic in Brazil by mitigating oral PrEP attrition. However, its success depends on decentralizing administration beyond central SAEs, implementing peer-educator strategies, and securing rigorous testing logistics to maintain diagnostic windows during bimonthly clinic visits.

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PT.166

HIV PRE-EXPOSURE PROPHYLAXIS PLUS DOXY-PEP: A NEW PARADIGM IN SEXUALLY TRANSMITTED INFECTION PREVENTION

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The synergy between HIV Pre-Exposure Prophylaxis (PrEP) and Doxycycline Post-Exposure Prophylaxis (Doxy-PEP) represents a groundbreaking paradigm shift in comprehensive sexual health. Aligned with recent 2026 CONITEC federal reports expanding doxycycline within the Brazilian Unified Health System (SUS), this study analyzes the epidemiological impact and microbiological bottlenecks of this dual strategy. While clinical evidence indicates that 200 mg of doxycycline taken within 72 hours post-intercourse sustains a 60-80% reduction in chlamydia and syphilis, recent 2025-2026 real-world data demonstrates that protection against Neisseria gonorrhoeae is non-durable. Molecular surveillance reveals a rapid loss of gonococcal efficacy (dropping to negative values) driven by the selection of high-level tetracycline resistance via the `tetM` gene and cross-susceptibilidade shifts linked to the mosaic `penA34.007` allele. Metagenomic profiles also confirm a dose-dependent enrichment of resistance genes in the gut resistome and a significant increase in tetracycline-resistant Staphylococcus aureus colonization (rising from 5% to 13%). Furthermore, mathematical models project a complete clinical efficacy loss within 1.6 to 12 years depending on population adoption rates. In conclusion, combining HIV PrEP with Doxy-PEP offers a highly effective shortcut to curb syphilis and chlamydia, mitigating oral PrEP-associated STI attrition. However, translating this paradigm into long-term public health policies within the SUS depends on transitioning from indiscriminate prescription to strict clinical targeting, combined with robust, decentralized antimicrobial resistance (AMR) surveillance networks to balance individual prophylaxis benefits against global evolutionary risks.

Financial Support: None.

PT.167

Molecular Investigation of Influenza A/B Viruses, Herpesviruses, and Adenoviruses in Marine Mammals from the Coast of Alagoas State, Brazil

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In the context of emerging zoonotic diseases and the One Health concept, viral circulation among different species highlights the importance of surveillance of pathogens with the potential for interspecies transmission. Influenza viruses (*Orthomyxoviridae*), herpesviruses (*Orthoherpesviridae*), and adenoviruses (*Adenoviridae*) have been reported in marine mammals and are associated with a range of clinical manifestations. This study aimed to investigate the presence of Influenza A and B viruses, herpesviruses, and adenoviruses in stranded marine mammals from the coast of Alagoas, Brazil, using molecular techniques. A total of 113 samples from 50 specimens, provided by the Biota Institute of Conservation, were analyzed. Samples included skin, lung, reproductive organs, lymph nodes, central nervous system, and intestinal tissues preserved in 10% formaldehyde, 100% ethanol, or stored at −20 °C. RNA and DNA were extracted and analyzed by RT-qPCR for Influenza A and B viruses and by nested PCR for pan-herpesvirus and pan-adenovirus detection. No samples tested positive for Influenza A or B viruses. However, two tissue samples were positive for herpesviruses and seven for adenoviruses. Positive samples were purified, quantified, and prepared for Sanger sequencing. These findings demonstrate the circulation of herpesviruses and adenoviruses in stranded marine mammals from the coast of Alagoas, while no evidence of Influenza A or B virus infection was detected. Sequencing will allow viral species identification, contributing to the epidemiological characterization of these pathogens and supporting future One Health surveillance strategies. Financial support: CAPES, CNPq, FAPEAL and FINEP.

PT.168

ASSESSMENT OF THE IMPACT OF THE COVID-19 PANDEMIC ON THE CIRCULATION OF RESPIRATORY VIRUSES IN CHILDREN.

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Introduction: The COVID-19 pandemic affected the circulation of respiratory viruses and may have altered their seasonal patterns, particularly among hospitalized children. **Objective:** To assess the impact of the COVID-19 pandemic on the circulation of respiratory viruses in pediatric patients. **Methods:** A total of 16,977 nasopharyngeal swabs from 13,176 children admitted to six public hospitals in São Paulo, Brazil, between February 2020 and February 2023 were analyzed. Patients were stratified into five age groups: newborns (708 samples), early childhood (3,355), second childhood (4,647), children (3,764), and adolescents (701). Respiratory viruses were detected by RT-qPCR. **Results:** SARS-CoV-2 was detected in 586 samples (3.4%), with similar positivity among boys (3.5%) and girls (3.4%). The highest positivity occurred in second childhood (20.7%) and early childhood (14.7%). Overall, 9,771 samples (57.6%) tested positive for at least one respiratory virus. Respiratory Syncytial Virus (RSV) was the most frequently detected virus (39.7%), followed by Bocavirus (13.6%), Adenovirus (12.8%), Parainfluenza (11.0%), Rhinovirus/Enterovirus (6.2%), seasonal coronaviruses (5.3%), Metapneumovirus (3.7%), and Influenza (1.4%). **Conclusion:** All respiratory viruses were detected across age groups, although hospitalizations were most frequent among children aged 31 days to 2 years. SARS-CoV-2 circulated throughout the study period, peaking in 2022. Influenza showed the lowest incidence, while RSV, Metapneumovirus, and Influenza exhibited changes in seasonal circulation compared with the pre-pandemic period, indicating that the COVID-19 pandemic altered the epidemiological pattern of respiratory viruses in children.

Keywords: SARS-CoV-2; Respiratory viruses; Seasonality; Lockdown; Pandemic.

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Prevalence of Human T-lymphotropic virus 1 (HTLV-1) infection among people living with HIV/AIDS in São Luís, Maranhão

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Human T-lymphotropic viruses 1 and 2 (HTLV-1 and HTLV-2) remain a public health problem despite their association with serious diseases such as adult T-cell leukemia/lymphoma and HTLV-1-associated myelopathy. People living with HIV-1 (PLHIV) constitute a group with greater vulnerability to HTLV infection due to the sharing of transmission routes and the possible clinical repercussions of concomitant infection. Although the state of Maranhão is considered an area of high endemicity for HTLV in Brazil, studies investigating its occurrence among PLHIV remain scarce. In this context, this study aimed to determine the seroprevalence of HTLV infection in PLHIV treated at a reference service in São Luís, Maranhão. A cross-sectional observational study was conducted among individuals treated at the aforementioned center who underwent serological screening for HTLV using the enzyme-linked immunosorbent assay (ELISA) and were confirmed by qPCR. Three hundred and seven PLHIV were evaluated, of whom eight tested seropositive for HTLV, and seven were confirmed as HTLV-1, corresponding to a prevalence of 2.28%. This frequency highlights the persistence of HTLV circulation in vulnerable populations, highlighting the urgent need to implement routine testing. These findings provide unprecedented data for the state and support the incorporation of systematic serological screening as a strategy to enable early diagnosis, adequate clinical monitoring, and the improvement of public policies aimed at sexually transmitted infections.

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PT.170

HTLV-EXPOSED PREGNANT WOMEN AND CHILDREN IN AN ENDEMIC REGION OF NORTHEASTERN BRAZIL: CHALLENGES FOR SCREENING AND PREVENTING VERTICAL TRANSMISSION IN MARANHÃO.

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Human T-lymphotropic virus (HTLV) infection remains a significant public health challenge in endemic regions of Brazil, including the state of Maranhão. Vertical transmission, particularly through breastfeeding, is one of the main routes sustaining HTLV dissemination, highlighting the importance of prenatal screening within the Brazilian Unified Health System (SUS). This study describes the clinical and epidemiological characteristics of four pregnant women initially identified as HTLV-reactive during prenatal care in an endemic region of Northeastern Brazil, as well as their HTLV-exposed children. Initial screening was performed using enzyme-linked immunosorbent assay (ELISA), followed by serological and molecular confirmatory testing. Among the four investigated cases, HTLV infection was confirmed in two pregnant women, whereas two presented initial reactivity without confirmation by complementary tests. The women were aged between 26 and 30 years, reported sexual debut between 14 and 18 years of age, had a family income of one to two minimum wages, and predominantly had higher education. Both confirmed cases had a family history of HTLV infection, including a previously diagnosed mother and a seven-year-old HTLV-reactive daughter, suggesting intrafamilial transmission. In one case, HTLV-2 was molecularly identified. All pregnant women were diagnosed during prenatal care, underwent cesarean delivery, and were advised not to breastfeed. Two HTLV-exposed infants, evaluated at four and eight months of age, tested non-reactive by ELISA. These findings highlight the importance of prenatal HTLV screening, diagnostic confirmation, and intrafamilial investigation as key strategies to prevent vertical transmission in endemic areas.

Financial Support: Coordination for the Improvement of Higher Education Personnel (CAPES), Brazil.

PT.171

MOLECULAR EPIDEMIOLOGY OF ASTROVIRUS IN ACUTE GASTROENTERITIS IN BRAZIL: A THREE-YEAR SURVEILLANCE STUDY (2022-2024)

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Acute gastroenteritis (AGE) is a leading cause of malnutrition, stunting growth and death in children under 5 years old worldwide. Among the several agents, gastroenteric viruses are responsible for over 75% of cases. However, the knowledge about epidemiological impact and genotype diversity from emerging gastroenteric viruses remains limited. In this study, we aim to evaluate the role of Human astroviruses (HAstV, *Astroviridae* family, genus *Mamastrovirus*) in the etiology of AGE in Brazil between 2022 and 2024. We investigated the prevalence and genetic diversity of HAstV from AGE cases with a previous negative result for rotavirus and norovirus. Using TaqMan®-based RT-qPCR, we screened 608 stool samples followed by genetic characterization of positive cases. Samples with Ct value ≤35 showing a characteristic sigmoid curve were considered positive. Viral loads were expressed as genome copies per gram (GC/g) of stool. For Sanger sequencing, an ABI Prism BigDye Terminator v3.1 ® kit was used. HAstV was detected in 8,15% (116/1424) of samples, with viral load ranging from 1,03 x 10^4 to 8,99 x 10^{12} GC/g. The highest viral loads were detected in children under 6 years old. Partial ORF2 gene sequencing determined genotypes in 81,2% (39/48) of positive samples. The most prevalent genotype was HAstV-1 (69,2%) followed by HAstV- 2 (17,9%). Minor genotypes (HAstV-3, HAstV-4 and HAstV-5) were detected at frequencies of 2.6% (genotypes 3 and 4) and 7,3%, respectively. The findings identify astrovirus as agent of AGE in Brazil and highlight the importance of enlarged surveillance to clarify sporadic and outbreak cases.
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DENV-3 REFERENCE GENOME CHOICE CAN AFFECT CONSENSUS QUALITY BUT NOT CLADE CLASSIFICATION

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Reference-based genome assembly is fundamental to dengue virus genomic surveillance, particularly with the recent re-emergence of DENV-3 in the Americas, yet it remains unclear how reference genome choice affects consensus quality. We evaluated this effect using 32 dengue virus serotype 3 genomes: 31 curated from NCBI as the basal representative of each lineage across clades I, II, III, and V, plus NC_001475.2, RefSeq surveillance reference used worldwide. Paired-end reads were simulated in silico for each genome (150 bp; 500x coverage) and mapped against all 32 references using the ViralFlow pipeline (fastp, BWA-MEM, iVar), producing 1,024 combinations. Assembly quality was assessed using average nucleotide identity (ANI, pwalign method) between reference/consensus and each sample, seeking associations with sequencing coverage. Genome integrity was additionally assessed through Nextclade's quality control (QC). Agreement between observed and expected clade classification was also evaluated. Mean ANI was approximately 92% between references and samples and 97.6% between consensus and sample genomes (32x32), and the two were positively correlated across genomes (R^2 = 0.67, n = 32). Consensus ANI weakly correlated with sequencing coverage (Spearman's ρ = 0.23), and among Nextclade's QC indicators, missingData and privateMutations corresponded to a reduction in ANI. All reconstructed DENV-3 genomes were assigned to the correct clade, indicating that reference genome choice did not impact a surveillance-based workflow, even though consensus quality itself was shaped by these mapping- and structure-related factors.

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A SCOPING REVIEW ON ARBOVIRUSES IN EQUIDS IN BRAZIL: SURVEILLANCE AND VIGILANCE CHALLENGES

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BACKGROUND: Arboviruses represent a major global threat, particularly in Brazil. In 2024, the country experienced its largest arboviral epidemic, with more than 6.5 million reported dengue cases alone. Because arboviruses can spill over from enzootic to epizootic transmission cycles, surveillance of sentinel animals is crucial for early detection and epidemiological monitoring. Equids are valuable sentinels within the One Health framework, as they are widely distributed, inhabit the urban–rural interface and are used in diverse settings. Moreover, equids play a key role in the surveillance of neurotropic arboviruses, as infections in horses can serve as indicators of viral circulation. **METHODS:** This study is a scoping review of all records regarding arbovirus detection in equids in Brazil, following the PRISMA 2020 guidelines. **RESULTS:** 31 articles were reviewed, encompassing 8,107 sampled equids and 1,537 positive detections of 15 different arboviruses (Bussuquara, Cacipacoré, Eastern Equine Encephalitis, Ilhéus, Madariaga, Maguari, Mayaro, Mucambo, Pixuna, Rocio, Saint Louis Encephalitis, Venezuelan Equine Encephalitis, Western Equine Encephalitis, West Nile, and Xingu). The most researched arbovirus was West Nile Virus (45%), the most detected was Saint Louis Virus (40%) and the most common diagnosis method was PCR (52%). Studies were concentrated in coastal and developed Brazilian regions (South, Southeast, and Northeast) and were scarce in Central-West and North regions. **CONCLUSION:** These findings underscore the need for broader and constant surveillance, mainly in states with high population density and mobility to anticipate and mitigate future outbreaks.
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ENTOMOLOGICAL SURVEILLANCE OF *Culicidae* IN A MUNICIPALITY IN NORTHWESTERN SÃO PAULO: ASSOCIATION BETWEEN VECTOR INDICES, CLIMATIC VARIABLES, AND THE OCCURRENCE OF ARBOVIRAL DISEASES.

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Introduction: Urban arboviral diseases constitute a public health challenge in Brazil, driven by the widespread of the vector *Aedes aegypti*. São José do Rio Preto presents a favorable conditions for the transmission of these diseases, with viral cocirculation, recurrent epidemics, and high vector infestation rates. Between 2020 and 2025, the municipality recorded more than 148,000 dengue cases and its largest chikungunya epidemic. This study aimed to develop entomological surveillance actions to evaluate culicid vector infestation indices in urban areas at high risk for arboviral diseases.
Materials and Methods: The study was conducted between October/2023 and April/2025, with monthly monitoring of 46 residences using ovitraps and BG-Sentinel™ traps, in addition to collections in two periurban areas and one forest fragment area. The entomological indices were correlated with climatic and epidemiological variables using Pearson correlation and multiple linear regression.
Results: A total of 7,824 eggs and 2,307 adult mosquitoes were collected, with a predominance of the genera *Aedes* and *Culex* in the urban area and greater species richness in natural fragments. The entomological indicators tracked one chikungunya epidemic peak and two dengue peaks. Precipitation was the main predictor of dengue, while the mean female index showed better performance in predicting chikungunya.
Conclusion: Integrated monitoring of entomological indices, habitat characteristics, and climatic variables proved to be a robust tool for understanding vector dynamics and supporting more precise surveillance and control actions.

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ABSENCE OF ACTIVE EQUINE HERPESVIRUS TYPE 1 (EHV-1) DETECTED BY QPCR IN ASYMPTOMATIC HIGH-RISK HORSES FROM SOUTHERN BRAZIL

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Equine herpesvirus type 1 (EHV-1) is a highly contagious pathogen of major clinical and epidemiological relevance in horses, capable of establishing lifelong latent infection with the potential for reactivation under stress conditions. This study aimed to investigate the circulation of EHV-1 in horses exposed to elevated epidemiological risk, particularly animals participating in large-scale events, through clinical monitoring, hematological assessment, and molecular diagnosis. The study was conducted between July and September 2025, 111 unvaccinated horses from Southern Brazil were evaluated clinically, and blood and nasal swab samples were collected. Hematological analyses were performed in parallel to support clinical interpretation. DNA extraction was carried out using a magnetic bead-based protocol, and real-time polymerase chain reaction (qPCR) targeting the glycoprotein B (gB) gene was employed for molecular detection of EHV-1. No hematological alterations suggestive of active viral infection were identified in any animal. Positive controls consistently yielded low cycle threshold (CT) values (mean 20.09), whereas field samples (plasma: 38.56; buffy coat: 37.93; and nasal swabs: 37.53) exhibited significantly higher CT values (overall mean 38.13; $P < 0.05$). Although no active infection was detected, these findings may reflect low viral circulation or latent infection with viral loads below the qPCR detection limit. The results underscore the importance of continuous molecular surveillance and sensitive diagnostic approaches to support biosecurity measures and prevent EHV-1 outbreaks, especially in populations subjected to transportation, stress, and aggregation.
Financial Support: FAPERGS, CAPES e PPG Feevale.

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IDENTIFICATION OF BOVINE PESTEVIRUS AND BOVINE HERPES VIRUS TYPE-1 IN CATTLE LOCATED ON RURAL PROPERTIES IN THE PARANHANA VALLEY REGION, RIO GRANDE DO SUL, BRAZIL.

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Bovine pestivirus, the causative agent of Bovine Viral Diarrhea Virus (BVDV), and bovine herpesvirus type 1 (BoHV-1), the etiological agent of Infectious Bovine Rhinotracheitis (IBR), are responsible for significant reproductive losses in Brazilian cattle herds. The aim of this study was to investigate the circulation of these viruses in dairy and beef herds in the Paranhana Valley, Rio Grande do Sul. Blood and nasal swab samples were collected from 151 animals across 17 farms (11 dairy and 6 beef). Serological screening was performed using ELISA, and positive samples were subjected to virus neutralization (SN) to determine antibody titers, classified as follows: ≥512 indicative of active infection; 4–512 consistent with vaccine-induced antibodies; and ≤4 considered negative. Samples classified as active infection by SN were processed and analyzed by conventional PCR and submitted for Sanger sequencing. Active circulation of BVDV was detected in 23.8% of dairy farms and 36% of beef farms ($P < 0.05$). Among vaccinated animals, 64.4% showed no evidence of BVDV, while 35.6% exhibited signs of viral circulation ($P < 0.05$). For IBR, 84.4% of vaccinated animals presented vaccine-induced antibodies, whereas 21.7% of unvaccinated animals tested positive for IBR antibodies ($P < 0.01$). Molecular analysis by conventional PCR corroborated the serological results for IBR; however, sequencing results were inconclusive for viral genome identification. These findings indicate continuous viral exposure in cattle herds, with partial immunization against IBR and stronger immune responses to BVDV in vaccinated animals. The results highlight the need to improve vaccination strategies to reduce reproductive losses in Brazilian cattle production. Financial Support: FAPERGS, CAPES

PT.177

Circulation of RSV (A and B) in Children Under 5 Years Old Hospitalized in Public Hospitals in the City of São Paulo: Pre- and Post-Maternal Vaccination.

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Respiratory Syncytial Virus (RSV) is the leading cause of acute lower respiratory tract infections in young children and represents a major public health concern due to its seasonal epidemics and high burden on healthcare services. In Brazil, maternal vaccination against RSV was incorporated into the SUS in late 2025 for pregnant women from the 28th week of gestation. This study evaluated the circulation of RSV-A and RSV-B before and after vaccine implementation. A total of 11,230 nasopharyngeal/oropharyngeal swab samples collected from patients attending five public hospitals in São Paulo were analyzed by RT-qPCR for 15 respiratory viruses. Among them, 2,578 (22.9%) were positive for RSV, including 59.19% RSV-A, 40.42% RSV-B, and 0.39% RSV-A/RSV-B coinfections. RSV activity remained concentrated during the autumn and winter seasons. A total of 339 cases were detected between May and December 2024, 1,227 throughout 2025, and 1,011 from January to July 2026. Infants aged 29 days to 6 months accounted for the highest proportion of cases in both 2024 (48.67%) and 2025 (45.23%). However, a comparison of January–June 2025 and 2026 revealed a reduction in cases among infants aged 29 days to 2 months (42.50% vs. 31.65%) and an increase among children aged 7 months to 2 years (44.17% vs. 55.97%). These findings suggest an early epidemiological shift following maternal RSV vaccination, reinforcing the importance of continuous molecular surveillance to assess vaccine impact and support public health strategies.

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Keywords: Respiratory Syncytial Virus (RSV); Maternal vaccination; Molecular surveillance; Epidemiology; Public health.

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PREVALENCE OF HTLV-1 AND HTLV-2 IN PREGNANT WOMEN IN THE MUNICIPALITY OF TOMÉ-AÇU, PARÁ

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Human T-lymphotropic virus (HTLV) is a globally distributed retrovirus, with prevalence rates varying according to the population studied, and mother-to-child transmission through breastfeeding representing one of its primary routes. This study aimed to investigate the prevalence of HTLV-1 and HTLV-2 infection among pregnant women in the municipality of Tomé-Açu, Pará. The study included 212 pregnant women aged 18 to 42 years (mean = 27.0; SD = 5.8) receiving prenatal care at 12 Family Health Strategy centers in Tomé-Açu, Pará, during October and November 2025. Following informed consent and the completion of an epidemiological questionnaire, peripheral venous blood samples were collected and separated into plasma and leukocytes. Plasma was used for serological screening by ELISA, while leukocytes from reactive samples were subjected to proviral DNA extraction, followed by qPCR for confirmation and differentiation of the infection. The results showed one positive case for HTLV-1 (a prevalence of 0.47%) and no cases of HTLV-2 infection. The positive woman was 26 years old, self-identified as mixed-race, married, had not completed elementary education, and had a family income of less than one minimum wage. She had initiated sexual activity at age 14 and reported breastfeeding her four children for more than six months. In the studied, high frequencies of early sexual initiation (73.11%), low regular condom use (28.30%), and prolonged breastfeeding (>6 months) (58.49%) were observed in the study population. Our findings underscore the importance of prenatal screening and the strengthening of surveillance, prevention, and maternal-child monitoring efforts in the municipality and throughout Brazil.

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MOLECULAR SCREENING AND VIRAL ISOLATION OF DENGUE VIRUS FROM CLINICAL SAMPLES IN WESTERN BAHIA, BRAZIL, BETWEEN 2023 AND 2025

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Arboviruses remain a major public health challenge in Brazil, particularly in tropical regions where environmental conditions favor vector proliferation and sustained viral circulation. Continuous molecular surveillance associated with viral isolation strategies is essential for epidemiological monitoring and the establishment of viral stocks for experimental studies. This study aimed to perform molecular screening, viral isolation, and preliminary characterization of arboviruses detected in Barreiras, Bahia, Brazil, between 2023 and 2025. Clinical serum samples were analyzed by RT-qPCR for Dengue virus (DENV), Chikungunya virus (CHIKV), and Zika virus (ZIKV). Positive samples with lower Ct values were selected for viral isolation in C6/36 mosquito cells, followed by monitoring for cytopathic effect (CPE) and molecular confirmation by RT-qPCR. A total of 650 clinical samples were screened, of which 158 (24.3%) were positive for arboviruses, including 134 DENV-positive and 24 CHIKV-positive samples, while no ZIKV-positive cases were detected. DENV was the predominant arbovirus throughout the study period. Viral isolation assays successfully recovered four DENV isolates confirmed by CPE and RT-qPCR. No successful isolation of CHIKV or ZIKV was achieved. These findings reinforce the predominance of DENV circulation in Western Bahia and demonstrate the usefulness of combining molecular diagnosis with viral isolation approaches for arbovirus surveillance and future experimental studies.
Keywords: arbovirus, RT-qPCR, epidemiology, western Bahia, viral isolation
Financial support: CNPq, FINEP, and FAPESB.

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INVESTIGATION OF THE EXPOSURE TO ILHEUS VIRUS OF DOMESTIC ANIMALS OF INDIGENOUS COMMUNITIES OF THE PANTANAL, MATO GROSSO, BRAZIL

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Ilheus virus (*Orthoflavivirus ilheusense* or ILHV) is a zoonotic arbovirus classified to the family *Flaviviridae.* ILHV was first isolated in 1944 in the city of Ilheus, state of Bahia, northeast Brazil. Since then, ILHV has been recognized as enzootic in South America, maintaining zoonotic cycles involving avian hosts as amplifying hosts and mosquitoes as vectors. The great diversity of non-human animals, including wildlife and arthropod vectors, in the Pantanal favors the circulation of arboviruses, which may lead to the exposure of local indigenous communities. In this context, a total of 79 domestic animals had blood collected in 2025 in two indigenous communities of the Pantanal, located in the state of Mato Grosso, west-central Brazil. All sera were screened by the plaque reduction neutralization test ($PRNT_{90}$)to detect the presence of specific neutralizing antibodies against ILHV. A total of 25 (31.64%) samples were reactive for ILHV, namely domestic dogs (14), domestic birds (3), equids (6), bovine (1), and swine (1). All samples will be retested by a confirmatory $PRNT_{90}$ for ILHV and other related orthoflaviviruses. Despite preliminary, these results may indicate exposure of non-human animals to ILHV in indigenous communities of the Pantanal. Given that arbovirus potential for dissemination and associated public health risks, active surveillance becomes a critical tool for monitoring ILHV circulation. Limited access and logistical difficulties in reaching remote locations, such as indigenous villages in the Pantanal, hinder the implementation of healthcare programs. Thus, it is essential to ensure that arboviral surveillance actions effectively reach these populations. Financial Support: FAPEMIG, CAPES, CNPq and Funarbe.

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Investigation of Saint Louis encephalitis virus in indigenous communities of the Pantanal of Mato Grosso, Brazil, 2025

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Saint Louis encephalitis virus (*Orthoflavivirus louisense* or SLEV) is an arbovirus of zoonotic importance that can cause infections ranging from asymptomatic cases to severe encephalitis. In its enzootic cycle, transmission occurs primarily through blood feeding of *Culex* spp. mosquitoes, with wild birds serving as amplifying hosts. SLEV is widely distributed throughout the Americas, including Brazil. Despite its widespread circulation in the country, little is known about SLEV circulation in indigenous communities in Brazil. These territories are susceptible to zoonotic viruses due to greater contact with non-human animals and limited access to healthcare services. In this context, blood samples were collected in 2025 from 79 domestic animals from indigenous territories of Guató and Bororo in the Pantanal, state of Mato Grosso. All serum samples were screened by $PRNT_{90}$ for SLEV. Preliminary results indicate that 42 (53.16%) animals had neutralizing antibodies for SLEV, including 16 domestic dogs, 16 domestic birds, seven equids, two pigs, and one bovine. All reactive samples will be retested by a confirmatory $PRNT_{90}$ for SLEV. These preliminary results indicate potential cryptic circulation of SLEV in these territories underscoring the need of continued surveillance of arboviruses in the indigenous communities of the Pantanal. Financial Support: FAPEMIG, CAPES, CNPq and Funarbe.

Keywords: Domestic animals; zoonotic; surveillance; arbovirus; one health.

Thematic area: Veterinary Virology; Viral Epidemiology and Public Health.

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QUANTITATIVE MICROBIAL RISK ASSESSMENT: A CASE STUDY APPLIED TO THE RECREATIONAL USE OF THE BEIRA-MAR NORTE BATHING AREA, FLORIANÓPOLIS, SANTA CATARINA, BRAZIL

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Microbiological water quality has traditionally been assessed by using bacterial indicators, although other pathogens may pose significant risks to human health. This study aimed to evaluate the microbiological risk associated with the recreational use of the Beira-Mar Norte bathing area, Florianópolis, Santa Catarina, Brazil, using the QMRA approach. *Escherichia coli* data was provided by the Santa Catarina Environmental Institute, and Human Adenovirus (HAdV) concentrations were obtained from previous studies for the period of 2009 to 2011. The analysis considered three scenarios: comparison between *E. coli* and HAdV risks, evaluation of *E. coli* before and after the implementation of the Environmental Recovery Unit (URA) in 2019, and analysis of the limits established by CONAMA Resolution No. 274/2000. The results indicated that children engaged in primary contact to water presented the highest probabilities of illness in all scenarios. HAdV showed a significantly higher risk than *E. coli*, reaching probabilities close to 70% in primary contact activities. After the implementation of the URA, there was a partial reduction in the risk associated with *E. coli* and an increase in the number of samples compliant with the legislation. However, even for waters classified as “excellent” according to CONAMA Resolution No. 274/2000, the estimated risks remained above the tolerable limit of 0.01% recommended by the World Health Organization. We concluded that monitoring based exclusively on bacterial indicators may underestimate microbiological risk, highlighting the importance of including enteric viruses, such as Adenovirus or other indicators, in bathing water quality criteria.

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MOLECULAR SURVEILLANCE OF ARBOVIRUSES IN PATIENTS WITH SUSPECTED ARBOVIRAL INFECTION ATTENDING PUBLIC HOSPITALS IN SÃO PAULO, BRAZIL, 2025 – 2026

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Arboviruses transmitted by *Aedes* mosquitoes, particularly dengue virus (DENV), chikungunya virus (CHIKV), and Zika virus (ZIKV), remain major public health threats in tropical and subtropical regions. Their geographic expansion has been driven by urbanization, climate change, globalization, and the widespread distribution of competent vectors, resulting in recurrent outbreaks and increasing pressure on healthcare systems. The overlapping clinical manifestations of arboviral infections and the co-circulation of multiple viruses complicate clinical diagnosis, reinforcing the importance of laboratory-based molecular surveillance for timely case detection and public health response.To evaluate the circulation of DENV, ZIKV, CHIKV, Oropouche virus (OROV), and Yellow Fever virus (YFV) among patients with suspected arboviral infection attending five public hospitals in São Paulo, Brazil, between January 2025 and June 2026. Clinical samples (serum, swab, and/or urine) from 320 patients with suspected arboviral infection were tested by RT-qPCR for DENV, ZIKV, CHIKV, OROV, and YFV. Patients were stratified into five age groups. Overall, 9/320 (3.0%) samples were positive for at least one arbovirus. Among adolescents (11–17 years), 2/33 (6.1%) samples were positive for DENV-2. Among adults (≥18 years), 6/120 (5.0%) samples were positive, including five DENV-2 and one CHIKV infection. No cases of ZIKV, OROV, or YFV were detected. Although the overall positivity was low, the detection of DENV-2 as the predominant circulating arbovirus and the identification of CHIKV highlight the continued circulation of arboviruses in São Paulo, reinforcing the importance of continuous molecular surveillance.

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Keywords: Arboviruses, Denguevirus, Chikungunya virus, Epidemiological surveillance, Public health

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Incidence of Dengue Fever in the Belém Metropolitan Area: 2025

Luany Cruz

Introduction: Dengue fever (DF) is an acute febrile illness caused by an arbovirus. The Belém Metropolitan Region (RMB) comprises the municipalities described in the results of this study. The objective of this abstract is to analyze the incidence of dengue fever during the 2020–2025 period. Material and Methods: This is an observational, analytical, ecological epidemiological study employing a quantitative approach based on secondary data. All collected data pertain to the year 2025. Dengue incidence figures were obtained from the Arbovirus Monitoring Dashboard maintained by the Ministry of Health (MS). Population data for the municipalities involved were collected from the Brazilian Institute of Geography and Statistics (IBGE) website. The ratio between these two parameters yielded the Incidence Rate (IR). Results: A total of 2,594 cases were recorded in the region. Although Belém showed the highest absolute number of notifications (894), Castanhal had the highest Incidence Rate (310.82), followed by Santa Izabel (286.63) and Barcarena (278.72). In contrast, Ananindeua presented the lowest IR (43.98), despite ranking second in terms of case volume. Other recorded IRs were: Benevides (211.71), Santa Bárbara (164.23), and Marituba (72.84). Conclusion: Epidemiological heterogeneity was observed within the RMB. According to InfoDengue system parameters, Castanhal reached the critical incidence level (≥300/100,000 inhabitants), signaling an alert situation, whereas Ananindeua remained at a moderate transmission level, highlighting the need for tailored vector control strategies for each locality.

Keywords: viral epidemiology; dengue; epidemiological surveillance.
Financial Support: Amazon Foundation for Studies and Research Support (FAPESPA)

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HUMAN METAPNEUMOVIRUS IN ALAGOAS, BRAZIL: SEASONAL CIRCULATION, COINFECTIONS, AND IMPLICATIONS FOR PUBLIC HEALTH SURVEILLANCE

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Human metapneumovirus (HMPV) is a pathogen that causes illnesses ranging from mild upper respiratory tract infections to acute bronchitis and pneumonia. Despite its clinical relevance, information on HMPV circulation in Alagoas, Brazil, remains limited. This study aimed to characterize the epidemiological profile and temporal distribution of HMPV cases detected in Alagoas between 2024-2026. We conducted a descriptive observational study using laboratory records of HMPV-positive cases identified by RT-PCR provided by the Central Public Health Laboratory of Alagoas (LACEN-AL). Demographic characteristics, temporal trends, and Ct values were analyzed using descriptive statistics and nonparametric tests. During the study period, 408 HMPV-positive cases were identified among 29.907 samples. Case detection peaked between July and September of 2024 and 2025, with the highest number of cases recorded in 2025. Nearly half of the cases occurred among residents of Maceió (46%). Viral coinfections were detected in 45 (11,0%) cases, most commonly involving HRV (40,8%) and RSV (22,4%); other viruses were also detected, though less frequently: ADV, INFA, HBoV, SARS-CoV-2, HCoV-NL63, and INFB. A modest association between sex and age group was also identified ($\chi^2 = 18,3$, df = 5, p = 0,003; Cramér's V = 0,21), with males predominating among children younger than five years and females among adults aged 18-39 years. Overall, these findings provide an updated overview of the epidemiological profile and seasonal dynamics of HMPV in Alagoas and may help guide respiratory virus surveillance strategies in the state.
Financial support: CAPES, FINEP, CNPq and FAPEAL

PT.186

Spatiotemporal Progression of the Inland Expansion of Highly Pathogenic Avian Influenza Outbreaks in Brazil, 2023–2026

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Highly pathogenic avian influenza (HPAI) outbreaks in Brazil were initially concentrated along the coast, but recent records indicate expansion toward inland areas. This study evaluated the spatiotemporal progression of this inland expansion between 2023 and 2026. An ecological study was conducted using secondary data from the Brazilian Ministry of Agriculture and Livestock, deduplicated by investigation number. Outbreaks were classified as occurring in coastal or non-coastal municipalities, and the shortest approximate distance between their coordinates and the coastline was calculated. Proportions of non-coastal outbreaks and distance distributions were compared between 2023–2024 and 2025. Data from 2026 were analyzed descriptively because the year was incomplete. A total of 189 unique HPAI-confirmed investigations were identified. The proportion of outbreaks in non-coastal municipalities increased from 7.2% in 2023–2024 to 90.0% in 2025 (OR = 115.5; 95% CI = 23.9–557.7; $p < 0.001$). Median distance from the coast increased from 0.76 km to 83.2 km ($p < 0.001$), while the 90th percentile rose from approximately 5.5 km to 912.5 km. This difference remained significant among free-ranging wild birds. The upper edge of the distribution showed an apparent progression of 35.9 km/month. These findings demonstrate a marked shift in the spatial pattern of detected outbreaks, with extensive involvement of inland municipalities from 2025 onward. This should be interpreted as the spatiotemporal expansion of recorded outbreaks, not as the speed of viral transmission.

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DETECTION OF AVIAN PARAMYXOVIRUS TYPE 1 AND AVIAN INFLUENZA VIRUS IN MIGRATORY SHOREBIRDS FROM COAST OF SAO PAULO

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Newcastle disease virus (NDV), also known as *Orthoavulavirus javaense* or APMV-1, and avian influenza virus (AIV) are highly relevant avian pathogens with sanitary, economic, and zoonotic importance. Wild migratory birds may contribute to the maintenance and dissemination of these viruses, highlighting the need for active surveillance along migratory routes. This study aimed to investigate the occurrence of NDV and AIV in migratory shorebirds from coastline of São Paulo, southeastern Brazil, as part of a surveillance program for emerging viruses. A total of 428 swab/fecal samples collected from coastal birds/animals on the coastline of São Paulo, between 2024 and 2026, were analyzed by Real time RT-PCR for viral detection. Five samples tested positive for NDV, representing an overall detection rate of 0.85% and five samples tested positive for AIV, with the same rate. All positive samples were associated with shorebirds with the exception of one sample positive for AIV from a sea lion (*Otaria flavescens*). These results will be confirmed by conventional PCR according to the laboratory protocol, followed by Sanger or next-generation sequencing to determine the viral genotype and phylogenetic relationships. Continuous surveillance in migratory stopover has enabled the early detection of emerging viruses circulating in wild bird populations. The identification of NDV and AIV in wild birds and AIV in mammals reinforces the role of wildlife virus ecology and underscores the importance of integrating wildlife surveillance into national animal health strategies, particularly in light of recent HPAI outbreaks in commercial poultry in southern Brazil. Financial Support: FUSP.

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ARBOVIRUS IN BIRDS ACROSS BRAZIL: A SCOPING REVIEW ON THE INVESTIGATION OF SILENT VIRAL CIRCULATION

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Introduction: Arboviral diseases have traditionally been studied from the perspective of vectors and their control. However, birds can serve as sentinels of viral circulation, supporting early detection and surveillance of emerging arboviruses. Given Brazil's exceptional avian diversity, this scoping review synthesized evidence on arbovirus occurrence in birds in Brazil and identified key knowledge gaps. **Methods:** A PRISMA-guided scoping review searched six databases (BVS, CAPES, Google Scholar, PubMed, SciELO, and Scopus) through February 2026, without language or initial date restrictions. Studies reporting natural viral infections in native, migratory, or introduced birds in Brazil were included; experimental infections were excluded. Eligible studies underwent geographic, statistical, and qualitative analyses. **Results:** Of 629 records identified, 23 studies met the eligibility criteria. Published since 1966, these studies covered six Brazilian states (AM, CE, MA, PA, RS, and SP), predominantly within the Amazon biome. Overall, 76,870 birds were captured, of which 61,213 were tested for arboviruses, yielding approximately 4,342 positive samples, 97.5% detected by serology. Arboviruses from the families *Peribunyaviridae*, *Togaviridae*, *Flaviviridae*, and *Sedoreoviridae* were identified, predominantly orthoflaviviruses. Twenty-five viruses were identified, with studies mainly focusing on Oropouche virus (26.1%), equine encephalitis viruses (Venezuelan, Western, and Eastern- 21.7%), West Nile virus (17.4%), and St. Louis encephalitis virus (17.4%). **Conclusions**: Research on arboviruses in birds in Brazil remains limited and geographically concentrated. This review highlights the need for more comprehensive reporting of sampling and study results, including both positive and negative findings, to strengthen future surveillance and research efforts. **Financial Support**: São Paulo Research Foundation (FAPESP).

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IN SILICO CHARACTERIZATION OF A PHOSPHATIDYLINOSITOL 3-KINASE-LIKE PROTEIN (PI3K) ENCODED IN THE TUPANVIRUS GENOME: EXPANDING PERSPECTIVES ON THE FUNCTIONAL EVOLUTION OF EUKARYOTIC SIGNALING PATHWAYS

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Viruses have traditionally been viewed as genetically streamlined entities that rely extensively on host-cell machinery to support their replication. However, giant viruses challenge this view by encoding large and complex gene repertoires, including proteins with similarities to cellular enzymes, metabolic factors, and signaling-related components. In this study, we performed an *in silico* characterization of a PI3K-like protein encoded by *Tupanvirus salinus*. Domain prediction and remote-homology analyses were performed using InterProScan, HHpred, CD-Search, BLAST-based similarity searches, Alphafold structural modelling, PYMOL-based structural comparison, and phylogenetic reconstruction using IQTree. The viral candidate was compared with amoebal homologs and with related sequences retrieved from broader similarity searches. The analyses supported the presence of a PI3/4-kinase-like catalytic core in the viral protein, with structural similarity to members of the PI3K/PI4K superfamily, including VPS34/class III PI3K templates. In addition, the viral protein contains a predicted FYVE-like zinc finger region, a domain typically associated with PI3P recognition and membrane targeting, which was not detected in the amoebal homolog analysed. The viral protein showed low sequence identity with the host homolog, while similarity searches also recovered related proteins in other giant viruses and a highly similar PI3K-like sequence from an archaeal metagenome-derived assembly. Structural and phylogenetic analyses suggest that the tupanvirus protein is evolutionary divergent from the host homolog and may represent part of an underexplored viral repertoire of PI3K-like proteins. Although these results do not demonstrate catalytic activity, lipid substrate specificity, or PI3P binding, they provide a framework for future residue-level analyses, expression and functional validation studies.

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INTRAHOST GENETIC DIVERSITY OF CHIKUNGUNYA VIRUS (CHIKV) AND ITS ASSOCIATION WITH HOSPITALIZATION STATUS

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Chikungunya virus (CHIKV), like other RNA viruses, exhibits a high mutation rate during genome replication because its RNA-dependent RNA polymerase lacks proofreading activity. This intrinsic characteristic generates intra-host genetic diversity, leading to the accumulation of intra-host single nucleotide variants (iSNVs). Although intra-host diversity is considered an important mechanism of viral evolution, its relationship with clinical outcomes remains poorly understood. Here, we investigated the intra-host genetic diversity of CHIKV using next-generation sequencing of 410 serum samples collected between January and April 2024. Viral genomes were analyzed to identify iSNVs across the coding regions, and a subset of 56 samples, including 28 hospitalized and 28 non-hospitalized patients, was selected for comparative analyses. Intra-host genetic diversity showed a homogeneous distribution across structural and non-structural proteins, with a predominance of nonsynonymous substitutions. No significant difference was observed in the number of iSNVs between hospitalized and non-hospitalized patients, indicating that intra-host viral diversity alone is unlikely to explain disease severity. To evaluate selective pressures, dN/dS ratios were estimated across CHIKV coding regions. Most coding regions exhibited dN/dS values below 1, consistent with predominant purifying selection. CHIKV genomes from hospitalized patients showed lower dN/dS ratios in NSP1, NSP2, NSP4, and E1 than those from non-hospitalized individuals, suggesting stronger purifying selection in these genomic regions. These findings expand our understanding of CHIKV intra-host evolutionary dynamics and provide a foundation for future studies investigating the relationship between viral diversity, selective pressures, and clinical outcomes.

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DIVERSITY OF GIANT VIRUSES IN BRAZIL: ISOLATION AND CHARACTERIZATION OF A NEW MARSEILLEVIRUS

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Giant amoeba viruses have intrigued science due to their dimensions of up to 2.6 micrometers, particles with diverse symmetries, and genomes of up to 3 megabase pairs that encode proteins never before associated with these entities. In this context, viral prospecting studies are of great importance for the description of these microorganisms. Therefore, this abstract aims to report the prospecting of 1338 samples, 360 of which were seawater samples from Trindade Island in Espírito Santo tested for *Vermamoeba vermifomis*, and 978 samples of water from the Paraguay River in the Brazilian Pantanal tested for *Acanthamoeba castellanii*. In the prospecting, only one sample from the Paraguay River showed a cytopathic effect on *A. castellanii*, this being a new strain of Marseillevirus called "Marseillevirus pantanense". Transmission and scanning electron microscopy revealed icosahedral particles with approximately 190 nm in diameter. Genomic and phylogenetic analyses show that M. pantanense has a genome of 379,900 bp with 496 open reading frames (ORFs), including 12 ORFans and two tRNAs. Furthermore, phylogenetic analysis places M. pantanense within lineage A, group I, of Marseilleviruses. The results of this study highlight the importance of isolating new viruses to broaden our understanding of the abundance and distribution of giant viruses in Brazilian biomes.

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FROM THE SÃO FRANCISCO TO THE PARAGUAY RIVER : DIVERSITY AND EVOLUTIONARY ANALYSIS OF PANDORAVIRUS ISOLATES FROM BRAZIL

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Pandoraviruses are giant amoeba-infecting viruses distinguished by their exceptionally large genomes, which can reach approximately 2.77 Mb, the largest described in the virosphere. Since their first isolation in 2013 in the Americas and Oceania, they have been identified in different regions worldwide. This study aimed to isolate and perform comparative genomic analyses of Pandoravirus isolates obtained from the São Francisco and Paraguay rivers in Brazil. Viral isolation was performed by screening environmental samples using *Acanthamoeba castellanii*. The isolates were purified, sequenced, assembled, annotated, and analyzed through comparative nucleotide and amino acid identity analyses (ANI and AAI), phylogenetic reconstruction, and additional genomic approaches. Four pandoraviruses were isolated. ANI and AAI analyses suggested that pandoraviruses are organized into eight genera and two subfamilies to be recognized by ICTV. Most analyzed viruses exhibited considerable nucleotide and amino acid divergence, with identities ranging from 58% to 98.9%. Two Brazilian isolates, Pandoravirus São Francisco 2 and Pandoravirus São Francisco 3, showed high genomic similarity, likely because they were recovered from the same location three months apart. Another isolate, Pandoravirus pantanense, was identified as a novel species, sharing a maximum nucleotide identity of 81.84% with Pandoravirus macleodensis, its closest relative. All isolates contained core genes of the phylum *Nucleocytoviricota*, including DNA polymerase family B and D5 helicase-primase, as well as gene encoding major protein 2, a structural component abundant in the viral particle. These findings expand current knowledge of the genomic diversity and evolution of Brazilian pandoraviruses.

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PROSPECTING FOR GIANT VIRUSES IN ENVIRONMENTAL WATER SAMPLES USING ACANTHAMOEBA CASTELLANII AS A CELL MODEL

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The identification of the mimivirus marked the beginning of the discovery of numerous giant viruses, highlighting their broad diversity and distribution across different environments. These microorganisms are characterized by large particles, often detectable by light microscopy, and by possessing extensive genomes that encode genes related to essential cellular functions, such as aminoacyl-tRNA synthetases and tRNAs, among others. For the isolation of these viruses, the amoeba *Acanthamoeba castellanii* stands out due to its permissiveness to a broad range of viruses and ease of cultivation under laboratory conditions. In this context, biodiverse environments are considered promising for investigating the diversity and ecology of these viruses. Thus, this study aimed to isolate, identify, and characterize giant viruses using *A. castellanii* as an experimental model from environmental samples. A total of 760 water samples were analyzed, including 80 from P. E. Brejinho, 460 from the Amapá region, 160 from L. Pampulha and 60 from Florianópolis. Amoebae cultivation was performed in PYG medium supplemented with antimicrobials. Screening was carried out in 96-well plates containing 125 µL of supplemented PYG medium, inoculated with 4×10^4 amoebae and 100 µL of each sample, followed by incubation at 30 °C. To date, a giant virus belonging to the genus *Cedratvirus* has been isolated from sample SC2.1, and its morphology was confirmed by scanning and transmission electron microscopy, with a viral titer of 2,14 x 10^8 $TCID_{50}$/mL. Additionally, sample SC3.1 induced cell rounding and lysis, suggesting the presence of another giant virus, although its morphological characterization is still underway.

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ECOLOGICAL AND EVOLUTIONARY ANALYSIS OF OROPOUCHE VIRUS REVEALS LINEAGE-SPECIFIC CODON USAGE PATTERN WITH VECTOR- AND HOST-ADAPTATIONS

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Oropouche virus (OROV) has gained prominence due to the increase in cases and the expansion of its circulation since 2024. With a segmented genome, OROV exhibits a high potential for genetic reassortment, an event possibly associated with adaptation and emergence of new variants. Understanding the mechanisms of viral adaptation to hosts and vectors is essential to understanding its evolution and transmission dynamics. Here, we investigated the codon adaptation to OROV and related viruses to OROV (rOROV) through relative synonymous codon usage (RSCU) analysis, principal component analysis, and codon adaptation index (CAI), considering different hosts and vectors. Complete genomic sequences of OROV and rOROV retrieved from GenBank were analyzed, selected based on the availability of the three genomic segments (L, M, and S) and sequence quality. Codon usage patterns revealed differences between the segments and group of viruses. RSCU results show the M segment clearly separated OROV and rOROV into distinct clusters, in contrast with other segments. Looking within OROV sequences, the divergence patterns observed in the RSCU results were reflected in the groupings observed in the phylogenetic analysis. CAI results revealed high adaptation to Bradypus tridactylus and Culicoides brevitarsis (an model to C. paraensis), corroborating the roles described for these species in transmission cycle and highlighting codon adaptation as a complementary tool for investigating virus-host and -vector relationships. These results indicate that the M segment constitutes the main component associated with the differentiation between OROV and rOROV, while codon adaptation analysis recovers previously established ecological associations for the main hosts and vectors of the virus.

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Fungi currently available on the market for biological control contain mycoviruses

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Mycoviruses are viruses that infect fungi, predominantly comprising ssRNA and dsRNA genomes. They have been described in phytopathogenic, endophytic, and insect-associated fungi, exhibiting complex interactions that can modulate fungal physiology, metabolism, and ecology, including hypovirulence or hypervirulence. Despite increasing knowledge of mycovirus diversity in natural fungal populations, little is known about the viromes of fungi used in commercial biocontrol products. In this study, fungal isolates were recovered from eight commercial products and screened for mycoviruses. Total nucleic acids were extracted, treated with S1 nuclease and RQ1 DNase, and analyzed by electrophoresis. Banding patterns suggestive of dsRNA molecules were observed in Beauveria bassiana and Trichoderma harzianum isolates. Libraries were sequenced on the Illumina platform, and reads were assembled *de novo* with rnaSPAdes. Contigs were filtered by size, ORFs predicted with Prodigal, and translated proteins compared against viral databases using DIAMOND and BLASTx. Ten contigs matched viral RdRp proteins: six related to mitoviruses (37–60% amino acid identity) and four to hypoviruses (69–85% identity), with query coverage ranging from 50% to 98%. These findings expand the known viral diversity associated with commercial biocontrol fungi and underscore the need to evaluate the biological effects of these infections on fungal fitness and biocontrol efficacy, as well as the potential for inter-strain transmission in formulated products.
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A novel strategy for dsRNA-based reverse genetic system applied to mycoviruses

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Polymycoviridae is a family of multi-segmented double-stranded (ds)RNA mycoviruses that lack a conventional capsid and are infectious as naked dsRNA. Their members have 4 to 9 dsRNA segments, of which four are conserved among all isolates. This conserved segment encodes the RdRp, Scaffold-Protein, Methyltransferase, and Proline-Alanine-Serine-rich-protein (PASrp). The other segments, when present, share no homology with any other sequences available in public databases, such as NCBI and UniProt. The common features within the cognate segments are the relatively conserved 5' and 3' untranslated regions (UTRs). Because of this, we are trying to understand the mechanism by which dsRNA polymycoviruses can acquire and lose genomic segments. Thus, we developed a clone harboring the Hygromycin B resistance gene flanked by the Aspergillus fumigatus polymycovirus 1 (AfuPmV1) UTRs using Gibson Assembly, and it was checked by Sanger sequencing. Then, we amplify the construction using two PCRs; in each, we add the T7 promoter sequence at the 5' or 3' ends. Each amplicon was used as a template for in vitro transcription (IVT), producing the positive-sense (+) and negative-sense (-) ssRNA. Subsequently, the ssRNAs was mixed in equimolar ratio and hybridized, mimicking the AfuPmV1 dsRNA. The synthetic-artificial segment was transfected into an AfuPmV1-infected *A. fumigatus* protoplast. It is anticipated that AfuPmV1 replication machinery will recognize the UTRs, resulting in the replication of the synthetic segment and expression of the hph selection marker. Our experimental design represents the first dsRNA-based reverse-genetic system and is facilitated by the unique features of AfuPmV1.

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GIANT VIRUSES IN THE ARCTIC: THEIR PRESENCE AND THE ISOLATION ATTEMPTS

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Giant viruses' complex particles contain large genomes encoding many proteins with unknown functions, a possible source for new biotechnological breakthroughs. They are ubiquitous, but despite their abundance in the ocean worldwide, few giant viruses have been found in cold-climate regions. Additionally, most known giant viruses to date were isolated from amoeba, creating a host bias that hinders the chance of finding novel viral groups. This study aims to isolate giant viruses from the Arctic using different groups of microalgae and amoeba as hosts. Ninety-four environmental samples were collected from latitude 84ºN and Svalbard in 2025. The samples were screened for giant viruses using amoeba and microalgae hosts and tested by qPCR for the detection of main giant virus families. qPCR analysis showed positive readings of *Marseilleviridae*, Cedratviruses, and Pandoraviruses from most of the freshwater (85%) and sea ice (73.3%) samples, particularly from glacial river water and seawater-ice interface. However, the isolation attempts yielded few suspected viruses: one from glacial river water in an *Acanthamoeba polyphaga* host, one from under-ice water samples in a mixed dinoflagellates culture, and two suspected from sea surface and glacial river water samples in a Chlorella-like algae host. Metagenomics sequencing of the suspected samples is being done to confirm the results. The findings highlight low recovery of giant viruses from polar samples despite being a hotspot for viral diversity. Isolations in more microalgae host types are underway. This project has received funding from the European Union's Marie Skłodowska-Curie Actions (MSCA) under grant agreement No. 101150485.

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ECOLOGICAL AND EVOLUTIONARY ANALYSIS OF ORTHOEBOLAVIRUS REVEALS SPECIES-SPECIFIC CODON USAGE PATTERNS AND HOST ADAPTATIONS

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Ebolaviruses, members of the genus Orthoebolavirus, circulate as re-emerging zoonotic viruses, but very little is known about their natural host associated with transmission dynamics and ecology, as well about the intra-host interaction to humans. Although Orthoebolavirus zairense has historically been responsible for the best-known outbreaks of Ebola virus disease, recent outbreaks caused by Orthoebolavirus bundibugyoense in the Democratic Republic of Congo demonstrate that other species can also emerge and cause severe disease in humans. To investigate these patterns, we analyzed genomic sequences of all Orthoebolavirus genus, performing phylogenetic analyses and examining codon usage patterns. Data sets were obtained from GenBank and analyzed using Relative Synonymous Codon Usage (RSCU) analysis, principal component analysis, and codon adaptation index (CAI) analysis. Briefly, the phylogenetic results demonstrate a congruence among the different genes. Codon usage analyses revealed specific signatures associated with virus species and geographic distribution. The inter-host adaptation indicated high CAI values to Pan troglodytes and Homo sapiens. Interestingly, Pteropodidae and Miniopteridae families exhibit lower CAI values when compared to primates, suggesting that these low values may be associated with a distinct infection pattern compared to that presented by primates. Considering the intra-host analysis to humans, high CAI values were observed to testes, liver, kidney, and skin. In summary, the results indicate patterns of codon usage for Orthoebolavirus species to their putative natural hosts, corroborating with a long period of coexistence between the organisms and also evidence of previous adaptation to Orthoebolavirus to humans as its dead end host.

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Identification of a viral sequence from the *Polycipiviridae* family in the midgut of *Nyssomyia neivai*.

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Nyssomyia neivai (Diptera: *Psychodidae*) is a New World hematophagous insect species of scientific interest due to its potential to transmit *Leishmania*. However, *N. neivai* virome remains unknown. Taking advantage of three public RNA-seq libraries, we identified a putative viral sequence of 9,918 nucleotides related to elements from *Polycipiviridae* family. This sequence showed no similarity at the nucleotide level when aligned against the nr database; at the protein level, it shared 30.83% identity over 32% coverage with a nonstructural protein from an unclassified *Polycipiviridae* isolate recovered from a bat metagenomic study (WWV87153.1). Genomic characterization revealed five ORFs: the first four (ORF1-4) are associated with the structural proteins of the capsid, and ORF5 encodes the replicative apparatus, which contains the conserved RNA-dependent RNA polymerase (RdRp) and helicase domains. Phylogenetic analysis of this viral family, associated with Hymenoptera, suggests a broader host range than previously described. Sequence characterization and phylogenetic analysis showed that this putative virus forms a distinct lineage within *Polycipiviridae*, diverging from the three currently recognized genera. It is placed as a sister lineage to a clade comprising unclassified at the genus level viral sequences recovered from bird and bat metagenomic studies 100% bootstrap support.To the best of our knowledge, this is the first virus described for *N. neivai*. Thus, knowledge regarding the species' virome is expanded, given the scarcity of genomic data and the lack of evidence concerning its vector activity. Investigating its virome is essential to understand its role in transmission dynamics and ecological interactions.
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PT.200

THE YELLOW FEVER VIRUS EXHIBITS GENOTYPE-SPECIFIC CODON USAGE PATTERNS THAT CORRELATES WITH THE VIRAL RNA QUANTIFICATION IN HUMAN TISSUES

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The yellow fever virus (*Orthoflavivirus flavi*, YFV), an arthropod-borne *Flavivirus*, comprises a single serotype and four distinct genotypes: East Africa, West Africa, South America I, and South America II. The diversification between African and American genotypes reflects long-term adaptation to distinct ecological niches. Despite its public health importance, the genomic diversity underlying YFV adaptation remains poorly understood. Here, we investigated genomic and ecological patterns associated with inter- and intra-host adaptation. We analyzed 327 complete YFV and YFV-related genomes retrieved from *GenBank*, focusing on genomic composition, codon usage, and evolutionary biases in relation to putative hosts and vectors. We also compared codon usage patterns with RT-qPCR data from seven human tissues collected from 69 fatal-YFV cases during the 2017–2019 Brazilian outbreak. Relative Synonymous Codon Usage (RSCU) and Codon Adaptation Index (CAI) analyses revealed genotype-specific clustering, with American genotypes showing greater codon usage diversity than African genotypes, likely reflecting adaptation to a broader range of hosts and vectors. To humans, CAI values were higher for the American and West African genotypes and lower for East African genotype, which clustered closer to YFV-related viruses. Within human tissues, the highest adaptation was observed for liver and kidney. Correlation analyses between median CAI values and median viral RNA concentrations according to the tissues demonstrated consistent positive correlations across all viral genes, ranging from strong to very strong. These findings provide the first biological evidence that viral genomic composition is associated with tissue tropism, highlighting codon usage adaptation as a potential determinant of YFV host and tissue specificity.
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PT.201

PARAMYXOVIRUSES IN NEOTROPICAL BATS: GENETIC DIVERSITY AND ECO-EPIDEMIOLOGICAL INSIGHTS FROM THE BRAZILIAN ATLANTIC FOREST

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Bats are recognized reservoirs of paramyxoviruses, including viruses of public health importance such as Hendra and Nipah. However, data regarding the occurrence of these viruses in Neotropical bats remain scarce. This study aimed to investigate the genetic diversity and eco-epidemiology of paramyxoviruses in bats from urban, peri-urban, and rural landscapes, and from Atlantic Forest areas in the state of São Paulo, Brazil. Samples were obtained through active field expeditions and passive surveillance conducted by partner institutions. Necropsies were performed, and tissues and swabs were collected. These were analyzed using nested RT-PCR assays targeting a conserved region of the L gene, followed by Sanger sequencing and phylogenetic analysis. Positive samples were further characterized using next-generation sequencing on an Illumina platform. To date, 26 bat species belonging to three families have been sampled, with seven animals testing positive for viral RNA. Detections occurred predominantly in kidney tissue, suggesting a possible renal tropism for some lineages, although positive results were also identified in rectal swabs, intestinal tissue, and lung tissue, expanding the spectrum of viral biodistribution. Phylogenetic analyses grouped the detected sequences into two genera, Jeilongvirus and Morbillivirus, with the recovery of two complete viral genomes. Sequences obtained from vampire bats clustered with bat morbilliviruses previously described in Brazil, whereas sequences from frugivorous bats clustered with Jeilongvirus lineages reported in the Brazilian Amazon. The data obtained contribute to the understanding of paramyxovirus diversity in Brazilian bats and highlight the relevance of these animals in the maintenance and dispersal of viruses with zoonotic potential.

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PT.202

MULTIPLE HUMAN-TO-SWINE TRANSMISSION EVENTS SHAPE THE EVOLUTION OF PANDEMIC H1N1 INFLUENZA VIRUS IN BRAZILIAN SWINE

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Pandemic influenza A(H1N1)pdm09 viruses have been repeatedly transmitted from humans to swine, where they continue to evolve through sustained circulation and reassortment. This study investigated the evolutionary dynamics of the six internal gene segments (PB2, PB1, PA, NP, MP and NS) of Brazilian swine influenza viruses collected between 2009 and 2024. A total of 209 swine isolates were sequenced using the Illumina MiSeq platform and analyzed together with more than 2,500 human H1N1pdm09 genomes. Maximum likelihood phylogenetic analyses were performed to identify human-to-swine transmission events, characterize transmission clusters, evaluate temporal dynamics and investigate genomic clustering profiles. At least 19 independent human-to-swine introductions were identified since the 2009 pandemic. Approximately half of these introductions resulted in sustained transmission within swine populations, whereas the remaining events represented self-limited infections. Most successful introductions occurred before 2018 and persisted for several years. Sampling intensity strongly correlated with swine herd size, indicating that the dataset broadly reflects the geographic distribution of Brazilian swine production. Genomic clustering profiles revealed recurrent constellations of internal gene segments shared among distinct influenza subtypes endemic in pigs, and across different states, supporting ongoing reassortment and interstate viral dissemination. Additionally, phylogenetic evidence identified a previously undescribed swine-to-human transmission event in São Paulo. These findings demonstrate that repeated reverse zoonotic transmission has established persistent H1N1pdm09 lineages in Brazilian swine, creating opportunities for viral diversification and zoonotic emergence. Continuous integrated surveillance at the human–swine interface is essential to detect emerging influenza variants and strengthen One Health preparedness. **Financial Support:** EMBRAPA (SEG 22.16.05.004.00.02), CNPq and FUNARBE/ARS/USDA (CT Postdoctoral Fellowship No. 13856).

PT.203

ISOLATION AND IDENTIFICATION OF TWO *KLEBSIELLA PNEUMONIAE* BACTERIOPHAGES FROM SEWAGE

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Klebsiella pneumoniae is a Gram-negative bacterium and an opportunistic human pathogen that has increasingly attracted attention due to its broad spectrum of antibiotic resistance, highlighting the need for alternative strategies to combat this microorganism, such as phage therapy. In this context, the present study identified two bacteriophages infective to *K. pneumoniae* ATCC using the liquid culture enrichment technique with urban and hospital sewage samples collected in Brasília, Federal District, Brazil, for 48 h. The filtered enrichment cultures were used in spot tests against *K. pneumoniae* ATCC at two incubation temperatures, 30 and 37°C. During isolation using the double-layer agar method, both translucent and opaque lysis plaques were observed, isolated, collected, and subjected to transmission electron microscopy (TEM). The results indicated a higher infectivity of the bacteriophages at 30°C compared with 37°C. TEM analysis revealed that the translucent and opaque plaques corresponded to two distinct bacteriophages, suggesting the presence of a lytic and a lysogenic phage, respectively. Therefore, these findings expand the current knowledge of bacteriophages and demonstrate a biotechnological potential for combating antibiotic-resistant *K. pneumoniae*. Further studies will be conducted to characterize the isolated bacteriophages, including genome sequencing, host range determination, and stability and treatment assays.

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PT.204

POSITIVITY RATE OF *Chlorella variabilis*-INFECTING VIRUS DETECTION BETWEEN 2024 AND 2025 IN TWO SOUTHEASTERN BRAZILIAN STATES

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Chloroviruses are large dsDNA viruses associated with three known host species used for research: *Chlorella variabilis*, *Micractinium conductrix*, and *Chlorella heliozoae*. In two years following the establishment of a plaque assay for *C. variabilis* in 1983, the collection of 55 samples from 16 cities in the United States led to the isolation of 17 chloroviruses, with a reported 31% positivity rate and titers up to 10^4 PFU/mL. Since 2024, our group has been isolating chloroviruses in Brazil, and here we report the outcome of a two-year effort aimed at detecting *C. variabilis*-associated viruses from 50 water samples randomly collected in 21 cities from 13 regions across Minas Gerais and São Paulo. Processing and testing of samples were conducted via filtration through 0.45 µm membranes, followed by plaque assays and liquid culture tests with host cultures. Fifteen chloroviruses were isolated from 9 of the 13 cities in Minas Gerais and 3 of the 8 cities in São Paulo. The maximum concentration of viable particles in environmental samples reached up to 10^6 PFU/mL at two sites in the metropolitan region of São Paulo. This 30% success rate and high viral titers suggest that *C. variabilis*-infecting viruses may be as abundant in Brazil as in the United States, where hundreds of virus strains have already been isolated. A plethora of environmental viruses in Brazilian ecosystems remain to be unveiled, and continued efforts in prospecting them might provide insights into how abundant and widespread chlorella-associated viruses are in our vast territory.
Financial support: FAPEMIG, CAPES, CNPq, PRPq

PT.205

ISOLATION AND MORPHOLOGICAL CHARACTERIZATION OF CHLOROVIRUS BR-MG-N08 INFECTING *CHLORELLA VARIABILIS* NC64A, THE FIRST ISOLATE FROM SOUTHERN MINAS GERAIS, BRAZIL

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Chloroviruses are large double-stranded DNA viruses that infect chlorella-like green algae and are known to occur in Brazilian freshwater environments, although studies on their diversity and distribution are still limited. Here, we report the isolation and initial morphological characterization of chlorovirus BR-MG-N08, obtained from a freshwater sample collected in the Sapucaí River in Santa Rita do Sapucaí, Minas Gerais, Brazil, using Chlorella variabilis NC64A as host. Environmental samples were centrifuged and the supernatant was filtered using a 0.45 µm membrane. The sample was then subjected to a double-layer agar plaque assay consisting of 1.5% bottom agar and 0.75% top agar containing $1x10^8$ NC64A cells and the filtered sample. The appearance of lytic plaques within 48 hours indicated the presence of infectious viral particles capable of replicating in C. variabilis NC64A. Once detected, the lysis plaques were subsequently purified through successive rounds of enrichment and plaque assay. Morphological analysis of BR-MG-N08 by negative-staining transmission electron microscopy (TEM) revealed icosahedral particles approximately 150 nm in diameter, consistent with the typical morphology of members of the genus Chlorovirus. The new virus will be prepared for DNA sequencing and genome characterization. These findings expand the current knowledge of chlorovirus occurrence in southern Minas Gerais, Brazil, and support further investigations into the diversity, distribution, and biology of chloroviruses in the region. Financial Support: FAPEMIG, CAPES, and CNPq.

PT.206

ESCHERICHIA PHAGE POLARIA – A NOVEL PHAGE WITH ADHERENCE TO MUCUS ADAPTATION

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Bacteriophage Adherence to Mucus (BAM) model proposes a symbiotic interaction between phages and metazoan hosts that relies on phage adherence phenotype to provide a non-host derived protection against bacterial infections. For the benefit of phages, weak binding to mucin fibers concentrates virions within the mucosal layer and enhances the chances for encounters with bacterial hosts. Replication efficiency on bacteria exposed to mucins, likely by exploiting virulence factors upregulated in the bacterial invasive phenotype, has been shown as another BAM-associated phenotype. Aiming to widen the scope of evidence that corroborates with the BAM model, and to highlight the importance of the associated phenotypes we isolated and investigated mucosal adaptations of *Escherichia phage* Polaria, a newly isolated phage from the Arctic. Polaria was isolated from an aquarium wastewater in Tromsø (Norway) and characterized by testing adherence and replication efficiency, as well as serial passage selection of adherent phages in mucin. Genome analysis focused on the detection and characterization of mucin-binding domains. Additionally, Polaria was tested against *E. coli* strains isolated from patients. We found that Polaria adheres to and grows better in the presence of mucin. Selection of phages that bind better to mucin can be achieved after a few passages, even in the absence of the canonical HOC protein associated with mucin binding in BAM model. Host-range tests revealed pathogenic bacteria strains susceptible to wild-type and to mucin adapted Polaria phages. Our work supports and expands the BAM model and paves the way for prophylactic phage-based practices based on mucosal adaptations.

PT.207

METAGENOMICS AS A TOOL FOR VIRUS DISCOVERY IN WILD ANIMALS

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Virus surveillance in wild animals is essential for discovering agents with the potential for cross-species transmission and for supporting conservation and One Health initiatives. This study combined three investigations to demonstrate the use of metagenomics as a tool for viral detection and characterization in seabirds and bats. Fecal samples from birds in the South Shetland Islands, Antarctica; frigatebirds and boobies from the Cagarras Islands Archipelago Natural Monument; and bats from the urban Atlantic Forest of Rio de Janeiro, Brazil, were analyzed. Viral RNA was extracted and screened by RT-PCR for coronaviruses and influenza A virus, and selected samples were subjected to metatranscriptomic sequencing on Illumina platforms. Sequences were filtered, assembled, compared against reference databases, and subjected to phylogenetic analysis. In Antarctic samples, a Deltacoronavirus was detected and its complete genome was recovered, along with 13 contigs related to rotaviruses, picornaviruses, and caliciviruses. In bats, complete genomes of Alpha- and Betacoronavirus were recovered, expanding knowledge of poorly characterized lineages. In birds from the Cagarras Islands, coronaviruses were detected in frigatebirds and boobies. Two complete and eight partial Deltacoronavirus genomes were obtained, in addition to picornaviruses and riboviruses. Metagenomics broadens investigations beyond the viruses initially targeted, enabling the recovery of complete genomes, the identification of potentially novel species, and the investigation of evolutionary relationships among viruses from different hosts and geographic regions. This approach strengthens wildlife surveillance and the early detection of risks relevant to One Health.

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PT.208

ISOLATION AND IDENTIFICATION OF GIANT VIRUSES FROM PANTANAL BIOME

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Since the discovery of the first giant amoeba virus, *Acanthamoeba polyphaga mimivirus*, in 2003, giant viruses have challenged classical concepts of virology due to their remarkable structural, genetic, evolutionary complexity. Consequently, the discovery of new giant viruses is essential to improve our understanding of their diversity and ecological roles. This study aimed to isolate giant viruses from environmental samples collected in the Pantanal wetland (Mato Grosso, Brazil), including water, sediment, and sewage, totaling 1,165 samples obtained during an expedition conducted in December 2025 as part of the project NAVIO. Additionally, 180 water samples from artificial and biological filtration aquaria at the School of Veterinary Medicine of the Federal University of Minas Gerais (UFMG) were analyzed. Viral screening was performed using *Acanthamoeba castellanii* cultured in peptone-yeast-glucose (PYG) medium supplemented with antimicrobial agents. Environmental samples were inoculated at a 1:5 dilution, and three seven-day isolation cycles were carried out. Viral infection was inferred by the observation of cytopathic effects compared with uninfected control cells. All aquarium samples and approximately 20% of the Pantanal collection were screened. No viral isolates were recovered from the aquaria. In contrast, ten viral isolates were obtained from the Pantanal samples, morphologically characterized by scanning electron microscopy. The isolates exhibited either icosahedral or ovoid particles. Three displayed fibrils resembling those of mimiviruses and megaviruses, five showed morphology compatible with cedratviruses, and two resembled marseilleviruses. These findings highlight the Pantanal as a promising environment for the discovery of novel giant viruses.

Financial Support: CAPES, CNPq , FAPEMIG e NAVIO.

Keywords: Giant viruses; Pantanal; Prospecting; Isolation; Electron microscopy.

PT.209

Phylogenetic panorama of Brazilian hantaviruses: genetic diversity and implications for surveillance

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Hantaviruses are zoonotic RNA viruses responsible for severe human diseases. In Brazil, the genetic diversity of circulating genotypes remains only partially characterized. This study aimed to assess the phylogenetic diversity of Orthohantavirus in Brazil using publicly available sequences. We retrieved 140 S segment sequences (encoding the nucleocapsid protein) from GenBank, restricted to Brazil (2000-2026). Sequences underwent quality control, alignment (MAFFT), and trimming (MEGA12). Duplicates were removed, resulting in a final dataset of 64 unique sequences. A Maximum Likelihood phylogenetic tree was inferred using IQ-TREE (v.2.4.0), with 1000 bootstrap replicates. The analysis identified seven well-supported clades corresponding to the main Brazilian genotypes: Araraquara, Juquitiba, Laguna Negra, Jabora, Araucaria, and Castelo dos Sonhos/Rio Mamoré-like lineages. Araraquara was the most abundant clade (26 sequences, bootstrap 97%), predominantly from São Paulo state. Laguna Negra (99%) was restricted to Mato Grosso state. Juquitiba and Araucaria formed a closely related Juquitiba-like clade, consistent with current ICTV taxonomy. The Andes virus, a genotype endemic to Argentina/Chile, was also identified as a single sequence from São Paulo (2022). Although the length of our segment (170 bp) limited the resolution of deeper relationships, our analysis reveals the considerable genetic diversity of orthohantaviruses in Brazil, highlighting the need for continued genomic surveillance in order to timely detect the emergence of new strains.

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This study did not receive any financial suport.

PT.210

MULTIPLATFORM SCIENCE COMMUNICATION ON MPOX REACHES DISTINCT YOUNG AND PROFESSIONAL AUDIENCES

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Since 2022, monkeypox virus (MPXV) has spread globally, becoming a major public health concern. Its continued circulation and misinformation underscore the need for evidence-based communication adapted to different audiences. This study evaluated a multiplatform science communication project on MPXV. Four educational posts addressing epidemiology, viral biology, transmission, and vaccination were created in Canva after a literature review and published on Instagram, TikTok, and LinkedIn. Metrics analyzed were views, reach, interactions, audience characteristics, traffic sources, and video retention. As identical posts were shared across platforms, engagement was compared using the Friedman test followed by Bonferroni-corrected Wilcoxon signed-rank tests. Chi-square or Fisher's exact tests, when expected counts were below five, compared engagement rates, demographics, and geographic distribution. Instagram accumulated 10,496 views, reached 5,719 accounts, and generated 285 likes, 87 comments, 84 shares/reposts, and 77 profile visits, mainly among women aged 18–34 years. TikTok exceeded 4,000 views, with 83.2%–95.8% of traffic from the "For You" feed and up to 78% of viewers aged 18–24 years. Mean viewing time was below six seconds, except for the vaccination video (11.65 s). LinkedIn generated 616 impressions and reached 273 users, mainly professionals in higher education, research, and healthcare. Total engagement differed significantly across platforms (Friedman $\chi^2=8.00$, $p=0.018$), consistently ranking Instagram>TikTok>LinkedIn. Platform-specific strategies may expand access to reliable virology information by combining youth outreach with professional dissemination.

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PT.211

A practical low-cost course for teaching bacteriophage isolation to undergraduate students

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Bacteriophages are viruses that infect bacteria and have gained attention as promising tools against antimicrobial-resistant pathogens. Despite their relevance, phage biology remain underrepresented in undergraduate curricula. This study describes a low-cost course designed to introduce students to fundamental virology concepts through hands-on phage isolation, characterization, and genome analysis. Participants collected environmental water samples, performed phage enrichment, and detected viral plaques using the double-layer agar technique. The course also incorporated web-based bioinformatics activities using BLASTN and PhageScope, enabling students to explore genome size, GC content, gene annotation, and taxonomy without the need of coding knowledge or powerful computers. These activities strengthened the integration between laboratory and computational virology. Students had access to key concepts such as viral diversity, lytic cycles, biosafety, host specificity, and plaque quantification. This accessible educational model promotes active learning, democratizes access to virology training, and helps prepare future professionals for research in bacteriophage biology and phage therapy.

Funding: FAPEMIG, CAPES, CNPq, CANS.

PT.212

Development of a Culturally Adapted Puppet Show for Virology Science Communication in Paraguay

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In Paraguay, public communication about viruses has focused primarily on prevention campaigns during epidemics, while science communication initiatives that explain virology to the general public remain limited. To address this gap, a culturally adapted puppet theater production was developed to communicate fundamental concepts of virology through an engaging narrative integrating medically important viruses, scientific research and elements of Paraguayan cultural identity. The production resulted from a collaborative process involving virologists, a puppet designer and a theater actress. Viruses were selected based on their public health relevance in Paraguay, and scientific concepts were translated into an accessible narrative featuring culturally meaningful characters, visual elements, and dialogues in *jopará* (the everyday mixture of Spanish and Guaraní, both official languages in Paraguay). The play is set in a laboratory where Dr. Valeria, a young scientist, and Ñati (from "*ñati'ũ*," Guaraní for mosquito), an anthropomorphized female *Aedes aegypti* mosquito, guide the audience through the story. Virus characters (dengue virus, chikungunya virus, influenza virus, SARS-CoV-2, and rotavirus) appear individually through a computer screen window incorporated into the stage set, representing the microscopic world. Each character was designed to reflect the distinctive biological and epidemiological characteristics of the corresponding virus. The story concludes with an interactive activity in which the audience applies preventive measures to contain virus transmission while highlighting the role of scientific research in protecting public health. The play provides a resource for virology science communication and establishes a foundation for future theatrical performances and evaluation of its educational impact. No funding was received.

PT.213

GAMING FOR PUBLIC HEALTH: DESIGNING A VISUAL NOVEL TO EQUIP HEALTHCARE AGENTS IN PERNAMBUCO

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Rabies is a fatal zoonotic viral encephalomyelitis, making timely post-exposure prophylaxis (PEP) a critical public health priority given the disease's near 100% case-fatality rate. Despite robust national guidelines, PEP management in Brazil faces systemic challenges, including clinical protocol non-compliance, patient misdirection, and difficulties in exposure risk assessment among frontline workers. To address these gaps, an immersive gamification strategy was developed for professional training. Content creation was informed by a systematic review of Brazilian rabies case studies (2021–2026) indexed in CAPES and PubMed, ensuring alignment with Ministry of Health protocols. Branching narrative scripts featuring interactive dialogues and clinical decision points were developed and coded using the Ren'Py engine. This process yielded two serious games: a clinical decision-making module simulating six routine public health unit scenarios, and a biosafety-focused virus spillover simulation. The interventions were integrated into ongoing health education programs across the Agreste, Sertão, and Zona da Mata regions of Pernambuco. To date, this ongoing initiative has completed 9 public dissemination events and 4 training workshops, qualifying 144 healthcare professionals, with active plans for geographic and multi-disease scaling.Leveraging interactive visual novels proves to be a highly effective methodology for continuous medical education. This gamified approach reinforces long-term retention of prophylactic protocols, mitigates clinical errors, and strengthens local epidemiological surveillance.

Financial Support: Fundação de Amparo à Ciência do Estado de Pernambuco (FACEPE).

PT.214

Quantification of HTLV-1 Proviral Load by dPCR: Prospects for Monitoring Clinical Progression

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Human T-cell lymphotropic virus type 1 (HTLV-1) is a deltaretrovirus found worldwide and is associated with severe diseases, such as adult T-cell leukemia/lymphoma (ATLL) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). Proviral load (PVL) is an important marker related to the pathogenesis and clinical progression of HTLV-1 infection. However, accurately quantifying low PVLs may be challenging with conventional quantitative methods. In this context, digital PCR (dPCR) emerges as a promising approach by providing greater analytical sensitivity, thereby favoring the monitoring of people living with HTLV-1 who present PVL values below the limit of detection by traditional methods, while also allowing the absolute quantification of the DNA target without requiring a standard curve. This study aimed to perform a preliminary dPCR assay for the detection and quantification of HTLV-1 proviral DNA in clinical samples. dPCR was conducted using the QIAcuity® EG PCR Kit with 100 ng of gDNA extracted from peripheral blood leukocytes from HTLV-1-positive and HTLV-1-negative individuals. Specific primers targeting the HTLV-1 pol gene were used, and human albumin was included as an endogenous control. Proviral DNA was detected in all positive samples, and absolute quantification was expressed as copies/µL. Together, these preliminary results demonstrate the technical feasibility of dPCR and support further assay standardization using samples from people living with HTLV-1, aiming to evaluate its future application in detecting low PVLs and monitoring infection.

Financial support: FAPEMIG, CAPES, and CNPq.

PT.215

LONGITUDINAL CLINICAL AND MOLECULAR RESPONSE TO MOLNUPIRAVIR IN A KITTEN WITH FELINE CALICIVIRUS-ASSOCIATED SYSTEMIC DISEASE

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Feline calicivirus (FCV) can cause severe, potentially fatal systemic disease, for which no specific antiviral treatment has been established. This report describes the longitudinal clinical and molecular response of a one-month-old kitten with FCV-associated systemic disease treated with molnupiravir. The kitten presented with fever, lethargy, weight loss, generalized edema, polyarticular pain and swelling, multifocal cutaneous ulceration, tongue-tip necrosis, pleural effusion, and a diffuse alveolar pulmonary pattern. FCV RNA was detected by RT-qPCR in an oral swab (quantification cycle [Cq] 16) and whole blood (Cq 15), whereas tests for feline immunodeficiency virus (FIV), feline leukemia virus (FeLV), and feline herpesvirus type 1 (FHV-1) were negative. Molnupiravir was administered orally at 20 mg/kg every 12 h for 21 days together with supportive therapy. Serial testing showed increasing Cq values in oral swabs (16, 18, 21, 28, and 31 on days 0, 1, 2, 7, and 10) and blood (15, 25, and 31 on days 0, 7, and 10), followed by undetectable FCV RNA. Normal cat's activity resumed by day 3, marked regression of systemic and cutaneous signs occurred by day 10, and complete clinical recovery was achieved by day 21. Transient neutropenia and thrombocytopenia on day 10 resolved by day 21. At day 51, the kitten remained healthy, both samples were RT-qPCR-negative, thoracic abnormalities had resolved, and body weight had increased by 172%. The temporal association between treatment, clinical recovery, and declining FCV RNA supports controlled evaluation of molnupiravir for severe FCV-associated disease.

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PT.216

WHY RABIES MUST ALWAYS BE INVESTIGATED: MADARIAGA VIRUS–RABIES CO-INFECTION IN HORSES

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Madariaga virus (MADV) has emerged as an important cause of equine encephalitis in South America, increasing the use of molecular assays for its diagnosis. However, detection of a single neurotropic virus may lead to premature closure of the diagnostic investigation, particularly in regions where rabies remains endemic. This study describes horses with neurological disease in which molecular assays detected both MADV and rabies virus (RABV), highlighting the diagnostic and epidemiological implications of viral co-infection. Horses presenting neurological signs were investigated using molecular assays targeting MADV, RABV, equine herpesvirus type 1, and flaviviruses. MADV-positive samples were confirmed by sequencing. Clinical presentation, sample type, and laboratory findings were reviewed. Coinfected animals presented neurological signs indistinguishable from those observed in single-virus infections. Detection of MADV alone would not have identified the concurrent rabies infection, a zoonosis of major public health importance requiring mandatory notification and specific control measures. These findings demonstrate that identification of MADV should not interrupt the investigation of other neurotropic pathogens, particularly rabies virus. Comprehensive laboratory testing and submission of central nervous system samples remain essential for definitive diagnosis, epidemiological surveillance, and implementation of appropriate public health measures in horses with neurological disease.

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Epidemiological and Molecular Characterization of Seasonal Human Coronaviruses and SARS-CoV-2 in Pediatric Respiratory Infections During and After the COVID-19 Pandemic in São Paulo, Brazil

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Respiratory diseases are a major cause of morbidity and mortality in children, particularly those under five years of age. Human coronaviruses (HCoVs), including seasonal strains (229E, NL63, OC43, and HKU1) and SARS-CoV-2, contribute significantly to respiratory infections. This study evaluated the incidence and genetic variability of these coronaviruses in pediatric patients with respiratory diseases in São Paulo, Brazil, during and after the COVID-19 pandemic. A retrospective analysis was conducted using 24,408 respiratory samples collected from patients aged 0 to under 18, between 2020 and 2025, from six public hospitals. Overall, 1,885/24,408 (7.7%) samples were positive for coronaviruses, including 1,065/1,885 (56.49%) seasonal HCoVs and 820/1,885 (43.51%) SARS-CoV-2. Positive cases were classified into six pediatric age groups: neonatal period (0?28 days), infant 1 (29 days?6 months), infant 2 (7 months?2 years), preschool (2?6 years), school age (7?10 years), and adolescence (11?17 years, 11 months, and 29 days). Genotyping was performed by TaqMan® RT-PCR in 184 previously positive samples. SARS-CoV-2 showed peaks associated with the emergence of new variants, including Omicron in January 2022, whereas seasonal HCoVs predominated during winter and early spring. Among typed seasonal HCoVs, OC43 was the most prevalent (108/184; 58.69%), followed by HKU1 (37/184; 20.1%), NL63 (24/184; 13.04%), and 229E (13/184; 7.06%). Eight coinfections involving OC43 were identified, mainly with HKU1 (50%), followed by NL63 (37.5%) and 229E (12.5%). One HKU1/229E coinfection was also detected. We acknowledge FAFESP Financial Support - N.2025/00179-8; 2025/09636-2; 2023/07746-0. Also FUNDEP Financial Support - N. 1882891 and CAPES 88887.162419/2025-00

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INVESTIGATION OF SEROLOGICAL RESPONSE AGAINST YELLOW FEVER VIRUS (YFV) AND ITS INFLUENCE ON PATIENTS INFECTED WITH DENGUE VIRUS (DENV).

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Dengue (DENV) and yellow fever (YFV) viruses share structural similarities that could induce immunological cross reactivity, a feature described for *Flaviviridae* family. However, there is low information about how previous humoral immunity against YFV could influence a subsequent infection by DENV. This study evaluates the influence of previous serological immunity against YFV in the evolution of DENV infection. In this context, 144 serum samples were collected from individuals with confirmed DENV infection and separated into 2 severity groups (with and without warning signs) and neutralizing antibodies against YFV were quantified by virus neutralization test (VNT). Samples were equally distributed between sex, with 52% of adults, 29% of elderly and 19% of children. Until now, 98 samples were submitted to VNT (67 without warning signs and 31 with warning signs), in a serial dilution (1:20 to 1:2560) to calculate the VNT_{50}. Populational arithmetic and geometric mean titers were 208.06 and 18.68, respectively. When stratified by DENV severity, for the warning signs group, the respective arithmetic and geometric mean titers were 217.13 and 30.47, while for the group without warning signs, values were 203.86 and 14.89, in that order. According to literature, the general antibody mean titer against YFV follows the expected values for vaccinated individuals (between 40 and 320). Regarding severity, a higher neutralization titer in the warning signs group was observed, but without statistical significance until now. After VNT conclusion, a major correlation analysis will be performed to check any association among YFV antibodies, demographic factors and DENV clinical evolution.

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INFECTION OF PLACENTAS AND EXPRESSION OF THE CYCLIC AMP-RESPONSIVE ELEMENT-BINDING PROTEIN 3 REGULATORY FACTOR (CREBRF) IN EXPERIMENTAL OROPOUCHE VIRUS INFECTION.

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Oropouche virus (OROV) is the agent of Oropouche fever, occurring in outbreaks recorded since the 1960s, mainly in the Amazon region. In 2023 a new OROV reassortant (OROV-AM0088) emerged and caused a wave of human cases in several regions of Brazil and other countries, including congenital infections with fetal anomalies and abortion. Previous data from our group showed that deletion of the non-structural protein encoded by the medium segment of OROV RNA genome (NSm), which originated rOROV-ΔNsm, resulted in greater virulence in an experimental mouse model. The present study was done to investigate the transplacental transmission of OROV-BeAn19991 (ancestral strain), rOROV-ΔNsm, and OROV-AM0088 (reassortant) in C57BL/6 mice, and the expression of CREBRF, a cellular protein involved in autophagy, in OROV-infected trophoblast in vitro. We showed that OROV infects placental tissue in vivo and that rOROV-ΔNSm was more virulent, suggesting that NSm plays an attenuating role in transplacental transmission. JEG-3 human trophoblast cell line is susceptible and permissive to OROV infection in vitro, and the different OROV strains used caused different CREBRF expression patterns. These findings have implications for the role of OROV nonstructural proteins in fetal pathogenesis. Financial Support: FAPESP, CNPq and FAEPA

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ASSESSING HTLV-1 TRANSCRIPTIONAL ACTIVITY THROUGH REAL-TIME PCR AND DIGITAL PCR-BASED QUANTIFICATION OF VIRAL STRUCTURAL AND ACESSORY GENE EXPRESSION

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Human T cell leukemia virus type 1 (HTLV-1) is a retrovirus that causes two major diseases: adult T-cell leukemia/lymphoma (ATLL) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). Approximately 90% of the infected individuals remain asymptomatic carriers (ACs) during their lives. HTLV-1 proviral load (PVL) is an important risk marker for HAM/TSP, however, the frequency of ACs with variable PVL in peripheral blood hampers its usage as a potential prognostic marker. Therefore, the evaluation of HTLV-1 mRNAs in peripheral blood cells might provide useful insights on future HAM/TSP prognostic biomarkers. Our objective is to standardize and compare real-time PCR (qPCR) and digital PCR (dPCR) for the quantification of viral transcripts in peripheral blood mononuclear cells (PBMCs) from HTLV-1-infected individuals, focusing on potential differences in sensitivity, specificity, and detection performance. For further characterization of mRNA in clinical samples, total RNA will be extracted and purified combining TRIzol® reagent (Invitrogen) and RNeasy Mini Kit® (QIAGEN), followed by quantification (NanoDrop), DNase treatment, cDNA synthesis, and amplification of cDNA derived from HTLV-1-specific tax/rex and gag/pol mRNAs, using GAPDH as an endogenous control. Preliminary data using MT-2 cell line, HTLV-1-infected cell line, has given positive detection signals for both viral targets. Gag-pol was significantly more expressed as compared to tax-rex in MT-2 infected cell line using conventional real-time PCR. Future studies are yet required to verify the prognostic power of *gag-pol* and *rex-tax* mRNA accumulation using either real-time PCR or digital PCR.

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NEUTRALIZING AUTOANTIBODIES AGAINST TYPE I INTERFERONS IN ARBOVIRAL INFECTIONS.

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Arboviruses represent a major global public health problem, accounting for thousands of deaths each year. Although most infections are asymptomatic or self-limiting, a subset of patients progresses to severe clinical manifestations. Type I interferon (IFN-I)-mediated responses constitute one of the main mechanisms of innate antiviral defense. However, the presence of neutralizing autoantibodies against type I interferons (AAN-I-IFN) can impair this response, increasing susceptibility to severe disease. In this study, we investigated the association between AAN-I-IFN and the severity of infections caused by the chikungunya virus (CHIKV), Oropouche virus (OROV), and yellow fever virus (YFV). We analyzed 76 samples of serum, whole blood, and cerebrospinal fluid (CSF) from patients with acute, fatal, and neurological syndrome cases, as well as healthy controls, using indirect ELISA to detect autoantibodies against IFN-α2a, IFN-β1b, and IFN-ω1. The highest prevalence of AAN-I-IFN was observed in fatal CHIKV cases. These cases exhibited significantly higher levels of autoantibodies against all three IFN-I subtypes than acute CHIKV cases, with additional differences observed between serum and CSF samples and in comparison with healthy controls. In OROV infections, significant differences were observed primarily between healthy controls and infected patients, with IFN-ω1 standing out in cases with neurological syndrome. In contrast, in YFV infections, autoantibodies were detected exclusively against IFN-ω1, with no significant differences between the clinical groups. These findings indicate that IFN-I autoantibodies exhibit distinct profiles across the arboviral infections evaluated, highlighting their potential as biomarkers associated with disease severity and clinical outcomes.

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IMMUNOPATHOLOGICAL FEATURES OF SARS-COV-2-INFECTED PLACENTAS: EVIDENCE OF INFLAMMATION AND MATERNAL–FETAL INTERFACE DISRUPTION

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At the end of 2019, a novel coronavirus, designated Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), belonging to the Coronaviridae family emerged in Wuhan, Hubei Province, China. On March 2020, the World Health Organization (WHO) declared COVID-19 a pandemic. Previous studies had already associated infections caused by other coronaviruses, including SARS-CoV and MERS-CoV, with several obstetric complications. In this context, the present study aimed to investigate the histopathological alterations in placental samples obtained from pregnant women infected with SARS-CoV-2 as well as characterize the immunological profile in these samples. Five placentas samples from unvaccinated pregnant women infected with SARS-CoV-2 and five placentas from uninfected pregnant women used as controls were analyzed. Histopathology was performed using routine and special histological staining techniques. Spike protein detection and immunological characterization were carried out by immunoperoxidase and immunofluorescence assays. Histopathological analysis revealed extensive mononuclear inflammatory infiltrates associated with widespread fibrin deposition and trophoblastic cell necrosis. Spike protein was detected within the intervillous space, trophoblasts, and $CD163^+$ cells. The inflammatory infiltrate was predominantly composed of $CD163^+$, $CD68^+$, $CD4^+$, and $CD8^+$ cells. Additionally, increased expression of the pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 was observed in both maternal and fetal cells. Collectively, these findings suggest that SARS-CoV-2 infection is associated with an intense placental inflammatory response capable of disrupting maternal–fetal barrier homeostasis, thereby contributing to a better understanding of the potential impacts on fetal development and pregnancy outcomes. This study was approved by the Research Ethics Committee (CEP/FIOCRUZ) under protocol number 38365420.4.0000.5248. Financial support: UERJ, FIOCRUZ, CNPq and FAPERJ.

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MOLECULAR AND PATHOLOGICAL CHARACTERIZATION OF BRAZILIAN PORCUPINEPOX VIRUS IN TWO FREE-RANGING *COENDOU SPINOSUS* FROM SOUTHERN BRAZIL

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Brazilian porcupinepox virus (BPoPV) is an emerging poxvirus associated with severe cutaneous disease and fatal outcomes in porcupines *(Coendou spinosus),* although molecular data remain scarce. This study investigated two cases of severe cutaneous disease in free-ranging porcupines from Rio Grande do Sul, Brazil, combining pathological and molecular analyses. Both animals presented with marked swellings of the nasal region, eyelids, and genitalia associated with ulcerative and proliferative lesions that progressed to severe respiratory distress. Histopathological examination revealed epidermal hyperplasia, ballooning degeneration, eosinophilic intracytoplasmic inclusion bodies, and marked dermal expansion by abundant myxoid matrix containing proliferating mesenchymal cells, confirmed by Alcian blue staining. Viral DNA extracted from lesional tissues was subjected to PCR targeting the metalloprotease gene, followed by Sanger sequencing and phylogenetic analysis. Both samples shared 100% nucleotide identity with previously described Brazilian BPoPV isolates and grouped within the BPoPV clade in the phylogenetic analysis. Combined pathological and molecular findings revealed a previously unreported myxomatous dermal proliferative pattern resembling lesions of rabbit myxomatosis. These findings expand the pathological spectrum of BPoPV infection and reinforce the value of integrating histopathology and molecular characterization for diagnosing emerging poxvirus infections in wildlife.

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PREVALENCE AND CLINICAL PROFILE OF HEPATITIS E VIRUS INFECTION IN PREGNANT PATIENTS FROM SOUTHERN BRAZIL

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The Hepatitis E Virus (HEV) poses a critical threat to pregnant women, potentially causing up to 30% mortality in the third trimester due to fulminant hepatic failure and obstetric complications, often worsened by the physiological, immunological, and hormonal changes that accompany pregnancy. To evaluate the prevalence, clinical features, and hepatic function markers of active HEV infection in this population, 278 serum samples from pregnant patients at a reference hospital in Santa Catarina, Brazil, were analyzed using RT-qPCR to detect viral genomes, alongside a review of electronic medical records. HEV prevalence was 3.2% (9/278), and the predominant profile of positive patients was adult, white women with no prior comorbidities. Despite the report of initial symptoms such as vomiting and fever, all positive patients had favorable clinical outcomes, normal pregnancies, and healthy newborns. Hepatic function markers (ALT, AST, total bilirubin, and alkaline phosphatase) remained within reference ranges and showed no difference compared to the non-detected group. However, significant lymphopenia was observed in most pregnant women in both groups, reflecting physiological immunomodulation during gestation. These findings indicate that HEV circulated with a mild clinical presentation in this cohort, without producing the classic liver enzyme peaks typically associated with infection. This underscores the limitations of relying solely on biochemical markers for diagnosis and emphasizes the urgent need for standardized molecular testing to monitor and protect high-risk pregnancies.
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MOLECULAR SURVEILLANCE AND OSELTAMIVIR RESISTANCE PROFILING OF INFLUENZA A VIRUSES IN PEDIATRIC PATIENTS IN SÃO PAULO CITY, BRAZIL

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Infection by Influenza A and B viruses (Orthomyxoviridae) causes severe complications and high hospitalization rates, particularly in pediatric populations. Continuous monitoring is essential due to high mutation rates in surface glycoproteins. Emerging neuraminidase inhibitor (NAI) resistance mutations, such as H275Y in H1N1 and E119V/R292K in H3N2, threaten Oseltamivir Phosphate efficacy, highlighting the need for genomic surveillance. We evaluated the molecular epidemiology and mapped key NAI resistance markers isolated from pediatric clinical samples in São Paulo, monitoring Oseltamivir-resistant strains. Between August 2025 and July 2026, 5,497 naso/oropharyngeal swabs were collected from five public pediatric hospitals in São Paulo. Automated magnetic bead-based extraction was followed by multiplex RT-qPCR for 15 respiratory viruses. Influenza A-positive samples were genotyped (H1N1 vs. H3N2) through Neuraminidase fragment amplification via conventional PCR, agarose gel electrophoresis, and Sanger sequencing to assess resistance markers. Out of 5,497 processed samples, 251 tested positive for Influenza A by RT-qPCR. Our initial results showed 31 samples successfully amplified by conventional PCR, genotyped as A(H1N1)pdm09 (n=7) and A(H3N2) (n=24). Subsequently, 23 samples were successfully sequenced and evaluated for Oseltamivir resistance profiles. This molecular surveillance workflow enabled precise identification and subtype characterization of circulating Influenza A strains, providing key molecular data to monitor antiviral resistance in hospitalized pediatric patients.

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Keywords: Influenza A; Oseltamivir Resistance; Molecular Surveillance.

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STANDARDIZATION OF RT-LAMP WITH SIMPLIFIED EXTRACTION FOR THE DIAGNOSIS OF DENGUE AND CHIKUNGUNYA

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The growing demand for rapid, sensitive, and accessible diagnostic methods has driven the development of alternatives to qRT-PCR, current gold standard. Among these, loop-mediated isothermal amplification (RT-LAMP) stands out for its speed, operational simplicity, low cost, and visual readout of results. RT-LAMP operates at a constant temperature, which eliminates the need for thermocyclers and supports diagnostic decentralization, especially in limited infrastructures. However, RNA extraction remains a bottleneck due to its high cost and need for specialized equipment.This study aimed to optimize and standardize RT-LAMP-based diagnosis using simplified extraction protocols for dengue (DENV) and chikungunya virus (CHIKV). Primers previously described in the literature and rapid extraction protocols were selected and evaluated. RT-LAMP reactions were standardized at 63°C for 45 minutes using the WarmStart® colorimetric kit (NEB). For the simplified extraction step, the SwiftX™ kit was evaluated and is still under optimization.Clinical samples of dengue and chikungunya, provided by the Belo Horizonte health secretary (MG), were subjected to conventional and simplified extraction methods, followed by qRT-PCR and RT-LAMP for performance comparison. Samples extracted by the conventional method showed satisfactory results for both qRT-PCR and RT-LAMP. In contrast, simplified extraction resulted in late Ct values in qRT-PCR and RT-LAMP assays yielded indeterminate results, suggesting low recovery of genetic material. New clinical samples and extraction protocols will be evaluated to improve the sensitivity of the method. The improvement of this strategy may enable a rapid, affordable, and decentralized diagnostic platform, with potential impact on clinical management and surveillance of arboviral diseases in resource-limited settings.

Key-words: LAMP, arborvirus, simplified extraction.

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Active Infection or Viral Persistence? Re-evaluating Parvovirus B19 Detection in Acute Febrile Illness

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Parvovirus B19 (B19V) has been reported in individuals presenting with acute-febrile illness (AFI) and is frequently co-detected with other pathogens. This study analyzed B19V co-detections among AFI-individuals. We conducted a descriptive cross-sectional study (2010–2023) analyzing 957 population-proportional AFI serum from Amapá, Alagoas, Maranhão, Mato Grosso do Sul, Piauí, Paraíba, Tocantins, and São Paulo. Samples were collected at state Central Public Health Laboratories (LACENs) as part of routine screening protocols, including tests for arboviruses, enteroviruses, and Leptospira. Subsequently, samples were sent to the Laboratory of Virology, School of Medicine, University of São Paulo (USP) for real-time PCR targeting B19V, Dengue, Zika, Chikungunya, Mayaro, Oropouche, Yellow-fever, and Enterovirus. At USP, samples were also tested for B19V-IgM/IgG antibodies (VIRCELL®). Positive diagnosis (PCR and/or IgM) were established in 367 individuals. Detection rates were: Dengue (21.3%, n=204), B19V (11.4%, n=109), Chikungunya (6.8%, n=65), Zika (3.0%, n=29), Enterovirus (0.2%, n=2), and Leptospira (0.2%, n=2). Isolated detections of EBV, CMV, HBV, and rubella accounted for 0.4% (n=4). Co-detections were observed in 11.9% (44/367). Among B19V-positive cases, co-detected pathogens represented 26.6% (29/109). Among cases diagnosed as dengue-positive, B19V was co-detected in 10.7% (22/204). In chikungunya- and Zika-positive cases, B19V was co-detected in 9.2% (6/65) and 6.9% (2/29), respectively. Additionally, B19V-IgG was detected in 60.5% (66/109) of B19V-positive cases. These co-detections challenge our understanding of B19V AFI role, whether it represents an active infection or mere viral persistence, given the concomitant detection of B19V-IgG antibodies, which indicate long-lasting immunity. Potential synergism with co-detected agents should be considered.

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NOVEL BROAD-SPECTRUM ANTIVIRAL CANDIDATES AGAINST OROPOUCHE VIRUS AND OTHER EMERGING RNA AND DNA VIRUSES

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The emergence of viral pathogens and the lack of specific antivirals highlight the need for broad-spectrum therapies. Yellow fever (YFV), Chikungunya (CHIKV), Oropouche (OROV), Human Herpesvirus 1 (HHV-1), and Vaccinia virus (VACV) are clinically relevant viruses from distinct families (RNA and DNA viruses). Notably, OROV has become a major public health concern, for which there are no approved vaccines or antiviral drugs. Here, we aimed to evaluate the broad-spectrum antiviral activity of seven candidate compounds against these viruses. Cytotoxicity was assessed by AlamarBlue and antiviral activity was determined through virucidal assays. Viral suspensions were incubated with the compounds (100–0.001µM) for 1 hour at 37°C, followed by viral titration using plaque-forming unit (PFU) assays. Mechanistic studies further explored compound activity across different stages of the viral replication cycle. Among the tested compounds, PROSP-N7 exhibited a broad-spectrum antiviral profile, with 100% of viral inhibition against YFV, OROV, HHV-1, and VACV, and a 97.97% of CHIKV reduction. PROSP-N2 also demonstrated potent activity, with complete inhibition of YFV and HHV-1. Against OROV, PROSP-N7 showed superior potency (IC_{50} = 0.86 µM) compared to PROSP-N2 (IC_{50} = 3.53 µM). Although selectivity index (IS) were >28.32 and 2.66, respectively, PROSP-N7 maintained complete virucidal activity at concentrations up to 10 µM, indicating that direct viral inactivation is likely the dominant mechanism of action. Collectively, these findings reveal a broad-spectrum antiviral activity across diverse viruses, highlighting PROSP-N7 and PROSP-N2 as a promising lead compound. It supports the development of studies on broad-spectrum antiviral therapy, particularly against emerging viruses.

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THE CONTEXT MATTERS: DIAGNOSTIC PERFORMANCE OF RAPID DENGUE TESTS ACROSS CLINICAL AND IMMUNOLOGICAL SCENARIOS

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Introducion: Dengue diagnosis remains challenging in endemic settings, particularly where RT-qPCR is not routinely available. Rapid tests expand diagnosis access, but he performance of NS1, IgM and IgG is not fixed: it varies with illness duration, previous immune status for dengue, patient characteristics, and clinical presentation. **Objective:** To evaluate whether integrating rapid-test markers with immune status, symptom duration, and clinical characteristics improves dengue diagnostic discrimination compared with interpreting each marker alone. **Methods:** We conducted a retrospective diagnostic accuracy study in a hospital-based cohort of patients with suspected arboviral infection in a dengue-endemic region of Brazil. NS1, IgM, and IgG rapid-test results were assessed individually and in AND/OR combinations, stratified by immune status and symptom duration. Multivariable clinical-laboratory models were developed using RT-qPCR as the reference standard. **Results:** The performance of individual markers varied substantially across clinical and immunological scenarios. NS1 was the most consistent single marker, whereas IgM and IgG performance depended on immune status and symptom duration. The NS1-or-IgG combination provided the most balanced rapid-test strategy. Incorporating rapid-test results into a clinical-laboratory model markegly improved discrimination within the study cohort, increasing the area under the receiver operating characteristic curve from 0.667 to 0.903. **Conclusion:** Rapid dengue tests are clinically valuable, but their results should not be interpreted as stand-alon evidence. Integrating NS1, IgM, and IGG with the timing of illness, immune background, and clinical characteristics substantially improves diagnostic interpretation. This context-based approach may support more reliable dengue assessment where molecular testing is limited or unavailable.

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Genomic Surveillance and Phylogenetic Characterization of Respiratory Syncytial Virus in Hospitalized Children from Southern Brazil

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Respiratory syncytial virus (RSV) is a leading cause of severe acute respiratory infections in children worldwide and exhibits substantial genetic diversity that may influence viral transmission, immune escape, and clinical outcomes. This study investigated the molecular epidemiology, genomic diversity, and phylogenetic relationships of RSV detected in hospitalized pediatric patients in Southern Brazil between 2018 and 2023. A retrospective genomic surveillance study was conducted using respiratory samples collected from children and adolescents (0–18 years) hospitalized at a tertiary university hospital. RSV-positive samples identified by RT-qPCR with adequate viral load were selected for whole-genome or partial genome sequencing, focusing on the G and F genes. Consensus sequences were classified according to the unified RSV nomenclature, and phylogenetic analyses were performed to identify circulating clades and temporal distribution patterns. Among 2,500 respiratory samples analyzed, 512 (20.4%) were RSV-positive, and 95 genomes were successfully characterized. RSV-A and RSV-B co-circulated throughout the study period without evidence of lineage replacement. Phylogenetic reconstruction revealed substantial genetic diversity, with predominance of RSV-A clade A.D.1 (31.6%) and RSV-B clade B.D.E.1 (80.0%). Multiple RSV-A and RSV-B lineages were detected, demonstrating continuous viral diversification across seasons. RSV-A was significantly more frequent among severe cases requiring intensive care or advanced respiratory support ($p = 0.0011$). These findings provide a comprehensive genomic description of RSV circulation in Southern Brazil and reinforce the importance of continuous genomic surveillance to monitor viral evolution, support molecular epidemiology, and guide prevention strategies, including vaccine and monoclonal antibody implementation. **Keywords:** Respiratory syncytial virus; Genomic surveillance; Phylogenetics; Molecular epidemiology; RSV-A; RSV-B. **Support:** Fundação Oswaldo Cruz (FIOCRUZ) – Rede Genômica

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METABOLOMICS ANALYSIS OF INDIVIDUALS INFECTED WITH HTLV-1

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Human T-lymphotropic virus 1 (HTLV-1) is a retrovirus associated with T-lymphocyte infection, affecting between 5 and 10 million people worldwide. Despite its public health relevance, studies investigating metabolic alterations associated with the infection remain scarce. Therefore, this study aimed to evaluate metabolites in individuals infected with HTLV-1. An untargeted metabolomic analysis was performed on plasma samples from 73 patients infected with HTLV-1 and 87 individuals from the non-infected control group. Data were obtained by mass spectrometry and processed using specific software. Metabolite selection was performed by applying an 80% frequency filter. Statistical analysis considered Fold Change ≥ 2 and an unpaired *t*-test with $p < 0.05$. Principal Component Analysis (PCA) was performed to visualize clustering among the studied groups. Metabolomic analysis identified 739 metabolites, of which 12 were selected after filtering; four showed differential expression after statistical analysis between controls and HTLV-1-infected individuals: PE(20:4/21:0), 2-cyanopyridine, PE(17:0/22:2), and PE(19:0/0:0). Among these, phosphatidylethanolamines (PEs) were identified as endogenous human metabolites. PE(20:4/21:0) showed higher abundance in the control group (FC = 68.99; $p = 1.42 \times 10^{-6}$), whereas PE(17:0/22:2) was more abundant in the HTLV-1 group (FC = 4.72; $p = 6.59 \times 10^{-5}$). PE(19:0/0:0) also showed a significant difference between the groups (FC = 1.13; $p = 0.01$). PCA did not indicate clustering between the studied groups. Alterations in PEs suggest membrane remodeling during HTLV-1 infection. The absence of discrimination between the groups reinforces the need for greater stratification in future studies.

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OROPOUCHE VIRUS INFECTION SHOWS STRAIN-DEPENDENT DIFFERENCES IN ZEBRAFISH EMBRYOS AND LARVAE

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Oropouche virus (OROV) is an emerging midge-borne arbovirus responsible for recurrent outbreaks of Oropouche fever in Latin America. Although infection is usually associated with an acute febrile illness, neurological complications and adverse pregnancy outcomes have recently raised concern about OROV pathogenesis. However, *in vivo* models to study infection, developmental damage, and immune pathogenicity remain limited. Here, we established zebrafish (*Danio rerio*) embryos and larvae as models for OROV infection. Embryos were first infected at the 4-cell stage with the prototype TRVL9760 strain. Infection impaired embryonic development, increasing the proportion of malformed embryos compared with controls. Analysis using DanioScope revealed reduced tail coiling activity at 24 hours-post-fertilization, followed by smaller eye size, reduced body length, and increased heart rate at 3 dpf. In contrast, infection of 3 days-post-fertilization larvae supported viral replication up to 7 days post-fertilization without evident morphological abnormalities, indicating that disease outcome depends on the developmental stage at infection. We then assessed the pathogenicity of the recently isolated OROV SCDC-2024 strain, obtained from a traveler returning from Cuba to Italy. Embryos infected at the 4-cell stage showed increasing viral RNA levels up to 48 hours post-infection, which remained elevated at 72 hpf. Unlike TRVL9760, SCDC-2024 induced marked embryonic lethality, with increased mortality already evident at 24 hours post-fertilization. Since the larval model supports sustained infection, we are currently using it as a platform to evaluate the antiviral activity of repurposed compounds. Overall, zebrafish provides a useful model to investigate OROV replication, developmental impairment, and strain-dependent differences in pathogenicity.

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GENETIC POLYMORPHISMS AND SOLUBLE LEVELS OF FAS AND FASL IN SYMPTOMATIC AND ASYMPTOMATIC HTLV-1-INFECTED INDIVIDUALS

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Human T-lymphotropic virus 1 (HTLV-1) causes disease in only a minority of infected individuals, and host genetic factors may influence this outcome. The Fas/FasL pathway regulates apoptosis and lymphocyte homeostasis, making its polymorphisms plausible candidates for modulating disease expression. This study aimed to evaluate the association of *FAS* and *FASL* polymorphisms and soluble FAS/FASL plasma levels with clinical status in people living with HTLV-1 (PLHTLV). Four polymorphisms (*FAS* rs1800682 and rs2234767; *FASL* rs7631110 and rs503077) were assessed in 108 PLHTLV and 162 uninfected controls by real-time PCR, and soluble FAS and FASL plasma levels were measured by ELISA. Among infected individuals, 50 were symptomatic and 35 asymptomatic. Genotypic and allelic frequencies were compared using chi-square or Fisher's exact tests, with odds ratios estimated by logistic regression; plasma levels were compared using the Mann-Whitney test. The T allele of *FASL* rs7631110 was less frequent among infected individuals than controls (38.8% versus 53.4%; p=0.002). Within the infected group, carriers of at least one T allele were more likely to be symptomatic (OR 3.21; 95%CI 1.25–8.56; p=0.028), whereas the remaining three polymorphisms showed no association with clinical status. Soluble FAS levels were higher in symptomatic than in asymptomatic individuals (median 363 versus 299 pg/mL; p=0.0048), a difference that persisted after exclusion of extreme values (p=0.0019), while soluble FASL did not differ between groups (p=0.351). These findings indicate that *FASL* rs7631110 and circulating soluble FAS are associated with clinical status in HTLV-1 infection, possibly reflecting impaired apoptotic regulation in symptomatic individuals.

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Transcriptional profiling of HIF1A and PFKFB3 in human macrophages and cell lines during arbovirus infection.

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Viral infections often trigger profound metabolic reprogramming in host cells, a process tightly regulated at the transcriptional level. The Hypoxia-Inducible Factor 1-alpha (HIF1A) and the glycolytic enzyme PFKFB3 are crucial mediators of cellular metabolism, hypoxia response, and inflammation. Understanding how arboviruses transcriptionally modulate this immunometabolic axis is essential for elucidating viral pathogenesis and host-pathogen interactions. The aim of this study was to evaluate the transcriptional profile of HIF1A and PFKFB3 in human macrophages (CD14+ isolates and monocytic cell lines THP-1 and U937) following infection with Chikungunya (CHIKV) and Mayaro (MAYV) viruses. Cells were infected with an MOI of 1, and the relative gene expression of HIF1A and PFKFB3 was quantified via RT-qPCR 24 hours after infection. Transcriptional changes were analyzed using the 2^-ΔΔCt method, comparing infected cells to uninfected controls. Transcriptional analysis revealed a widespread downregulation of HIF1A across all cell types upon infection, with the most severe repression observed in primary CD14+ cells infected with MAYV (27-fold decrease). PFKFB3 expression was similarly
downregulated in primary CD14+ and THP-1 cells by both viruses. However, the U937 cell line exhibited a highly divergent transcriptional response, showing an upregulation of PFKFB3 with a 143-fold increase during MAYV infection. From this preliminary data, we could conclude that CHIKV and MAYV infections predominantly suppress the HIF1A-PFKFB3 transcriptional axis in primary human macrophages. Transcriptional heterogeneity among immortalized cell lines emphasizes the importance of primary models when investigating the metabolic and transcriptional landscape of arbovirus infections.

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IDENTIFICATION AND CHARACTERIZATION OF OROPOUCHE VIRUS GLYCOPROTEINS: ELUCIDATION OF Gc-MEDIATED PATHOGEN–HOST INTERACTIONS

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Oropouche virus (OROV) is the etiological agent of Oropouche fever. Between 2023 and 2025, the number of reported cases increased, with infections documented across all regions of Brazil. Among the proteins encoded by Orthobunyaviruses, the surface Gc glycoprotein plays an important role in host cell receptor that may be regulated by post-translational modifications (PTMs), such as N-glycosylation. Therefore, this study aimed to identify the OROV Gc, focusing on the presence of N-glycosylation sites and its interaction with host cell proteins. In silico analyses were performed to identify differences in the Gc between the reference strain BeAn19991 and the emerging strain AM0088. Subsequently, the AM0088 strain was purified and subjected to proteomic analysis by LC-MS. Predictions identified differences in the Gc amino acid sequence associated with distinct N-glycosylation between the two strains. Experimentally, treatment of purified viral particles with PNGase F confirmed the presence of a conserved N-glycosylation site at residue N141 in the Gc of both strains, while revealing the loss of the glycosylation site at residue 79 in the AM0088 strain. To characterize Gc interactions with host proteins, the coding sequence of the Gc protein was cloned into two mammalian expression vectors, pcDNA-FRT-to-FLAG and pcDNA-TurboID. The constructs were validated by restriction enzyme digestion and Sanger sequencing. Ongoing experiments of LC-MS are being performed to characterize the interactome between Gc and membrane proteins, which will contribute to advancing our understanding of the molecular mechanisms underlying OROV virus infection and supporting the identification of potential therapeutic targets.

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ROLE OF IFNL1 IN ENDOTHELIAL CELL REPROGRAMMING BY GLIOBLASTOMA CELLS: IMPACTS OF ZIKA VIRUS INFECTION

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Glioblastoma (GBM) is the most common and aggressive primary cancer of the central nervous system (CNS). Current treatment consists of surgical resection of the tumor, radiotherapy, and chemotherapy. Treatment is ineffective, and most patients experience recurrence. GBM presents a heterogeneous tumor microenvironment (TME) composed of tumor and non-tumor cells, which play an important role in tumor progression. Endothelial cells play a central role in the TME by mediating angiogenesis, supplying the tumor with oxygen and nutrients for cell proliferation. Zika virus (ZIKV) exhibits oncolytic activity in glioblastoma cells *in vitro* and *in vivo*. ZIKV induces the death of GBM cells and promotes antitumor immunity. Previous data from our group identified IFNL1 as the most differentially expressed gene in GBM cell lines during ZIKV infection. Here, we aim to produce IFNL1 knockout GBM cells, and IFN-unresponsive endothelial cells by knocking out the IFNL1 receptor (IFNLR1) using the CRISPR-Cas strategy to generate IFNL1 and IFNLR1 knockout lines. At first, we evaluated the IFNL1 expression by mock and ZIKV-infected GBM cells using RT-qPCR and found that ZIKV-infected cells produce IFNL1 at MOIs of 1 and 3, while no expression was detected in mock-infected cells. Additionally, after cloning the IFNL1 and IFNLR1 sgRNAs in vectors, we transfected them in GBM and endothelial cells, and sorted the positive populations utilizing cell sorting. By using knockout cell lines, we plan to perform phenotypic assays to assess the role of the IFNL1 in the oncolytic effect induced by the ZIKV infection in GBM cells.

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COMPARATIVE ANALYSIS OF PRIMARY DENV-2 AND DENV-3 INFECTION REVEALS DISTINCT DISEASE PROFILES IN AG129 MICE

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Severe dengue is a multifactorial disease influenced by host factors as well as viral serotype and genotype. Although seroepidemiological studies have consistently associated DENV-2 and DENV-3 with an increased risk of severe disease, their relative contribution to disease severity remains unclear. To compare the pathogenesis of primary DENV-2 and DENV-3 infections, AG129 mice were inoculated with either serotype and clinical, hematological, biochemical, macroscopic, and histopathological analyses were conducted at 24, 48, 72, and 96 hours post-infection (hpi), and at 7 and 14 days post-infection (dpi). DENV-2 infection resulted in reduced weight gain, greater splenomegaly, and increased spleen weight compared with both MOCK and DENV-3 groups. Histopathological examination of the lung, liver, heart, and kidney revealed similar lesion patterns in both infections, although lesions developed earlier and were consistently more severe in DENV-2-infected mice. Biochemical analyses demonstrated persistently elevated AST and ALT levels in the DENV-2 group, whereas significant increases were detected in DENV-3-infected mice only at 96 hpi. Hematological evaluation showed lower hematocrit values and marked thrombocytopenia in DENV-2-infected mice, while platelet counts remained unchanged following DENV-3 infection. Leukocyte counts increased in both groups but were consistently higher in DENV-2-infected animals, particularly at 7 dpi. Collectively, these findings suggest that primary DENV-2 infection elicits a more severe systemic disease than DENV-3, reinforcing serotype-specific differences in dengue pathogenesis.

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A HETEROLOGOUS SECONDARY DENGUE INFECTION MODEL RECAPITULATES HEMORRHAGIC MANIFESTATIONS AND HEMOSTATIC DYSFUNCTION IN AG129 MICE

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Although AG129 mouse models have substantially advanced dengue (DEN) pathogenesis research, they predominantly reproduce severe disease following primary infection, limiting their ability to investigate hemorrhagic manifestations and coagulopathy associated with heterologous secondary DEN infection. To address this gap, we established a heterologous secondary infection model by inoculating AG129 mice with DENV-3 followed by DENV-2. Macroscopic, histopathological, hematological, and rotational thromboelastometry (ROTEM®) analyses were performed at 24, 48, 72, and 96hpi. Secondary-infected mice exhibited reduced hematocrit, erythrocyte, and hemoglobin levels at 72hpi, accompanied by significant thrombocytopenia at 72 and 96hpi. Hemorrhagic manifestations and signs of impaired hemostasis were observed through 7 dpi. Macroscopic analysis revealed extensive right-sided pulmonary hemorrhage (24–72hpi), upper gastrointestinal bleeding (24–72hpi), testicular hyperemia (48–72hpi), and cerebral lesions characterized by frontal and occipital hyperemia and a subdural temporal clot at 7dpi. These findings have not, to our knowledge, been previously reported in murine DEN models. Histopathological analysis revealed extensive pulmonary hemorrhage involving adjacent bronchioles, foci of hepatic, renal (cortical region), and subendocardial hemorrhage. To further investigate the mechanisms underlying these hemorrhagic manifestations, ROTEM® was performed. Secondary-infected mice exhibited a hypercoagulable profile, including dynamic, structural, and FIBTEM hypercoagulability. Together, these findings suggest that the coexistence of hypercoagulability, thrombocytopenia, and hemorrhagic manifestations reflects a compensatory hemostatic response associated with hepatic dysfunction, hyperfibrinogenemia, elevated D-dimer and acute-phase proteins levels. This heterologous secondary infection model consistently reproduces hemorrhage, vasculopathy, coagulopathy, and multiorgan involvement, providing a robust platform for investigating the mechanisms underlying hemostatic dysfunction in severe DEN.

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ZIKA VIRUS INDUCES ALTERATIONS IN ORGANS OF STILLBIRTH AND UMBILICAL CORDS OF INFECTED PATIENTS

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Zika fever is an arboviral disease caused by the Zika virus (ZIKV), in which a link has been found between Zika cases and an increase in diagnoses of Guillain Barré Syndrome (GBS), congenital effects and miscarriages. The study was approved by the Ethics Committee (CAEE: 56089116.2.0000.5248). A pregnant woman infected with ZIKV developed GBS and then was admitted to hospital, subsequently the ultrasound revealed a retained fetus. Fetal brain, lung, kidney, skin and liver were processed for immunohistochemistry (IHC) and transmission electron microscopy analysis. Additionally, umbilical cords from five ZIKV-infected pregnant women and five controls were also processed for IHC. ZIKV E and NS1 proteins were detected in fetal tissues, confirming viral presence and replication. Immune cells were identified by labeling CD68, CD8, CD4 and CD20 proteins. Ultrastructural analysis revealed plasma membrane rupture in lung and kidney cells; pyknotic nucleo in the kidney; indented nucleo and cytoplasmic rarefaction in the skin; loss of tissue organization with lipid bodies and vesicles in the liver. Residual bodies were observed in all fetal tissues. In the umbilical cords, ZIKV NS1 was detected in all infected cases, confirming viral presence and replication. CD68 and CD8 proteins were identified in greater quantities in the umbilical cord blood of infected cases. In addition, the pro-inflammatory cytokines TNF-α and IL1-β were found in all infected cases. These samples are important for investigating the relationship between ZIKV infection, GBS, vertical transmission, inflammation and miscarriage. Financial support: UERJ, FAPERJ and CNPq.

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SARS-CoV-2 Drives EpCAM Upregulation in Respiratory Epithelial Cells Associated with a Hypercellular Profile in Severe COVID-19

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In December 2019, severe acute respiratory syndrome caused by SARS-CoV-2 emerged as a major global health threat. This study investigated the phenotypic profile and activation status of epithelial and immune cells in tracheal aspirates from patients with severe COVID-19. A total of 48 samples were obtained from 28 mechanically ventilated patients and analyzed by flow cytometry and conventional microscopy. We observed high prevalence of basal, ciliated, undifferentiated epithelial cells, and pneumocytes, many displaying morphological abnormalities. Based on total cell counts, samples were stratified into hypocellular and hypercellular profiles, which correlated with clinical outcomes. Hypercellular samples were frequently associated with fatal cases and were characterized by increased frequencies of basal and undifferentiated epithelial cells, as well as pneumocytes. These samples also exhibited higher proportions of $CD45^+$ cells with elevated HLA-DR expression, suggesting an influx of activated leukocytes and antigen-presenting cells. In contrast, hypocellular samples showed a predominance of $CD45^-PanCK^+$ epithelial cells. Hypercellular samples further demonstrated increased $EpCAM^+PanCK^+$ epithelial populations under conditions of heightened inflammation. Furthermore, SARS-CoV-2 infection, identified by the expression of Spike protein, was significantly higher in EpCAM+ cells regardless of the granularity, and in EpCAM+PanCK+ cells with higher granularity, corroborating with these findings. Additionally, ICAM-1 expression was elevated in $CD45^+$ cells but reduced in $CD45^-$ cells within hypercellular samples, indicating differential regulation between immune and epithelial compartments. Together, these findings define two distinct cellular profiles in the lower respiratory tract that are associated with disease severity and clinical outcome, highlighting their potential utility as prognostic indicators in severe COVID-19.

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EVIDENCE OF HOMOTYPIC DENGUE VIRUS REINFECTION IN A HYPERENDEMIC REGION

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Infection with a dengue virus (DENV) serotype is traditionally associated with the induction of long-lasting homotypic immunity. However, the occasional identification of homotypic infections raises questions about the duration and quality of this protection, which may vary across different epidemiological and immunological contexts. This study investigated the association between previous infections with different DENV serotypes and acute infection, aiming to identify possible cases of homotypic reinfection. Between January and December 2021, 1,926 serum samples were collected from patients treated at a tertiary hospital located in a dengue-endemic region with co-circulation of other arboviruses in southeastern Brazil. All samples were tested by RT-PCR for the detection of acute DENV infection. Among them, 279 acute DENV-positive cases were analyzed using the focus reduction neutralization test (FRNT) to characterize previous immunity. Samples were classified as homotypic (immune response against the same infecting serotype) or heterotypic (immune response against a different infecting serotype). Among individuals with a homotypic profile, seven samples showed acute DENV-1 infection with previous neutralization against DENV-1, whereas twelve showed acute DENV-2 infection with previous neutralization against DENV-2. To minimize interference from acute-phase antibodies, IgM and IgG depletion was performed, followed by repeat FRNT and ELISA analyses, confirming two cases with a homotypic DENV-2 profile. These findings suggest that homotypic immunity may be more complex than previously recognized and highlight the importance of serotype-specific studies to improve the understanding of dengue dynamics in endemic areas, supporting the development of prevention strategies.
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INVESTIGATION OF ARBOVIRUSES IN BATS FROM THE PANTANAL OF MATO GROSSO DO SUL AND THE ATLANTIC FOREST OF RIO DE JANEIRO

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Arboviruses are important for public health in Brazil and worldwide. Investigating arbovirus circulation in synanthropic and wild vertebrates is essential to understand viral maintenance at the urban–wildlife interface. Bats represent the second most diverse order of mammals, and Brazil harbors one of the greatest bat diversities worldwide. Habitat loss may increase contact among bats, humans, and vectors, favoring arbovirus transmission and spillover. This study aimed to investigate arbovirus circulation in bats from the peri-urban Maciço da Pedra Branca, Rio de Janeiro State, and the wild environment of Corumbá, Mato Grosso do Sul State, using reverse transcription polymerase chain reaction (RT-PCR) and plaque reduction neutralization tests (PRNT). A total of 291 bats were captured in Corumbá, Mato Grosso do Sul (2025), and 234 in Rio de Janeiro (2023–2024). Tissue samples (kidney, liver, and brain) were analyzed by RT-PCR targeting the NS5 region for pan-orthoflavivirus detection, followed by semi-nested PCR for specific orthoflavivirus detection. Serum samples were tested by PRNT for Mayaro virus (MAYV) and Yellow fever virus (YFV). Molecular analysis included 280 liver and 222 kidney samples from Corumbá. One liver sample from *Noctilio albiventris* and seven from *Platyrrhinus lineatus* showed amplicons compatible with DENV-2 based on expected product size. All RT-PCR-tested samples from Rio de Janeiro (234 brain, 158 liver, and 142 kidney) were negative. PRNT90 was performed on 190 sera (131 from Corumbá and 59 from Rio de Janeiro). Neutralizing antibodies against MAYV (titer 1:20) were detected in one *Noctilio albiventris* from the Pantanal region. These findings suggest possible DENV-2 circulation and prior MAYV exposure in bats from Corumbá.

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PRECLINIAL ANALISE OF CROTOXIN FROM SNAKE VENOM, AGAINST MONKEYPOX VIRUS IN VITRO INFECTION

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Crotoxin, a phospholipase A_2 complex derived from ***Crotalus durissus*** venom, has shown immunomodulatory activity in viral infections, but its effects against monkeypox virus (MPXV) remain unknown. This study evaluated whether crotoxin pretreatment protects epithelial cells from MPXV infection. Crotoxin did not reduce Vero E6 cell viability by 50% at the highest concentration tested (500 µg/mL), indicating a CC_{50} >500 µg/mL by MTT assay. Vero E6 cells were pretreated with crotoxin (50 µg/mL) or PBS for 24 h and infected with MPXV at a multiplicity of infection of 1. After inoculation under biosafety level 3 conditions, cell monolayers were harvested in TRIzol for nucleic acid extraction. Viral load was quantified by qPCR targeting the MPXV genome and normalized to RNase P. IL6, IL10, and IFNB1 expression was assessed by SYBR Green qPCR using GAPDH as the reference gene. Crotoxin- and PBS-treated infected cells showed comparable viral loads, with an approximately 1.47-fold difference based on raw Ct values (PBS: 21.21 ± 1.32; crotoxin: 20.66 ± 0.25), indicating no detectable antiviral activity. However, crotoxin altered the inflammatory profile: IL6 expression increased 7.3-fold, whereas IL10 and IFNB1 decreased 3.3-fold and 7.8-fold, respectively, compared with PBS-treated controls. These changes occurred without reduced viral load, suggesting an imbalanced inflammatory response rather than antiviral protection. Overall, crotoxin did not protect Vero E6 cells against MPXV and modulated host inflammatory gene expression, highlighting the need for virus-specific efficacy and toxicity assessments before therapeutic consideration.

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PT.244

HLA-B Alleles Are Associated with Viral Blips During Suppressive Antiretroviral Therapy

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Background: Viral blips are transient episodes of detectable plasma HIV RNA (<200 copies/mL) during suppressive antiretroviral therapy (ART). Host genetic factors, particularly HLA-B alleles, may influence virological control, although their association with viral blips remains poorly understood. We evaluated the relationship between HLA-B alleles, viral blips, and immunological parameters in people living with HIV receiving ART. **Methods**: A retrospective cross-sectional study included 70 individuals receiving ART (55 men and 15 women; mean age 55.5 ± 10.2 years). Clinical, virological, immunological, and HLA-B genotyping data were analyzed using nonparametric tests and correlation analyses. **Results**: Forty-seven (67.1%) participants experienced at least one viral blip, and 37 (52.9%) carried protective HLA-B alleles. Protective alleles were associated with longer blip-free intervals ($p = 0.0085$). HLA-B44 showed the strongest association with improved virological control, including fewer viral blips ($p = 0.018$), lower blip frequency ($p = 0.021$), reduced viral load variability ($p = 0.043$), and higher median CD4 counts ($p = 0.017$). HLA-B57 was also associated with longer blip-free intervals ($p = 0.026$), confirming its established protective role. Conversely, HLA-B35 was associated with shorter intervals between blips, consistent with poorer HIV control. HLA-B15, HLA-B07, and HLA-B49 showed exploratory associations with virological or immunological outcomes. **Conclusions**: Protective HLA-B alleles were associated with sustained virological control during suppressive ART, whereas conventional immunological markers were not associated with viral blips. These findings suggest that host genetic factors may contribute more to long-term virological stability than the immunological parameters evaluated.
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Coronavirus Infection and Type 1 Diabetes Mellitus Development in Genetically Susceptible Hosts: A Descriptive and Experimental Approach

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COVID-19 has been associated with an increased incidence of type 1 diabetes mellitus (DM1), although the mechanisms remain poorly understood. Plasmacytoid dendritic cells (pDCs) play key roles in antiviral immunity and autoimmunity through type I interferons (IFN-I), which may enhance cytotoxic T-cell activation and MHC-I expression on pancreatic β cells, promoting autoimmune responses associated with DM1. This study aimed to investigate the relationship between coronavirus infection and DM1 diagnosis and evaluate metabolic alterations in an experimental model. A descriptive study was conducted using a questionnaire administered to individuals with DM1 and controls recruited from the Pediatric Outpatient Clinic of the Hospital das Clínicas, Ribeirão Preto Medical School, University of São Paulo (HCFMRP-USP). In parallel, NOD mice, a murine model genetically predisposed to DM1, were infected with MHV-59, a model used to study betacoronavirus immunopathogenesis. Body weight and blood glucose were monitored throughout infection. In the descriptive study, 7 of 26 participants reported previous SARS-CoV-2 infection. Most participants adhered to social distancing (24/26), were vaccinated (22/26), and experienced mild COVID-19. Among individuals with DM1 and COVID-19, diagnosis occurred predominantly after the pandemic. In the experimental model, infected NOD mice exhibited early increases in blood glucose and greater weight loss compared with controls. From the sixth week post-infection, higher DM1 incidence was observed in infected animals. These findings support the hypothesis that coronavirus infection may contribute to DM1 development in genetically predisposed hosts. Ongoing studies will investigate pDCs, IFN-I, and pancreatic MHC-I expression to elucidate immunological mechanisms.

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CHIKUNGUNYA VIRUS INFECTION MODULATES THE MICRORNA CARGO OF EXTRACELLULAR VESICLES RELEASED BY HUMAN FIBROBLAST-LIKE SYNOVIOCYTES: AN *IN VITRO* AND *IN SILICO* STUDY

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Alphavirus chikungunya (CHIKV) is an arbovirus that causes polyarthralgia. Virus-host interactions involve post-transcriptional regulation of gene expression, including microRNA (miRNA)-mediated mechanisms. Extracellular vesicles (EVs) may contribute to this process by mediating intercellular miRNAs transfer. This study investigated whether CHIKV infection alters miRNA cargo of EVs released by human fibroblast-like synoviocytes (HFLS). HFLS were infected with CHIKV for 48 hours (MOI 0.5), and EVs were isolated from supernatants. EVs were characterized by nanoparticle tracking analysis (NTA) and cryogenic electron microscopy (cryo-EM). RNA was extracted, and miRNA expression was profiled using RT-qPCR OpenArray. Differentially expressed (DE) miRNAs ($p \leq 0.05$) were analyzed *in silico* to identify experimentally validated target genes using miRTarBase v.2025. Functional network and pathway enrichment analysis were performed using Cytoscape v.3.10.1, ClueGO v.2.5.10, and WebGestalt v.2024. EVs exhibited heterogeneous morphology and size, with mean diameters of 223.4 nm in uninfected HFLS and 274.5 nm in CHIKV-infected HFLS. Among 239 detected miRNAs, 30 were DE, including 16 upregulated and 14 downregulated. *In silico* analysis identified 268 experimentally validated target genes, including 170 targets of upregulated miRNAs (188 interactions) and 122 targets of downregulated miRNAs (131 interactions). Pathway enrichment analysis highlighted associations with osteoclast differentiation, regulation of actin cytoskeleton, MAPK, PI3K-Akt, and VEGF signaling. These findings reveal that CHIKV infection remodels the miRNA cargo of EVs released by HFLS, highlighting EV-associated miRNAs as potential mediators of virus–host interactions that may modulate host cellular pathways and provide new insights into the molecular mechanisms underlying CHIKV infection.

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SYSTEMIC TISSUE DISTRIBUTION OF A CITLODAVIRUS INFECTING *THEOBROMA CACAO* L.

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Viral infections represent significant threats to cacao cultivation in the main producing countries. Although previously known cacao viruses are not endemic to Brazil, a new virus of the family *Geminiviridae* (genus Citlodavirus, species name *Citlodavirus theobromae)* whose presence is associated with major symptoms, was recently discovered specifically in crops in Southern Bahia. In order to characterize its distribution in different tissues of *Theobroma cacao* L. and to investigate the possibility of vertical transmission, samples of branches, leaves, root and fruits were collected from healthy and symptomatic plants. Genetic material from the tissue samples was extracted using the Doyle & Doyle methodology with modifications. DNA and RNA samples were respectively subjected to PCR and RT-PCR to identify the presence of the virus. Amplification was carried out using specific primers for fragments of the Geminivirus coat protein and replicase genes. The results show the systemic distribution of the new virus and reinforce horizontal transmission, since the virus is not present in cacao seeds. Viral DNA and RNA were detected across different plant tissues. These data provide novel insights into the pathogen–host relationship associated to the new cacao Geminivirus, highlighting the need to strengthen surveillance of emerging plant viruses in the region.

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ANALYSIS OF SUSCEPTIBILITY TO SEVERE DENGUE IN OFFSPRING OF Zika virus-INFECTED MICE

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The Zika virus (ZIKV) caused an epidemic in Brazil in 2015, during which an increase in microcephaly cases in newborns was observed. Infection by ZIKV can result in a series of fetal malformations known as Congenital Zika Syndrome. Recently, a significant increase in severe dengue cases has been reported. Infection with dengue virus (DENV) can result in symptoms ranging from self-limiting illness to clinical presentations of dengue with warning signs, which include hemodynamic and hematological alterations. Thus, DWS cases may be linked to prior flavivirus infections, particularly considering the cocirculation of DENV and ZIKV and intrauterine ZIKV exposure. The objective of this study was to investigate the association between intrauterine ZIKV infection and susceptibility to DWS in adulthood. For this, pregnant C57BL/6 female mice were infected with ZIKV or injected with PBS via the intraperitoneal route on embryonic day 5.5. Six weeks after the natural birth of the offspring, the animals were injected intravenously with DENV or PBS and euthanized after 48 hours. Four groups were obtained: NI;NI/DENV;ZIKV/NI;ZIKV/DENV. Blood, spleen, and liver samples were collected for analysis. Platelet, red blood cell, hemoglobin, and hematocrit were reduced in the ZIKV/DENV group compared to all other groups, indicating possible hematological alterations. Macrophage recruitment in the liver and CCL5 levels in the spleen were also increased in ZIKV/DENV, while splenic vascular permeability was lower. Thus, these findings suggest that intrauterine ZIKV exposure exacerbates the pathophysiological response to DENV infection in adulthood.

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MONKEYPOX VIRUS DISPLAYS DISTINCT VIRAL BURDEN PROFILES IN PRIMARY AND DIFFERENTIATED HUMAN MONONUCLEAR CELL lineage

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Monkeypox virus (MPXV) can disseminate systemically and interact with circulating monocytes and tissue macrophages; however, the influence of myeloid differentiation on MPXV infection remains incompletely understood. This study compared MPXV infection and inflammatory gene expression in THP-1 cells maintained in a monocyte-like state or differentiated into macrophage-like cells with phorbol 12-myristate 13-acetate (PMA). Differentiation was confirmed by indirect immunofluorescence staining for CD68. Untreated and PMA-differentiated THP-1 cells were infected with MPXV at a multiplicity of infection of 1. Viral DNA was quantified at 24 and 48 hours post-infection by TaqMan real-time PCR targeting the viral G2R gene, whereas viral antigen expression was assessed by indirect immunofluorescence. Cytokine gene expression at 48 hours post-infection was evaluated by SYBR Green reverse-transcription quantitative PCR using the $2^{-}\Delta\Delta Ct$ method. MPXV DNA was detected under both experimental conditions; however, PMA differentiation markedly increased the viral burden at 48 hours post-infection, reaching approximately 4×10^7 genomic equivalents, compared with approximately 6×10^6 in undifferentiated cells ($p < 0.001$). Cytoplasmic staining for viral antigens further confirmed infection in differentiated THP-1 cells. In parallel, IL1B and IL6 expression was significantly upregulated in infected PMA-differentiated cells, whereas IL18 expression did not change significantly. These findings indicate that macrophage-like differentiation enhances MPXV-associated viral DNA accumulation and promotes a stronger pro-inflammatory transcriptional response. Therefore, PMA-differentiated THP-1 cells represent a useful model for investigating MPXV–myeloid cell interactions and evaluating antiviral or host-directed interventions.

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FOLIC ACID RESTRICTION DURING PREGNANCY ASSOCIATED WITH Zika virus INFECTION IN A MURINE MODEL COMPROMISES EMBRYONIC DEVELOPMENT AND NEURODEVELOPMENT IN ADULTHOOD

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In 2015, Brazil was hit by an outbreak of infections caused by the Zika virus (ZIKV), an arbovirus associated with neurological complications, microcephaly and fetal malformations. The most serious cases occurred during pregnancy and in regions of social vulnerability. This may be associated with poor nutrition during pregnancy, such as low intake of folic-acid, which is important for neurological development. Therefore, the objective was to investigate the effects of folic-acid deficiency on embryonic development and neurodevelopment in the offspring of ZIKV-infected mice. Pregnant female C57BL/6, previously fed a diet with or without folic-acid, were injected with PBS or infected with ZIKV on embryonic day (ED) 5.5, resulting in four experimental groups: control diet, PBS and ZIKV (C;C/ZIKV); diet without folic-acid, PBS and ZIKV (AF^-;AF^-/ZIKV). Fetal tissue was removed on DE15.5 or the offspring were waited for natural birth, and they were followed up until 12 weeks. In the embryonic phase, AF^-/ZIKV animals presented placental insufficiency with increased inflammation in the organ, which generated a restriction in the development of embryos compared to other groups. In adulthood, the offspring presented impaired neurological development and behavioral changes associated with memory, anxiety and hyperactivity in AF^-/ZIKV. Furthermore, there was a loss of neurons and glial cells in adult animals, with brain inflammation characterized by an increase in astrocytes and expression of IL-1β/IL-18/IL-33. These results suggest increased maternal immune activation due to ZIKV infection associated with maternal folic acid deficiency during pregnancy, which is reflected in the development of offspring until adulthood.

Keywords: Folic acid, Maternal immune activation, Zika virus.

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PT.251

CHIKUNGUNYA AND MAYARO VIRUS-INDUCED OXIDATIVE STRESS IMPAIRS SKELETAL MUSCLE CELLS INTEGRITY AND MYOFIBER FORMATION.

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Chikungunya (CHIKV) and Mayaro (MAYV) viruses are arthritogenic alphaviruses that induce acute and chronic joint and muscle inflammation. In addition, CHIKV and MAYV infections impair long-term skeletal muscle repair and recovery following injury. To investigate the direct effects of arthritogenic alphavirus replication on skeletal muscle, we performed an in vitro study of CHIKV and MAYV infection in skeletal muscle precursor, differentiating, and mature cells. We found that both viruses induce extensive skeletal muscle cell death at all stages of differentiation, potentially compromising muscle integrity and regenerative capacity. Notably, direct muscle cell damage occurred in the absence of a robust inflammatory cytokine response but was associated with marked production of reactive oxygen species (ROS). Moreover, we observed early mitochondrial dysfunction accompanied by reduced ATP production, indicating that metabolic impairment is an early event during CHIKV and MAYV infection. Supporting a central role for oxidative stress in virus-induced muscle injury, treatment with monomethyl fumarate, an inducer of antioxidant responses, significantly reduced myoblast and myofiber damage. Together, our findings demonstrate that CHIKV- and MAYV-induced oxidative stress is a major driver of skeletal muscle cell death, providing new insights into the mechanisms underlying arthritogenic alphavirus-associated muscle pathology.

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PT.252

Defining antigenicity and immune recognition of Anelloviruses

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Anelloviruses (ANVs) are ubiquitous, non-enveloped ~3 kb ssDNA viruses that persistently colonize human blood and bone marrow, being part of the human virome. Their genome primarily encodes the capsid protein ORF1, the principal immunogenic structure. The ORF1 protein comprises an N-terminal DNA-binding region, a jelly-roll domain, spike-forming P1/P2 subdomains, and a cleaved C-terminal region. Despite their ubiquity and persistence, a central challenge in ANV biology is the lack of molecular tools to characterize their antigenicity and host interactions. This study aims to characterize human humoral responses against ANVs using ORF1 recombinant proteins. To assess domain-specific reactivity, two proteins, one covering the P1/P2 subdomain and another covering the C-terminal region, were designed based on the sequence of a *Betatorquevirus*, the most immunogenic human ANV genus. We expressed both proteins at high yields in mammalian cells. *In silico* prediction using BepiPred-2.0 (threshold = 0.5) identified eight linear B-cell epitopes in the P1/P2 protein (six in the P1 and two in the P2 subdomain) and five within the C-terminal construct. Epitope accessibility was visually analysed by predicting the structures in AlphaFold, then evaluating their conformational structure using UCSF ChimeraX. To assess epitope exposure within the complete viral particle, the structure of P1/P2 protein was matched against a previously published ANV virus-like particle structure (PDB ID: 8V7X). To validate the antibody reactivity *in vitro*, the proteins are being tested against pediatric and adult human sera. In conclusion, we are developing ANV-specific reagents that can be applied in future research to investigate these understudied viruses.
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PT.253

INVESTIGATION OF THE IN VITRO ROLE OF THE AGONIST (CABERGOLINE) AND THE ANTAGONIST (ETICLOPRIDE) OF DOPAMINE RECEPTOR 2 IN CHIKUNGUNYA VIRUS INFECTION

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Dopamine (DA) is a neurotransmitter that acts through interaction with dopamine receptors (DRs). Perhaps DRs are mainly expressed in the central nervous system, they are also expressed in the muscles, one of the principal tissues affected by Chikungunya virus (CHIKV). One of the DR2 roles is to activate mTOR, blocking autophagy which can be unfavorable to the establishment of CHIKV infection, since CHIKV inhibits these processes. We aimed to analyze the in vitro role of DR2 signaling on CHIKV infection. Human embryonic kidney cells (HEK293T) treated post viral adsorption with 50 μM of DA showed inhibition by 86% of CHIKV replication at 24 hpi by plate assay. The same was observed in mouse myoblasts (C2C12). In neuroblastoma cells (SH-SY5Y), CHIKV replication was inhibited at 24 hpi by 69% in 5 minutes of pre-treatment with 10 μM of DA, and by 68% in post-treatment. C2C12 cells post-treated with DR2 agonist (Cabergoline) inhibited CHIKV replication by 91% at 24 hpi (1.25 to 10 μM). The post-treatment with DR2 antagonist (Eticlopride) stimulated CHIKV infectious progeny production by 18.69-fold at 24 h.p.i. (1.25 to 250 nM). In SH-SY5Y cells, any Cabergoline concentrations analyzed (0.03906 to 10 μM) affect CHIKV replication. However, the post-treatment with Eticlopride enhanced 16.41-fold CHIKV replication at 24 hpi (39.06 and 78.125 nM). The pre-treatment with both composts had no effect on CHIKV infection. We conclude that the DA anti-CHIKV activity possibly occurs by DR2 engagement.

Financial Support: CAPES, CNPQ, FAPERJ, FECD

PT.254

Evaluation of the Presence of Bovine Papillomavirus in *Rhipicephalus (Boophilus) microplus* Ticks.

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Bovine papillomatosis, caused by Bovine Papillomavirus (BPV), generates severe economic and sanitary losses in livestock. Although its direct transmission is known, the role of hematophagous vectors such as the tick Rhipicephalus (Boophilus) microplus in the epidemiological chain remains unclear. This study aimed to investigate the presence of BPV in adult ticks and nymphs collected in Paraíba. The methodology included genomic DNA extraction using the Qiagen DNeasy Blood and Tissue commercial kit, quantification by spectrophotometry, and molecular detection via polymerase chain reaction (PCR) employing specific oligonucleotides for the viral type BPV-2 conditions: 94°C for denaturation, 57°C for annealing, and 72°C for extension, followed by analysis in 2% agarose gel electrophoresis. The results revealed a significant positivity rate for BPV-2 DNA, identified in 90.9% (20/22) of adult tick samples and in 82.6% (19/23) of the analyzed nymph pools. It is concluded that R. microplus shows a high prevalence of the virus in different stages of its life cycle. Since *R. microplus* spends its entire life cycle on a single host, the presence of the virus in nymphs indicates its relevance in the dissemination of BPV within cattle herds, potentially leading to impacts on the livestock industry.

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PT.255

Oropouche Virus Renal Tropism and Pathogenesis in Experimentally Infected Mice: Involvement of Convoluted Tubular Epithelium

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Oropouche virus (OROV) is an arbovirus that caused multiple outbreaks of febrile illness across tropical Central and South Americas. More recently the OROV circulation area has expanded to other regions of Brazil and reached even other countries, with severe cases including maternal-fetal transmissions and fatalities, including renal failure and renal damage in post mortem studies. Detection of OROV in the urine of patients and in postmortem renal tissue corroborates the OROV renal pathogenesis. We have previously reported the presence of OROV BeAn19991 in kidneys of experimentally infected mice, and now present recent progress in this ongoing investigation. Neonatal and 3-week-old BALB/c, and adult IFNR-/- C57/BL6 mice were infected with rWT, ΔNSs, or ΔNSm. Renal epithelial cell lines were also used as *in vitro* models. In neonatal renal tissue, quantification of OROV RNA by qRT-PCR evidenced reduced loads in animals of the ΔNSs group compared to the rWT and ΔNSm groups, suggesting a role of NSs in the OROV ability to infect the kidney. Furthermore, western blot analysis demonstrated significant reduction in pan-cytokeratin expression in kidneys infected with rWT and ΔNSm, indicating kidney epithelial damage. These findings were further confirmed in IFNR-/- model, which displayed expression of active caspase-9 and the kidney injury biomarker NGAL. NGAL was also shed and detected in the urine of rWT-infected mice. The findings confirm the tropism of OROV for the renal tubular epithelium with direct cytopathic consequences and highlight the roles of the OROV non-structural proteins in OROV pathogenesis.

Financial support: FAPESP; CNPq

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SARS-COV-2 REPROGRAMS MITOCHONDRIAL HOMEOSTASIS IN PRIMARY CULTURES OF HUMAN B LYMPHOCYTES.

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SARS-CoV-2 infects human B lymphocytes in lymphoid organs, but its effects in the host cell responses and mitochondrial homeostasis are still unknown. We investigated the effects of modulating of the SphK/S1P/S1PR1 signaling axis on mitochondrial homeostasis and viral replication in primary human B lymphocytes. Purified B cells from human tonsils were treated with an S1PR1 antagonist (W146) or SphK1/2 inhibitor (SKI-II) and subsequently infected with SARS-CoV-2 (MOI=1). Viral load determined by RT-qPCR in cells and supernatants were significantly reduced at 48 hpi in cells treated with SKI-II ($p<0.0001$). This reduction was accompanied by increased expression of genes associated with mitochondrial homeostasis (DRP1, MFN1, MFN2, PINK1, TFAM, and PGC1A) and antiviral responses (MAVS, cGAS, STING, IFNA, and IFNB). In contrast, W146 did not cause reduction in intracellular viral load, but decreased viral RNA in supernatants, suggesting that S1PR1 modulation and SphK1/2 inhibition have different effects in response to SARS-CoV-2 infection in human B lymphocytes. The SphK/S1P/S1PR1 axis affect viral production, mitochondrial homeostasis in human B cells infected in vitro with SARS-CoV-2.

SARS-CoV-2
SphK/S1P/S1PR1 signaling
Human B cells
Mitochondrial homeostasis

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EVALUATION OF THE ASSOCIATION OF THE *IL18* rs1946518 (T/G) POLYMORPHISM WITH HBV INFECTION

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Introduction: Hepatitis B virus (HBV) infection remains a public health problem, with approximately 240 million infected individuals worldwide. Alterations in the host's immune response can influence disease progression. Polymorphisms in cytokine genes may contribute to variations in the immune response, such as interleukin-18 (IL18), a pro-inflammatory cytokine involved in the antiviral immune response. This study investigated the association of the *IL18* rs1946518 (T/G) polymorphism with susceptibility to HBV infection. **Methodology:** The study included blood samples from 89 patients with hepatitis B and 92 individuals without the infection (control group). Genomic DNA was extracted using the Puregene kit, and genotyping of the *IL18* rs1946518 (T/G) polymorphism was performed by real-time PCR using the TaqMan assay. Statistical analyses were performed using the chi-square test. **Results:** The polymorphic genotype (GG) and allele (G) showed similar frequencies in both groups (HBV: 27.6%; Control: 25.0%). There was no statistically significant difference in genotype distribution ($p = 0.7816$) or allele frequencies ($p = 0.9566$) between HBV individuals and the control group. **Conclusion:** The *IL18* rs1946518 (T/G) polymorphism showed no association with susceptibility to HBV infection in the studied population. This study expands the knowledge of the distribution of this polymorphism in individuals from the Amazon Region and its possible impact on HBV infection. However, increasing the sample size and evaluating IL-18 expression are necessary to understand the role of the *IL18* rs1946518 (T/G) polymorphism in the immune response against HBV. Financial Support: INCT-VER #406360/2022-7, INCT-UroGen #408576/2024-3 and FAPESPA/SESPA/DECIT-MS/CNPQ #001/2026.

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SUBGENOME-BIASED EXPRESSION OF GHBPP1 LINKS MICROTUBULES TO COTTON LEAFROLL DWARF VIRUS RESISTANCE

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Cotton-leafroll-dwarf-virus (CLRDV), causal agent of Cotton Blue Disease, is a major limitation to cotton production worldwide, yet the molecular basis of host resistance remains poorly understood. GhBPP1, a basic-proline-rich-1 microtubule-associated protein (BPP-MAP), was identified within a candidate CLRDV-resistance locus, suggesting investigation of the BPP gene family across allotetraploid cotton (*Gossypium hirsutum*) and its diploid progenitors. We combined comparative genomics, phylogenetics, and protein characterization with functional assays. BLASTp searches, chromosomal mapping, and maximum-likelihood phylogeny were used to identify and classify BPP genes in *G. hirsutum, G. arboreum*, and *G. raimondii*. GhBPP1 physicochemical properties, conserved motifs, and intrinsic disorder were assessed via ProtParam, MEME, AlphaFold, and D²P². Subgenome-biased expression was examined using public RNA-seq datasets, while virus-induced gene silencing (VIGS) and RT-qPCR were applied to evaluate *GhBPP1* transcriptional responses during CLRDV infection and aphid herbivory in resistant (Delta Opal) and susceptible (FiberMax 966) cultivars. Fourteen BPP genes, unevenly partitioned between At and Dt subgenomes into four subfamilies, were identified in *G. hirsutum*, with A-subgenome members showing broader tissue expression. *GhBPP1* displayed characteristics features of an intrinsically disordered MAP with liquid-liquid phase separation propensity. VIGS silencing produced developmental defects, specially in stem diameter and cotton height. Critically, *GhBPP1* expression remained elevated in the resistant cultivar but was transiently downregulated shortly after CLRDV contact in the susceptible cultivar, while herbivory-only responses were comparable between genotypes. These findings support a model in which *GhBPP1* contributes to a cytoskeleton-linked defense layer restricting early CLRDV spread, positioning BPP-MAPs as candidates for engineering virus resistance in polyploid crops.

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EFFECTS OF A DIET RICH IN REFINED CARBOHYDRATES AND ITS IMPACT ON PERSISTENT PAIN IN A MOUSE MODEL INFECTED WITH THE CHIKUNGUNYA VIRUS.

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Chikungunya is virus belonging to the *Togaviridae* family and the *Alphavirus* genus, whose main symptom is joint involvement and the development of persistent pain. One way to induce basal inflammation is through diet, due to metabolic changes and dysbiosis. In this context, this study aims to evaluate the impact of a diet rich in sugar and refined carbohydrates on the genesis and maintenance of persistent pain induced by infection. Three-week-old C57BL/6 mice were fed the HC diet for 2 weeks prior to infection and continued on this diet until the end of the experiment. The infection was performed intraplantarly with 1.0×10^6 PFU/animal; on days 1, 7, 14 and 21 post-infection, the animals were euthanized. Total blood cell count, cytokine, chemokine and NAG levels were measured in adipose tissue samples and body weight, adiposity index, edema and hypernociception measurements were also performed. Our results showed that the HC diet caused an increase in body weight only prior to infection. However, when comparing infected animals on the standard diet with those on the high-calorie diet, the high-calorie group exhibited edema through the 7th and 14th days post-infection, greater hypernociception only on the 3rd dpi, higher body fat index on the 21st dpi, and an increased total white blood cell count. There was no increase in the concentration of cytokines, chemokines, and NAG in adipose tissue. These results suggest that a 2-week high-carbohydrate diet does not induce low-grade inflammation and that the duration or dietary components need to be adjusted.

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PT.260

TEMPORAL PROTEOMICS OF OROPOUCHE VIRUS INFECTION REVEALS MECHANISMS OF HOST SHUTOFF AND IMMUNE EVASION

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Oropouche virus (OROV) is an emerging arbovirus of the genus *Orthobunyavirus* that causes recurrent outbreaks across South America and represents an increasing public health concern. Despite its clinical importance, the molecular mechanisms underlying OROV–host interactions and viral immune evasion remain poorly understood. To address this question, we performed quantitative proteomic analysis of OROV-infected human fibroblasts and identified extensive remodeling of the host proteome, including significant changes in proteins involved in innate immunity, transcriptional regulation, and extracellular matrix organization. Selected alterations were validated by western blot analysis. Notably, OROV specifically induced depletion of the RNA polymerase II catalytic subunit POLR2A, whereas RNA polymerases I and III remained largely unaffected. In addition, we identified multiple components of the JAK–STAT signaling pathway as targets of OROV infection, with early depletion of JAK1 followed by reduced STAT2 levels. Consistent with these findings, IFN-α-induced phosphorylation of STAT1 and STAT2 was inhibited before detectable changes in their total protein abundance, indicating that OROV suppresses interferon signaling at an early stage of infection. Using an inducible NSs-expressing cell line, we further demonstrate that the viral nonstructural protein NSs is sufficient to induce depletion of POLR2A and JAK1, thereby impairing host transcription and antiviral signaling. Collectively, these findings identify key mechanisms by which OROV promotes host shutoff and immune evasion, providing new insights into OROV pathogenesis and potential targets for antiviral intervention.

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ZIKA VIRUS INFECTION ALTERS AMYLOID PRECURSOR PROTEIN TRAFFICKING AND PROMOTES LYSOSOME-DEPENDENT DEGRADATION

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Zika virus (ZIKV) is a neurotropic flavivirus associated with congenital neurological abnormalities and neurological complications in adults. Although ZIKV infection profoundly remodels host cell membranes and trafficking pathways, its impact on the amyloid precursor protein (APP), a key regulator of neuronal homeostasis and Alzheimer's disease pathology, remains poorly understood. Here, we investigated the effects of ZIKV infection on APP expression, trafficking, and degradation using primary mouse cortical neurons and human fibroblasts. ZIKV efficiently infected both cell types and induced a marked reduction in full-length APP levels. Inhibition of lysosomal acidification with NH_4Cl or bafilomycin A1 restored APP levels, whereas proteasome inhibition had no significant effect, demonstrating that APP depletion is mediated by the endolysosomal pathway. Expression of the viral nonstructural protein NS4A alone reproduced the perinuclear accumulation of APP observed during infection, suggesting that NS4A contributes to the remodeling of APP intracellular trafficking. Interestingly, despite the reduction of full-length APP detected by western blotting, immunofluorescence revealed prominent perinuclear APP staining, raising the possibility that C-terminal APP fragments accumulate in these compartments during infection. Together, these findings demonstrate that ZIKV disrupts APP homeostasis by altering its intracellular trafficking and promoting lysosome-dependent degradation. Our results identify APP as a novel cellular target of ZIKV infection and suggest that viral-induced perturbation of APP processing may contribute to the neuropathogenesis associated with ZIKV infection.

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Differential susceptibility to RNA silencing-based defense underlies fitness contrasts between CiLV-C lineages

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Citrus leprosis caused by citrus leprosis virus C (CiLV-C; *Cilevirus leprosis*) is the most important viral disease affecting the Brazilian citriculture. The virus is transmitted by *Brevipalpus yothersi* mites and establishes non-systemic infections in sweet orange plants, producing localized lesions on fruits, branches, and leaves. CiLV-C populations comprise two major lineages: Cordeirópolis (CRD) and São José do Rio Preto (SJP). While CRD viruses are widely distributed across Latin America, they are rarely detected in the main Brazilian citrus belt, where the SJP lineage is predominant. Their contrasting distribution suggests differences in biological fitness, potentially influenced by vector transmission and viral accumulation, vector transmission and viral accumulation. This work hypothesizes that host defense modulation is also involved in differential biological fitness. To investigate this hypothesis, sap extracts from *Nicotiana benthamiana* plants agroinoculated with infectious clones of CRD and SJP were mechanically inoculated onto healthy wild-type (wt) and transgenic HC-Pro-expressing *N. benthamiana* plants. Lesion size, lesion number, and viral loads were evaluated by RT-qPCR. Both lineages produced larger lesions and accumulated to higher levels in HC-Pro plants than in wt plants. However, the HC-Pro/wt viral load ratio was markedly higher for CRD (4.7-fold) than for SJP (1.2-fold), suggesting that CRD is more susceptible to host RNA silencing than SJP. Ongoing experiments using *N. benthamiana* lines deficient in Dicer-like (DCL) proteins or expressing salicylate hydroxylase will further investigate the contribution of RNA silencing and salicylic acid-mediated defenses to the lineage-specific fitness of CiLV-C. These findings provide new insights into the molecular basis of CiLV-C pathogenicity and epidemiology.

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GENOMIC CHARACTERIZATION OF ENVIRONMENTAL LYTIC BACTERIOPHAGES INFECTING PAN-RESISTANT *PSEUDOMONAS AERUGINOSA* IN THE DOMINICAN REPUBLIC

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Pseudomonas aeruginosa is a critical-priority pathogen due to its high levels of antimicrobial resistance, particularly to carbapenems, highlighting the need for alternative therapeutic strategies such as bacteriophage therapy. This study aimed to isolate and biologically and genomically characterize environmental lytic bacteriophages active against pan-resistant *P. aeruginosa* in the Dominican Republic. Environmental water samples collected from rivers, streams, and lakes were screened using *P. aeruginosa* ATCC 27853 as the primary host by the double-layer agar method. Lytic activity was subsequently evaluated against a pan-resistant clinical isolate exhibiting resistance to carbapenems, cephalosporins, fluoroquinolones, aminoglycosides, and β-lactam/β-lactamase inhibitor combinations, with carbapenemase production confirmed phenotypically. Representative phages were subjected to whole-genome sequencing, assembly, annotation, and comparative genomic analyses. Eight environmental bacteriophages exhibited lytic activity against the reference strain, and four retained activity against the pan-resistant clinical isolate. Comparative genomic analyses performed using multiple independent bioinformatic classification approaches consistently identified two distinct lytic bacteriophage species, represented by ALM, a putative novel *Pakpunavirus*, and AMH, classified within the genus *Luzseptimavirus*. Both phages possessed complete genomes lacking antimicrobial resistance-, virulence-, and lysogeny-associated genes, consistent with a strictly lytic lifestyle and supporting their therapeutic potential. These findings expand the current knowledge of environmental bacteriophage diversity in Caribbean aquatic ecosystems and provide a strong foundation for future phage cocktail development and therapeutic applications against multidrug-resistant *P. aeruginosa*.

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STUDY OF HUMAN AICHI VIRUS AND HUMAN ADENOVIRUS GASTROENTERIC VIRUSES WITH EMPHASIS ON VIRAL ISOLATION AND PROTEIN CHARACTERIZATION

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Introduction: Acute gastroenteritis (AGE) is a major cause of childhood morbidity, and its viral etiological agents include Human Adenovirus (HAdV) and Aichi virus (AiV). This study aimed to isolate HAdV and AiV from stool samples and characterize their protein and antigenic profiles using SDS-PAGE and Western blot. **Methods:** A total of 131 stool samples were collected from children up to 10 years of age with AGE in Salvador, Bahia, Brazil. HAdV and AiV were detected by molecular diagnosis (PCR). Positive samples were subjected to viral isolation in Vero (CCL-81) cells. Protein profiles were evaluated by SDS-PAGE and Western blot. **Results:** Among the 131 samples, HAdV was detected in 13, of which three were successfully isolated from stool samples. AiV was identified in 29 samples; however, viral isolation was unsuccessful, and a reference strain (GenBank accession no. DQ028632) was used instead. For HAdV, SDS-PAGE identified the major capsid proteins Hexon, Penton Base, and Fiber, while Western blot revealed higher immunoreactivity for Penton Base, V, and VI proteins. For AiV, SDS-PAGE identified the capsid proteins VP0, VP1, and VP3. **Conclusion:** This study expands current knowledge on the protein and antigenic characteristics of HAdV and AiV. The successful isolation of HAdV from stool samples is particularly relevant given the high microbiological complexity of this type of specimen. The isolated strain enabled the identification of immunoreactive proteins, while the characterization of AiV provided information on its protein profile, contributing to future investigations on diagnosis and immune response.

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EVALUATION OF THE OCCURRENCE OF NUCLEOCAPSID PROTEIN (N) PROTEOFORMS AND THEIR IMPACT ON THE REPLICATION EFFICIENCY ACROSS DIFFERENT SARS-COV-2 VARIANTS

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The continuous evolution of SARS-CoV-2 has led to the emergence of variants with distinct replication efficiencies and phenotypic characteristics. The nucleocapsid (N) protein plays essential roles in viral RNA packaging, ribonucleocapsid assembly, viral replication, and modulation of host cell responses, making it a potential contributor to viral adaptation and evolution. Recent evidence has identified distinct N proteoforms whose origin and biological significance remain poorly understood. Here, we investigated the occurrence of N proteoforms among SARS-CoV-2 variants and their association with viral replication dynamics. Vero/hACE2/hTMPRSS2, RPMI-2650/hACE2, and Calu-3 cells were infected with Delta, Omicron (BA.1), and Omicron subvariants to evaluate viral replication kinetics. Supernatants were collected for viral titer determination by plaque assay and viral RNA quantification by qPCR, while lysates were collected for analysis of N expression, proteoforms, and phosphorylation patterns by Western blotting and Phos-tag SDS-PAGE. Preliminary results indicate that differences in replication dynamics between variants are associated with variant-specific changes in N protein processing and phosphorylation. Although viral RNA levels were comparable among variants, infectious viral titers differed markedly, suggesting that N modifications influence post-replicative stages of the viral life cycle. Delta and BA.1 displayed higher viral titers, greater proteoform diversity, and increased phosphorylation, whereas sub-Omicrons exhibited fewer proteoforms and delayed but sustained replication kinetics. These findings suggest that N proteoforms contribute to viral adaptation and fitness, potentially driving phenotypic differences among SARS-CoV-2 variants. Ongoing studies will characterize these proteoforms and their origin using PCR followed by Sanger sequencing, fluorescence microscopy, and transmission electron microscopy.

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INVESTIGATION OF POTENTIAL *ALPHAVIRUS* MUTATIONS HOTSPOTS AND ITS IMPACT ON CHIKV *IN VITRO* ADAPTATION DINAMICS.

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Chikungunya virus (CHIKV) is an arbovirus transmitted between vertebrate and invertebrate hosts, whose adaptation involves genetic changes that impact its viral fitness. Thus, the present study aims to evaluate how mutations detected in Mayaro virus (MAYV) may influence the *in vitro* replicative cycle of CHIKV, as well as the function and structure of viral proteins and its ability to adapt to vertebrate and invertebrate hosts, describing possible mutation hotspots shared by different viruses of this group. For this purpose, a recombinant CHIKV-GLuc virus derived from the LR2006-OPY1 ECSA strain was used, and the mutations W456G and W456R in nsP1, D13N in nsP3, E162K and G95S in E2, and M296V in nsP4 were individually introduced. Transfections were performed in C6/36 and U2OS cell lines, where increased viral titers were observed for the E162K, G95S, and W456R variants (2.71×10^5 PFU/mL) compared to the wild type virus (1.02×10^5 PFU/mL), while G13N showed no alteration. Growth kinetics curves were performed in U2OS cells, and the E162K mutation showed a marked increase in replication, reaching 1.2×10^5 at 48 h, higher than the wild type. In C6/36 cells, G95S exhibited greater replicative efficiency (8.0×10^5 at 48 h), suggesting possible adaptation to the invertebrate host. These preliminary data suggest that mutations described in MAYV may modulate CHIKV replication in a host-dependent manner, indicating possible shared adaptation genome hotspots between them. Additional experiments, including the conduction of *in vitro* intra and interhost infectious cycles, are ongoing to further evaluate the impact of these mutations on viral adaptation.

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Isolation of a lytic bacteriophage for *Pseudomonas aeruginosa* PAO1: physicochemical and biological characterization

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While some bacterial species form symbiotic relationships, others are pathogenic. The study reported herein aimed at characterizing lytic bacteriophages for the pathogen *Pseudomonas aeruginosa*, which can cause some diseases just like some skin infections in burnt skin and in newborns, hospitaller infections in immunosuppress patients and even pneumonia. Bacteriophages, or simply phages, represent good and specific alternatives for the treatment of the pathogens, without the need to use antibiotics, taking into consideration that *P.A.* is a Mult resistant bacteria. The project led to a search for these lytic phages against *Pseudomonas aeruginosa,* that was found in a steam in the city of Sorocaba. One Phage was isolated and characterized via UV-Vis, spectral scans for determination of their molar extinction coefficients, Transmission Electron Microscopy (TEM), so we can visualize the presence of phages and define which type the phage is. Some biological characterizations were necessary for the determination of their host range and efficiency of plating on susceptible bacterial species, determination of phage growth parameters via production of their one-step growth curves, determination of the rate of adsorption onto their susceptible bacterial host cells and potential for biocontrol of Pa via planktonic cell inactivation at different MOI values. The study shows some of the potential of the bacteriophages for future uses against Pa for skin diseases.

Keywords: *Pseudomonas aeruginosa (Pa*); Bacteriophage particles; Physicochemical and biological characterization; Phage-based inactivation of bacteria.

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Potential of a biopolymeric hydrogel integrating ionic liquid and lytic bacteriophage virions for *Pseudomonas aeruginosa* PAO1 in the treatment of deep skin infections: transdermal permeation studies

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One of the main concerns in today's clinical practice is the increasing number of skin infections caused by multidrug-resistant bacteria. In this scenario, bacteriophage-based therapies have emerged as a promising alternative. Therefore, this study aimed to develop and evaluate a biohydrogel containing a bacteriophage for the treatment of skin infections. A lytic bacteriophage against *Pseudomonas aeruginosa* was isolated and incorporated into a biohydrogel composed of sodium alginate, calcium carbonate, gluconolactone, an ionic liquid. The ionic liquid was incorporated to enhance transdermal permeation. The system was characterized by X-ray tomography (XRT) to examined its internal structure and scanning electron microscopy (SEM) to investigated its surface morphology. Permeation studies were conducted using porcine skin in Franz diffusion cells. Samples were collected over time and analyzed by plaque assay to assess phage viability and permeation. XRT and SEM revealed a porous hydrogel structure. The formulation maintained phage lytic activity and allowed progressive release over time, from 60 to 180 min. The porous structure may facilitate bacteriophage incorporation and release, indicating the potential of the formulation for localized delivery in skin infections caused by *P. aeruginosa*. Overall, permeation studies demonstrated maintained phage viability and sustained release over time, suggesting its potential applicability in topical therapies.

Keywords: *Pseudomonas aeruginosa*; Bacteriophages; biohydrogel; transdermal permeation.

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STRATEGIES FOR THE PRODUCTION AND PURIFICATION OF OROPOUCHE VIRUS FOR STRUCTURAL STUDIES

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Structural virology is essential for understanding viral organization, infection mechanisms, and virus–host interactions. Progress in this field is limited by the requirement for highly purified and concentrated samples, particularly for pleomorphic viruses such as Oropouche virus (OROV). This study aims to standardize an OROV purification protocol compatible with structural and proteomic analyses. To this end, viral growth curves were performed in BHK-21 and HuH-7.0 cells under different culture conditions. Additionally, purification strategies were evaluated, including ultracentrifugation through a 22% (w/v) sucrose cushion and sucrose and tartrate gradients. The resulting samples were titrated by plaque assay (PFU/mL), and their protein profiles were analyzed by SDS-PAGE. Our results indicated that HuH-7.0 cells infected at an MOI of 0.01 yielded viral titers up to tenfold higher than those obtained in BHK-21 cells in 24-well plates. However, at larger volumes (500 and 1,000 mL), samples purified from BHK-21 cultures exhibited higher viral titers and better quality after purification, as evidenced by the detection of structural proteins (L, Gn, Gc, and N) with lower host protein contamination. Furthermore, the use of a tartrate gradient resulted in an up to 100-fold reduction in viral infectivity compared with the sucrose gradient. In conclusion, we established that OROV purification from BHK-21 cells using sucrose gradients was the most suitable strategy for obtaining viable viral particles while preserving their infectivity. These results provide a promising foundation for the standardization of protocols aimed at structural studies of OROV, such as cryo-electron microscopy and atomic force microscopy (AFM).

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THE STRUCTURES OF MATURE AND IMMATURE ST. LOUIS ENCEPHALITIS VIRUS REVEAL KEY HISTIDINE RESIDUES FOR ORTHOFLAVIVIRUS FITNESS

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Orthoflaviviruses undergo significant structural changes through maturation and infection, yet the molecular mechanisms remain incompletely understood. Using St. Louis encephalitis virus (SLEV) as a model, a reemerging mosquito-borne orthoflavivirus endemic in the Americas, we elucidated the structures of immature and mature SLEV particles at resolutions of 4.4 Å and 3.3 Å, respectively, using cryo-EM. SLEV is characterized by glycosylated E and prM proteins, the presence of lipid pockets, and is stabilized by an intricate network of inter- and intra-protein interactions between E and (pr)M across maturation stages. Several interactions were mediated by histidines that play different roles depending on SLEV maturation and pH. Non-lethal single mutations of H285 and H443 in E protein affect SLEV replication in mammalian and mosquito cell lines, and delay death in mouse models of infection. His residues are conserved across orthoflaviviruses, illustrating the complexity of orthoflavivirus particles and providing a rationalized strategy for attenuation.

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MAPPING THE TOLERANCE OF THE ZIKA VIRUS NS2B-NS3 PROTEASE CRYSTAL SYSTEM FOR CO-CRYSTALLIZATION AND SOAKING WITH SMALL MOLECULES

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Zika virus (ZIKV) remains a public health concern and no approved antiviral therapy is available. The viral NS2B-NS3 protease is essential for polyprotein processing and is a validated antiviral target, but structure-guided design requires protein–ligand complexes, which in turn require crystallization conditions that remain permissive in the presence of a compound and its solvent carrier. Here, the tolerance of this crystal system to the main co-crystallization variables was mapped. The recombinant protease was expressed in *Escherichia coli* cepa and purified by immobilized-metal affinity and size-exclusion chromatography. Crystals were grown by sitting-drop vapor diffusion at 20 °C from a reference condition containing PEG 2000, ammonium sulfate and sodium acetate pH 4.8. Titration of PEG 2000 from 21% to 29% in the absence of compound showed that lower precipitant concentrations reduced nucleation density and yielded larger crystals. DMSO titration up to 15% showed a progressive reduction in crystal size with increasing solvent, identifying the carrier as a determinant of morphology independent of the compound, whereas crystals still grew at compound concentrations up to 5.5 mM. Diffraction data were collected at the Manacá beamline (LNLS/CNPEM) for 19 apo crystals and 28 crystals grown with six compounds previously evaluated against the protease, by co-crystallization (22% and 30% PEG) and by soaking, with resolutions between 1.7 and 2.5 Å. Automatic processing assigned tetragonal space groups to 43 of 47 datasets, consistent with the apo lattice. Data processing and structure determination are ongoing.
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PT.272

Human cytomegalovirus (HCMV) increases kallikrein 6 (KLK6) expression and promotes its subcellular redistribution to the viral assembly compartment

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HCMV is a globally prevalent β-herpesvirus that establishes latent infection for life and causes serious diseases in immunocompromised individuals. Evidence links HCMV to cancer progression. Various studies, including ours, show viral components in glioblastoma multiforme (GBM) tissues. Moreover, functional assays demonstrate that viral proteins can be oncogenic in cell line and animal models. We show that HCMV (strain TB40/E) significantly increases kallikrein 6 (KLK6) mRNA and protein expression in primary fibroblasts (MRC-5) and GBM cell lines (U138 and U251). We also demonstrate that during the late times of viral infection KLK6 is localized to juxtanuclear regions that coincide with the viral assembly compartment (cVAC) in fibroblasts, confirmed by its colocalization with pp28 a viral protein that functions during viral envelopment. Since KLK6 is a serine protease with trypsin-like specificity for basic amino acids (Arg/Lys) at P1 and P1', precisely matching the furin recognition site, and considering that gB is cleaved by furin during maturation, KLK6 may participate in viral maturation and assembly process during the HCMV replicative cycle. In GBM disease, KLK6 overexpression is associated with reduced sensitivity to temozolomide and cisplatin, radiotherapy resistance, and poor prognosis. Our working hypothesis is that the HCMV-induced increase in KLK6 expression may facilitate viral replication via glycoprotein processing and, additionally, contribute to tumor progression and resistance to standard therapies. This work was funded by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

PT.273

WHOLE-GENOME SEQUENCING OF *LYSSAVIRUS RABIES* FROM URBAN NON-HEMATOPHAGOUS BATS

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Non-hematophagous bats play an important role in the maintenance and spread of rabies virus (RABV) in urban environments. Although distinct antigenic variants (AgVs) have been associated with specific natural reservoirs, few is known about the whole genome of the virus, due to little sequences available. In this context, next generation sequencing has become an essential tool for the genetic characterization of different viruses. Accordingly, this study aimed to perform genomic surveillance of RABV through whole-genome sequencing of positive samples obtained from non-hematophagous bats collected in urban areas of Campo Grande, Mato Grosso do Sul, Brazil. Ten RABV-positive samples from 2025, previously diagnosed by the direct fluorescent antibody test (DFA) and RT-qPCR (Ct < 30), were selected for sequencing. cDNA synthesis was performed using the SMART-9N protocol, followed by library preparation with the Illumina Microbial Amplicon Prep (IMAP) kit and sequencing on the Illumina MiSeq i100 platform. Three samples generated high-quality assemblies, yielding genomes ranging from 11,956 to 12,115 bp, consistent with the expected genome length of RABV. The recovered genomes showed 99–100% genome coverage and 97.1–98.8% nucleotide identity relative to publicly available complete RABV genomes, confirming successful recovery of near-complete viral genomes. These findings demonstrate the feasibility of recovering near-complete RABV genomes from non-hematophagous bats and highlight the potential of genomic surveillance to support phylogenetic analyses, characterize circulating viral lineages, and improve monitoring of rabies virus circulation in urban environments.

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001. INCT Vigilância Genômica de Vírus e Saúde Única (INCT-One).

PT.274

Immobilization of tequatrovirus phage virions in a calcium alginate biopolymeric matrix for mercury (II) removal from the aquatic environment by biosorption onto capsid proteins: *proof-of-concept*

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Contamination of water with mercury constitutes a serious public health problem, especially in locations where the use of Hg occurs improperly/illegally and negligently, as is the case in the Amazon region (Brazil), where its riverside populations are frequently invaded by illegal gold mining and which has exposed these populations to significant risks via contamination by heavy metals such as mercury (Hg^{2+} ions). The aquatic biome is extremely important for the local economy and population, being drastically affected by Hg^{2+} contamination and its effects. Therefore, bioremediation/biomitigation methods that are effective and less harmful to the environment are of paramount importance, aiming to remove Hg^{2+} from water. Hence, we can highlight the possibility of using bacteriophage virions to remove Hg^{2+} ions from water by electrostatic biosorption onto their viral capsids using their negative Zeta Potential for this purpose. In this sense, the main goal of the research work undertaken was to test the possibility of mitigating the presence of Hg^{2+} ions in water through the immobilization of a T4 miovirus bacteriophage (EcoM021, *Straboviridae* family and genus *Tequatrovirus*), on a chitosan-coated Ca-alginate microparticle support, through which water contaminated with Hg^{2+} ions was percolated. The system developed in microparticle form integrating trapped phage virions showed to be very promising for retaining mercury ions through biosorption (electrostatic attraction), thus enabling the removal of these ions from water.

PT.275

DNA VACCINES ENCODING THE ECTODOMAIN OF THE E AND NS1 PROTEIN FROM THE FOUR DENGUE SOROTYPES

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Dengue virus comprises four antigenically distinct serotypes (DENV1–DENV4) sharing ~60% genomic similarity. Envelope glycoprotein (E) is the main target of neutralizing antibodies, while non-structural protein 1 (NS1) plays a key role in viral replication and immune evasion, making both promising vaccine candidates. Although three dengue vaccines are licensed in Brazil, limitations remain regarding safety and efficacy against all four serotypes. Previously, our group constructed two DENV2 DNA vaccines (pE1D2 and pcTPANS1) that induced antibody and T-cell-mediated protection against lethal challenge in BALB/c mice. However, their plasmid vector is no longer recommended for human use. Therefore, this study aimed to develop a second generation of DNA vaccines encoding E and NS1 genes from all four dengue serotypes. Recombinant protein expression was confirmed *in vitro* in transfected HEK293-T cells, detecting E and NS1 proteins across all serotypes via immunofluorescence. Cellular immune responses were evaluated by ELISPOT, showing that DENV2-targeted vaccines (pVD2E and pVD2NS1) successfully induced IFN-γ production, as did the remaining constructs (pVD1NS1, pVD3NS1, and pVD4NS1). Subsequently, mice immunized with DENV2 vaccines were subjected to a lethal DENV2 challenge. The new vaccines (pVD2E and pVD2NS1) generated protection (90%) comparable to that observed with the original constructs (pE1D2 and pcTPANS1). Future studies will evaluate neutralizing antibody levels elicited by the various E protein–based vaccines.
Financial Support: CNPq, FAPERJ, IOC, INCTV, CAPES.

PT.276

EVALUATION OF SYNTHETIC PHENYLPROPANOID DERIVATIVES REVEALS A PROMISING ANTIVIRAL LEAD AGAINST MAYARO VIRUS

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Arthropod-borne viruses (arboviruses), transmitted mainly by *Aedes*, *Culex*, and *Haemagogus* mosquitoes, are widely distributed and represent a major global health burden. Among the most relevant are *Alphavirus chikungunya* (CHIKV), *Orthoflavivirus zikaense* (ZIKV), and *Alphavirus mayaro* (MAYV), for which no specific antiviral therapies are currently available. Natural products are important sources of bioactive compounds and often serve as scaffolds for synthetic drug development. This study evaluated the in vitro antiviral activity of twenty-two synthetic phenylpropanoid derivatives (DL-1 to DL-22) against CHIKV, MAYV, and ZIKV. Cytotoxicity was assessed by the MTT assay (CC_{50}), and antiviral activity was determined by EC_{50} and selectivity index (SI). Antiviral effects were confirmed by cytopathic effect (CPE) inhibition, plaque reduction assays, and RT-qPCR. Among the tested compounds, DL-22 showed the most promising profile, with no cytotoxicity at 100 µg/mL. It exhibited potent antiviral activity against all three viruses, with the strongest effect against MAYV (EC_{50} = 1.49 µg/mL; SI > 67.1), surpassing reference drugs. Viral titers were reduced by up to 8 $\log_{10}$ PFU·mL^{-1} at 48 h post-infection (5 µg/mL), and RT-qPCR confirmed a marked reduction in viral replication. No virucidal activity was observed, suggesting inhibition of viral replication. These findings identify DL-22 as a promising lead for anti-MAYV drug development and support further mechanistic and in vivo studies. Financial support: FAPEMIG, CNPq, UFOP, Rede Mineira de Antivirais.

PT.277

In Vitro Antiviral Activity of Quercetin Against Oropouche Virus

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Oropouche orthobunyavirus (OROV) is an emerging arbovirus responsible for recurrent outbreaks in Latin America, posing a growing public health concern due to its expanding geographic distribution and the lack of approved antiviral therapies. Natural compounds have attracted attention as potential antiviral agents, and quercetin has demonstrated antiviral effects against several RNA viruses. This study evaluated the antiviral activity of quercetin against OROV in Vero cells (ATCC CCL-81) using virucidal and anti-infection assays. Antiviral activity was assessed by light microscopy of cytopathic effects, while viral RNA levels were quantified by RT-qPCR. Quercetin exhibited antiviral activity in both approaches. In the virucidal assay, activity was detected at 156 µg/mL, whereas the lowest biologically active concentration in the anti-infection assay was 312 µg/mL. Although activity was detected at a lower concentration in the virucidal assay, the anti-infection assay produced greater increases in Cq values (ΔCq ≈ 5–8) than the virucidal assay (ΔCq ≈ 4), indicating greater viral RNA reduction during the early stages of infection. RT-qPCR findings were supported by microscopy, showing preserved cell viability with minimal or no cytopathic effect in quercetin-treated cultures. Because the virucidal protocol did not include compound neutralization before cell inoculation, a direct virucidal effect cannot be conclusively demonstrated. These findings suggest that quercetin primarily interferes with early stages of the OROV infectious cycle, although a partial virucidal effect cannot be ruled out. Overall, quercetin is a promising antiviral candidate against OROV, warranting further investigation

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PT.278

DNA vaccines based on SARS-CoV-2 nucleocapsid (N) protein and the immune response elicited in mice

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Coronavirus Disease 2019 (COVID-19), caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), led to a pandemic characterized by hundreds of millions of cases and more than 7 million deaths. Vaccines against SARS-CoV-2 were rapidly developed following the significant social and economic impact caused by the rapid spread of the virus. Although these vaccines have helped to reduce the number of COVID-19 cases and deaths, the virus continues to circulate and mutate, resulting in variants that evade the immune response induced by vaccination or previous infection. Most vaccines target the Spike protein, which is essential for viral entry and highly immunogenic, but it's also the viral protein that accumulates the greatest number of mutations among circulating variants. Therefore, including more conserved proteins in COVID-19 vaccines is a promising strategy to minimize the impact of viral mutations on vaccine-induced immunity. This study evaluated DNA vaccines based on the SARS-CoV-2 nucleocapsid (N) protein, a highly conserved and immunogenic structural protein. The full-length and truncated N genes were inserted into a mammalian expression vector, generating the plasmids pCOV-N and pCOV-N1. Gene integrity was evaluated by electrophoresis and sequencing, while protein expression was evaluated by immunofluorescence. Both plasmids successfully mediate the expression of the N protein in HEK-293T cells. C57BL/6 mice were immunized with two doses of pCOV-N or pCOV-N1 and the specific immune response was evaluated by ELISA for anti-N antibodies and by ELISpot for IFN-γ-secreting cells. pCOV-N induced higher antibody titer than pCOV-N1, whereas both vaccines elicited similar cellular immune responses.

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PT.279

BIOTECHNOLOGICAL VALORIZATION OF AMAZONIAN CACAO RESIDUES: PRODUCTION OF RHAMNOLIPIDS WITH POTENT ANTIVIRAL AND ANTIOXIDANT ACTIVITIES

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Biosurfactants are microbial amphipathic molecules that mirror synthetic surfactants while offering superior biodegradability and biocompatibility. Leveraging Amazonian agro-industrial residues is a strategic approach to valorize biomass and strengthen the regional bioeconomy. This study evaluated the antiviral and antioxidant properties of rhamnolipids produced by *Pseudomonas aeruginosa* using *Theobroma cacao* residue as a substrate. *In vitro* antiviral activity was tested against respiratory syncytial virus (RSV) and herpes simplex virus type 1 (HSV-1). Cytotoxicity was assessed in HEp-2, Vero, and L929 cells (MTT assay) to determine the 50% cytotoxic concentration (CC50). Antiviral efficacy was measured to establish the 50% inhibitory concentration (IC50) and selectivity index (SI), followed by a 1-hour virucidal assay. The rhamnolipids showed low cytotoxicity (CC50> 1,000 $\mu g \cdot mL^{-1}$), IC50 values were 53.87 $\mu g \cdot mL^{-1}$ (RSV) and 157.6 $\mu g \cdot mL^{-1}$ (HSV-1), with SI values of 18.56 and 6.34. Virucidal testing at 500 $\mu g \cdot mL^{-1}$ inhibited RSV by 87% and HSV-1 by 82%. Antioxidant activity (ABTS) reached 87.44% at 8.6 $mg \cdot mL^{-1}$. These results underscore the potential of these multifunctional compounds for sustainable high-value bioproducts.

PT.280

ANTIVIRAL ACTIVITY OF PEQUI AND ANNATTO OIL-BASED NANOEMULSIONS AGAINST MAYARO VIRUS (MAYV)

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Mayaro virus (MAYV) is an emerging alphavirus with the potential for urbanization through adaptation to transmission by Aedes aegypti. In the absence of specific antiviral therapies and given the possibility of chronic sequelae, such as persistent polyarthralgia, the search for new therapeutic strategies is essential. This study evaluated the in vitro antiviral activity of nanoemulsions (NEs) formulated with pequi oil obtained by three extraction methods (solvent extraction, cold pressing, and boiling) and annatto oil against the complete replication cycle of MAYV. The cytotoxicity of the formulations was determined in BHK-21 cells using the MTT assay after 24 h of exposure, at concentrations ranging from 1000 to 7.8 µg. The maximum non-toxic concentration was 1000 µg for pequi NEs and 7.8 µg for annatto NE. Subsequently, the antiviral activity of the formulations was assessed in cells infected with CMV-MAYV-Nanoluc (MOI = 0.05) for 24 h. The annatto nanoemulsion (ANNE) exhibited approximately 69.4% inhibition of viral replication, whereas the pequi nanoemulsion obtained by solvent extraction (PSNE) inhibited viral replication by approximately 54%. Pequi formulations obtained by cold pressing and boiling showed low antiviral activity. These findings demonstrate the promising antiviral activity of ANNE and PSNE against MAYV and highlight the influence of the extraction method on the biological activity of pequi oil. To our knowledge, this is the first study to evaluate nanoemulsions against MAYV and to report antiviral activity for pequi and annatto oils, underscoring their potential for the development of novel antiviral strategies.

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PT.281

FRUTICULIN A FROM *SALVIA PROCURRENS* INHIBITS EARLY STAGES OF HSV-2 INFECTION AND ENHANCES ACYCLOVIR ACTIVITY

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Herpes simplex virus type 2 (HSV-2) causes genital herpes, and acyclovir-resistant strains reinforce the need for antivirals with alternative mechanisms. We investigated the anti-HSV-2 activity of *Salvia procurrens* exudate and the icetexane diterpenoids 7-hydroxyfruticulin A, demethylfruticulin A, and fruticulin A. Cytotoxicity was assessed in Vero cells by AlamarBlue, and antiviral activity by plaque reduction under simultaneous, virucidal, pre-treatment, post-infection, and attachment conditions. Extracellular viral DNA was quantified by qPCR, and fruticulin A was combined with acyclovir using an effect-matched design. Experiments comprised three independent biological replicates performed in technical triplicate. The exudate showed a CC50 of 4.6 µg/mL, an IC50 below 1 µg/mL, and a selectivity index above 4.6. Fruticulin A was the most active compound, with an IC50 below 5 µM, CC50 of 118.7 µM, and selectivity index above 24. It produced dose-dependent inhibition during simultaneous and virucidal treatments and reduced productive infection in the attachment assay. No significant activity was detected after cell pre-treatment for 1 or 6 h or when fruticulin A was added at 3, 8, or 24 h post-infection, indicating a predominantly extracellular, early-stage mechanism. qPCR showed reduced extracellular viral DNA, although viral genomes remained detectable. Fruticulin A combined with acyclovir achieved greater than 90% viral inhibition, consistent with a strong additive effect. Exploratory docking supported glycoprotein D as a candidate target for further validation. These findings identify fruticulin A as a promising anti-HSV-2 lead with a mechanism complementary to acyclovir. Financial Support: FAPERGS, INCT-PROBIAM, and CAPES.

PT.282

SEMISYNTHETIC CARDENOLIDES AS PROMISING ANTIVIRALS AGAINST HSV-1 IN NEURONAL CELLS: A POTENTIAL STRATEGY FOR ALZHEIMER'S DISEASE PREVENTION

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Herpes Simplex Virus 1 (HSV-1) is a prevalent pathogen that causes a range of clinical manifestations and infects about 90% of the world's population, posing a global health problem, particularly because it can cause latent and recurrent infections and has the potential to develop resistance to available antiviral drugs. Besides, scientific and epidemiologic evidence demonstrates that HSV-1 infection can contribute to the development of neurodegenerative diseases, such as Alzheimer's disease (AD), reinforcing the need for new antiviral alternatives with potential indirect impact on AD. Cardenolides are metabolites from natural sources, traditionally used to treat cardiac diseases, and in recent years have been studied for new therapeutic possibilities, particularly due to their remarkable antiviral properties. In this context, cardenolides emerge as promising candidates. In this study, SH-SY5Y neuroblastoma cells were used to evaluate the antiviral activity of cardenolides against the acyclovir-resistant HSV-1 strain 29-R, using the plaque reduction assay to determine the IC_{50} values and the sulforhodamine B assay to determine CC_{50} values in the same cell line. Two compounds, 6b and 6d, showed IC_{50} values below 60 nM, with CC_{50} values of 11.93 μM and 18.83 μM, respectively, resulting in selectivity indices above 199 and 314. Therefore, these semisynthetic cardenolides deserve further investigation as bioactive compounds with potential applications against acyclovir-resistant HSV-1 strains, contributing to the advancement of antiviral drug discovery. Given the increasing evidence linking HSV-1 infection to Alzheimer's disease, these compounds may also represent promising candidates for future studies exploring antiviral approaches to Alzheimer's disease prevention.

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PT.283

HYBRID VIRUS-MIMICKING NANOSTRUCTURES AS A VACCINE CANDIDATE FOR CHIKUNGUNYA VIRUS VACCINATION DEVELOPMENT

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Chikungunya virus (CHIKV) imposes a significant epidemiological burden, demanding innovative and safe vaccine platforms. This study aimed to develop hybrid nanostructures based on viral lysates to generate a robust immune response against CHIKV. To establish the immunization protocol, female AG129 mice lacking type I and II interferon receptors were intraperitoneally immunized with CHIKV lysate dilutions (100%, 25%, 5%, 0,5% and 0.05%) prepared by freeze-thaw cycles and sonication. Daily monitoring and RT-qPCR detected CHIKV RNA in livers, spleens, and kidneys of animals that succumbed, confirming systemic viral progression despite viral inactivation. Sera from surviving mice were evaluated for neutralizing antibodies using plaque reduction assays in Vero E6 cells. High lysate concentrations (100%, 25%, 5%) were uniformly lethal to AG129 mice within 4-20 days, likely due to uncontrolled viral replication exploiting the absence of type I and II interferon signaling. In contrast, the 0.05% concentration was identified as a safe, non-lethal dose, allowing survival throughout the 30-day period. However, sera from mice immunized with lower doses (0.05% and 0.5%) failed to prevent cytopathic effects in Vero E6 cells (0.01 MOI of CHIKV), indicating insufficient neutralizing antibody responses. These findings establish a safe antigenic dose for subsequent vaccine development while highlighting limitations of the current lysate preparation. Ongoing studies focus on optimizing lysate production and incorporating it into liposome-based hybrid virus-mimetic nanostructures to improve multiepitope presentation and promote robust humoral immunity against CHIKV.

Financial Support: PIBIC - CNPQ, FAPESP.

PT.284

NS1 INTERACTION AND ANTIVIRAL ACTIVITY OF A CHALCONE ANALOG AGAINST HRSV IN CELL CULTURE

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Human respiratory syncytial virus (hRSV) remains a major cause of respiratory disease in children and older adults, while therapeutic options are still limited. Chalcones are bioactive compounds with antiviral and anti-inflammatory potential. We evaluated the post-infection activity of the synthetic chalcone analog L1323 against hRSV and investigated whether its activity could involve the viral nonstructural protein NS1. In HEp-2 monolayers, L1323 inhibited viral replication with EC_{50} values of 65 µM at 24 hpi and 11 µM at 48 hpi, with selectivity indices of 1 and 5, respectively. In A549 cells, IC_{50} values were 20 µM and 11 µM at 24 and 48 hpi, with selectivity indices of 3 and 5, respectively. Preliminary assays in A549 3D spheroids showed that L1323 was tolerated at 20-40 µM and reduced rgRSV fluorescent foci, suggesting activity in a more complex respiratory epithelial model. Metabolomic profiling in HEp-2 cells indicated that L1323 counteracted hRSV-induced changes, especially in mitochondrial and energy metabolism. Since NS1 is associated with modulation of antiviral responses and mitochondrial-related processes, recombinant hRSV NS1 was produced and purified for fluorescence spectroscopy analysis. L1323 quenched NS1 intrinsic fluorescence, with K_{SV} values of 1.3×10^5 and 2.3×10^5 M^{-1} and K_b values of 8.3×10^4 and 34.9×10^4 M^{-1} at 20 °C and 37 °C, respectively. Thermodynamic analysis supported spontaneous, entropy-driven binding compatible with hydrophobic interactions. Together, these findings support L1323 as a post-infection hRSV inhibitor and suggest that direct association with NS1, potentially antagonizing its functions, may contribute to its antiviral activity and metabolic effects.

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PT.285

In Vitro Efficacy and Protein Binding Interactions of Natural Phenolic Compounds as hRSV Inhibitors

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Human respiratory syncytial virus (hRSV) is a leading global cause of severe pneumonia and bronchiolitis in infants and the elderly. Treatment options are currently limited to nonspecific antivirals, such as ribavirin, and vaccines recently authorized for specific groups, emphasizing the necessity of targeted antivirals. In this context, our research group is working to identify antiviral candidates against the syncytial virus. This study investigated the antiviral potential of natural phenolic compounds such as hesperetin and coumarins (1,2-benzopyrone, esculetin, and 4-methylesculetin) against hRSV in cell cultures (A549 cells and differentiated HBE cultures) and their interactions with the hRSV nucleoprotein. The results revealed a specific interaction of 1,2-benzopyrone and hesperetin with a specific region of the nucleoprotein, to which the hRSV phosphoprotein binds to enable viral replication. In A549 cell assays, esculetin, 4-methylesculetin, and hesperetin exhibited inhibition of 70%, 80%, and 50%, respectively. In addition, when encapsulated with β-cyclodextrin, coumarin and esculetin exhibited hRSV inhibition in HBE cell culture of approximately 25% and 7%, respectively. The study demonstrated that coumarins and hesperetin inhibit hRSV; however, we are conducting further research to try to identify the target of these compounds and at which stage of the hRSV life cycle they act.
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PT.286

EVALUATION OF SAFETY OF POLY(I:C)-ADJUVANTED BSA NANOPARTICLES IN MURINO MODEL OF IMMUNIZATION

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Vaccines represent one of the most effective public health interventions, and adjuvants play a critical role in enhancing immunogenicity by promoting host responses to antigenic stimuli. Despite their widespread use, the number of available adjuvants remains limited, and some are associated with adverse effects, low tolerability, or high production costs. In this context, the development of safe and effective novel adjuvant systems is essential. This study evaluates the safety of a pathogen-mimetic nanoparticulate adjuvant developed by our research group (patent: BR10 2022 002426 0), composed of bovine serum albumin (BSA) nanoparticles combined with polyinosinic–polycytidylic acid (Poly I:C). C57BL/6 mice were immunized with formulations containing the nanoparticulate adjuvant at different doses (5mg/40μL, 1mg/40μL, 0,1mg/40μL). Safety was assessed through clinical, macroscopic, histopathological, and hematological analyses. No significant body weight loss or behavioral alterations associated with disease were observed in any experimental group. Macroscopic examination of target organs revealed no evidence of toxicity. Histopathological analysis of muscle tissue at the injection site showed preserved tissue architecture without signs of inflammation or damage. Additionally, hematological parameters remained within normal reference ranges up to 15 days post-administration. These findings demonstrate that the BSA/poly(I:C) nanoparticulate system exhibits a favorable safety profile in a preclinical model, supporting its potential application as a safe adjuvant platform for vaccine development.

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Evaluation of the Antiviral Activity of *Chamaecrista diphylla* Against Mayaro Virus

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Mayaro Fever is a neglected arboviral disease caused by Mayaro virus (MAYV), *Alphavirus*. It manifests with symptoms such as fever, headache, eye pain, muscle aches, and severe joint pain and arthritis. First isolated in Trinidad and Tobago in 1954 and later detected in Brazil in 1955, MAYV is mainly transmitted by *Haemagogus* mosquitoes. Currently, there is no vaccine or specific antiviral treatment available. In this context, plant-derived natural compounds are being explored as alternative treatments. The *Chamaecrista* genus, traditionally used in folk medicine, is rich in flavonoids with potential antiviral properties. This study investigated the antiviral activity of extracts and fractions from *Chamaecrista diphylla* leaves and branches against MAYV. The cytotoxic concentrations (CC50) and effective antiviral concentrations (EC50) was determined. CC50 values ranged from 20.29 µg/mL to 478.53 µg/mL, while EC50 values ranged from 13.42 µg/mL to 203.26 µg/mL, confirming antiviral activity across all samples. The ethanolic extract of the branches showed a higher selectivity index (13.34) and was selected for investigation of the mechanisms of antiviral action. In the virucidal assay, the extract showed 84% of reduction in the viral load. Furthermore, the treatment fully inhibited viral adsorption to host cells, resulting in a 100% reduction in viral load; however, it had no effect on viral internalization. This result indicates that the extract somehow interferes with the binding of the virus to the host cell. These findings highlight the antiviral potential of *C. diphylla* extracts and encourage future studies investigating these extracts as a promising treatment for Mayaro fever.

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RECOMBINANT AVIAN INFLUENZA H5 INDUCES HIGH ANTIGENICITY MONOCLONAL ANTIBODY PRODUCTION AND CAN BE USED AS A TOOL FOR DIAGNOSTIC TESTING

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Avian influenza, caused by the *Alphainfluenzavirus influenzae* virus, is a global animal health issue due to the high pathogenicity of certain subtypes, such as H5. This virus has a broad host range with zoonotic potential. Disease surveillance relies on clinical signs, viral isolation, molecular, and serological testing. However, the use of current diagnostics is limited by their laboratory-infrastructure dependence, making them unfeasible for remote areas. Therefore, point-of-care tests are essential to overcome underreporting in continental-sized countries with limited infrastructure, such as Brazil, and to enable case identification and subtype surveillance. The goal was to obtain an avian recombinant H5 protein and evaluate its role in inducing high antigenic monoclonal antibody (mAb) production and its potential as an innovative tool for a rapid diagnostic test. The recombinant protein was expressed in Sf9 cells employing a baculovirus expression system and used to obtain 5 hybridomas, then the antigenicity of purified monoclonal antibodies (mAbs) was evaluated by recognition of inactivated avian H5 virus and recombinant protein using immunoassays such as indirect ELISA, sandwich ELISA, and Dot Blot. Results showed a high yield of the recombinant antigen based on an intense band on the SDS gel, and high specific recognition of the mAbs for both inactivated avian H5 virus and H5 recombinant protein. In conclusion, the results suggest that recombinant avian H5 protein and specific antigenic mAbs can be used as a tool for a rapid diagnostic test to overcome the underreporting of Avian influenza A virus.

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PT.289

DEVELOPMENT OF A MULTIPLEX PCR FOR THE DETECTION OF CANINE PAPILLOMAVIRUS COINFECTIONS IN PAPILLOMAS AND ORAL SQUAMOUS CELL CARCINOMA.

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Canine papillomavirus (CPV) is known for its ability to infect keratinocytes, leading to the development of benign epithelial neoplasms (papillomas and pigmented plaques) and malignant neoplasms (squamous cell carcinoma - SCC) in dogs. For molecular diagnosis, multiplex PCR has the advantage of amplifying multiple CPV types in the same sample, which may be associated with different degrees of pathogenicity. Therefore, this study aimed to design specific primers and establish a multiplex PCR assay for the diagnosis of CPV in different types of lesions. The primers were designed based on the L1 gene sequences of 23 CPV types using the Primer3plus software. Thirty-three samples, obtained from 10 dogs in Sergipe, were analyzed. Of these dogs, 7 had exophytic papillomas, and 3 had oral SCC. With degenerate primers (FAP59/FAP64), CPV1 was amplified in all samples (~480 bp). With the specific primers, divided into 5 Blocks, CPV1 (182 bp) was identified in all samples in Block I; CPV20 (270 bp) in oral papillomas and CPV19 (214 bp) in oral SCC in Block II; Block III amplified CPV8 (173 bp) and CPV12 (224 bp) in oral papillomas and CPV12 in oral SCC; and Block IV amplified CPV21 (165 bp) in SCC and CPV21, CPV22 (216 bp), and CPV23 (359 bp) in oral papillomas. The multiplex PCR strategy enabled the identification of co-infections. These findings highlight co-infection with CPV1/12/19/21 in oral SCC, reinforcing the importance of this tool in improving our understanding of the roles of different CPV types and their association with cancer risk.

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PT.290

STANDARDIZATION OF REAL-TIME PCR WITH HIGH-RESOLUTION MELTING (PCR-HRM) FOR GENOTYPING THE PNPLA3 GENE rs738409 POLYMORPHISM IN PATIENTS WITH CHRONIC HEPATITIS C AND METABOLIC DYSFUNCTION-ASSOCIATED STEATOHEPATITIS

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Metabolic dysfunction-associated steatohepatitis (MASH) is an important condition associated with the progression of liver disease. In patients with chronic hepatitis C (CHC), both viral and host-related factors may contribute to its development. Among the genetic factors, the rs738409 C>G single nucleotide polymorphism (SNP) in the *PNPLA3* gene, which results in an isoleucine-to-methionine substitution (I148M), has been associated with an increased risk of liver disease. Real-time polymerase chain reaction with high-resolution melting (qPCR-HRM) analysis has shown potential for the differentiation of genetic variants. Therefore, we aim to standardize an alternative methodology based on PCR-HRM for genotyping the *PNPLA3* gene rs738409 SNP in patients with CHC and MASH. Initially, 37 samples from a cohort of 78 individuals were selected for qPCR-HRM analysis. These samples were previously characterized by nucleotide sequencing. Up to now, the melting curves from 18 samples, representing the three possible genotypes were analyzed in parallel by qPCR-HRM. Distinct melting curves were generated, allowing discrimination among the three evaluated genotypes. So far, qPCR-HRM demonstrated potential as an alternative methodology for genotyping the *PNPLA3* gene rs738409 SNP, successfully discriminating the CC (wild-type), CG, and GG (mutant) genotypes. These findings indicate the potential applicability of this technique as a faster and more cost-effective alternative for routine analyses.

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PT.291

Establishment of a reporter cell line for basic and applied virology studies of Oropouche virus

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Oropouche virus (OROV) infections have historically affected northern Brazil. More recently, OROV has re-emerged, raising concerns due to its expanding geographic distribution and its potential for vertical transmission and teratogenicity. Despite its longstanding public health importance, OROV has remained neglected for decades, and important knowledge gaps persist regarding its biology and the development of preventive and therapeutic strategies. Herein, we describe the establishment of a reporter cell line for basic and applied virology studies of OROV. Initially, we constructed the pJG_Neo_Vero_OROV_MGLuc plasmid by homologous recombination in *Saccharomyces cerevisiae* (strain YPH252). The plasmid contains the neomycin resistance gene for cell selection and the Gaussia luciferase (GLuc) reporter gene flanked by the 5′ and 3′ untranslated regions (UTRs) of the OROV M segment and the Vero cell (CCL-81) RNA polymerase I promoter at the 3′ end. The plasmid was transfected into Vero (CCL-81) cells using the FuGENE transfection reagent. Three days post-transfection, the cells were selected with G418 (700 µg/mL). After selection, the recombinant reporter cell line, termed Vero_Neo_OROV_MGLuc, was maintained in medium containing G418 (500 µg/mL) and evaluated for GLuc expression following OROV infection at multiplicities of infection (MOIs) of 0.1 and 1. At 24 and 48 h post-infection, GLuc activity increased by approximately 100-fold under both infection conditions, demonstrating the functionality of our reporter cell line. Finally, following its complete characterization, we believe that Vero_Neo_OROV_MGLuc will represent a valuable tool for basic and applied OROV research, including studies of virus biology and antiviral drug screening.

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PT.292

EVALUATION OF MORIN AND PENTA-ACETYLATED MORIN AS POTENTIAL ANTIVIRAL AGENTS AGAINST HRSV IN A549 CELLS

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Human respiratory syncytial virus (hRSV) is a global public health problem and one of the leading pathogens causing acute respiratory infections in infants up to two years, the elderly, and immunocompromised individuals. Currently, the only licensed antiviral is ribavirin, however, it is a broad-spectrum drug that causes side effects. Given this scenario, there is a notable need for alternative therapies that are safe and more specific to hRSV. The objective of this study was to use natural molecules with potential antiviral, morin and penta-acetylated morin, to evaluate their antiviral effects against hRSV in A549 cells. The cytotoxicity of molecules was evaluated using the MTT assay to calculate CC_{50}. The inhibition tests (post infection treatment and virucidal assay) was carried out on rgRSV (recombinant green fluorescent protein expressing hRSV) (MOI 0,01) to calculate IC_{50} and selectivity index (SI). The cytotoxicity results showed that morin had a CC_{50} of 1158 µM (24 h), 632 µM (48 h), and 425 µM (72 h), while the modified morin exhibited elevated toxicity, with CC_{50} values of 315 µM (24 h), 250 µM (48 h), and 142 µM (72 h). Furthermore, morin exhibited inhibitory potential in the virucidal assay, with estimated SI values of 826 (24 h), 575 (48 h), and 850 (72 h), unlike penta-acetylated morin, which presented no antiviral potential in any of the inhibition assays. It is concluded that morin is a promising candidate for future research.
Financial Support: CNPq, FAPESP and FINEP.

PT.293

IN VITRO EVALUATION OF THREE LIPID NANOPARTICLE FORMULATIONS ENCAPSULATING RABIES VIRUS GLYCOPROTEIN SARNA

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Rabies is a highly fatal zoonotic viral encephalitis and a major global public health threat, underscoring the need for scalable vaccines. Lipid nanoparticles (LNPs) are a promising platform for mRNA-based immunization formulations, mostly made up of four lipids: ionizable, phospholipid, sterol, and PEGylated lipid. This study evaluated three different LNP formulations with self-amplifying mRNA (saRNA) encoding the rabies virus glycoprotein (RVGP). Formulations were characterized by hydrodynamic size and polydispersity index (PDI) using Dynamic Light Scattering (DLS). Protein expression was assessed by indirect immunofluorescence (IFI) in BHK-21 and HEK-293 cells at 24 and 48 hours post-transfection. Formulations had a mean size of 137.7 ± 9.5 nm, ranging from 128 nm (F3) to 147 nm (F2), and a mean PDI of 0.098 ± 0.04, from 0.068 (F2) to 0.139 (F3), indicating homogeneity. At 24 hours, F1 induced RVGP expression in both cell lines, while F3 showed fluorescence only in HEK-293 cells. At 48 hours, protein expression appeared for all formulations in both cell types, except F2 in BHK-21 cells. These results show that despite different ionizable lipids, all formulations effectively delivered saRNA, with peak expression at 48 hours. Transfection efficiency varied, likely due to differences in endosomal escape and cellular kinetics. In conclusion, these findings support saRNA-loaded LNPs as a promising platform for alternative rabies vaccines, meriting further optimization and in vivo study.

Financial support: Finep, FAPESP, CAPES, CNPq, Fundação Butantan, Instituto Butantan

PT.294

DEVELOPMENT OF AN RT-qPCR PROTOCOL FOR RABIES VIRUS VIRAL LOAD ASSESSMENT DURING VACCINE PRODUCTION

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Rabies is a fatal zoonosis that can be prevented through vaccination. The Rhabdocell rabies vaccine is widely used by the Brazilian Ministry of Health in national vaccination campaigns for dogs and cats. Large-scale vaccine production requires rigorous quality control and reliable methods for monitoring viral replication during manufacturing. This study aimed to develop an RT-qPCR method for rabies virus load quantification during vaccine production. A double-stranded DNA fragment of 705 base pairs containing the RT-qPCR target within the viral nucleocapsid gene was generated from the vaccine rabies virus strain, with the addition of flanking M13 primer-binding sites and T7 promoter sequence for *in vitro* RNA transcription. The fragments were purified and quantified fluorometrically. A standard curve was generated, using Log10 serial dilutions, from 5,0 x 10^7 equivalent copies of the viral genome per reaction, down to 5,0 copies, each tested in five replicates for each dilution point. Samples were amplified using the TaqPath 1-Step RT-qPCR Master Mix (ThermoFisher) on a Real-Time PCR System. The standard curve showed an R^2 of 0.99 and an efficiency of 100.29%, and the limit of detection (LoD) was between 50 and 5 equivalent copies of the viral genome per reaction. Samples from 10 Rhabdocell vaccine production processes, up to 11 days post-infection, were quantified using the RT-qPCR standard curve, yielding consistent results across processes. By enabling the absolute quantification of viral genomes using a standard curve, this approach provides a quantitative molecular tool for quality control throughout the manufacturing process.

Keywords: Rabies virus; RT-qPCR; Vaccine quality control

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PT.295

MOLECULAR DYNAMICS ASSESSMENT OF VIRTUAL SCREENING-DERIVED ANTIVIRAL CANDIDATES TARGETING THE MAYARO VIRUS nsP3 PROTEIN

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The Mayaro alphavirus (MAYV), a member of the Togaviridae family, is an arbovirus associated with sylvatic cycles, yet it is also capable of urban transmission via *Aedes aegypti*. Currently, there are no approved vaccines or antiviral therapies available. The viral protein nsP3 represents a promising therapeutic target because its macrodomain is essential for replication. This study aimed to validate a previously constructed nsP3 model and to identify potential antiviral candidates against MAYV through molecular docking, toxicity, pharmacokinetic predictions, and molecular dynamics simulations. Therefore, the MAYV nsP3 protein model was initially subjected to energy minimization and quality assessment. Subsequently, the active site was identified, and virtual screening of DrugBank compounds was carried out through targeted molecular docking. The best-performing ligands were filtered by toxicity prediction and absorption, distribution, metabolism, and excretion (ADME) properties. Finally, Gibbs free energy of ligand–protein binding was estimated using the linear interaction energy (LIE) method. The MAYV nsP3 model exhibited good structural quality and was validated for subsequent steps. Docking of pyrimidone (template 6w0t.1.D) yielded a score of −6.9 kcal/mol. In total, 198 similar compounds were docked. Among them, six were selected based on predicted non-toxicity, favorable pharmacokinetics, and commercial availability. Molecular dynamics simulations of the flumequine–nsP3 complex indicated energetic stabilization, with a binding free energy of −96.44 kJ/mol, suggesting a favorable interaction. Besides providing a reliable nsP3 model for MAYV, our results identify promising candidates for experimental validation, advancing rational antiviral drug development against MAYV. Financial Support: FAPEMIG and UFSJ.

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MOLECULAR DYNAMICS STUDIES OF PROTEIN–LIGAND INTERACTIONS OF THE nsP3 PROTEIN FROM CHIKUNGUNYA VIRUS WITH CANDIDATE ANTIVIRAL MOLECULES AGAINST MAYARO VIRUS IDENTIFIED BY VIRTUAL SCREENING

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The occurrence of Mayaro fever is endemic to tropical Latin America, with increasing spread of Alphavirus mayaro (MAYV) into previously unaffected regions, further intensified by its potential transmission via Aedes aegypti. Based on the hypothesis that antivirals targeting Alphavirus chikungunya (CHIKV) may also inhibit other Alphavirus species, this study aimed to computationally prospect candidate antivirals against MAYV. CHIKV proteins containing co-crystallized ligands were retrieved from the Protein Data Bank (PDB), and structurally similar compounds were selected from DrugBank through 2D/3D similarity searches. These molecules were subjected to molecular docking using Python Prescription 0.8 (PyRx), followed by pharmacophoric analysis (Discovery Studio), ADME profiling (SwissADME), and toxicity prediction (ProTox-3.0), evaluating hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity. The most promising compounds were further assessed by molecular dynamics simulations (310 K, 20 ns) and binding free energy calculations using the LIE method. The nsP3 protein of CHIKV (PDB ID: 6W0T), with 1.95 Å resolution and pyrimidone as the crystallographic ligand, was used as the target, with docking validation via redocking (RMSD = 0.8267 Å, <2 Å). Approximately 400 pyrimidone-like molecules (-8.1 kcal·mol^{-1}) were screened, yielding 38 with favorable binding affinities. Ten compounds showed superior docking scores and interaction conservation, of which four exhibited safe toxicological profiles and no Lipinski violations. Two molecules emerged as promising antivirals, with Rebamipide (DB11656) showing stable protein–ligand interactions and spontaneous binding (ΔG = -5.48 kJ/mol), supporting its potential for future in vitro studies. Financial support: FAPEMIG, UFSJ.

PT.297

LONGITUDINAL CHARACTERIZATION OF FUNCTIONAL NEUTRALIZING ANTIBODIES IN BREAST MILK FOLLOWING BUTANTAN-DV VACCINATION DURING LACTATION: A CASE STUDY

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Data on functional neutralizing antibodies in breast milk following vaccination with the Butantan Institute's live-attenuated tetravalent dengue vaccine during lactation are currently unavailable. We longitudinally characterized functional neutralizing antibodies in breast milk from a lactating woman accidentally vaccinated. The study was approved by CEP/CONEP. Breast milk samples were collected daily from days 1-21 post-vaccination. Maternal serum was collected before vaccination and on days 11, 15, 22, and 36, whereas infant serum was collected on days 11 and 40. Neutralizing antibodies against DENV1-4 were measured by $FRNT_{50}$, and dengue virus RNA was detected by RT-qPCR. Before vaccination, maternal serum contained neutralizing antibodies only against DENV2, consistent with pre-existing immunity. After vaccination, neutralizing antibodies against all four serotypes were detected in breast milk from day 1 throughout follow-up. Activity was highest against DENV2, followed by DENV1-4-3. Maternal serum also presented neutralizing antibodies against all four serotypes, with DENV2 remaining predominant, consistent with an anamnestic response. Vaccine-derived DENV4 RNA was detected only in maternal serum on day 11 and confirmed by sequencing. No viral RNA was detected in breast milk or infant serum. Transient DENV2-neutralizing activity was detected only in infant serum on day 11. This case study provides the first longitudinal characterization of neutralizing antibodies in breast milk following accidental Butantan-DV vaccination during lactation. Although vaccine-derived viral RNA was not detected in breast milk or infant serum, these findings do not establish vaccine safety or passive antibody transfer and support larger studies of viral shedding and maternally derived antibodies in breastfed infants.

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PT.298

Evaluation of Immune Responses Induced by mRNA-LNP H5 Vaccine Candidates in Human Tonsil Organoids

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Avian influenza is caused by subtypes of influenza A virus, classified by hemagglutinin (HA) and neuraminidase (NA) glycoproteins. Transmission was previously restricted to contact with infected birds, but H5N1 outbreaks (2024 and 2025) in dairy cattle suggest possible mammal-to-mammal transmission. Human cases remain mostly zoonotic, but potential human-to-human transmission and mortality rates approaching 50% are raising public health concerns. The development of effective vaccines is essential and mRNA encapsulated in lipid nanoparticles (LNPs) represents a promising strategy. Therefore, HA gene sequences from A/Astrakhan/3212/2020 (H5N8, clade 2.3.4.4b) were cloned into replicating and non replicating plasmid vectors. Plasmids were used as templates for in vitro mRNA transcription. Transcripts were encapsulated in a lipid solution by microfluidic. Encapsulation efficiency (EE%) was determined by fluorometric quantification, and physicochemical characterization was performed by dynamic light scattering (DLS) to assess particle size and polydispersity. mRNA-LNPs were transfected into HEK-293T cells, followed by immunoassays to confirm protein expression. Vaccine-induced immune responses are evaluated using human tonsil-derived immune organoids and subsequent flow cytometry analysis of B- and T-cell responses. As a result, mRNA was successfully synthesized and particle sizes (≈139–184 nm) were within the optimal range for mRNA delivery (<200 nm). Polydispersity index (PDI) values (0.23–0.29) indicated acceptable size distribution. High encapsulation efficiency demonstrated effective incorporation of genetic material. Immunoassays confirmed protein expression in HEK-293T cells. Both mRNA vaccines induced plasmablast development in human tonsil-derived immune organoids. Antigen specific B- and T- cells are currently being evaluated by ELISpot.

PT.299

DESIGN AND IN VITRO VALIDATION OF CHIMERIC MRNA-LNP VACCINES AGAINST HPAI H5

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Since 2014, highly pathogenic avian influenza (HPAI) viruses belonging to clade 2.3.4.4b (H5N1, H5N6, and H5N8) caused outbreaks in poultry across Asia, Europe, and America, posing a major threat to the poultry industry and public health due the potential human-to-human transmission and mortality rates approaching 50%. To broaden immune coverage against diverse HPAI variants, three chimeric vaccine candidates (Q1, Q2, and Q3) were designed based on conserved epitope regions (CERs) of the hemagglutinin (HA) protein. The pipeline combined sequence data from NCBI and GISAID, AlphaFold structural prediction, and the NetMHCpan, NetMHCstabPan, NetMHCII, and NetChop tools to identify epitopes with high MHC-I/MHC-II binding affinity, immunogenicity, conservation, and broad HLA allele coverage. Selected sequences were cloned into the pVAX vector, transcribed in vitro into mRNA, and encapsulated in lipid nanoparticles (LNPs). The formulations were characterized for encapsulation efficiency, particle size, and polydispersity index, and protein expression was confirmed following transfection of HEK-293T cells. Three chimeric constructs comprising combinations of nine conserved epitope regions distributed throughout the HA protein were generated. mRNA was successfully synthesized and particle sizes (≈161-173 nm) were within the optimal range for mRNA delivery (<200 nm). Polydispersity index (PDI) values (0.07–0.14) indicated acceptable size distribution. High encapsulation efficiency demonstrated effective incorporation of genetic material. Immunoassay results indicate that sequence engineering preserved epitope exposure. The Q1-expressed protein was recognized by both anti-H5N1 and anti-H5N8 antibodies, supporting the potential of this construct as broadly protective mRNA vaccine candidates against HPAI. Financial Support: Fundação Butantan, FAPESP, CAPES and CNPq.

PT.300

DEVELOPMENT OF A SEROLOGICAL DIAGNOSTIC TEST FOR *CHIKUNGUNYA VIRUS* INFECTION BASED ON IMMUNOGENIC EPITOPES IDENTIFIED BY BIOINFORMATIC

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Chikungunya fever is caused by the *Chikungunya virus*, a member of the genus *Alphavirus* within the family *Togaviridae*, and is transmitted by mosquitoes of the genus *Aedes*. Serological diagnosis, although sensitive, may exhibit low specificity due to cross-reactivity with other arboviruses. To address this limitation, the present study employed a reverse vaccinology *in silico* approach to identify CHIKV-specific immunogenic epitopes. Structural and non-structural polyproteins were retrieved from GenBank, and epitope prediction was performed using the IEDB platform, with antigenicity evaluated through VaxiJen. Epitopes were selected based on conservation criteria (>90%), specificity (<60% identity with other alphaviruses), population coverage, as well as toxicity, allergenicity, and solvent accessibility (SAS) analyses. Four peptides identified in structural proteins were selected, synthesized, and resuspended at a concentration of 2 mg/mL. Indirect ELISA standardization for detection of IgM anti-peptides antibodies was carried out using pools of human sera from CHIKV IgM positive and IgM negative individuals, evaluating different experimental conditions (blocking, antibody dilutions, and washing steps) to establish optimal assay parameters. The best-performing condition for all peptides consisted of blocking with 5% skim milk, primary antibody at a 1:200 dilution (30 min), secondary antibody at a 1:10,000 dilution (1 h), and washing with 0.1% PBS-T, resulting in improved discrimination between positive and negative samples and reduced nonspecific reactivity. Thus, the results demonstrate that sera from naturally infected patients show reactivity (IgM) against the predicted epitopes, highlighting their immunological relevance and potential application in the development of more specific serological tests for the diagnosis.

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PT.301

PREDICTION AND ANALYSIS OF EPITOPES FROM THE E5 AND E7 ONCOPROTEINS OF HIGH-RISK ONCOGENIC HUMAN PAPILLOMAVIRUS GENOTYPES TO DEVELOP THERAPEUTIC VACCINE STRATEGIES AGAINST HPV-RELATED CANCERS

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Long-term infection with high-risk HPVs (hrHPVs) is directly associated with the development of cervical cancer. Although prophylactic vaccines are available, their efficacy is limited in individuals who are already infected, highlighting the need for new treatment strategies. In this context, the present study aimed to predict and analyze epitopes derived from the E5 and E7 oncoproteins of high-risk HPVs to develop vaccine strategies for the treatment of HPV-related cancers. Initially, the sequences of the E5 and E7 proteins from hrHPVs were obtained from GenBank and the HPV Information Center. Based on the protein sequences, MHC class I epitopes were predicted using the NetMHCpan 4.1 EL and MHCflurry 2.0 methods, taking into account the 27 most common HLA alleles in the global population and the murine haplotypes. Immunogenicity, antigen processing, clustering of similar sequences, toxicity, conservation among hrHPVs, and population coverage were also evaluated. The selected epitopes were modeled and subjected to molecular docking with HLA. From 3,720 predicted E7 epitopes and 5,471 E5 epitopes, 17 epitopes from both proteins were selected. These peptides proved to be promiscuous, as they exhibited high binding affinity for various HLAs; they were non-toxic and showed high population coverage. Molecular docking of the epitope-HLA complexes demonstrated that they exhibited strong binding affinity, indicating favorable structural stability when the selected epitopes interact with the corresponding HLA molecules. The results reinforce the potential of immunoinformatics approaches in identifying candidate immunogens for the development of vaccine antigens broad spectrum against multiple oncogenic HPV genotypes.

PT.302

ANTIVIRAL ACTIVITY OF NATURAL COMPOUNDS AGAINST THE HUMAN RESPIRATORY SYNCYTIAL VIRUS IN A549 CELLS

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Human Respiratory Syncytial Virus (hRSV) is a negative-sense, single-stranded RNA virus belonging to the *Pneumoviridae family* and a major cause of Severe Acute Respiratory Syndrome (SARS), especially in children. Despite recent advances in immunization and prophylaxis, ribavirin remains the only approved antiviral treatment; however, it is a broad-spectrum antiviral. Therefore, the search for new therapeutic candidates remains essential, and natural compounds have attracted attention due to their diverse biological activities. In this study, the antiviral activity of a licochalcone (L1323) and syringaldehydes (S1215 and S1218) against hRSV was evaluated in A549 cells. Cytotoxicity assays were conducted to determine concentrations that maintained cell viability above 80%. Antiviral activity was investigated using the recombinant viruses rgRSV and rA2-Rluc in post-infection treatment and virucidal assays. Among the tested compounds, licochalcone L1323 demonstrated significant antiviral activity in a concentration- and time-dependent manner, reaching a inhibition of 80.7% at 48 hpi. The IC_{50} values were 22.3 µM and 8.6 µM at 24 and 48 hpi, respectively, while the selectivity index increased from 1.7 to 4.5 over the same period, indicating enhanced antiviral action in 48 hpi. In contrast, syringaldehydes S1215 and S1218 showed no significant antiviral activity in the systems studied. These findings identify licochalcone L1323 as a promising antiviral candidate against hRSV, acting after viral entry and potentially targeting intracellular stages of viral replication.

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PT.303

ANTIVIRAL ACTIVITY OF AN AMANTADINE DERIVATIVE AGAINST MAYARO VIRUS: AN IN VITRO STUDY

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Mayaro virus (MAYV) is a neglected alphavirus transmitted by hematophagous arthropods, responsible for Mayaro fever, a disease characterized by fever, myalgia, and debilitating chronic arthralgia. In the absence of specific antiviral drugs or licensed vaccines, therapeutic options remain limited to symptomatic management, underscoring the urgent need for novel therapeutic strategies. Amantadine, a well-established antiviral agent against influenza A virus, represents a promising molecular scaffold for the development of new antivirals. In this study, we evaluated the antiviral potential of an amantadine derivative against MAYV using Vero cells as an in vitro model. Cytotoxicity was assessed by the MTT assay, revealing a CC50 of 1,253 µM, indicating low cellular toxicity. In an initial screening at 20 µM, the compound reduced viral replication by 91%. Dose-response analysis yielded an EC50 of 2.4 µM and a selectivity index of 522, reflecting an excellent safety margin. Time-of-drug-addition experiments demonstrated that antiviral activity was most pronounced when the compound was administered between 0 and 2 hours post-infection, suggesting interference with early stages of the viral replication cycle. Consistent with this, viral adsorption assays revealed dose-dependent inhibition of MAYV adsorption, reaching approximately 90% at 20 µM, while virucidal assays demonstrated moderate but significant inactivation of viral particles (40–45% at 20 µM). Collectively, these findings identify this amantadine derivative as a highly promising antiviral candidate against MAYV, warranting further in vivo investigation.

PT.304

POTENTIAL ANTI-CHIKUNGUNYA ANTIVIRAL APPROACH BASED ON PLANT EXTRACTS FROM THE GENUS *BANISTERIOPSIS*

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Chikungunya fever (CHIKF) is an arboviral disease characterized by symptoms that can become chronic and debilitating. The etiological agent of CHIKF is chikungunya virus (CHIKV), an emerging arbovirus of major public health concern for which no specific antivirals are currently available, highlighting the need for novel therapeutic strategies. In this context, plant extracts from the genus *Banisteriopsis*, known for their anti-inflammatory and antioxidant properties, may represent promising sources of antiviral compounds. This study aimed to evaluate the antiviral activity of stem plant extracts from three *Banisteriopsis spp.* against CHIKV and investigate their mechanisms of action. To this purpose, Vero E6 cells were infected with CHIKV-*nanoluc*, a virus inserted with the *nanoluciferase* reporter gene, and treated with extracts from *Banisteriopsis anisandra*, *Banisteriopsis argyrophylla*, and *Banisteriopsis stellaris*. Dose-response assays were performed to determine the effective concentration of 50% (EC_{50}), cytotoxic concentration of 50% (CC_{50}), using luminescence and MTT assays, respectively, and selectivity index (SI) was calculated. Time-of-drug-addition assays were applied to elucidate the mechanism of action of each extract. The results indicate that all three extracts possess strong antiviral activity, with EC_{50} values in the low-hundreds µg/mL range, with no significant cytotoxicity effects. The SI values were higher than 4.6, indicating favorable antiviral profiles. Moreover, inhibitory activity was observed in all stages of the viral replication cycle investigated, reducing viral replication by over 87%. In conclusion, these findings highlight the potential of *Banisteriopsis spp.* extracts as novel anti-CHIKV candidates and support further studies to elucidate their mechanisms of action and therapeutic applicability.

Financial support: CNPq; FAPEMIG; CAPES.

PT.305

Evaluation of the anti-herpetic activity of *Cecropia glaziovii* Snethl. extracts

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Herpes simplex viruses (HSV-1 and HSV-2) remain a problem affecting more than half of the global population despite the availability of antiviral therapies. The constant use of acyclovir and its derivatives has led to treatment resistance and natural products are emerging as sources of new compounds of therapeutic interest. *Cecropia glaziovii*, popularly known as “Embaúba”, was primarily studied for its antiviral potential against HSV-1 and HSV-2, and the antiviral effect was attributed to flavonoids (isoorientin and isovitexin) and others phenolics compounds (chlorogenic acids and procyanidins). Using VERO cells, the sulforhodamine B assay was employed to assess cytotoxicity (CC_{50}) and the plaque reduction assay was used to evaluate antiviral activity against HSV (IC_{50}). Five samples derived from *C. glaziovii* were evaluated for antiviral activity against HSV-1 (KOS strain and the acyclovir-resistant strain 29-R) and HSV-2 (333 strain): crude extract, MeOH fraction without partition, MeOH fraction with partition, BuOH fraction, and the aqueous residual fraction. CC_{50} values ranged from 1,013 to above 2,500 µg/mL across these samples. The MeOH fraction with partition showed the most favorable selectivity profile, with IC_{50} values of 26.94, 30.65, and below 12.5 µg/mL against strains KOS, 29-R, and 333, respectively, yielding selectivity indices of 66.6, 58.6, and 143.6, representing the highest SI observed among the samples. The aqueous residual fraction, in contrast, presented the lowest selectivity overall (SI 12.3 - 51.6 across strains).The results indicate that *C. glaziovii*, especially the partitioned MeOH fraction, is a promising source of antiviral compounds with high activity against both acyclovir-sensitive and acyclovir-resistant HSV strains.

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PT.306

GRAPHENE OXIDE–MEDIATED MODULATION OF RESPIRATORY VIRUS DETECTION: A PRELIMINARY EXPERIMENTAL STUDY

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The development of innovative strategies to improve viral detection is essential for advancing diagnostic performance in respiratory infections. This study aimed to evaluate the interaction between graphene oxide (GO)–based nanomaterials and influenza virus and its impact on RT-qPCR detection. Influenza samples were incubated with GO under controlled conditions, including virus-only and material/buffer controls to assess nonspecific amplification and background interference. After incubation, samples were centrifuged to separate supernatant and pellet fractions, and viral RNA was extracted from each fraction for RT-qPCR. Preliminary results showed consistent differences in amplification profiles between GO-treated and untreated samples, with Ct shifts suggesting altered viral distribution between fractions. These findings indicate a possible adsorption or concentration effect mediated by GO, influencing viral availability for detection. This approach represents a potential application of nanomaterials as adjuncts in influenza sample processing. Further assays are ongoing to compare GO formulations, optimize experimental conditions, validate reproducibility, and explore the mechanism of interaction. Financial Support: This study was supported by Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG)

PT.307

Optimization of Recombinant Baculovirus Stock Production Carrying the Structural Protein Genes of Mayaro Vírus

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Mayaro virus (MAYV) is an emerging arthritogenic alphavirus considered a potential public health threat due to its expanding geographic distribution and the lack of licensed vaccines or antiviral therapies. The production of high-titer recombinant baculovirus stocks is a critical step for the successful application of the Baculovirus Expression Vector System (BEVS), directly influencing the efficiency and reproducibility of downstream applications. This study aimed to optimize the production of recombinant baculovirus stocks carrying the genes encoding the structural proteins (C, E3, E2, 6K, and E1) of MAYV in insect cells. A recombinant bacmid was generated using the Bac-to-Bac® system and transfected into Spodoptera frugiperda (Sf9) cells to produce the initial viral stock (L1). Subsequent stocks (L2 and L3) were obtained by serial amplification using a multiplicity of infection (MOI) of 0.1. Viral supernatants were harvested at different time points based on cell viability, and viral titers were determined by plaque assay in Sf9ET cells. The initial stock (L1) reached 6.65×10^5 PFU/mL. During L2 production, the highest titer (1.09×10^6 PFU/mL) was obtained at 82 h post-infection, corresponding to approximately 35% cell viability. For L3, harvesting at 72 h post-infection with approximately 40% cell viability yielded the highest titer (7.45×10^7 PFU/mL), representing a 112-fold increase over L1 and a 68-fold increase over L2. These findings demonstrate that harvest timing based on cell viability is critical for maximizing recombinant baculovirus production, establishing an optimized and reproducible strategy for generating high-titer viral stocks for downstream BEVS applications.

Financial Support: FAPESP; FINEP; CNPq; Fundação Butantan.

PT.308

POTENT ANTI-INFLUENZA ACTIVITY OF AMAZONIAN BIOSURFACTANTS

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Microbial biosurfactants are promising sustainable antiviral agents. This study investigated the anti-influenza (IAV) potential of biosurfactants (BS) produced by *P. aeruginosa* BM02, an Amazonian isolate, using diverse carbon sources. Activities against H1N1 (WSN strain) and H5N1 (A/Thailand/KAN-1/2004 strain) were assessed via Focus Forming Assay. While Amazonian residues (Açaí, Andiroba, Babaçu) yielded non-active metabolites, glycerol-derived BS (BSg) exhibited potent, dose-dependent inhibition, at different stages of IAV replication. Notably, BSg sustained robust inhibition (~95%) of H1N1 infection when added up to 6 hours post-infection, accompanied by a downregulation of NP and M1 protein expression, at the lowest concentration tested (25 µg/mL). BSg demonstrated rapid virucidal activity (99.99%) within 1 hour at 25 µg/mL, with its inactivation potential accelerating to a 1-minute interval at higher concentrations (250–500 µg/mL). It exhibited a Selectivity Index of 7.14 (CC50: 100 µg/mL; IC50: 14 µg/mL). Crucially, BSg demonstrated 99.99% efficacy against the H5N1 strain when administered up to 1-hour post-infection at 50 µg/mL, whereas efficacy declined to 78% at 25 µg/mL. In conclusion, these findings underscore the potent, multi-stage inhibitory action of BSg against IAV. The combination of rapid virucidal kinetics and high selectivity positions BSg as a promising, sustainable candidate for the development of novel biotherapeutics to mitigate both H1N1 and H5N1 infections, highlighting the significant biotechnological value of Amazonian microbial resources.
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PT.309

EFFECT OF CHROMONE AND THIAZOLIDINONE DERIVATIVES AGAINST VARICELLA-ZOSTER VIRUS REPLICATION: EVIDENCE OF VIRAL DNA POLYMERASE INHIBITION

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Nucleoside analogues remain the standard treatment for Alphaherpesviruses, yet emerging resistance demands novel antivirals. Chromone and thiazolidinone derivatives (LMed 36, 74, 75, 76, and 77) selected via virtual screening against herpes simplex DNA polymerase and previously active against HSV-1 and HSV-2, were evaluated against the varicella-zoster virus (recombinant pOKA strain - VZV-GFP). All compounds yielded CC_{50} values >50 µg/mL in MRC-5 cells (MTT assay). In antiviral assays (3.12–25 µg/mL), VZV-GFP replication was quantified via relative fluorescence units (RFU) 3 days post-infection (pi) during simultaneous (0 h) and late-addition assays (4, 8, and 24 hpi; MOI 0.1). LMed 36, 74, and 76 were the most potent against VZV (0h), with IC_{50} values ranging from 8.93 to 11.34 µg/mL and selectivity indices from 4.41 to 5.60. Anti-VZV activity was maintained up to 8 hpi, with an abrupt reduction in efficacy (<14%) at 24 hpi. This was confirmed by a 56% reduction in viral genome copies, consistent with phenotypic data. LMed 75 and 77 exhibited less than 50% inhibition at the highest concentration tested (IC_{50} >25 µg/mL). These results demonstrate the anti-VZV activity of LMed 36, 74, and 76, particularly within the first 8 hpi, suggesting a mechanism of action that coincides with the expression and activity phases of DNA polymerase. Further studies will be conducted to confirm this enzymatic mechanism.

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PT.310

BACTERIOPHAGE 2.1.1.1 AGAINST *Pseudomonas aeruginosa* ARE VERY STABLE TO PH AND TEMPERATURE VARIATIONS.

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The World Health Organization predicts that by 2050, infections caused by multi-resistant bacteria to antibiotics could result in approximately 10 million deaths annually. This is exacerbated by opportunistic multi-resistant bacteria such as *Pseudomonas aeruginosa*. Therefore, the use of bacteriophages became a promising method due to its capacity to use the natural replication mechanism to provoke bacterial cell lysis and thus control the replication of these pathogenic organisms. This work aimed to analyze the stability of a phage isolated from environmental water samples under different pH and temperature conditions. The phage was amplified and titrated using the double-layer agar technique. To evaluate stability of the phage at different pH levels, 15µL of the virus were added to 1485µL of LB broth (*Luria Broth*) and exposed to the respective pHs of 3, 5, 7, and 9 for 30 minutes. Parallelly, for thermal stability, 15µL of the phage were added to 1485µL of 0.9% saline solution and subjected to temperatures of 23°C, 37°C, 50°C, 60°C, 70°C, 80°C, and 90°C for 60 minutes. Subsequently, samples were titrated to verify viability. Results showed that pH 3.0 was able to completely inactivate the virus, while temperature tests revealed that the phage maintains its viability at temperatures up to 60°C; however, exposure to temperatures of 70°C or higher resulted in complete inactivation of viral particles. In conclusion, the analyzed bacteriophage 2.1.1.1 maintains its lytic activity across various pH and temperature and is highly resistant to variations in these conditions.
Financial support: FAPEMIG, CAPES, CNPq and UNIFAL-MG.

PT.311

EVALUATION OF THE ANTIVIRAL ACTIVITY OF THE COMBINATION OF RESVERATROL AND RIBAVIRIN IN CELL CULTURE AGAINST HUMAN RESPIRATORY SYNCYTIAL VIRUS (HRSV)

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Human respiratory syncytial virus (hRSV) is among the leading causes of mortality worldwide, causing approximately 100,000 deaths in children under five years of age each year. The only approved treatment is ribavirin, a broad-spectrum antiviral drug that has several adverse effects. In this context, it is necessary to develop a specific antiviral drug against hRSV. Given this, resveratrol which has antioxidant and antiviral properties, may be a promising candidate to mitigate the side effects of ribavirin. Therefore, the objective of this study was to evaluate the effect of the combination of ribavirin and resveratrol on A549 cell cultures infected with hRSV. First, the cytotoxicity of the molecules was evaluated individually and in combination, using the MTT assay. After determining the safe concentrations, the post infection treatment assay was performed with resveratrol. The CC_{50} of the polyphenol was 226 µM (24 h) and 218 µM (48 h), while ribavirin proved to be toxic at high concentrations. The combined compounds assay showed a minimum viability of 75.5%. In the post infection treatment assay, resveratrol exhibited viral inhibition of 43% at 24 hpi and 48 hpi, at the highest concentration. The IC_{50} of resveratrol was 35 µM (24 h) and 36 µM (48 h), associated with selectivity index of 6.4 and 6.1, respectively. These results indicate the antiviral potential of this antioxidant against hRSV and suggest new approaches for combination therapies, aiming for a synergistic effect between the molecules while reducing the deleterious effects of ribavirin. **Financial Support:** CNPq, FAPESP and FINEP.

PT.312

EXPLORING THE HRSV MATRIX PROTEIN AS AN ANTIVIRAL TARGET USING MODIFIED FLAVONOIDS AND SPECTROSCOPY

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Human Respiratory Syncytial Virus (hRSV) is a major causative agent of severe respiratory infections in infants, the elderly and immunocompromised individuals. The hRSV Matrix protein is responsible for a fundamental step in the process of virus assembly, budding across the plasma membrane, forming elongated membrane filaments through oligomerization and activity of the matrix protein (M). In this context, it was observed that natural products interact with key proteins and in the case of M protein can prevent the budding of mature hRSV virions. The aim of this study was to investigate the interaction parameters between the flavonoids chrysin (C), quercetin (Q), pentacetylated quercetin (Q1), tetracetylated quercetin (Q2) and M protein of hRSV. The M protein was expression in Escherichia coli, purified by affinity and molecular exclusion chromatography. The M/flavonoids interaction assays were verified to spectroscopy titration by fluorescence spectroscopy. Fluorescence experiments showed binding constants of the M/compounds complexes on order of 103-104 M-1, with one ligand per monomeric unit. The thermodynamic analysis for quercetins revealed values of $\Delta G<0$, $\Delta H>0$ and $\Delta S>0$, suggesting that hydrophobic interactions play a role in the formation of the complexes, and for chrysin Van der Waals interaction with $\Delta G<0$, $\Delta H<0$ and $\Delta S<0$. These results of viral protein interaction with flavonoids enable us to interfere with potential effects on viral assembly or the formation of mature viruses, both of which are mediated by the M protein.

Financial support: CAPES, CNPq, FAPESP and Finep.

PT.313

Synthetic 4-Quinolones Derivatives as Potential Inhibitors of Dengue Virus Type 2 NS5 RdRp: An *in silico* and *in vitro* approach

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The dengue virus (DENV) is an arbovirus transmitted by *Aedes* mosquitoes and associated with symptoms of high fever and haemorrhage. In Brazil, more than 250,000 cases were reported in 2026. There are no approved drugs for DENV and vaccination faces some challenges. In this scenario, the quinolone class is known by their antiviral activity. The aim of this study is to investigate the *in silico* and *in vitro* antiviral potential of novel quinolone derivatives (**M01**–**M10**) against DENV-2. The derivatives were first evaluated for pharmacokinetic parameters using computational tools. Cytotoxicity was then assessed in Vero cells using the MTT assay, followed by antiviral evaluation using the plaque reduction assay after a 72h treatment at 50 μM. To investigate their putative target, molecular docking studies using AutoDock 4.2.6 were performed with the DENV-2 NS5 RNA-dependent RNA polymerase (RdRp). All derivatives showed favourable pharmacokinetic profiles and oral bioavailability. Moreover, **M01**-**M09** demonstrated low cytotoxicity (CC_{50} > 400 μM), while **M01** (R_1=H, R_2=2Cl), **M07** (R_1=Cl, R_2=Cl), and **M08** (R_1=Me, R_2=Me) exhibited notable antiviral activity, inhibiting over 90% of viral replication. The most active derivatives interacted via hydrogen bonds and π-anion interaction with the NS5 RdRp catalytic residues Asp663 and Asp664, but their interaction profile did not resemble that of a known ligand in the absence of viral RNA. Their binding mode with NS5-RNA complex remains to be investigated. These findings suggest that the derivatives are promising candidates for the development of antivirals possibly targeting DENV NS5 protein.

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PT.314

Evaluation of Kaempferol Derivatives Against Human Respiratory Syncytial Virus: In Silico and Cell Culture Approaches

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Human respiratory syncytial virus (hRSV) is one of the main causes of acute respiratory infections, affecting children under two years of age, the elderly, and immunocompromised individuals. Currently, treatment options are limited to ribavirin. In the search for a treatment, kaempferol has stood out for its biological activities against various pathogens. The aim of this study was to evaluate the antiviral potential of kaempferol 3-β-D-glucopyranoside and tetra-acetylated in A549 cell cultures against hRSV, as well as to conduct molecular docking studies on the viral F and M proteins. An MTT cytotoxicity assay was performed to determine the CC50 of the compounds, followed by viral inhibition and virucidal activity assays. Molecular docking studies were conducted to identify ligand-binding regions. The results indicated that kaempferol 3-β-D-glucopyranoside showed no cytotoxicity up to 100 µM and tetra-acetylated kaempferol demonstrated 60% cytotoxicity at 50 µM. In inhibitory assay, kaempferol 3-β-D-glucopyranoside presented in 3 µM 64 and 35% of inhibition at 24 and 48h, respectively, and tetra-acetylated 70% showed inhibition at 50 µM to 24 and 48 h. In molecular docking analyses, tetra-acetylated kaempferol exhibited stable conformations at the interfaces between the M and F protein monomers. In contrast, kaempferol 3-β-D-glucopyranoside showed better stabilization in the region near the interface of the F protein trimer. In summary, the in silico and in vitro results demonstrated that kaempferol derivatives have significant antiviral effects against hRSV, reinforcing their potential as a therapeutic strategy.

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PT.315

IN VITRO EVALUATION OF THE LARVICIDAL POTENTIAL OF C*YMBOPOGON DENSIFLORUS* ESSENTIAL OIL ON C6/36 CELLS

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The spread of arboviral diseases, such as dengue and zika, has been favored by disordered urbanization and climate change, increasing the proliferation of mosquitoes of the genus *Aedes,* vectors of these diseases. Synthetic insecticides are widely used in the prevention of these diseases; however, they may trigger environmental pollution and vector resistance, highlighting the need for natural alternatives, such as *Cymbopogon densiflorus* essential oil (CDEO), described in the literature as promising, although still poorly explored. For this reason, the present study aimed to evaluate the chemical composition of CDEO and test its larvicidal action on C6/36 cells, derived from *Aedes albopictus*. The chemical composition of CDEO was analyzed by gas chromatography coupled to mass spectrometry (GC-MS). C6/36 cells were treated with different concentrations of CDEO (10 mg/mL; 1 mg/mL; 100 µg/mL; 0.01 µg/mL), in addition to the control group, maintained in the absence of the essential oil. After incubation, the cells were treated with fresh medium containing MTT (5 mg/mL). Absorbance was determined in a spectrophotometer at 570 nm. The main compounds identified in CDEO were trans-p-mentha-2,8-dien-1-ol (9.93%), 8-dien-2-ol (16.61%) and cis-piperitol (7,44%). The results showed a reduction in the viability of cells exposed to CDEO at concentrations of 10 and 1 mg/mL (in both, $p<0.05$). These findings indicate a potential bioinsecticidal action of CDEO against cells derived from *A. albopictus,* pointing to it as an alternative to conventional synthetic insecticides, contributing to the development of new approaches for the vector control of arboviral diseases.
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PT.316

ANTIVIRAL ACTIVITY OF A OBLONGIFOLIN ISOLATED FROM RED PROPOLIS ON OROPOUCHE VIRUS INFECTION

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Oropouche virus (OROV) is an emerging *orthobunyavirus* capable of causing severe systemic febrile illness and neurological complications. Its potential to cause neurological complications, such as meningitis and encephalitis, represents a significant threat to public health. Despite its impact, no approved antiviral therapies are currently available. In this context, the phytochemical diversity of the Brazilian flora offers a valuable reservoir for drug discovery. In this study, we investigated the antiviral potential of oblongifolin, a compound isolated from Brazilian red propolis, against OROV infection. The maximum non-toxic concentration of the compound was determined in Vero E6 cells using the MTT cell viability assay. For the antiviral evaluation, cells were infected with OROV (MOI 0.001) and simultaneously treated with oblongifolin for 1 hour, followed by a 5-day incubation period with carboxymethylcellulose (CMC)-supplemented media in the presence of oblongifolin. After this period, the media was removed, and the cells were fixed with 4% formaldehyde and stained with crystal violet. Viral plaques were then counted, and the results were normalized with the DMSO 0.1% vehicle control. Our results demonstrated that oblongifolin significantly impaired OROV replication, reducing viral plaque formation by up to 99%. These findings suggest that oblongifolin from Brazilian red propolis is a promising candidate for the development of new antiviral agents against OROV. Additional studies are under development to elucidate the molecular mechanisms underlying its antiviral activity and to further characterize its mode of action.

Keywords: Antiviral, Oropouche virus, Brazilian red propolis, oblongifolin, Natural compounds

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A DUAL-REPORTER VIRUS–HOST CELL PLATFORM FOR HIGH-CONTENT ANTIVIRAL SCREENING OF MAYARO VIRUS

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The Amazon Basin is a hotspot for arbovirus emergence, and Mayaro virus (MAYV), an *Alphavirus* (*Togaviridae* family), is increasingly recognized as a candidate for urban emergence due to environmental change, human encroachment into forests, and competence in urban mosquito vectors. MAYV causes acute febrile illness, often associated with prolonged and debilitating arthralgia. Yet, no specific antiviral therapies or licensed vaccines are currently available. This gap highlights the need for preparedness strategies, and antiviral screening platforms are essential to rapidly identify candidate compounds that could support outbreak response and mitigation. This study aimed to develop a MAYV-reporter based application in a High Content Screening (HCS) assay. For that, synthetic DNA fragments covering the complete viral genome (strain AF237947) harboring eGFP in the subgenomic region, were amplified and assembled into a pcDNA3.1Zeo (+) vector using a Gibson Assembly. The recombinant virus was rescued by transfection of BHK-21 cells. The assay was established in BHK-21J-H2B-miRFP670 reporter cells. Optimization led to 79.6 ± 1.4% infection at 2 days post-infection with minimal early cytopathic effects <5%), resulting in excellent assay robustness ($Z' = 0.95$). Functional validation with reference antivirals Functional validation with reference antivirals showed expected sigmoidal dose–response curves, with EC_{50} values of 0.178 ± 0.03 µM for brequinar, 0.783 ± 0.13 µM for EIDD-1931, and 4.07 ± 0.35 µM for chloroquine. Together, these results establish a robust fluorescence-based MAYV infection platform suitable for high-throughput antiviral screening and future drug discovery efforts against this emerging arbovirus. Financial Support: CNPq (446550/2024-8).

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Development of a yeast-delivered DNA-based therapeutic vaccine strategy containing the wild-type E5 oncogene of HPV16 and evaluation of its antitumor effect

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Cervical cancer is associated with long-term infection with the human papillomavirus (HPV). Conventional treatments are not very effective and have many side effects. In this context, immunotherapies based on DNA vaccines containing the E5 oncogene can be delivered via an immunostimulatory delivery system, such as yeast, and emerge as a new treatment alternative by stimulating the body's own immune cells to fight cancer. Thus, the objective of this study was to develop a yeast-delivered DNA vaccine strategy containing the HPV16 E5 oncogene and to evaluate its antitumor effect. Recombinant yeast strains containing the HPV16 E5 DNA vaccine were obtained and incubated with PBMCs for an in vitro immunostimulation assay to assess the Th1/Th2/Th17 response profile. Subsequently, mice bearing HPV-positive tumors were immunized intramuscularly with two doses of the recombinant yeast. After euthanasia, the tumors underwent histopathological analysis, and the supernatant from splenocytes was evaluated for immunophenotyping and cytokine profile. The recombinant yeasts were shown to be capable of stimulating TNF, IL-6, and IL-10 in vitro, indicating a pro-inflammatory Th1 and regulatory profile. When the antitumor effect was evaluated in vivo, smaller tumors with edema and areas of fibrosis were observed in the immunized animals. In addition, an increase in TCD3+/TCD8+ was detected, which are markers of lymphocytes that induce cytotoxicity in tumor cells. Thus, it was demonstrated that the vaccine developed can help create a favorable environment for the treatment of HPV-positive cancers.

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HUMORAL IMMUNE RESPONSE AGAINST YELLOW FEVER VIRUS VACCINE AND WILD-TYPE STRAINS IN VACCINATED CHILDREN AND ADULTS

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The yellow fever vaccine (17DD) is a live-attenuated virus vaccine derived from the Asibi strain isolated in 1929 from a patient in Ghana. Following multiple viral passages, several genomic mutations were identified in the vaccine virus, including in the envelope protein (E), the primary target for neutralizing antibodies. Recent studies have demonstrated significant differences between the E protein of the vaccine strain and the wild-type virus (WT), which may affect the immune response against these viruses. In this context, the objective of this study was to evaluate the humoral immune response against the 17DD and WT viruses in vaccinated children and adults. For this purpose, we analyzed samples collected 30-45 days upon primary 17DD-YF vaccination from infants (9-23 months; n=8), children (2-4 years; n=23), and adults (n=14) from Paraíba (Brasil). The Plaque Reduction Neutralization Test (PRNT) was used to measure neutralizing antibody titers. The results showed that infants and children had lower levels of neutralizing antibodies against WT compared to 17DD. In contrast, this difference was not observed in adults. Furthermore, infants and children showed lower antibody levels against WT when compared with adults.These findings reveal age-related differences in humoral immune responses among vaccinees, providing relevant insights for immunization guidelines.

Financial support: FAPEMIG; FIOCRUZ; CNPq; CAPES; NIH.

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DEVELOPMENT AND OPTIMIZATION OF A MULTI-SPECIES INDIRECT ELISA USING RECOMBINANT ANTIGEN FOR RATHEV SERODIAGNOSIS IN SWINES AND CAPYBARAS IN BRAZIL

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Rat hepatitis E virus (ratHEV) is an emerging zoonotic threat associated with acute hepatitis in humans, with rodents acting as primary reservoirs and swines as potential hosts. However, tracking its circulation remains challenging due to the lack of commercial assays, highlighting the need for efficient diagnostic tools across these populations. From this perspective, this study aimed to standardize and evaluate a multi-species indirect ELISA using a recombinant antigen for ratHEV serological detection in swines and capybaras. For this purpose, the ratHEVCP antigen was heterologously expressed under optimized conditions and purified. The indirect ELISA was standardized (optimizing antigen, serum, and conjugate) for swines (n = 212) and capybaras (n = 25) samples, in accordance with ethics committee approvals. For comparison, samples were also evaluated using commercial kits and in-house ELISA with swHEVCP2 recombinant antigen for anti-HEV IgG detection. The discriminatory capacity of the assay was confirmed by clear distinction between control sera. No swine samples reacted with ratHEVCP antigen. Similarly, only 1 of 25 capybara samples showed low reactivity to ratHEVCP (OD = 0.185), contrasting with its high reactivity to swHEVCP2 antigen (OD = 3.492). These findings suggest limited recognition of the ratHEV recombinant antigen by sera reactive to HEV genotype 3 in the evaluated animal populations, supporting its potential use as a specific antigen for ratHEV serological investigations. As future perspectives, available human and wild rodent samples will be tested using the developed ELISA. This study was funded by the Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco.

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Antiviral activity assessment of Mycophenolic Acid Against Mayaro Virus

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The Mayaro virus (MAYV, family *Togaviridae,* genus *Alphavirus*), is an arbovirus transmitted by mosquitoes in tropical forests in South and Central America, where it establishes a sylvatic cycle. The increasing contact between humans and sylvatic vectors, as well as the transmission by mosquitoes of the genus *Aedes*, has the potential to spread MAYV, causing new outbreaks, similarly to what occurred to Dengue, Zika, and Chikungunya viruses. Despite this, MAYV remains neglected and without antiviral treatments available. Therefore, the objective of this study was to assess the antiviral activity of mycophenolic acid (MPA) against MAYV. The assays were performed with baby hamster kidney cells (BHK-21) and human hepatoma cells (Huh-7), and a complete viral construction with a *nanoluciferase* reporter. MTT, antiviral screening and dose-dependent assays were performed to evaluate cytotoxicity, viral inhibition, and to establish CC_{50}, IC_{50}, and SI values. The results were analyzed with the *software* GraphPad Prism 8. The maximum non-toxic dose of MPA in both cell lines was 5 µM, used to perform the antiviral screenings. MPA inhibited 96.3% (BHK-21) and 89.3% (Huh-7) viral particles. MPA also showed a dose-dependent effect in BHK-21 cells (CC_{50} = 102.8 µM, IC_{50} = 0.2 µM, and SI = 514). So far, MPA demonstrated high viral inhibition and low cytotoxicity in both cells, and a high selectivity index in BHK-21 cells, indicating that it may be a potent inhibitor against the Mayaro virus.

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Large-scale Expression, Optimized Purification, and Antiviral Screening of Arbovirus nsP2 Protease Using a Baculovirus System

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Arboviruses (Togaviridae), possessing enveloped positive-sense single-stranded RNA genomes, are emerging global pathogens causing severe morbidities without approved therapies. Viral replication strictly depends on the non-structural nsP2 protease, making it a promising pharmacological target. This study aimed to establish a platform for the heterologous expression, purification, and enzymatic screening with specific substrate of the nsP2 protease against potential natural antiviral molecules. The viral genes were cloned into the pFastBac1 vector to generate recombinant baculoviruses for insect cell infection. Initial standard bench purification attempts were unsuccessful. To overcome this, the process was optimized and scaled up using 500 ml of cell suspension culture. To ensure enzymatic stability, the entire protocol was conducted strictly at 4° and the extraction buffer was to an ideal pH 8.0 for enzymatic activity. Automated affinity chromatography using the ÄKTA pure system successfully isolated the His-tagged protease. Initial nsP2 proteolytic activity was confirmed directly in the culture supernatant utilizing two specific nsP2 substrates—a control and a fluorogenic substrate for cleavage via the FRET method—to systematically assess the inhibitory activity of a control molecule and a promising natural antiviral candidate . Following optimized chromatography, the active enzyme was successfully detected across eluted fractions via Western blot, with one specific fraction demonstrating a remarkably better degree of purity. Finally, the purified enzyme will be successfully employed on in-vitro functional assays to evaluate the inhibitory activity of a control molecule and a candidate natural antiviral molecule. This optimized production platform provides a vital tool for discovering new antiviral treatments against arboviruses.

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IN VITRO ANTIVIRAL ACTIVITY OF RUTHENIUM COMPLEXES AGAINST ZIKV

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Introduction: Zika fever is caused by Zika virus (ZIKV), an arbovirus transmitted by *Aedes* mosquitoes, associated with microcephaly, which has drawn global attention from health authorities. Currently, no antivirals are available against ZIKV, making essential the search for effective compounds. In this context, ruthenium (Ru) complexes have previously shown antiviral activity. Here, we evaluated the *in vitro* anti-ZIKV activity of ruthenium-based compounds. **Materials and methods:** Vero E6 cells were treated with Ru-BTA complex (BTA = 4,4,4-trifluoro-1-phenyl-1,3-butanedione), its free ligand (LBTA) and the Ru precursor (LP) for 72 h to determine non-cytotoxic concentrations by MTT assay. For antiviral screening, Vero E6 cells were infected with $ZIKV_{PE243}$ (MOI 0.01) in the presence of each compound at the established non-cytotoxic concentrations, and focus of infection (FFU) were quantified by immunofluorescence. The most active compounds were further evaluated in dose–response assays with concentrations ranging from 1.6 to 100 µM for 72 h for the determination of effective concentration of 50% (EC_{50}) and cytotoxicity concentration of 50% (CC_{50}) values, and calculation of selectivity index (SI). **Results:** LBTA, LP and BTA, at 50 µM, inhibited viral infection by 22%, 93% and 88%, respectively. In the dose–response assay, LP and BTA exhibited CC_{50} values > 100 µM, EC_{50} of 41.9 and 70.1, with SI values > 2.4 and > 1.4, respectively. **Conclusions:** Our findings highlight the potential of these molecules as inhibitors of ZIKV infection, suggesting that ruthenium-based compounds may serve as promising templates for developing antiviral agents against Zika fever.

Keywords: Zika virus; antiviral; ruthenium; metal complexes.
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PT.324

MELTING CURVE-BASED qPCR ASSAY FOR THE DETECTION OF HHV-1, HHV-2 AND VZV

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Infections caused by Human Alphaherpesvirus (HHV-1, HHV-2 and VZV) are clinically significant, especially in immunocompromised individuals, in which they can cause potentially fatal encephalitis. In addition, these viruses can cause severe complications in patients with neoplasms. Accurate identification of these agents is critical for the proper management of infections, since susceptibility to acyclovir varies among species, directly influencing therapeutic success. This study optimized a specific and cost-effective qPCR assay, based on SYBR Green and melting curve analysis, for the detection of HHV-1, HHV-2 and VZV, using *UL39* (ribonucleotide reductase), *gC* (glycoprotein C) and *UL30* (DNA polymerase) genes as targets, respectively. Specific primers were designed using reference nucleotide sequences retrieved from the GenBank database. The regions corresponding to each *amplicon* were synthesized and inserted into the pUC57 plasmid and used as positive controls in the reactions. The assays were performed in a Rotor-Gene Q Series thermocycler, in a final volume of 20 µL, using QuantiNova™ SYBR® Green PCR Kit, 1 µM of each primer and 10^6 copies of positive control. All viral targets were successfully amplified, generating dissociation curves with a single peak, indicating specific amplification. Melting curve analysis revealed distinct melting temperatures (Tm) of 66.0 °C, 89.5 °C, and 83.0 °C for HHV-1, HHV-2, and VZV, respectively, enabling reliable differentiation among the three viruses. These findings demonstrate the potential of the SYBR Green-based assay combined with melting curve analysis for application in both epidemiological surveillance and clinical diagnostics of these viruses.

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PT.325

MORPHOLOGICAL AND GENOMIC CHARACTERIZATION OF A BACTERIOPHAGE INFECTING *Yokenella regensburgei*

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Yokenella regensburgei is an environmental enterobacterium associated with diverse hosts, particularly hemipteran insects, yet the diversity of viruses infecting this bacterium remains unexplored. This study aimed to isolate and characterize, both morphologically and genomically, a bacteriophage infecting a *Y. regensburgei* strain isolated from the extracts of the brown stink bug *Euschistus heros*. The phage was isolated and purified using spot assays and the double-layer agar method, propagated in Luria-Bertani broth, concentrated by ultracentrifugation, and analyzed by transmission electron microscopy. The phage consistently produced opaque plaques, which prevented reliable assessment of additional parameters of phage-host interaction. Viral DNA was sequenced using the Illumina platform, followed by genome assembly, annotation, and comparative genomic and proteomic analyses. The virions exhibited an 82 ± 5 nm capsid and a tail-like structure approximately 89 ± 5 nm long, although tail measurements were considered approximate because of low image contrast. The genome encoded proteins associated with DNA replication and modification, nucleotide metabolism, virion structure and assembly, and genome packaging, together with a cluster of 17 tRNA genes. The highest nucleotide similarity was detected with Klebsiella phage vB_KaeM_KaOmega. Comparative genomic and proteomic analyses placed the phage near a group of large bacteriophages infecting members of the Enterobacterales, including Pectobacterium phage phiTE and Cronobacter phages CR9, CR8, CR3, and PBES02. To our knowledge, this is the first bacteriophage infecting *Y. regensburgei* to be isolated and characterized morphologically and genomically, expanding the known diversity of bacteriophages infecting Enterobacterales and providing new insights into phages associated with insect-associated bacteria.

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POPULATION GENETIC ANALYSIS OF CHIKUNGUNYA VIRUS SEQUENCES FROM CEREBROSPINAL FLUID AND SERUM DURING THE 2023 PARAGUAYAN OUTBREAK

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Paraguay experienced its largest chikungunya virus (CHIKV) outbreak in late 2022–2023, accompanied by an unprecedented increase in neurological complications. We investigated whether cerebrospinal fluid (CSF)-derived CHIKV genomes exhibited genetic signatures associated with neuroinvasive disease. Nineteen CHIKV-East/Central/South African genomes were generated from 2023 CSF samples using nanopore sequencing and analyzed alongside 182 publicly available genomes from the same outbreak (201 genomes: 42 CSF; 159 serum). No CSF-specific mutations were identified, as all variants detected in CSF were also present in serum genomes. Population genetic analyses restricted to genomes with >90% completeness revealed moderate genetic differentiation between CSF- and serum-derived viruses (AMOVA Φ_{ST}=0.163; Hudson's K_{ST}=0.081), with a significant correlation between genetic distance and viral compartment (Mantel r=0.308, p<0.001). However, the Slatkin–Maddison test was not significant (p=0.102), and maximum-likelihood phylogeny showed no CSF-specific clustering. Despite the absence of phylogenetic segregation, CSF-derived genomes exhibited higher within-compartment nucleotide diversity than serum-derived genomes (π=0.00238 vs. 0.00145). Together, these findings do not support CSF-specific adaptive mutations or a distinct neurotropic CHIKV lineage. Instead, they are more compatible with stochastic central nervous system invasion rather than with adaptive viral evolution. In parallel, neuroinvasive cases showed a U-shaped age distribution, with peaks among young children and older adults, suggesting a contribution of age-related host factors to neurological disease susceptibility. Further studies are needed to clarify the relative contributions of stochastic neuroinvasion, viral compartmentalization, and host susceptibility in CHIKV neurological disease.

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VIRALFETCH: A COMMAND-LINE TOOL FOR INTEGRATING VIRAL TAXONOMY, METADATA, AND SEQUENCE RETRIEVAL

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Virological research increasingly depends on cross-referencing authoritative but fragmented data sources: the International Committee on Taxonomy of Viruses (ICTV) defines the official taxonomy, while NCBI databases hold the associated sequences and metadata. Reconciling these resources manually is error-prone and time-consuming, particularly because NCBI taxonomy commonly lags behind ICTV revisions. We present Viralfetch, an open-source command-line tool that unifies four sources into a single, reproducible interface: (1) the ICTV Virus Metadata Resource (VMR), (2) NCBI E-utilities, (3) ICTV Report chapters, and (4) the phylogenetic trees and multiple sequence alignments (MSAs) bundled with the ICTV Report. Viralfetch embeds the VMR as a local taxonomic index, from which lineages, child taxa, and GenBank/RefSeq accessions are resolved entirely offline. On demand, it queries NCBI E-utilities for sequence metadata, nucleotide/protein FASTA, and full GenBank records, and can compare a taxon's ICTV lineage directly against its NCBI counterpart to find taxonomic divergences. Viralfetch also gives access to the family-level phylogenetic trees and alignments underlying the ICTV Report: a queried species or genus is automatically located and highlighted within its family's cladogram, and the corresponding multiple sequence alignment can be viewed with a per-column consensus row and a scrollable column window. By placing taxonomic lineage, sequence retrieval, and phylogenetic context behind a single reproducible command-line interface, Viralfetch reduces the manual effort of cross-referencing ICTV and NCBI resources and supports more reproducible workflows in viral genomics. All details and repository information are available in https://github.com/gabrielvpina/viralfetch/tree/main.

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PT.328

Immunoinformatics analysis of dengue virus epitopes associated with Qdenga immunity

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Dengue represents a major global public health issue and viral genetic variability can compromise vaccine efficacy by altering epitopes recognized by the immune system. In this context, this study evaluated the conservation of Qdenga-associated immune epitopes across DENV-1–4 variants circulating worldwide between 2024 and 2025. Therefore, we assessed a total of 473 viral polyprotein sequences obtained from the NCBI Virus database and 757 B- and T-cell epitopes retrieved from the Immune Epitope Database. The sequences underwent conservation analysis, population coverage assessment, and structural mapping of epitopes associated with E (envelope), and non-structural proteins NS3 and NS5, using bioinformatics tools. Results showed higher conservation of T-cell epitopes, particularly those related to non-structural proteins, whereas B-cell epitopes exhibited lower conservation among circulating variants. DENV-2 displayed the most conserved profile among the serotypes, a result attributed to its use as the non-structural protein backbone in the Qdenga vaccine. Population coverage analysis of conserved T-cell epitopes reinforced this pattern, showing estimated coverage of 93.72% for CD4+ T cells and 98.23% for CD8+ T cells for DENV-2, while DENV-4 showed the lowest values. Structural mapping revealed a concentration of conserved epitopes in domains II and III of the E protein and in the NS3 helicase and in the NS5 polymerase domains. These findings identify immunologically relevant regions and indicate potential impacts of recent viral polymorphisms on the breadth of the immune response induced by Qdenga, thereby contributing to the improvement of dengue vaccination strategies.

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PT.329

IN SILICO PREDICTION OF PANDEMIC H1N1 HEMAGGLUTININ VARIANTS IN SOUTH AMERICA USING RESTRICTED BOLTZMANN MACHINES AND STRUCTURAL MODELING

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The emergence of a new Influenza A pandemic remains a constant and catastrophic threat, with the potential to arise at any time and cause millions of fatalities worldwide. In this context, anticipating viral evolutionary trajectories has become a critical priority for global health security. This study presents an advanced computational pipeline integrating Restricted Boltzmann Machines (RBMs) and high-resolution structural modeling to predict high-risk viral variants. A robust dataset comprising 31,700 H1N1 hemagglutinin sequences was used, spanning 110 years of viral evolution with a strategic focus on South America. An RBM was trained to decode the complex co-evolutionary dependency patterns (epistasis) among 14 critical antigenic positions within the HA1 subunit. The model achieved strong discriminative performance, with an accuracy of 0.6727 and an AUC-ROC of 0.9674. Through an exhaustive search across a combinatorial space of 3,188,646 possible variants, the model identified 10 mutations not previously observed in nature with high dissemination potential, highlighting the motif "CSYRFINDVWIHQG" (Score: −0.000002) as the highest-ranked candidate. The physical feasibility of these predicted variants was subsequently validated through structural modeling using AlphaFold and ESMFold, confirming the preservation of protein folding and thermodynamic stability. The integration of unsupervised machine learning with structural biology establishes an effective framework for proactive genomic surveillance, enabling the early identification of influenza strains with pandemic potential in South America and supporting the strategic design of next-generation vaccines.

PT.330

LTR RETROTRANSPOSON REACTIVATION AND GENOMIC LOCALIZATION DURING HPV-DRIVEN CERVICAL CARCINOGENESIS

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Reactivation of transposable elements (TEs), whose dysregulated expression is linked to genomic instability and oncogene activation, is among the genetic and epigenetic mechanisms incompletely understood in cervical cancer, mainly driven by high-risk human papillomavirus (HPV) infection. This study characterized long terminal repeat (LTR) retrotransposon expression using RNA-seq data (GEO accession: GSE147009) from HPV16-associated normal cervical epithelium (NC), HSIL, and HPV-positive high-grade cell carcinoma (SCC) samples. Reads were aligned to hg38 using HISAT2, and gene quantification was performed using HTSeq-count. TE quantification was performed in R with the atena package, followed by differential expression analysis in DESeq2, applying two filters: count sum > 50; padj < 0.05, and genomic coordinates were intersected with host genes within 10 kb range using GENCODE annotations, concentrating on HPV/carcinoma integration hotspot: 3q28, 13q22.1, and 21p11.2. LTR relative abundance progressively increased from NC (71.05 ± 4.54%) to HSIL (76.36 ± 5.74%) to SCC (80.89 ± 6.60%). NC vs. SCC comparison revealed 27 neighboring genes, mostly in 3q28, near LPP (cell adhesion and motility) and GMNC (cell cycle and DNA replication regulation). No genes were found in NC vs. HSIL comparison. HSIL vs. SCC showed 15 genes, including MSTB near BAGE22 (21p11.2), a melanoma/testis antigen linked to cancer and MLT1B near KLF12 (13q22.1), regulation of transcription by RNA polymerase II and chromatin organization. Those findings indicate progressive LTR reactivation during cervical carcinogenesis, spatially associated with cancer-relevant genes, suggesting a regulatory role TE activity and malignant transformation.

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PT.331

GENOMIC IDENTIFICATION AND CHARACTERIZATION OF A NEW VIRUS ASSOCIATED WITH *PHYTOPHTHORA PALMIVORA*

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Phytophthora palmivora is an oomycete responsible for severe diseases affecting economically important crops, including *Theobroma cacao* (cocoa tree). Despite its agricultural relevance, little is known about the viruses associated with this organism, many of which remain taxonomically unclassified. This study aimed to identify and genomically characterize a virus associated with *P. palmivora* belonging to an unclassified viral lineage. A public RNA-seq library retrieved from the NCBI database was subjected to quality control using FastQC and Trimmomatic. Reads were mapped against the oomycete reference genome with Bowtie2, and unmapped reads were assembled into contigs using SPAdes. Viral sequences were identified through hidden Markov models (HMMs) and sequence similarity analyses. Among the detected viral sequences, one clustered within elements from the order Elliovirales but showed insufficient sequence identity to be assigned to any currently recognized family. In addition, no reports of viruses from this lineage associated with oomycetes were found. The best hits at aminoacid level exhibited high sequence identity and coverage, with e-values approaching zero. These sequences also shared a similar genomic organization, consisting of a single large open reading frame encoding only an RNA-dependent RNA polymerase (RdRp) domain, as well as comparable codon usage and dinucleotide frequency patterns. These findings provide evidence for a potentially novel viral taxonomic group associated with *P. palmivora*, expanding current knowledge of oomycete viromes and providing a foundation for future studies on viral evolution and their potential impact on cacao health.

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MUTATIONAL SCREENING AND STRUCTURAL CHARACTERIZATION OF H3N2 HEMAGGLUTININ THROUGHOUT ITS EVOLUTIONARY TRAJECTORY

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Alphainfluenzavirus influenzae, also known as Influenza A virus (IAV), relies on its surface glycoprotein hemagglutinin (HA) to mediate host cell entry. Since its emergence in humans in 1968, distinct H3N2 viruses have been described in other host species. Viral entry depends on the HA1 subunit's Receptor-Binding Domain (RBD), which recognizes terminal sialic acid residues (Neu5Ac) linked to host glycans (usually a galactose) via α-2,6 ("human" type) and α-2,3 ("avian" type) linkages. Because host-specific receptor preferences act as a barrier to cross-species transmission, with intermediate hosts like swine expressing both linkage types, characterizing RBD mutational trajectories is essential for understanding host adaptation. To explore mutational diversity over time and across different hosts, 28,334 HA of H3N2 sequences from 1968 to 2025 were analyzed. The mutational screening focused on the RBD region, comprising 130-loop, 150-loop, 190-helix, and 220-loop. Using a mutation frequency table and a threshold to identify key substitutions, representative sequences across different time periods and hosts (*Homo sapiens*, swine, avian, and canine) were selected for structural prediction in complex with α-2,6 and α-2,3 ligands using Boltz-2. The structural analyses in PyMOL revealed key RBD amino acid substitutions over time (such as the W222R substitution, which establishes additional polar contacts with the ligand), alongside highly conserved residues (*e.g.*, Y98), underscoring their critical role in H3's receptor engagement. Furthermore, host-specific structural variations modulated ligand-binding interactions, offering insights into the molecular mechanisms driving the H3N2 evolutionary trajectory since their emergence to humans.

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PT.333

Discovery of Small-Molecule P-Domain Inhibitor Candidates for Hepatitis E Virus via Virtual Screening

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Hepatitis E Virus (HEV) causes ~20 million infections annually, resulting in fatal fulminant hepatitis in pregnant women and chronic disease in immunocompromised individuals. The lack of approved antiviral therapies and severe side effects of off-label ribavirin treatment create an urgent need for novel therapeutics. Capsid-targeting antivirals have shown recent success, motivating evaluation of the HEV ORF2 P-domain as an underexplored target. To characterize this domain, we employed computational approaches to identify potential ligand-binding sites and screen a small-molecule commercial library, aiming to identify promising binding candidates. The HEV ORF2 P-domain dimer was reconstructed using SWISS-MODEL and refined through energy minimization. PrankWeb analysis identified potential ligand-binding pockets, and the highest-scoring pocket was selected for virtual screening. Screening 460,160 compounds from the Enamine library using AutoDock Vina yielded five lead candidates with binding affinities ranging from −9.2 to −9.6 kcal/mol. ProLIF analysis revealed specific interactions through hydrogen bonding, cation–π, and π–π stacking. Four of five leads anchored to PRO491, a highly conserved and functional residue, via Van der Waals contacts. Maximum Common Substructure analysis identified a conserved pharmacophore comprising an aromatic anchor, polar nitrogenous hinge, and aliphatic tail. All compounds demonstrated zero Lipinski violations, high gastrointestinal absorption, and safe toxicity profiles in ADMET assessment. These findings highlight the potential of the HEV ORF2 P-domain as an attractive and previously underexplored target, providing a foundation for the rational design of novel therapeutics. Molecular dynamics simulations and experimental validation are planned to assess lead compound stability and antiviral potential.

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PT.334

Phylogenetic Analysis and Amino Acid Mutations in the Glycoprotein Gene of Rabies Virus from *Desmodus rotundus* in Latin America

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Rabies virus (RABV) is the responsible agent of rabies, a severe zoonotic disease affecting mammals, including humans, and characterized by progressive acute encephalitis with a high case-fatality rate. RABV belongs to the genus *Lyssavirus* within the family *Rhabdoviridae*. Globally, the virus circulates in both urban and wildlife environments, particularly where interactions between domestic and wild animals facilitate transmission. Its genome is approximately 12 kb long and contains five genes encoding viral proteins. Phylogenetically, RABV is divided into two major clades: bat-related and dog-related. This study investigated amino acid mutations in the glycoprotein (G) gene of RABV isolates from the hematophagous bat *Desmodus rotundus* (DR) circulating in Latin America. A total of 2,434 G gene sequences were retrieved from the NCBI Virus database, assembled, and aligned using MAFFT v7.490. Metadata, including host species, sampling date, geographic location, and amino acid mutations, were compiled. Phylogenetic relationships were inferred using the maximum likelihood method implemented in IQ-TREE v2.1.3. Codon-level positive selection in the DR clade was assessed against the bat-related clade using HyPhy. Contrast-MEME identified six codons (56, 126, 186, 318, 321, and 500) with nominal differences in selective pressures ($p < 0.05$), but none remained significant after False Discovery Rate correction (FDR = 0.20). Overall dN/dS ratios were below 1, indicating strong purifying selection. However, we identified a DR-specific site within a distinct DR clade that has previously been associated with reduced RABV pathogenicity, highlighting an important target for further evolutionary and functional analyses. Financial Support CNPq.

PT.335

CENTRAL VRA CORE MOTIF PROVIDES THE MOLECULAR BASIS FOR FELV SUBGROUP CLASSIFICATION

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Feline leukemia virus (FeLV) comprises different subtypes historically defined by viral interference assays based on the cellular receptor utilized. Although this functional classification is widely adopted, genomic and phylogenetic analyses have not fully explained the molecular determinants responsible for subtype specificity. To address this gap, this study aimed to map and characterize the specific molecular signatures within the envelope glycoprotein that dictate receptor binding across different FeLV subtypes. FeLV envelope sequences were analyzed using machine learning to predict key functional positions, and structural models of different FeLV subtypes and their respective cellular receptors were subjected to molecular docking analysis using ClusPro. Based on these approaches, the Variable Region A (VRA) within the receptor-binding domain (RBD) was selected as the primary focus of analysis due to its structural relevance and high variability. The predicted receptor-interaction residues were mapped in a multiple sequence alignment of FeLV sequences (n=396) available. In this region comprising 38 amino acids, comparative analyses revealed that these residues concentrate in a short central hypervariable core. In contrast to their flanking residues, which are widely conserved, subtype-specific amino acid substitutions, together with insertions and deletions in this central core, consistently distinguished the FeLV-A, FeLV-B, FeLV-C, FeLV-E, and FeLV-T subtypes. This robust structural pattern provides a coherent molecular explanation for the classical subtypes historically defined by interference assays. Our results establish this central VRA core as a robust molecular signature for rapid, reproducible, and biologically founded classification of FeLV subtypes. Financial Support: CAPES.

PT.336

PHYLOGEOGRAPHIC RECONSTRUCTION REVEALS TWENTIETH-CENTURY TRANS-ATLANTIC DISPERSAL OF HEPATITIS DELTA VIRUS GENOTYPE 8 BETWEEN AFRICA AND BRAZIL

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Hepatitis delta virus genotype 8 (HDV-8) causes severe chronic hepatitis and has been reported predominantly in Africa, with a presence in Brazil. To investigate its African–Atlantic dispersal history, we analyzed 44 HDV-8 sequences sampled between 1999 and 2019 from ten countries, including eight from Brazil. Bayesian phylogenetic analyses were performed in BEASTX using discrete phylogeographic reconstruction at country and regional levels under an asymmetric diffusion model. Migration pathways, lineage residence times, and transition histories were evaluated using adjusted Bayes factors, Markov rewards, stochastic-history jumps, and Skygrid analyses, with inferred transitions assigned to historical periods. Africa-to-Brazil transitions showed very low posterior probability during the trans-Atlantic slave-trade era (<0.3% before 1850 and <1.5% before 1888). In contrast, 46–50% of the transition probability was assigned to periods after 1960, with median first-entry dates into Brazil between 1963 and 1966. Gabon showed the highest inferred lineage residence time and was frequently reconstructed as a source of early dispersal, although this may reflect broader West-Central African diversity and sampling structure rather than a definitive origin. The strongest supported routes were West Africa to Central Africa at the regional level and Brazil to Namibia at the country level, the latter occurring approximately 46 years after the first inferred entry into Brazil. Overall, the results support a deeply African HDV-8 lineage shaped by long-term West-Central African persistence, post-colonial trans-Atlantic dispersal to Brazil, and subsequent dispersal between Brazil and southern Africa. LH has a grant from the São Paulo State Research Support Foundation (FAPESP N°2025/27287-5).

PT.337

SPATIOTEMPORAL DYNAMICS, TRANSMISSION POTENTIAL, AND DEMOGRAPHIC SHIFTS OF DENGUE IN ESPÍRITO SANTO, BRAZIL (2023–2025)

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Espírito Santo has stood out in recent years due to the high incidence of arboviral diseases, including dengue. This study aimed to characterize the temporal, spatial, and demographic dynamics of dengue in Espírito Santo between 2023 and 2025 using RT-PCR-confirmed cases. Epidemiological, climatic, and transmission indicators, including the effective reproduction number (Rt) and the transmission potential index (Index P), were analyzed using R software. A total of 13,013 confirmed cases were recorded between January 2023 and August 2025. The highest incidence occurred in 2024, with epidemic peaks between epidemiological weeks 10 and 12, while peaks in 2023 and 2025 occurred in weeks 19 and 8, respectively. Transmission consistently concentrated in the first trimester of each year. Spatial analysis revealed marked heterogeneity, with the highest incidence rates observed in Central ES in 2024. Rt values were highest in 2023, whereas Index P peaked in 2024, both indicating favorable conditions for transmission during early weeks. Climatic patterns showed that higher temperature and precipitation preceded incidence peaks. Serotype analysis indicated predominance of DENV-1 with co-circulation of DENV-2 and occasional DENV-3 detection. No significant differences were observed between sexes over time ($p = 0.3391$), although a higher number of cases was consistently observed among females. In contrast, a significant shift in age distribution was observed ($p < 0.001$), with increasing incidence among adults and elderly populations in 2024 and 2025. These findings highlight strong seasonality, spatial heterogeneity, and evolving population susceptibility, emphasizing the need for integrated surveillance strategies.

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PT.338

Highly pathogenic avian influenza virus subtype 5 in Brazilian cetaceans: implications for surveillance in threatened species

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Influenza A viruses (IAVs) infect a wide range of hosts, including birds, humans, and marine mammals. In 2023, a mass mortality event involving South American sea lions and fur seals (*Otaria flavescens* and *Arctocephalus australis*), caused by highly pathogenic avian influenza virus (HPAIV H5N1), raised concerns about emerging transmission routes in Brazil. Although IAV has been detected in some marine mammals, infections in cetaceans remain rarely documented. In this context, the present study aimed to identify cetacean species along the Brazilian coast that are susceptible to IAV infection. Tissue samples were collected using sterile swabs from multiple anatomical sites of stranded cetacean carcasses along 1,482 km of coastline, spanning the states of Rio Grande do Sul, Rio de Janeiro, and Ceará. IAV detection was performed by reverse transcription quantitative polymerase chain reaction (RT qPCR) in samples from 45 individuals belonging to 11 small cetacean species, using assays targeting the matrix (M) gene and the hemagglutinin gene of the H5 subtype. Among the analyzed individuals, 18 from six species tested positive for IAV. Notably, this study reports the first detection of H5Nx in the franciscana dolphin (*Pontoporia blainvillei*) and the Clymene dolphin (*Stenella clymene*). Additionally, unidentified subtypes were detected in Lahille's bottlenose dolphin (*Tursiops gephyreus*), rough-toothed dolphin (*Steno bredanensis*), dwarf sperm whale (*Kogia sima*), and Fraser's dolphin (*Lagenodelphis hosei*). These findings demonstrate the susceptibility of cetaceans to IAV and highlight the critical need for continuous health surveillance to detect emerging threats to vulnerable species along the Brazilian coast.

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PT.339

EPIDEMIOLOGICAL DYNAMICS AND GEOGRAPHICAL SPREAD OF DENV-3 IN ALAGOAS IN 2025

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The co-circulation and alternating predominance of dengue virus serotypes can alter the epidemiological landscape and increase the risk of epidemics, which requires enhanced viral surveillance. This study characterized the temporal, demographic, and spatial dynamics of circulating DENV serotypes in Alagoas in 2025. Human serum samples from suspected cases of arboviral infections referred to LACEN-AL underwent viral RNA extraction and were analyzed by multiplex RT-qPCR for the simultaneous detection and serotyping of DENV-1, DENV-2, DENV-3, and DENV-4. Statistical and spatial analyses were performed in R, using Pearson's chi-square test or Fisher's exact test, as applicable, with a significance level of $p < 0.05$. A total of 260 cases of dengue were confirmed: 49.23% (n = 128) due to DENV-2, 28.85% (n = 75) due to DENV-3, and 21.92% (n = 57) due to DENV-1; DENV-4 was not detected. DENV-2 predominated in the first quarter, while DENV-1 increased between April and June. Beginning in August, DENV-3 showed sustained growth and became the predominant serotype in November, accounting for approximately 75% of monthly cases. No significant associations were observed between serotype and sex, age group, or race/ethnicity. The highest incidence rates were concentrated in the north and in the Zona da Mata region, with Ibateguara standing out at 350 cases per 100,000 inhabitants. DENV-3 was primarily established in the northern and central regions of the state. The findings demonstrate the rapid spread of DENV-3 and underscore the need for continuous laboratory surveillance to guide prevention and control measures.

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PT.340

EPIDEMIOLOGICAL AND MOLECULAR ASPECTS OF CHELONID ALPHAHERPESVIRUS 5 DETECTION IN GREEN SEA TURTLES

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Herpesviruses are double-stranded DNA viruses that infect a wide range of vertebrate hosts, including reptiles. Chelonid alphaherpesvirus 5 (ChHV5) is considered the primary etiological agent of fibropapillomatosis, a benign neoplastic disease that mainly affects green turtles (*Chelonia mydas*). This study investigated the presence of ChHV5 in renal, pulmonary, and ocular tissues from green turtles found dead along the coast of Paraná, Brazil, between 2019 and 2025. A total of 47 individuals were analyzed by quantitative PCR (qPCR). ChHV5 DNA was detected in 30 renal samples (63.8%), 21 pulmonary samples (44.7%), and 21 ocular samples (44.7%). A binomial generalized linear model revealed no significant association between viral detection and the biological characteristics of the turtles. In contrast, ChHV5 infection was strongly associated with the presence of fibropapillomatosis, and viral detection in multiple tissues was significantly associated with greater disease severity, suggesting that broader tissue distribution accompanies disease progression. These findings demonstrate the circulation of ChHV5 in green turtles from the Paraná coast and reinforce the association between viral dissemination and fibropapillomatosis. Additional molecular and virological analyses are needed to further characterize infection dynamics and viral replication in different tissues.

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SEROLOGICAL PROFILE OF SAINT LOUIS ENCEPHALITIS VIRUS (SLEV) EXPOSURE IN *TAPIRUS TERRESTRIS* POPULATIONS FROM THREE BRAZILIAN BIOMES

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The Saint Louis Encephalitis virus (SLEV) presents infectious cycles involving birds and mosquitoes, with mammals being incidental hosts. In this context, this study aims to analyze the serological profile of tapirs (*Tapirus terrestris*) from the Amazon, Cerrado, and Pantanal biomes by detecting neutralizing antibodies against SLEV using the focus reduction neutralization test (FRNT). Between 2017 and 2025, 88 serum samples were collected. Initially, a screening was performed for all samples single dilution of 1:20 to confirm seropositivity, where 48 samples were $FRNT_{50}$ positive (50% of SLEV neutralization at 1:20 dilution). To increase specificity, $FRNT_{90}$ was calculated and 22 samples were positive (90% of SLEV neutralization at 1:20 dilution). To measure the neutralization titer for each $FRNT_{50}$ positive sample at 1:20, a serial two fold dilution was performed for FRNT, until 1:320. Until now, 21 samples analyzed, and 9 samples neutralized SLEV until 1:40, 8 neutralized until 1:80, 1 presented neutralization capacity until 1:160 and 3 were still capable of inhibiting 50% of SLEV infection at the lowest tested concentration, 1:320. The tapirs whose samples presented this high neutralization profile are from Pantanal biomes, a known route for migratory birds from the north that could be contributing to SLEV introduction in South America. The same process will be conducted on the other positive samples, and the findings will bring some important insights on the real circulation of SLEV in sylvatic cycles in Brazil, a neglected arbovirus with enormous potential to emerge and impact public health.

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PT.342

VACCINATION STATUS, RISK FACTORS, AND DIAGNOSTIC CHARACTERISTICS OF VIRAL HEPATITIS IN THE BRAZILIAN AMAZON: A POPULATION-BASED STUDY

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Background: Viral hepatitis remains a major public health concern in Brazil despite the availability of effective preventive measures, including vaccination against hepatitis A and B. Understanding vaccination status, risk factors, and diagnostic characteristics is essential for improving disease control strategies. Methods: A population-based cross-sectional study was conducted using surveillance data on viral hepatitis cases reported in Pará, Brazil, from 2022 to 2025. Sociodemographic characteristics, vaccination status, exposure factors, diagnostic methods, and HIV coinfection were analyzed using descriptive statistics. Results: A total of 4,142 viral hepatitis cases were included. Hepatitis B and C accounted for 94.6% of notifications, and 69.6% of cases were classified as chronic or asymptomatic carriers. Vaccination coverage was low, with only 6.6% of individuals reporting complete vaccination against hepatitis A and 18.5% against hepatitis B, while a substantial proportion of records contained missing vaccination information. Most cases were laboratory-confirmed (97.7%), demonstrating the robustness of the surveillance system; however, incomplete laboratory records remained frequent for complementary serological markers. HIV coinfection was identified in 6.6% of cases, highlighting the clinical relevance of integrated screening strategies. High proportions of missing information regarding exposure history, including sexual contact and household transmission, limited a more comprehensive assessment of transmission pathways. Conclusions: Viral hepatitis in the Brazilian Amazon is characterized by a predominance of chronic hepatitis B and C, insufficient vaccination coverage, and important gaps in epidemiological records. Strengthening immunization programs, improving surveillance data quality, and integrating testing for sexually transmitted infections are essential to reduce disease burden and support effective public health interventions.

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PT.343

MOLECULAR DETECTION OF ARBOVIRUSES IN PATIENTS WITH ACUTE FEBRILE SYNDROME ATTENDED AT A MIXED PUBLIC-PRIVATE HOSPITAL IN SOUTHEASTERN BRAZIL

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Arboviruses remain a major public health concern in Brazil, where dengue, Zika, and chikungunya have overlapping clinical manifestations and require laboratory confirmation. This study aimed to describe the molecular detection and clinical profile of arboviruses among patients with acute febrile syndrome attended at a hospital in southeastern Brazil. A descriptive observational study was conducted using clinical and laboratory data from patients sampled between January and July 2026. Biological samples were collected from 131 patients and tested by real-time PCR for dengue virus serotypes 1–4, Zika virus, and chikungunya virus. The median age was 36.9 years, ranging from <1 to 96 years, and 83/131 patients (63.4%) were female. Fever was reported in 123/131 patients (93.9%), followed by myalgia (80.2%), headache (77.1%), nausea (60.3%), abdominal pain (36.6%), vomiting (34.4%), cough (32.1%), arthralgia (22.9%), and diarrhea (22.9%). The median interval between symptom onset and sample collection was 2 days. Among 121 patients with available arbovirus results, 3 (2.5%) were positive: 2 (1.7%) for dengue virus serotype 2 and 1 (0.8%) for dengue virus serotype 4. No dengue virus serotype 1 or 3, Zika virus, or chikungunya virus was detected. Despite early sample collection and a clinical presentation compatible with arboviral infection, molecular positivity was low. These findings demonstrate the concurrent circulation of dengue virus serotypes 2 and 4 and highlight the need for broader differential diagnosis and integrated laboratory surveillance of acute febrile syndromes.

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PT.344

GENOMIC EPIDEMIOLOGY AND REGIONAL DISPERSAL OF CHIKUNGUNYA VIRUS IN TOCANTINS, BRAZIL, 2017–2024

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Chikungunya virus (CHIKV) has caused recurrent outbreaks in Brazil, but its fine-scale circulation within individual states remains poorly characterized. We investigated the persistence and regional dispersal of CHIKV across the Health Regions of Tocantins state using 21 complete reference genomes sampled 2017–2024, and 49 genomes generated in this study. Sequences were assigned to six Health Regions and analysed using Bayesian time-scaled phylogenetics combined with an asymmetric discrete phylogeographic model. Epidemiological incidence data were used to contextualize the inferred transmission dynamics. Markov jumps, Markov rewards, and first-entry times were estimated to characterize lineage movement and persistence, while sampling-adjusted Bayes Factors (BFadj) were applied to account for spatial sampling imbalance. Capim Dourado had the highest posterior probability as the ancestral location (PP = 0.934) and accounted for 66.4% of total residence time, with a positive net export of 18.68 transitions; all other regions were predominantly net recipients. This source role aligns with recurrent high-incidence hotspots in Capim Dourado, supporting its function as a persistent transmission hub. Three dispersal routes remained supported after adjustment: Capim Dourado to Cantão (BFadj = 3.30), Capim Dourado to Ilha do Bananal (BFadj = 4.41), and Médio Norte Araguaia to Bico do Papagaio (BFadj = 4.69), with the first two movements dated to 2016–2017. These results identify Capim Dourado, encompassing the capital Palmas, as a persistent source of CHIKV dissemination, supporting the hypothesis that endemic transmission sustains viral spread in Tocantins.

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PT.345

ASSESSMENT OF A THREE-DIMENSIONAL CELL CULTURE MODEL FOR ISOLATION OF ENTERIC VIRUSES FROM FLOODWATER SAMPLES

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Extreme weather events, such as the historic 2024 flood in Porto Alegre, Brazil, extensively contaminate surface waters with untreated sewage, expanding enteric virus dissemination. While molecular environmental surveillance tracks viral circulation, PCR cannot distinguish infectious from inactive particles, and low viral loads can lead to false negatives. To assess actual infectious risks, integrating cell culture with molecular detection is essential. Thus, this study tested the efficiency of a 3D spheroid cell culture model against traditional 2D monolayers to optimize waterborne virus detection and isolation from 7 floodwater samples. Vero cells in both configurations were inoculated with positive environmental concentrates. Lysates and serial blind passages (P0–P4) were analyzed via RT-qPCR for Hepatitis A virus (HAV), Hepatitis E virus (HEV), and Norovirus genogroups I/II (NoV-GI/GII). After cell culture in both models, genomic load levels fluctuated near the detection limit with a low number of detectable replicates; therefore, productive replication could not be definitively confirmed. However, 3D spheroids generally demonstrated a superior performance than 2D culture and a trend of enhanced viral recovery for HAV and NoV-GI: HAV showed a twofold increase in viral load in spheroids for sample A, while, for sample G, both viruses were detected exclusively in spheroids. For NoV-GI, spheroids enabled persistent detection, showing a distinct Ct decrease from P1 to P3 in sample G. HEV and NoV-GII remained undetected. High dilution and the fastidious nature of these viruses impaired statistical significance. Nevertheless, spheroids represent a complementary tool to assess infection risk and optimize One Health surveillance.

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OUTBREAK OF CANINE DISTEMPER DISEASE IN A DOG SHELTER

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Canine distemper is caused by the *Morbillivirus canis*, especially important in shelter environments due to the density of dogs and increased risk of transmission. Accordingly, this study aimed to investigate the presence of canine distemper virus in dogs from a shelter located in Campo Grande, Mato Grosso do Sul, Brazil. Urine and ocular and nasal swabs samples were collected from dogs and analyzed by one-step RT-qPCR. Results were interpreted based on cycle threshold (Cq) values and melting temperature (Tm) as criteria for viral detection. A total of 19 dogs were evaluated in two different time points with a 20-day interval between collections. In the first evaluation, 13 dogs were sampled, and 12 animals (92.31%) were positive in both urine and secretion swabs. In the second evaluation, 14 dogs were assessed, including eight previously monitored animals and six new contact animals exposed to positive dogs. Among all evaluated animals, six (42.86%) were positive in urine samples and seven (50%) in secretion swabs. Viral detection decreased over time, with one dog remaining positive in urine and four in secretion swabs. Considering new contact animals, five were positive in urine and three in swabs, indicating viral circulation among exposed dogs. The detection of canine distemper virus in shelter dogs highlights the importance of preventive strategies, including vaccination, isolation of infected animals, and monitoring of asymptomatic animals to reduce transmission risks and improve adoption safety.

Keywords: RT-qPCR, canine, *Morbillivirus canis*.

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LYMPHOCYTIC CHORIOMENINGITIS VIRUS (LCMV) IN BRAZIL: CURRENT EVIDENCE AND EMERGING PERSPECTIVES FOR A NEGLECTED ZOONOSIS

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Lymphocytic choriomeningitis virus (LCMV) is a globally distributed rodent-borne pathogen associated with neuroinvasive disease, congenital infection, and severe disease in immunocompromised individuals. Despite its recognized public health relevance, LCMV is rarely included in surveillance programs, particularly in South America. In Brazil, knowledge of LCMV circulation has remained limited and fragmented, with previous evidence restricted to seroprevalence studies among mastozoologists and, more recently, the detection in *Mus musculus*. To address this gap, we integrated recent human, molecular, genomic, and ecological investigations conducted by our group to provide a comprehensive overview of LCMV circulation in Brazil. In Salvador, ELISA screening revealed anti-LCMV seroprevalence of 1.9% (13/651) among residents and 4.2% in *Rattus norvegicus*, together with the first molecular detection and characterization of LCMV in this species. In Rio de Janeiro State, serum and tissue samples from *M. musculus*, *R. norvegicus*, and *R. rattus* showed an overall seroprevalence, in ELISA, of 20% (49/236), and complete viral genome sequences were obtained from *M. musculus*, representing the first genomic characterization of LCMV in Brazil. Surveillance was further expanded to wild rodents from additional municipalities in Rio de Janeiro, where LCMV was detected for the first time in a native wild rodent species, indicating that virus circulation extends beyond synanthropic hosts. Together, these findings expand the known geographic distribution, host range, and genomic characterization of LCMV in Brazil, providing new insights into virus ecology and host dynamics. These data establish an important foundation for future surveillance and risk assessment, particularly for pregnant women and immunocompromised individuals.

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MOLECULAR DETECTION REVEALS DISTINCT ADENOVIRUS LINEAGES IN BRAZILIAN WILD FELIDS

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Wild felids are key components of natural ecosystems and may serve as sentinels for pathogen circulation at the wildlife–domestic animal interface. However, information on environmentally transmitted viruses in South American wild felids remains scarce. This study investigated the presence of adenoviruses (AdV) in 72 free-ranging and captive wild felids representing seven species in Brazil. Samples were screened by PCR, and AdV-positive amplicons were sequenced and analyzed. Adenovirus DNA was detected in 5/72 (6.94%) animals, including *Leopardus guttulus* (n = 2), *L. munoai* (n = 2), and *L. wiedii* (n = 1). Phylogenetic analysis identified two distinct adenoviral groups. Three sequences clustered with canine adenovirus type 2 (CAdV-2), sharing high nucleotide identity with previously described strains, while one sequence grouped with *Felid mastadenovirus* 3 (98.5% of nucleotide identity). A fifth sequence, detected in *L. wiedii*, exhibited 76.9% nucleotide identity with known *Felid mastadenovirus* 3 sequences and formed a distinct phylogenetic branch, suggesting the circulation of a divergent mastadenovirus in Brazilian wild felids. These findings expand the known diversity and host range of AdVs infecting South American wild felids and reinforce the importance of wildlife viral surveillance for understanding pathogen circulation at the wildlife–domestic animal interface. The occurrence of CAdV-2 in two individuals of the critically endangered *Leopardus munoai*, according to the Chico Mendes Institute for Biodiversity Conservation (ICMBio), highlights the need to integrate wildlife health surveillance into conservation efforts for threatened species.

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Investigation of respiratory viruses in free-roaming cats from the Municipal Park of Belo Horizonte, Minas Gerais, Brazil

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Dogs and cats are the most abundant domestic animals in urban areas. Cats, particularly in forest fragments, exert pressure on wildlife, generating conflicts with biodiversity and raising concerns about ecosystem health. Free-roaming cat colonies in urban parks may increase exposure to diverse microorganisms, favoring transmission, coinfections, and the potential to act as intermediate or amplifying hosts. Given the multifactorial nature of emerging viral diseases, surveillance requires a multidisciplinary One Health approach. Among these diseases, respiratory infections are notable due to their association with public health emergencies, yet they remain poorly investigated in felines. This study aims to investigate the circulation of respiratory viruses in domestic cats from Américo Renné Giannetti Municipal Park, in Belo Horizonte, Brazil. A total of 134 individuals had oral–rectal swab and serum samples collected between 2024-2025. Genetic material was extracted using the Chemagic DNA/RNA kit and subjected to qPCR analysis for the main agents associated with respiratory infections in humans: Adenovirus, Influenza A/B, Human metapneumovirus, SARS-CoV-2, Rhinovirus, and Respiratory syncytial virus. Molecular assays were performed using multiplex reactions with a commercial kit from Bio-Manguinhos/FIOCRUZ. For serology, 146 serum samples are being pre-treated and tested using the hemagglutination inhibition assay for anti-influenza virus H1N1 antibodies. In the molecular approach, no samples tested positive, whereas in the serological analysis, titers ranged from 1:80 to 1:320. The serological findings indicate exposure of felines to influenza viruses circulating in the human population and provide insights into the dynamics of interaction in urban parks.

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MOLECULAR SURVEILLANCE OF ROTAVIRUS A IN HOSPITALIZED CHILDREN WITH ACUTE GASTROENTERITIS IN BELÉM, PARÁ DURING THE POST-PANDEMIC PERIOD (2024-2026).

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Introduction: Acute gastroenteritis (AGE) remains a leading cause of pediatric mortality and morbidity, with Rotavirus A (RVA) as a major cause of severe disease in children under five years of age. Although vaccination has reduced the incidence of severe cases, molecular surveillance remains essential for monitoring circulating RVA genotypes and detecting potential changes in the epidemiological profile.
Methodology: A cross-sectional study was conducted at a pediatric hospital in Belém, Pará, Brazil to monitoring children aged 3 months to 10 years, hospitalized with AGE. Fecal samples were collected within 48 hours of hospital admission. Laboratory procedures included viral nucleic acid extraction using a commercial kit, RT-qPCR screening, RT-PCR genotyping, and nucleotide sequencing followed by phylogenetic analysis. **Results:** Between November 2024 and June 2026, a total of 193 fecal samples were collected and RVA positivity was 9.3% (18/193). Children aged 2 – 5 years (50.0%; 9/18) were the most affected by RVA infection and no significant differences were observed between gender. RVA was detected frequently during November to December/2024 and January to March/2025. With respect VP7 and VP4 molecular characterization, 14 samples have been sequenced and classified as G3P[8] equine like (72.2%) and G3P[9] (5.5%) genotypes. **Conclusion:** These findings reinforce the importance of continuous molecular surveillance to monitor genomic variations, guide potential updates to vaccination strategies, and strengthen regional epidemiological surveillance in the post-pandemic period.
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Epidemiological surveillance of Norovirus in the North and Northeast regions of Brazil

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Norovirus (NoV) is the leading cause of acute gastroenteritis across all age groups, frequently associated with outbreaks. Infection mainly causes diarrhea, commonly accompanied by vomiting and fever. This study conducted epidemiological surveillance of Norovirus in all states of the North Region and two states of the Northeast Region (Piauí and Maranhão) of Brazil. A total of 894 samples from the Rotavirus Surveillance Network in 2025 were analyzed, under ethical opinion 8.026.801. Fecal suspensions were prepared, followed by nucleic acid extraction using a commercial kit. For agent detection, the RT-qPCR technique was applied, targeting the junction region between ORFs 1 and 2 of the viral genome. Of the samples analyzed, 15.9% (142/894) showed positivity for NoV. Among the nine states included in the study, Amapá presented the highest percentage of positive cases with 26.2% (22/84), followed by Tocantins (23.5%; 43/183), Pará (18.8%; 28/149), Roraima (18.2%; 6/33), Amazonas (11.1%; 40/361), Rondônia (5.6%; 1/18), Piauí (3.3%; 2/61), Acre (0%; 0/4), and Maranhão (0%; 0/1). NoV infection was more frequent in females (17.9%; 68/380) compared to males (14.5%; 74/509). The most affected age group was children between 7 and 24 months old, comprising 21.8%. Regarding the analyzed symptoms, vomiting showed the highest frequency of positivity (19.2%; 102/532), followed by diarrhea (16.2%; 128/791) and fever (14.0%; 67/479). This study highlighted the importance of continuous epidemiological surveillance of Norovirus to support the creation of public policies focused on collective health and the effective combat against this etiological agent.

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PT.352

DETECTION AND TEMPORAL DISTRIBUTION OF ENTERIC VIRUSES IN URBAN CHANNEL OF BELÉM, BRAZIL, FROM 2024 TO 2026.

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Urban channels in Belém are impacted by sewage discharge and contributes for enteric viruses transmission such as norovirus (NoV) and rotavirus (RVA), highly stable in environmentaland associated with severe diarrhea. Monitoring the virological profile of these environments provides vital insights for epidemiological surveillance.To report the occurrence and temporal distribution of norovirus and rotavirus in urban channel waters in Belém, Pará, Brazil. A total of 160 water samples were collected between October 2024 and May 2026 from eight channels located in highly populated neighborhoods of Belém. Water samples weresubjected to skimmed-milk flocculation process for viral concentration, followed by viral nucleic acid extraction and detection by RT-qPCR targeting NSP3 (RVA) and NSP1/VP1 (NoV) in pure and diluted (1:10) samples. RVA was detected in 36.25% (58/160) of samples, (mean Ct:35.36, range: 31.41–37.94).The lowest RVA Ct occurred in the Sacramenta channel, with peak detection during the rainy season transition (Nov 2024–Jan 2025; 32.8%, 19/58). NoV positivity was 35.62% (57/160), (mean Ct: 32.42,range: 20.79-37.99), the lowest Ct value was in the Terra Firme channel. During the less rainy season (Oct–Nov 2024), 28.1% (16/57) of positives were detected. The detection of these viral agents highlights the impact of untreated sewage contamination in urban water bodies, which constitute important routes for the dissemination of enteric viruses. Furthermore, the findings reinforce environmental virology as an important tool for public health surveillance activities.

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FREQUENT DETECTION OF HUMAN ALPHAHERPESVIRUS 1 IN NON-HUMAN PRIMATES FROM MINAS GERAIS, BRAZIL: FINDINGS CONSISTENT WITH HUMAN-TO-WILDLIFE TRANSMISSION

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Urban and peri-urban environments create close interfaces between humans and wildlife, potentially facilitating pathogen transmission across species. In Brazil, non-human primates (NHPs) frequently inhabit parks, forest fragments, and other green areas where close interactions with humans may favor the transmission of human-associated viruses. Human alphaherpesvirus 1 (HHV-1) is of particular concern because infection in neotropical NHPs can cause severe disease and epizootics. This study investigated HHV-1 in NHPs submitted to the Yellow Fever Surveillance Program in Minas Gerais. Liver samples from 167 NHPs (*Callithrix* spp. and *Sapajus* spp.), collected in Belo Horizonte and other municipalities in 2023-25 were analyzed. Following DNA extraction, sample integrity was confirmed by β-actin amplification. HHV-1 DNA was detected by qPCR in 73 (Callithrix) samples (43.7%). Because samples were from NHP carcasses obtained through passive surveillance, the observed detection frequency should not be interpreted as prevalence among free-ranging NHP populations. Nevertheless, the frequent detection of HHV-1 is concerning given its potential to cause lethal infections and epizootics in susceptible primates. Since HHV-1 is primarily associated with humans its detection is consistent with human-to-NHP transmission at human–wildlife interfaces. These findings demonstrate the value of established wildlife mortality surveillance networks for investigating multiple pathogens and improving the differential diagnosis of NHP deaths. Integrating molecular surveillance, wildlife conservation, and responsible human behavior may reduce opportunities for pathogen transmission and support the early recognition and prevention of HHV-1-associated epizootics.

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PT.354

HANTAVIRUSES AND ARENAVIRUSES IN BATS FROM BRAZIL AND MEXICO

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Aiming to investigate the circulation and understand the macroevolution of hantaviruses and arenaviruses in Neotropical chiropterans, this study analyzed oral swab and intestines samples from diverse bat species and ecological niches of two countries of American continent. Viral RNA was extracted and subjected to RT-PCR targeting conserved regions of both families, followed by genetic sequencing and phylogenetic analyses. As results, we detected on distinct tissues of an *Artibeus planirostris* the arenavirus Tacaribe with the recover of both genome segments by NGS. Additionally, hantavirus RNA was detedted in five *Artibeus jamaicensis* individuals from two Mexican regions and preliminary classified as a a lineage at *Mobatvirus* genera. In conclusion, the characterization of the complete Tacaribe virus genome and the identification hantavirus in new hosts and locations expand the knowledge on viral diversity, evolution and biology on bats hosts. These findings reinforce the complexity of the eco-epidemiology of these agents in the Americas, providing fundamental data for virological surveillance and the prevention of public health emergencies.
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Keywords: Arenavirus, Hantavirus, Neotropical bats, Emerging zoonoses.

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BEYOND THE CENTRAL NERVOUS SYSTEM: ANTE-MORTEM DETECTION OF MADARIAGA VIRUS IN NASAL AND CONJUNCTIVAL SWABS FROM HORSES

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Madariaga virus (MADV) is an emerging alphavirus associated with severe neurological disease in horses and humans in South America. Although definitive diagnosis is traditionally based on post-mortem examination of central nervous system (CNS) tissues, information regarding the distribution of MADV in accessible ante-mortem clinical samples remains scarce. This study investigated the distribution of MADV RNA across multiple biological specimens collected from seven naturally infected horses presenting neurological disease. Horses were tested using a semi-nested RT-PCR targeting the non-structural region of the MADV genome, and positive amplicons were confirmed by sequencing. Viral RNA was detected in CNS tissues (4/4), whole blood (3/4), nasal swabs (3/4), cerebrospinal fluid (3/4), and conjunctival swabs (1/2). Consistent detection of MADV RNA in nasal swabs demonstrates that the virus can be identified in readily accessible ante-mortem specimens from naturally infected horses. In addition, MADV RNA was detected in a conjunctival swab collected ante mortem from a horse presenting neurological disease accompanied by ocular abnormalities. To our knowledge, this is the first report of molecular detection of MADV in a conjunctival swab from a naturally infected horse. Together, these findings expand the spectrum of biological matrices available for molecular investigation of equine Madariaga virus infection. Although the diagnostic performance of nasal and conjunctival swabs remains to be established, these readily obtainable specimens represent promising complementary samples for ante-mortem diagnosis and molecular surveillance of equine Madariaga virus and warrant further evaluation in larger case series.

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PT.356

FIRST REPORT OF ORTHOPOXVIRUS IN *Galea spixii* FROM RESERVA NATURAL SERRA DAS ALMAS, CEARÁ, BRAZIL: INSIGHTS FROM MOLECULAR DETECTION AND GENOMIC SEQUENCING

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Orthopoxvirus (OPV) species has drawn strong concern due to its zoonotic potential to adapt and spread. Some of these viruses, such as vaccinia virus (VACV) and monkeypox virus (MPV), can cause harm to a substantial host range, and understand how these viruses reach new animals is a key role in curbing disease spread and guiding effective public health responses. This study investigated, by real-time PCR, the OPV evidence of infection in rodents and marsupials liver samples captured in two environmentally protected areas, Reserva Natural Serra das Almas and Serra da Meruoca, both located in Ceará state, Brazil. A total of 40 small mammals were sampled, among which one spix's yellow-toothed cavy (*Galea spixii*) tested positive for the A56R (viral hemmaglutinin) gene in the molecular testing. The extracted DNA was submitted to a multiplex PCR and genomic sequencing, where this data yielded 249,627 reads, with 225 mapping to the VACV-WR reference genome. An 1,897-nucleotide consensus sequence of the D5R gene was obtained, which showed 100% identity and coverage to five Brazilian VACV group I strains. Phylogenetic analysis robustly placed the isolate within the VACV-BR Group I cluster. To our knowledge, this is the first documented detection of OPV in *Galea spixii*, a small free-living rodent, which is also a bushmeat hunting target in Northeast Brazil, and the first wildlife OPV detection in Ceará state. These findings underscore the necessity for continuous virological surveillance in the regional fauna and preventive public health strategies aimed at minimizing human exposure to orthopoxviruses.

Financial support: FAPEMIG, FUNCAP, CAPES, CNPq, INCT-POX.

PT.357

INVESTIGATION OF HUMAN AND WILDLIFE EXPOSURE TO ORTHOPOXVIRUS IN THE BRAZILIAN AMAZON: A SEROLOGICAL APPROACH

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In the context of the emergence of infectious diseases, orthopoxviruses are pathogens that exhibit a broad host range and geographic distribution. These viruses cause outbreaks of exanthematous diseases with clinical, social, and economic impacts. In Brazil, vaccinia virus stands out as the causative agent of a disease that mainly affects rural workers who have direct contact with infected dairy cattle. The Brazilian Amazon, a biome of high biodiversity, is constantly impacted by the expansion of anthropogenic activities such as agriculture. Human occupation accompanied by domestic animals and livestock results in ecosystem changes that favor interactions with wildlife. Thus, permissive conditions are established for the circulation of pathogens, such as orthopoxviruses. In this context, this study aims to investigate the presence of anti-orthopoxvirus antibodies in serum samples from humans and wildlife in the Brazilian Amazon. In this segment, 200 human and 60 wildlife serum samples collected from two communities in the state of Amazonas, Brazil, were screened for neutralizing antibodies using a plaque reduction neutralization test (PRNT) performed in 6-well plates. Among the tested individuals, 5/200 (2.5%) humans showed positive results, all born before 1980 and possibly vaccinated against variola. These individuals will be considered vaccinated, meaning their neutralizing antibodies will be interpreted as vaccine-derived antibodies. Additionally, 1/60 (1.7%) wildlife samples, corresponding to a canid, showed a positive result. Our findings reinforce the need for sustained ecoepidemiological surveillance to better understand the dynamics of orthopoxvirus exposure and circulation in these populations and associated animals.

Financial support: FAPEMIG; CNPq; ViVeRA.

PT.358

Rapid subclade replacement drives off-season resurgence of influenza A(H3N2) in Brazil

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Influenza A(H3N2) typically drives seasonal influenza epidemics in Brazil during the austral autumn and winter; however, an atypical increase in influenza activity occurred between August and December 2025. To investigate the mechanisms underlying this off-season resurgence, we integrated national epidemiological surveillance data with genomic analyses of hemagglutinin (HA) sequences collected between epidemiological weeks (EW) 31–49 of 2025. Weekly laboratory-confirmed influenza cases from Severe Acute Respiratory Infection (SARI) and Influenza-like Illness (ILI) surveillance systems were analyzed together with viral lineage assignments generated using Nextclade. During the study period, SARI cases increased from 21,798 to 28,061 and ILI cases from 9,990 to 12,944, with A(H3N2) accounting for the majority of detections while A(H1N1)pdm09 remained stable and influenza B/Victoria showed only a modest increase. Genomic analyses revealed a progressive decline in Shannon diversity despite stable lineage richness, consistent with a selective sweep rather than increased viral diversification. This pattern coincided with the rapid replacement of subclade J.2 by J.2.3, which increased from 10% to 80% of sequenced genomes within four epidemiological weeks and became the dominant lineage nationwide by late 2025. Regional analyses showed the same replacement pattern with differences in timing, whereas HA sequences consistently carried the antigenically relevant substitutions N158K and K189R characteristic of J.2.3. In contrast to the contemporaneous global expansion of subclade K, the Brazilian off-season epidemic was driven predominantly by local expansion of J.2.3. These findings demonstrate that rapid clonal expansion of a single A(H3N2) subclade sustained an atypical influenza resurgence and reinforce the importance of continuous genomic and epidemiological surveillance beyond the traditional influenza season.

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ECO-EPIDEMIOLOGICAL SURVEILLANCE OF AVIAN INFLUENZA IN *Spheniscus magellanicus* ALONG THE COAST OF RIO GRANDE DO SUL, BRAZIL

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The Magellanic penguin (*Spheniscus magellanicus*) is a migratory seabird found along the coast of Rio Grande do Sul during the austral winter. During migration, these birds may interact with other wild bird species and environments, potentially contributing to spread of infectious agents, such as the High Pathogenicity Avian Influenza, an important pathogen affecting animals and humans. Climate change and environmental disturbances may alter migratory routes and viral transmission dynamics. Therefore, eco-epidemiological surveillance of migratory birds is essential for early detection and monitoring of Influenza A virus (FLUAV). This study investigated the circulation of FLUAV in *S. magellanicus* along the coast of Rio Grande do Sul, Brazil. Fifty samples collected by partner institutions were processed and nucleic acids were extracted using the MagMAX™ kit, followed by RT-qPCR targeting the influenza A matrix (M) gene with the AgPath-ID™ One-Step RT-PCR kit. Positive samples for the M gene were subsequently tested by H5-specific RT-qPCR. Seven of the 50 samples were positive for the M gene; however, H5-specific RT-qPCR did not detect the H5 subtype. The detection of the matrix gene, coupled with the absence of the H5 subtype, may indicate the circulation of other HA subtype of FLUAV in this migratory population as waterfowl constitute the natural reservoir of the virus. Therefore continuous surveillance is essential to characterize viral diversity, detect emerging highly pathogenic strains, and support early warning systems within a One Health framework.

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PT.360

SURVEILLANCE OF INFLUENZA A VIRUS IN RESIDENT AND MIGRATORY BIRDS ON THE COAST OF RIO GRANDE DO SUL

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Waterfowl are natural reservoirs of Influenza A virus (FLUAV), driving its global dissemination via migratory flyways. The rapid spread of highly pathogenic avian influenza (HPAI) highlights the need for surveillance in these populations, particularly along the coast of Rio Grande do Sul (RS), where key stopover sites host massive numbers of resident and migratory birds. This work aimed to survey FLUAV between January and July on resident and migratory birds along the Rio Grande do Sul coast by RT-qPCR and high-throughput sequencing (NGS). A total of 416 samples from 43 species were collected and processed for RNA extraction using a magnetic beads kit. FLUAV detection was performed by RT-qPCR targeting the matrix gene, followed by H5-specific assay for positive samples. For sample enrichment, whole-genome amplification was conducted by RT-PCR using UNI12 and UNI13 primers. NGS libraries were prepared and run on the Ion Torrent GeneStudio S5 Plus, with raw reads processed and assembled using the IRMA pipeline. Out the 416 collected samples, 51 were positive for the FLUAV matrix gene, and 15 were subtyped as H5. Six M-positive samples, including four H5-positive cases, were selected for NGS; of these, one was confirmed as a H5N1 virus. These findings demonstrate the circulation of FLUAV along the coast of RS, which serves as an important migratory corridor, reinforcing the need for continuous surveillance of wild birds to monitor viral spread and assess the risk of spillover to local wildlife.

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PT.361

Comparative Evaluation of PMMoV and crAssphage as Human Fecal Contamination Markers in Commercial Mussels Using Metagenomic Sequencing and qPCR

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This study evaluated the occurrence of two indicators of human fecal contamination, Pepper mild mottle virus (PMMoV; *Tobamovirus capsicis*) and crAssphage (*Carjivirus communis*), in commercially produced mussels (*Perna perna*) from southern Brazil using two complementary molecular approaches. Sampling was conducted between December 2023 and January 2025. Digestive tissues from 10 pooled samples were processed using an enzymatic protocol based on ISO 15216-1:2017. Extracted nucleic acids were analyzed by shotgun metagenomic sequencing, and taxonomic classification was performed using quality-filtered reads aligned against the NCBI nucleotide (nt) database using BLASTn. In parallel, TaqMan quantitative PCR (qPCR) assays targeting PMMoV and crAssphage were performed to compare detection rates and viral loads. Shotgun metagenomic sequencing detected PMMoV in 30% of the samples and crAssphage in 20%, whereas qPCR detected these markers in 80% and 90% of the samples, respectively. Comparative analysis showed that both methods detected PMMoV and crAssphage in 20% of the samples. Exclusive detection of PMMoV by metagenomic sequencing was observed in 10% of the samples, whereas exclusive detection by qPCR occurred in 60% of the samples for PMMoV and 70% for crAssphage. Median viral loads were 8.35×10^2 genome copies (GC)/g for PMMoV and 2.59×10^2 GC/g for crAssphage. No significant association was observed between viral load and detection by the metagenomic approach (*p value* > 0,9999). These findings demonstrate the occurrence of PMMoV and crAssphage in commercially harvested mussels and support the complementary use of shotgun metagenomic sequencing and qPCR for environmental virological surveillance and food safety monitoring.
Financial Support: FAPERJ SEI-260003/001753/2023; CNPq 402987/2023-3

PT.362

DETECTION AND PHYLOGENETIC CHARACTERIZATION OF ENCEPHALOMYOCARDITIS VIRUS (EMCV) IN OUTBREAKS OF SUDDEN DEATH IN PIGS FROM THE CENTRAL-WEST REGION OF BRAZIL

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Encephalomyocarditis virus (EMCV) belongs to the family *Picornaviridae*, genus *Cardiovirus*, and species *Cardiovirus rueckerti*. It has zoonotic potential and infects a wide range of animal species. Few cases have been reported in Brazilian pigs, and rodents are considered the main natural reservoirs. This study aimed to detect EMCV in pigs from the Central-West region of Brazil. Six heart samples from 75–80-day-old pigs from the same farm, showing motor incoordination followed by sudden death, were collected in Sorriso, Mato Grosso, in February 2025. Four heart samples from pigs that died suddenly, originating from three farms in Lucas do Rio Verde, Mato Grosso, were received in May 2025. Heart tissues were macerated and subjected to RNA extraction and cDNA synthesis. Viral detection was performed by conventional PCR targeting a 286-bp fragment of the RNA-dependent RNA polymerase (RdRp) gene. All ten samples tested positive and were subsequently subjected to a second PCR assay targeting an approximately 500-bp fragment of the VP1 gene. All samples tested positive for both genomic regions and were subjected to Sanger sequencing. Phylogenetic analysis clustered the sequences with *Cardiovirus rueckerti* isolates. The clinical presentation, including sudden death in pigs, was consistent with previous reports of EMCV infection in swine. EMCV detection reinforces the need for epidemiological surveillance and biosecurity within a One Health framework.

Keywords: *Cardiovirus*; zoonosis; swine.
Financial Support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

PT.363

INTEGRATED SURVEILLANCE OF RESPIRATORY VIRUSES IN ANTARCTIC WILDLIFE FROM A ONE HEALTH PERSPECTIVE

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Antarctica is increasingly important for respiratory virus surveillance as migratory wildlife connects the continent with other regions, facilitating pathogen introduction. The emergence of highly pathogenic avian influenza (HPAI) H5N1 has highlighted the need for continuous surveillance of viruses with epidemic and pandemic potential. Coronaviruses are also important emerging pathogens, reinforcing integrated surveillance under a One Health framework. This study presents influenza A virus and coronavirus surveillance in wildlife from the Antarctic Peninsula and the South Shetland Islands. During the 2024-2025 austral summer expeditions, biological samples were collected from live and deceased wildlife, including oropharyngeal and cloacal swabs, serum, and tissues. Influenza A virus surveillance included real-time RT-PCR and serological assays, whereas coronaviruses were investigated by conventional RT-PCR. Positive samples were characterized by whole-genome sequencing. A total of 199 live birds representing six species were sampled. No molecular evidence of influenza A virus or coronavirus infection was detected; however, antibodies against influenza A virus were identified in 20/177 (11.3%) sera, including H6/H11 in *Pygoscelis adeliae*, H11 in *P. antarcticus*, and H1, H5, H6, and H9 in *Stercorarius antarcticus*, indicating previous viral exposure. Investigation of 31 carcasses from wild birds and marine mammals identified 18 HPAI H5N1-positive animals from eight species. Whole-genome sequencing revealed multiple independent viral introductions into Antarctica from South America. These findings highlight the importance of integrated respiratory virus surveillance in Antarctic wildlife to improve viral ecology knowledge, early detection, One Health preparedness, and conservation.

PT.364

MADARIAGA VIRUS EPIZOOTIC OUTBREAK IN EQUINES

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Alphaviruses causing equine encephalomyelitis have been increasingly reported because they infect animals and humans and can cause severe, often fatal, neurological disease. However, their prevalence remains uncertain in several regions of Brazil, including the Pantanal and Cerrado biomes, where equine deaths associated with neurological syndromes are frequently attributed to rabies or remain undiagnosed. This study investigated alphavirus infection in horses presenting neurological signs. Central nervous system samples from 62 horses that died between 2022 and 2025 with suspected *Lyssavirus* infection were analyzed. The animals originated from the Pantanal and Cerrado biomes of Mato Grosso do Sul, Brazil. Molecular testing detected *Alphavirus* infection in seven animals (11.3%). All positive cases were identified as Madariaga virus and originated from rural areas of the municipalities of Miranda (n = 4), Corumbá (n = 1), Bonito (n = 1), and Amambai (n = 1), between December 2022 and March 2024. Affected horses exhibited blindness, ataxia, tremors, behavioral alterations, paralysis, and muscle spasms. Histopathological examination of five cases revealed multifocal lymphoplasmacytic and neutrophilic meningoencephalitis or encephalomyelitis associated with gliosis, neuronal necrosis, and axonal degeneration, consistent with neurotropic viral infection. These findings provide the first evidence of the occurrence of Madariaga virus-associated equine encephalomyelitis and an epizootic in the Pantanal and Cerrado biomes of Brazil, highlighting the need for epidemiological surveillance and inclusion of this virus in the differential diagnosis of neurological syndromes in horses.

Financial Support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001. INCT Vigilância Genômica de Vírus e Saúde Pública (INCT-One).

PT.365

COMPARATIVE ANALYSIS OF RELATIVE RABIES VIRUS LOAD IN DIFFERENT CENTRAL NERVOUS SYSTEM REGIONS OF NATURALLY INFECTED EQUINES

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Rabies remains a fatal zoonotic disease of major public health and veterinary importance, and accurate laboratory diagnosis depends on the quality and representativeness of central nervous system (CNS) samples. This study compared the relative viral load of rabies virus in different CNS regions of naturally infected equines using real-time quantitative PCR (qPCR). CNS samples from equines submitted for rabies diagnosis to the official diagnostic laboratory of the Agência Estadual de Defesa Sanitária Animal e Vegetal de Mato Grosso do Sul, Brazil, were analyzed. Relative viral load was inferred from quantification cycle (Cq) values obtained by RT-qPCR. A total of 33 equine samples were received, out of which 12 (36.4%) were positive by the direct fluorescent antibody test (DFAT). The availability of CNS fragments varied among submissions and only one sample contained all seven requested CNS regions. In two samples, the lumbar spinal cord showed the lowest Cq values, indicating the highest viral load. Among the remaining samples, the lowest Cq were observed in the cerebral cortex (41.6%), thalamus (16.7%), cervical spinal cord (16.7%), and cerebellum (8.3%). No consistent pattern of viral distribution was identified across the CNS of the evaluated equines. These findings demonstrate heterogeneous rabies virus distribution within the equine CNS and reinforce the importance of collecting multiple CNS regions and the advance of integrating molecular techniques for rabies detection, improving the sensitivity and reliability of laboratory diagnosis.

Key words: diagnosis, RT-qPCR, surveillance.

Financial Support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001. INCT Vigilância Genômica de Vírus e Saúde Única (INCT-One).

PT.366

Microbiological monitoring of water samples through the AquaCID citizen science network: detection of bacteria and lytic bacteriophages

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Water is an important reservoir of microorganisms, including clinically relevant bacteria and bacteriophages. These bacteria may exhibit antimicrobial resistance, a global health challenge, while bacteriophages represent a potential therapeutic alternative. Citizen Science initiatives have proven effective in expanding data collection and promoting science education. Thus, this study implemented a Citizen Science network for the microbiological monitoring of national water sources. A total of 41 samples, collected by partner schools in 9 brazilian states, were evaluated and screened for the presence of bacteria and lytic bacteriophages. Enrichment was performed for ESKAPE pathogens, followed by a double-layer agar drop test to detect lytic phages. Bacteria were recovered on selective media, isolated, identified by MALDI-TOF, and the genera considered priority pathogens in public health were sent for susceptibility profiling. Lytic phages were identified in 4 samples, targeting *A. baumannii*, *E. coli*, *P. aeruginosa*, and *K. pneumoniae*. In addition, 134 bacterial isolates from 15 genera were identified, with a predominance of *Pseudomonas* and *Acinetobacter*. Among the 11 isolates selected for susceptibility testing, *E. ludwigii* and *P. aeruginosa* exhibited resistance profiles and were therefore selected for genome sequencing. The results will be shared with schools through presentations covering concepts such as One Health, AMR, and therapeutic alternatives, such as bacteriophages. The AquaCID network demonstrates Citizen Science as a promising strategy for microbiological monitoring and the generation of relevant data, while contributing to science education. Financial Support: CNPq - Conselho Nacional de Desenvolvimento Científico e Tecnológico.

PT.367

OCURRENCE, QUANTIFICATION AND MOLECULAR CHARACTERIZATION OF CRASSPHAGE IN WATER AND SEDIMENT SAMPLES FROM A TROPICAL COASTAL ESTUARINE SYSTEM IN BRAZIL

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The bacteriophage crAssphage has emerged as a promising molecular marker of human fecal contamination in environmental waters. This study aimed to quantify and characterize crAssphage in water and sediment samples from four rivers draining into a tropical coastal estuarine system in the metropolitan region of Rio de Janeiro, southeastern Brazil, including the Guandu River, the main drinking water source for the region. Surface water samples (2.5 L) were collected monthly throughout 2024 during spring tides, while sediment samples (~400 g) were collected quarterly. Viral particles were concentrated by ultracentrifugation, followed by nucleic acid extraction using magnetic beads (LOCCUS). CrAssphage genomic copies were quantified by qPCR using a TaqMan assay targeting the ORF24 region. Positive samples with Ct < 27 were further analyzed by conventional PCR and Sanger sequencing. CrAssphage was detected in 79.7% (51/64) of all samples, including 87.5% (42/48) of water samples and 56.3% (9/16) of sediment samples, with concentrations ranging from 1.74 to 6.57 log genomic copies/L. Detection occurred in all rivers, with highest water positivity in Canal Dr. Ari Chagas and Canal do Itá (100%), followed by Piraquê River (91.7%), while Guandu River showed lower positivity (58.3%). Sediment positivity reached 75% in impacted rivers, with no detection in Guandu. Phylogenetic analysis of eleven environmental sequences (835–910 bp) showed up to 99% identity with *Carjivirus communis* (crAssphage reference sequences) and low genetic distances (<0.015 substitutions/site), indicating high conservation. These findings reinforce crAssphage as a robust molecular indicator for monitoring human fecal contamination in tropical coastal aquatic systems.

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PT.368

Integrated Water Quality Monitoring through Physicochemical, Microbiological, and Viral Analyses in Rivers of the Rio de Janeiro Metropolitan Region

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This study evaluated the occurrence of CrAssphage and human mastadenovirus as molecular indicators of anthropogenic fecal contamination in rivers associated with the Sepetiba watershed, Rio de Janeiro, Brazil. Forty-eight surface water and 16 sediment samples were collected from the Piraque River, Dr. Ari Chagas Channel, Ita Channel, and the Guandu River. Physicochemical parameters (pH, temperature, turbidity, dissolved oxygen, oxidation-reduction potential, and total dissolved solids) were measured, while Escherichia coli were quantified by the multiple-tube fermentation method. Viral particles were concentrated by ultracentrifugation, followed by nucleic acid extraction and TaqMan® real-time PCR. The Guandu River exhibited the best environmental quality, with high dissolved oxygen (7.8 mg/L), low turbidity, and near-neutral pH, whereas the Piraque River and Ita Channel showed degradation, characterized by low dissolved oxygen, high turbidity, and negative oxidation-reduction potential values. All sampling sites exceeded the microbiological limits established by CONAMA Resolution No. 357/2005, with the highest bacterial loads observed in the Dr. Ari Chagas Channel. CrAssphage was detected in 87.5% (42/48) of water samples and 56% (9/16) of sediment samples, while human mastadenovirus was detected in 68% (33/48) and 18% (3/16), respectively. Lower Ct values for both viruses were observed in the most contaminated environments, whereas the Guandu River showed the highest Ct values. The physicochemical, microbiological, and virological analyses demonstrated an association between water quality indicators and viral occurrence. The prevalence of CrAssphage highlights its value as a robust marker of human fecal pollution, while human mastadenovirus complements environmental surveillance due to its persistence and public health relevance.

Financial Support: CNPq, FAPERJ and CAPES (Finance Code 001).

PT.369

ONE HEALTH APPROACH TO RABIES OUTBREAK CONTROL IN MUNICIPALITY OF CRUZ DAS ALMAS: AN EXPERIENCE REPORT

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Rabies is a highly lethal zoonotic disease that requires continuous surveillance and preventive measures to reduce transmission risk within a One Health framework. This study aimed to describe the activities carried out by a One Health and Infectious Diseases Service Program for the control, epidemiological investigation, and prevention of rabies virus circulation following an outbreak involving attacks by crab-eating foxes (*Cerdocyon thous*) in the municipality of Cruz das Almas, Brazil. This descriptive experience report presents the actions developed by a residency program in Animal Health integrated with public health in collaboration with the municipal epidemiological surveillance service during rabies outbreak control in the municipality between March and June 2026. The activities included active surveillance, monitoring of victims, outbreak containment measures, vaccination campaigns and technical-scientific discussions. Control measures were initiated following laboratory confirmation of the rabies virus by the Central Public Health Laboratory (LACEN) in three *Cerdocyon thous* specimens. Two human victims were referred for appropriate post-exposure prophylaxis; 998 dogs and cats were vaccinated between March and June 2026; additionally, continuing education activities on rabies pathophysiology and training sessions on rabies vaccination were held within the residency program. This experience demonstrates that intersectoral collaboration in surveillance and health education constitutes a relevant strategy for preventing outbreaks and supporting rabies virus control measures at the municipal level.

PT.370

Epidemiological and laboratory characterization of suspected dengue cases tested at a reference laboratory in southern Bahia.

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Dengue is one of the most significant arboviral diseases affecting public health, and laboratory diagnosis plays a crucial role in case confirmation and epidemiological surveillance. This study aimed to characterize the epidemiological and laboratory profile of samples submitted to the Laboratório de Farmacogenômica e Epidemiologia Molecular (LAFEM) for dengue investigation. This was a retrospective, descriptive, observational study based on the laboratory's database. Samples were screened using ZDC, ELISA NS1 antigen, or ELISA IgM tests according to the interval between symptom onset and sample collection. Each sample was counted only once in the positivity analysis. A total of 1,701 samples were analyzed, including 1,307 (76.8%) collected in 2024 and 394 (23.2%) in 2025. The IgM test was the most frequently performed (52.8%), followed by NS1 (24.6%) and ZDC (22.6%). In 2024, May accounted for the highest number of analyzed samples (n=649) and positive results (n=82), with a positivity rate of 12.6%. In 2025, April showed both the highest testing demand (n=92) and the highest positivity rate (51.1%). Ilhéus, Maraú, and Itajuípe were the municipalities that submitted the largest number of samples, with positivity rates of 12.7%, 17.9%, and 19.3%, respectively. Female individuals predominated (54.3% in 2024 and 64.7% in 2025), as residents from urban areas (74.8% and 89.1%, respectively). These findings indicate sustained dengue virus circulation throughout the region and reinforce the importance of strengthening regional epidemiological surveillance.

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PT.371

HTLV Infection Profile in Bahia: An Analysis of Reported Cases Over the Last Decade (2015–2024).

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Epidemiological studies indicate that Brazil has the largest absolute number of individuals living with the Human T-cell Lymphotropic Virus (HTLV) worldwide. This study aimed to evaluate and update the epidemiological profile of HTLV infections in the state of Bahia from 2015 to 2024 using data obtained from the Brazilian Notifiable Diseases Information System (SINAN). During the period, 3,847 laboratory-confirmed HTLV cases were reported in Bahia. Consistent with previous findings, a higher prevalence was observed among women and Black and mixed-race individuals. Regarding notification trends, a decline was observed in 2020, with a detection rate of 1.1 cases per 100,000 inhabitants, likely reflecting the impact of the COVID-19 pandemic. Notifications increased in subsequent years, reaching the highest detection rate in 2024 (4.67 cases per 100,000 inhabitants). Most notifications occurred in June, coinciding with Brazil's national blood donation campaign. No significant differences were observed in the epidemiological profile of infected individuals before and after the pandemic. Among the Regional Health Centers, the Eastern region, which includes Salvador, and the Southern region, encompassing Ilhéus and Itabuna, accounted for the highest number of reported cases. These findings are consistent with previous studies identifying Salvador and the Ilhéus–Itabuna microregions as areas of higher HTLV prevalence in Bahia. Additionally, substantial incompleteness in notification forms and the lack of information regarding clinical outcomes were identified, limiting data accuracy and posing challenges for effective surveillance and control of HTLV infection.

Financial Support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES; Fundação de Amparo à Pesquisa do Estado da Bahia - FAPESB;

PT.372

RESPIRATORY VIRUS DETECTION AND CLINICAL PROFILE OF PATIENTS ATTENDED AT TWO HOSPITALS IN JUIZ DE FORA, MINAS GERAIS, BRAZIL

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Respiratory viruses remain a major cause of acute respiratory illness, particularly in young children, and their clinical presentations frequently overlap across etiologies. This study aimed to describe the viral detection profile and clinical characteristics of patients tested for respiratory viruses in two hospitals in Juiz de Fora, Minas Gerais, Brazil. A total of 223 patients were evaluated by real-time PCR. Demographic, clinical, vaccination, and laboratory data were analyzed. The median age was 1.0 year, and females represented 51.1% of the sample. Overall, 171/223 patients (76.7%) had at least one virus detected, whereas 52/223 (23.3%) were negative. Monoinfections were observed in 148/223 patients (66.4%), and coinfections in 23/223 (10.3%), corresponding to 13.5% of virus-positive cases. Human rhinovirus was the most frequently detected virus (69), followed by respiratory syncytial virus (49), influenza A virus (45), adenovirus (16), influenza B virus (9), human metapneumovirus (5), and SARS-CoV-2 (1). The most frequent coinfection was RSV/rhinovirus (8/23; 34.8%). The most common symptoms were cough (84.8%), fever (78.5%), nasal congestion (68.2%), rhinorrhea (67.3%), nausea or vomiting (24.7%), and fatigue (23.8%). Influenza-like illness was identified in 52/223 patients (23.3%) and was more frequent among virus-positive than negative patients, although the difference was not statistically significant (26.3% vs. 13.5%; p=0.062). No significant differences were observed in age or interval from symptom onset to sample collection between positive and negative groups. These findings highlight the substantial burden and diversity of respiratory viral infections and reinforce the importance of multiplex molecular surveillance.

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PT.373

RISK-BASED PRIORITIZATION FOR AVIAN INFLUENZA SURVEILLANCE AT THE WILDLIFE–POULTRY INTERFACE IN ZONA DA MATA, MINAS GERAIS, BRAZIL

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Highly pathogenic avian influenza (HPAI) represents a One Health concern because of its effects on wildlife, poultry production and public health. This study aimed to develop a risk-based prioritization framework for avian influenza surveillance in 140 municipalities of Zona da Mata, Minas Gerais, Brazil. A database was constructed using 13,452 bird occurrence records representing 515 species, poultry population data from the 2017 Agricultural Census, georeferenced poultry establishments from SISBI/MAPA and IMA-MG, hydrographic and climatic variables, and the location of confirmed HPAI outbreaks in Minas Gerais. A priority index, designated H and ranging from 0 to 1, was calculated by integrating poultry pressure (30%), interface with aquatic environments (25%), interface with wild birds (30%) and proximity to confirmed outbreaks (15%). In the scenario, the highest-priority municipalities were Rio Pomba (H=0.45), Ubá (0.40), Rio Doce (0.36), São Miguel do Anta (0.33) and Juiz de Fora (0.33). Spatial autocorrelation was significant (Moran's I=0.29; p=0.002), indicating clustering of municipalities with similar priority levels. Sensitivity analysis showed that a ±20% variation in component weights produced limited changes in the final classification, with a Jaccard similarity index ≥0.87. However, replacing census-based poultry data with only georeferenced establishments changed the priority category of 26 municipalities (18.6%), demonstrating the influence of poultry data availability. In addition, 80 municipalities had no eBird occurrence records, indicating observation gaps. The proposed index does not estimate outbreak probability but identifies ecological and operational priorities for surveillance, field sampling, and resource allocation in a region without confirmed outbreaks. Financial Support: FAPEMIG

PT.374

WHEN INFECTION DOES NOT MEAN DISEASE: SILENT CIRCULATION OF NEUROPATHOGENIC EHV-1 IN COMPETITION HORSES

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Equine herpesvirus type 1 (EHV-1) is a major cause of respiratory disease, abortion, and equine herpesvirus myeloencephalopathy, particularly in high-density competition settings where animal movement facilitates viral dissemination. This study investigated a large outbreak of EHV-1 associated with a show-jumping event in Brazil. Following molecular confirmation of two neurological index cases, 417 samples from 283 horses were analyzed using real-time PCR targeting the ORF30 polymorphism to differentiate neuropathogenic and non-neuropathogenic strains. Viral DNA load was estimated using Ct values to support outbreak management. Among the horses investigated, 201 (71.0%) were positive for the neuropathogenic ORF30 D752 genotype, whereas only four horses carried the non-neuropathogenic genotype, all concomitantly infected with D752. Despite widespread circulation of the neuropathogenic strain, only 14 horses (4.9%) developed compatible clinical signs, including seven with neurological disease, demonstrating marked dissociation between molecular detection and clinical expression. Most positive horses exhibited low or suspect viral DNA loads, while animals with moderate or high viral loads were significantly more likely to develop clinical signs. Rapid molecular diagnosis enabled immediate implementation of biosecurity measures, Ct-guided segregation of infected horses, and structured follow-up testing, resulting in rapid decline of viral detection during quarantine. These findings demonstrate extensive silent circulation of neuropathogenic EHV-1 among competition horses and highlight the importance of integrating molecular diagnostics with epidemiological surveillance and biosecurity measures for effective outbreak control.

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PT.375

MOLECULAR DETECTION OF INFLUENZA VIRUSES IN LUNG SAMPLES FROM ROAD-KILLED WILD MAMMALS

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One Health is an integrated approach that plays a key role in the surveillance of zoonotic pathogens, including influenza viruses. Monitoring pathogens in wildlife is essential for understanding disease ecology. However, capturing free-ranging animals poses ethical challenges. In this context, road-killed wild animals represent an important alternative source for epidemiological surveillance. This study aimed to investigate the molecular occurrence of influenza viruses in lung samples from road-killed wild mammals collected across different Brazilian regions. A total of 158 lung samples from different species of mammals were analyzed. RNA was extracted and quality and purity were assessed by spectrophotometer. Sample integrity was confirmed by conventional polymerase chain reaction (PCR) targeting the vertebrate constitutive genes β-globin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Influenza viruses were detected by quantitative reverse transcription real-time PCR, targeting the matrix (M1/M2) and nonstructural (NS1/NS2) genes. Overall, 60% (96/158) of the samples showed adequate RNA purity values (1.80 – 2.04), and 51% (81/158) were positive for at least one constitutive gene, confirming the suitability of the extracted genetic material. Influenza virus RNA was detected in seven samples from *Dasypus novemcinctus* (n=2), *Euphractus sexcinctus* (n=1), *Cavia aperea* (n=1), *Tamandua tetradactyla* (n=1), *Sapajus* sp.(n=1) and Echimyidae (n=1). All positive samples presented low viral loads and will undergo sequencing for further characterization. These findings demonstrate the feasibility of using road-killed wild mammals as an ethical resource for molecular influenza surveillance, expanding and reinforcing their value as a practical and ethical resource for monitoring respiratory viruses within a One Health framework.

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PT.376

SEROLOGICAL INVESTIGATION OF WEST NILE VIRUS IN DOMESTIC ANIMALS FROM INDIGENOUS COMMUNITIES IN THE PANTANAL: PRELIMINARY DATA

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West Nile virus (*Orthoflavivirus nilense*, WNV) is considered one of the most geographically widespread arboviruses in the world. While the majority of infections in humans and animals are asymptomatic, severe and fatal neurological diseases have been documented. In Brazil, human and animal cases have been reported across different biomes. This study aims to investigate serological evidence of WNV in animals of the indigenous communities of the Guató and Bororo ethnic groups in the Pantanal of Mato Grosso. In February 2025, a field campaign was conducted to collect blood samples from domestic animals in the villages of Córrego Grande (Bororo) and Aterradinho (Guató) in the state of Mato Grosso, west-central Brazil. A total of 81 serum samples were collected: 44.4% from domestic birds (36/81), 35.8% from domestic dogs (29/81), 8.6% from equids (7/81), 7.4% from swine (6/81), 2.5% from cattle (2/81), and 1.2% from a domestic cat (1/81). All sera were screened for neutralizing antibodies against WNV by plaque reduction neutralization test (PRNT90). Preliminary results indicate that 12 samples (14.8%) were reactive, including five dogs, five equids, one bovine, and one pig. All reactive samples were from the Guató community. Confirmatory testing is ongoing, but preliminary results indicate that multiple domestic species from the Guató community may have been exposed to WNV. These preliminary findings underscore the critical need for continuous arbovirus surveillance in the Pantanal.
Financial Support: FAPEMIG, CAPES, CNPq and Funarbe.
Keywords: Pantanal biome; Mato Grosso; zoonotic; surveillance; arbovirus.

PT.377

INVESTIGATION OF OROPOUCHE VIRUS CIRCULATION IN DOGS FROM RURAL AND QUILOMBOLA COMMUNITIES IN THE VALE JEQUITINHONHA, MINAS GERAIS, BRAZIL

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Orthobunyavirus are potential agents of emerging and re-emerging diseases. Among them, Oropouche virus (OROV) stands out for having caused extensive outbreaks in the Amazon region and, in recent years, for its geographic expansion, with reported spread to different regions of Brazil. Although exposure to OROV has been detected in dogs, the extent of OROV circulation in these animals remains uncertain. This study aimed to investigate the circulation of OROV in dogs from rural and Quilombola communities in the Vale Jequitinhonha, Minas Gerais, Brazil. A total of 67 blood samples were collected from dogs in the municipality of Virgem da Lapa. Following virus inactivation, serum samples were tested using the plaque reduction neutralization test ($PRNT_{90}$) against an OROV strain isolated in Rio de Janeiro in 2024. Overall, 20 samples (29.85%) were considered presumptively positive, presenting $PRNT_{90}$ titers ≥20 in the screening assay. All samples will undergo confirmatory testing. If confirmed, these findings support the exposure of dogs to OROV and highlight the need for further studies to assess their potential use as sentinels for viral circulation. Strengthening the active surveillance of arboviruses such as OROV in vulnerable communities is essential for the early detection of viral circulation and for guiding prevention and control measures. From a One Health perspective, integrating human, animal, and environmental health is crucial for improving surveillance strategies and epidemiological response.

Financial Support: FAPEMIG, CAPES, CNPq e Funarbe.

PT.378

MOLECULAR DETECTION OF ARBOVIRUSES IN FEMALE *AE. AEGYPTI* BY RT-QPCR: ENTOMOLOGICAL SURVEILLANCE IN AN ENDEMIC AREA OF NORTHWESTERN SÃO PAULO STATE

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Anthropogenic processes associated with urbanization and globalization have increased the frequency of arbovirus emergence and re-emergence. Climate change has also been associated with increased transmission and geographic spread, contributing to epidemics such as the record number of dengue cases reported in Brazil in 2024, when approximately 5.9 million cases were confirmed.Therefore, epidemiological and entomological surveillance are essential for supporting public health decision-making and reducing the impact of arboviral diseases. In this study, 780 female Aedes aegypti were collected between December 2025 and April 2026 in São José do Rio Preto, São Paulo State, Brazil. RNA was extracted using TRIzol and screened by RT-qPCR for dengue virus serotypes 1–4 (DENV-1–4), chikungunya virus (CHIKV), zika virus (ZIKV), and the insect-specific viruses Humaita-Tubiacanga virus (HTV) and Phasi Charoen-like virus (PCLV). Four samples tested positive for DENV-3 (0.51%) and 11 for CHIKV (1.41%), most detected between January and March, although four CHIKV-positive samples were identified in December, marked by high temperatures and rainfall. No samples tested positive for ZIKV. Insect-specific viruses were widely detected: PCLV in 594 samples (76.15%) and HTV in 432 (55.38%), with 368 samples (47.17%) positive for both. Despite the absence of documented endemic transmission of DENV-3 and CHIKV during the study period, their detection in the mosquito population highlights the importance of continuous entomological surveillance. This enables early detection of viral introduction or re-emergence and support evidence-based public health strategies to prevent, control, and mitigate future arbovirus outbreaks.

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PT.379

SPATIAL DISTRIBUTION OF DENGUE AND IDENTIFICATION OF HOTSPOTS IN MUNICIPALITIES OF ALAGOAS, BRAZIL, 2014–2025

Silva LEA[1], de Oliveira MP[2], Leão LM[3], Souza AKP[4], Agulhan MHC[5], Silva-Junior A[6], Lobo CDP[7], de Almeida GPSB[8], Araújo MAS[9], da Silva TFCF[10] - [1]Universidade Estadual de Ciências da Saúde de Alagoas (UNCISAL) - Departamento de Microbiologia, [2]Centro Universitário CESMAC - Departamento de Medicina, [3]Faculdade Iguaçu (FI) - Departamento de Hemoterapia e Terapia Celular, [4]Universidade Estadual de Ciências da Saúde de Alagoas (UNCISAL) e Centro Universitário CESMAC - Departamento de Ciências da Saúde, [5]Universidade Federal de Alagoas (UFAL) - Faculdade de Medicina da UFAL, [6]Universidade Federal de Alagoas (UFAL) - Departamento de Bioquímica, [7]Universidade Estadual de Ciências da Saúde de Alagoas (UNCISAL) - Departamento de Inovações Terapêuticas, [8]Universidade Estadual de Ciências da Saúde de Alagoas (UNCISAL) - Departamento de Ciências Farmacêuticas, [9]Centro Universitário CESMAC - Departamento de Biologia de Fungos, [10]Universidade Federal de Alagoas (UFAL) - Departamento de Biologia de Parasitas e Microrganismos

Dengue is a major arboviral disease and a persistent public health challenge in Brazil, with heterogeneous spatial distribution. Spatial analysis can identify priority areas for surveillance and guide prevention and control measures. This study aimed to analyze the spatial distribution of temporal average dengue incidence and identify hotspots and spatial clusters in the municipalities of Alagoas, Brazil, from 2014 to 2025. This ecological, retrospective, and descriptive study used probable dengue cases from the Brazilian Notifiable Diseases Information System (SINAN). Temporal average incidence rates were calculated using municipal population estimates from the Brazilian Institute of Geography and Statistics (IBGE). Incidence values were log-transformed [log(incidence + 1)]. Spatial relationships were defined using a first-order Queen contiguity matrix, and incidence was smoothed by local spatial lag. Global spatial autocorrelation was assessed using Moran's I, while Local Moran (LISA) and Getis-Ord Gi* identified spatial clusters, hotspots, and coldspots. A total of 160,944 probable dengue cases were reported across 102 municipalities. Mean incidence ranged from 15.5 to 1,256 cases per 100,000 inhabitants. Significant global spatial autocorrelation was detected (Moran's I = 0.130; p = 0.025). Hotspots were concentrated in the eastern and coastal regions, with Marechal Deodoro showing the strongest hotspot (99% confidence), whereas coldspots predominated in the Sertão and Alto Sertão. LISA analysis identified High-High clusters in the eastern region. Dengue exhibited a significant spatial pattern in Alagoas. Identifying hotspots and clusters supports targeted surveillance and control strategies, improving resource allocation and public health interventions. Financial Support: None.

PT.380

SEASONAL CHARACTERIZATION OF ARBOVIRAL DISEASES ACCORDING TO CLIMATIC PERIODS IN ALAGOAS, 2017–2025

Silva LEA[1], de Oliveira MP[2], Leão LM[3], Souza AKP[4], Agulhan MHC[5], Silva-Junior A[6], Lobo CDP[7], de Almeida GPSB[8], Araújo MAS[9], da Silva TFCF[10] - [1]Universidade Estadual de Ciências da Saúde de Alagoas (UNCISAL) - Departamento de Microbiologia, [2]Centro Universitário CESMAC - Departamento de Medicina, [3]Faculdade Iguaçu (FI) - Departamento de Hemoterapia e Terapia Celular, [4]Universidade Estadual de Ciências da Saúde de Alagoas (UNCISAL) e Centro Universitário CESMAC - Departamento de Ciências da Saúde, [5]Universidade Federal de Alagoas (UFAL) - Faculdade de Medicina da UFAL, [6]Universidade Federal de Alagoas (UFAL) - Departamento de Bioquímica, [7]Universidade Estadual de Ciências da Saúde de Alagoas (UNCISAL) - Departamento de Inovações Terapêuticas, [8]Universidade Estadual de Ciências da Saúde de Alagoas (UNCISAL) - Departamento de Ciências Farmacêuticas, [9]Centro Universitário CESMAC - Departamento de Biologia de Fungos, [10]Universidade Federal de Alagoas (UFAL) - Departamento de Biologia de Parasitas e Microrganismos

Urban arboviruses are a major public health concern in tropical regions, where environmental and climatic conditions favor the proliferation of *Aedes aegypti*. Rainfall and temperature play important roles in viral transmission dynamics. This study aimed to evaluate the seasonality of dengue, Zika, and chikungunya and their association with climatic variables in Alagoas, Brazil, from 2017 to 2025. An ecological, retrospective, and descriptive study was conducted using secondary data from the Brazilian Notifiable Diseases Information System (SINAN). Probable cases of dengue, Zika, and chikungunya reported between 2017 and 2025 were analyzed. Monthly incidence rates per 100,000 inhabitants were calculated using population estimates from the Brazilian Institute of Geography and Statistics (IBGE). Monthly temperature and rainfall estimates were obtained from regional climatological patterns described by the National Institute of Meteorology (INMET). Pearson and Spearman correlation analyses were performed between arboviral incidence and climatic variables using lag 0, lag 1, and lag 2 models. Marked seasonality was observed, with the highest incidence rates occurring between April and July, corresponding to the rainy season in Alagoas. Rainfall showed significant positive correlations with dengue ($r=0.430$), Zika ($r=0.425$), and chikungunya ($r=0.355$) in the lag 0 model ($p<0.001$), with similar results in Spearman analyses. Correlations weakened in lagged models, particularly for dengue, suggesting a rapid epidemiological response to rainfall. Temperature showed weak negative correlations, especially for dengue ($r=-0.309$; $p=0.001$). The findings highlight the influence of rainfall on arboviral transmission and support integration of epidemiological surveillance with climate monitoring to improve outbreak prediction and vector control strategies. Financial Support: None.

PT.381

MOLECULAR DETECTION OF FELINE LEUKEMIA VIRUS (FeLV) IN FREE-LIVING AND CAPTIVE JAGUARS (*Panthera onca*) IN BRAZIL

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Feline Leukemia Virus (FeLV) is a major pathogen of domestic cats and potentially an increasing threat to wild felid conservation. Habitat fragmentation intensifies contact between domestic reservoirs and top predators, facilitating pathogen spillover. This study investigated FeLV occurrence in 82 samples from 52 jaguars (*Panthera onca*), including 46 free-living and 6 captive individuals (from Jaguar Conservation Fund). Molecular screening was performed via qPCR with SYBR Green, whereas 12 samples presented significant Cq values, but with non-specific melting curves. These were then subjected to confirmation by a TaqMan probe targeting the U3-LTR region. Only two samples were confirmed as positive. Both came from the same wild individual but were collected at different times. This jaguar was captured in a region of the Pantanal wetlands, near human settlements. These results demonstrate FeLV circulation in wild jaguars at the domestic-wildlife interface. The proximity to anthropogenic areas suggests spillover from domestic cats, the primary reservoirs of the virus. Given the logistical unfeasibility of continuous health monitoring in free-living jaguars, mitigation strategies must necessarily focus on the domestic reservoir. Effective protection of wild populations depends on large-scale testing and mandatory vaccination of domestic cats in areas surrounding natural habitats and conservation units. Preventing infection at the source through domestic animal health programs is the most effective "One Health" tool to safeguard vulnerable jaguars against domestic-origin viruses.

Key-words: *One health*; Domestic-wildlife interface; qPCR.

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PT.382

EPIDEMIOLOGICAL, CLINICAL AND LABORATORY PROFILES OF SEVERE AND NON-SEVERE DENGUE CASES IN MUNICIPALITIES IN SOUTHERN BAHIA (2024-2025)

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Dengue is a viral disease that can take a severe and fatal course. This study aimed to describe the epidemiological, clinical and laboratory profile of suspected dengue cases in municipalities in southern Bahia, between April 2024 and December 2025. A total of 1,640 samples, from health centers and hospitals, were tested for dengue, of which 321 (19.6%) were positive. Of these, 302 (94.1%) were from individuals presenting as non-severe dengue. Meanwhile, 19 (5.9%) were from individuals who presented at least one warning sign or sign of severe dengue, the most common being sudden decrease in platelet count (52.6%). On average, the time interval between symptom onset and sample collection was shorter in patients with severe dengue compared to those with non-severe dengue (5.8 days and 7.2 days, respectively). The predominant test used to confirm a positive result was serology for IgM, regardless of severity. The majority of patients in both groups were female (60.6% in the non-severe group and 57.9% in the severe group), and the predominant age group in both cases was up to 19 years (33.4% and 52.6%, respectively). The most common symptom was headache in severe cases (89.5%) and fever in non-severe cases (93.4%). These findings indicate that dengue virus circulates significantly in the region, causing mainly mild and moderate disease, with nonspecific symptoms, mostly affecting women and young people. Considering that everyone is vulnerable to severe disease, it can be concluded that preventing infection is the most important approach.

Keywords: dengue; severe; non-severe

Financial Support: FAPESB; PPGCS/UESC; LACEN/BA

PT.383

GENOMIC EPIDEMIOLOGY OF RABIES VIRUS IN SOUTHERN BRAZIL REVEALS FOUR NOVEL LINEAGES

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Rabies is a fatal zoonotic disease caused by viruses of the genus Lyssavirus (family Rhabdoviridae) and remains a major threat to animal and public health worldwide. Genomic surveillance provides valuable insights into viral evolution, transmission dynamics, and lineage diversity, supporting more effective epidemiological monitoring. In this study, we investigated the genomic epidemiology of rabies virus (RABV) circulating in Rio Grande do Sul, southern Brazil, using whole-genome sequencing of 268 central nervous system samples collected from five animal groups: Bovidae, Chiroptera, Equidae, Felidae, and Ovine. Samples were obtained during 2022, 2024, and 2025. Genome sequencing achieved a mean breadth coverage of 76.66%. Viral lineage designation was performed using the MADDOG pipeline. Fourteen bat-related lineages were identified, while 69 genomes could not be classified, suggesting the presence of previously undescribed genetic diversity. The most prevalent lineage was Bat_B1, representing 72 genomes (26.87%). Phylogenetic analysis also enabled the identification of four novel bat-associated lineages: Bats DR_G1.1.1 (n = 6; 2.24%), Bats DR_J1 (n = 3; 1.12%), Bats DR_J1.1 (n = 39; 14.55%), and Bats DR_J1.1.1 (n = 13; 4.85%). These findings expand the current understanding of RABV genetic diversity in southern Brazil and highlight the importance of continuous genomic surveillance for identifying emerging viral lineages, supporting more effective epidemiological monitoring and control strategies. The high proportion of unclassified genomes further suggests that the genetic diversity of RABV circulating in the region remains underestimated and underscores the need to improve current lineage classification frameworks.

PT.384

FIRST REPORT OF OROPOUCHE VIRUS CIRCULATION IN FREE-LIVING XENARTHRANS IN MATO GROSSO DO SUL, BRAZIL

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Oropouche virus (OROV) is an arbovirus that poses an emerging threat to public health on a global scale. With an unprecedented geographical spread beginning in 2023, OROV has reached all the 26 Brazilian states and the Federal District. Symbols of Mato Grosso do Sul's wildlife, the giant anteater (*Myrmecophaga tridactyla*), the giant armadillo (*Priodontes maximus*), and the six-banded armadillo (*Euphractus sexcinctus*) face various threats to their populations, including wildfires, agricultural expansion, roadkill, and infectious diseases. We investigated, for convenience, OROV infection in 35 free-living xenarthrans from two different biomes in Mato Grosso do Sul. The investigation consisted of a retrospective serological analysis using the Plaque Reduction Neutralization Test (PRNT) using the BeAn 19991 strain on serum samples collected from 2015 to 2024, including 16 giant anteaters, 16 giant armadillos, and 3 six-banded armadillos. The results revealed a seropositivity rate of 31.4% (11/35). Giant anteaters from Cerrado biome showed the highest seroprevalence (10/16; 62.5%) among the species analyzed. Only one giant armadillo (1/10; 6.2%) from the Pantanal biome was seropositive. All samples that tested seropositives were obtained between 2023 and 2024, during the epidemic of human cases in Brazil. To our knowledge, this is the first circulation evidence of OROV infection in xenarthrans, other than sloths (*Bradypus tridactylus*), and outside the Brazilian Amazon region, reinforcing the emergence of the Oropouche virus not only in humans but also wild endangered species.

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Keywords: arbovirus, *Orthobunyavirus*, zoonosis, wildlife, one health.

PT.385

EPIDEMIOLOGICAL SURVEILLANCE OF NEUTRALIZING ANTIBODIES AGAINST THE MAYARO VIRUS IN GIANT ANTEATERS AND GIANT ARMADILLO IN MATO GROSSO DO SUL, BRAZIL

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Mayaro virus (MAYV) is an enzootic virus of the genus *Alphavirus* transmitted by mosquito vectors. Viral infection in humans presents with nonspecific symptoms similar to those of yellow fever and chikungunya. Studies indicate that non-human primates, such as marmosets of the genus *Mico* (endemic to the Brazilian Amazon), serve as amplifying hosts for the virus. However, knowledge gaps remain regarding other potential vertebrate hosts in other Brazilian biomes. We investigated MAYV infection in a convenience sample of 35 free-living xenarthrans in Mato Grosso do Sul: 16 giant anteaters (*Myrmecophaga tridactyla*) from the Cerrado biome, and 16 giant armadillos (*Priodontes maximus*) and 3 six-banded armadillos (*Euphractus sexcinctus*) from the Pantanal biome. The study involved a retrospective serological analysis using the Plaque Reduction Neutralization Test ($PRNT_{50}$) to MAYV BeAr 20290 on serum samples collected between 2015 and 2024. None of the xenarthran samples tested positive in the $PRNT_{50}$ assay. However, these findings represent the first serological data on MAYV exposure in these xenarthran species, reinforcing the relevance of a One Health approach to wildlife surveillance in the prevention of arbovirus outbreaks in urban and peri-urban areas.

Keywords: arbovirus, *Alphavirus*, zoonosis, wildlife, one health

Financial Support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001. INCT Vigilância Genômica de Vírus e Saúde Única (INCT-One).

PT.386

Influenza A Virus Surveillance in Southern Elephant Seals (*Mirounga leonina*) from the Antarctic Peninsula

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Influenza A virus (*Alphainfluenzavirus influenzae; IAV*), a member of the family *Orthomyxoviridae*, is an enveloped virus with a segmented, negative-sense single-stranded RNA genome. IAV infects a wide range of hosts, including domestic and wild birds and mammals, including marine mammals.The migratory movements of Antarctic wildlife may have facilitated the introduction and spread of IAV within Antarctic ecosystems, leading to the detections reported in recent years. Given the ecological importance of Antarctica and the need to protect its wildlife, this study investigated the occurrence of IAV in southern elephant seals (*Mirounga leonina*). During the 2024/2025 and 2025/2026 austral summers, fecal samples were collected from six islands along the Antarctic Peninsula: Livingston, King George, Robert, Snow, Deception, and Nelson Islands, with logistical support from the Brazilian Navy, the Brazilian Antarctic Program (PROANTAR), and the Chilean Antarctic Institute (INACH). Nucleic acids were extracted using the MagMAX™ CORE kit on the KingFisher™ Flex automated platform. IAV RNA was detected by RT-qPCR targeting the conserved matrix (M) gene. A total of 99 fecal samples were analyzed, of which only four, all collected in 2024 in Snow Island, tested positive for IAV. These findings highlight the importance of continued IAV surveillance in Antarctic marine mammals to improve our understanding of viral circulation and support wildlife conservation.

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PT.387

Molecular surveillance of morbillivirus in pennipeds in Antarctica

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Historically, morbilliviruses have been responsible for several mass mortality events in pinniped populations as high-density breeding aggregations favors pathogen transmission. Antarctica hosts unique ecosystems and significant pinniped populations, whose historical isolation is increasingly threatened by climate change and anthropogenic pressures. This raises their vulnerability to emerging pathogens and makes the animal–environmental–human health interface critical in zoonotic spread. The presence and circulation of morbilliviruses in Antarctic pinnipeds remain largely unexplored. Thus, this study performed molecular surveillance of morbillivirus in fecal samples from Southern elephant seals (Mirounga leonina) and Weddell seals (Leptonychotes weddelli) in Antarctica during the 2024/2025 and 2025/2026 austral summers through the XLV Brazilian Antarctic Operation. In ten points of the South Shetland Islands, 154 fecal samples from these pennipeds were collected. Nucleic acids were extracted using MagMAX CORE Nucleic Acid Isolation Kit on the automated KingFisher Flex System. Detection of morbillivirus was performed by RT-qPCR, targeting the P gene of all genus members. No morbillivirus was detected in any of the samples. The absence of morbillivirus RNA in all samples suggests no active viral circulation at the time of sampling. This is consistent with findings from other remote pinniped populations, where molecular detection often yields negative results even when seroprevalence indicates historical exposure. Continued surveillance is therefore essential, and future efforts should include expanded temporal and multi-species sampling to improve understanding of viral circulation in the region.

Financial Support: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq No. 25/2551-0002524-9); CNPq grant 350733/2026-0

PT.388

Enteric Virus Surveillance in Children Hospitalized for Acute Gastroenteritis at a Sentinel Pediatric Hospital in Niterói, Rio de Janeiro, 2024-2026

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Acute gastroenteritis (AGE) is a leading cause of malnutrition, stunting growth and death in children under 5 years old worldwide. Among the several agents, gastroenteric viruses are responsible for over 75% of cases. In Brazil, recent studies focusing on the etiology of severe AGE disease among hospitalized pediatric patients remains limited. This study aims to determine the incidence of three major causes of AGE, rotavirus, norovirus and adenovirus, in children hospitalized for AGE in Niteroi, Rio de Janeiro, Brazil. From March 2024 to March 2026, diarrheic stool samples from children up to 12 years old were systematically collected in a sentinel hospital, and viruses were investigated by using quantitative TaqMan®-based RT-qPCR. A total of 71 stool samples were collected, and 42.2% (30/71) of samples was positive for at least one of the 3 screened viruses. The most prevalent agent was adenovirus, detected in 19.7% of samples, followed by rotavirus and norovirus, detected in 15.5% and 12.7% of samples, respectively. Additionally, we investigated sapovirus and astrovirus, and both were detected in 1.4% of samples each. Co-infections were observed in 16.7% of samples. The highest incidence was found in 12- to 23-month-old children (8/30, 27%). This two-year study demonstrates the circulation of multiple etiological agents of severe AGE cases, especially adenovirus, and highlights the importance of enlarged AGE surveillance in hospital settings.

Financial support: FAPERJ, CNPq PROEP/IOC 441653/2024-3, CGLab and Fiocruz.

PT.389

ENTERIC VIRUS SURVEILLANCE IN CHILDREN HOSPITALIZED FOR ACUTE GASTROENTERITIS AT A SENTINEL PEDRIATIC HOSPITAL IN NITERÓI, RIO DE JANEIRO, 2024-2026.

Calheiros LF[1], Silva LR[1], Assis RMS[1], Baduy GA[1], Mello MS[1], Fumian TM[1], Burlandy FM[1] - [1]Fundação Oswaldo Cruz - FIOCRUZ - Laboratory of Comparative and Environmental Virology - LVCA

Acute gastroenteritis (AGE) is a leading cause of malnutrition, stunting growth and death in children under 5 years old worldwide. Among the several agents, gastroenteric viruses are responsible for over 75% of cases. In Brazil, recent studies focusing on the etiology of severe AGE disease among hospitalized pediatric patients remains limited. This study aims to determine the incidence of three major causes of AGE, rotavirus, norovirus and adenovirus, in children hospitalized for AGE in Niteroi, Rio de Janeiro, Brazil. From March 2024 to March 2026, diarrheic stool samples from children up to 12 years old were systematically collected in a sentinel hospital, and viruses were investigated by using quantitative TaqMan®-based RT-qPCR. A total of 71 stool samples were collected, and 42.2% (30/71) of samples was positive for at least one of the 3 screened viruses. The most prevalent agent was adenovirus, detected in 19.7% of samples, followed by rotavirus and norovirus, detected in 15.5% and 12.7% of samples, respectively. Additionally, we investigated sapovirus and astrovirus, and both were detected in 1.4% of samples each. Co-infections were observed in 16.7% of samples. The highest incidence was found in 12- to 23-month-old children (8/30, 27%). This two-year study demonstrates the circulation of multiple etiological agents of severe AGE cases, especially adenovirus, and highlights the importance of enlarged AGE surveillance in hospital settings.

Financial support: FAPERJ, CNPq PROEP/IOC 441653/2024-3, CGLab and Fiocruz.

PT.390

Epidemiological Surveillance of Arboviruses in Mosquitoes (Diptera: Culicidae) in the State of São Paulo, Brazil

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BACKGROUND: Arboviruses are diseases whose clinical manifestations range from asymptomatic infections to severe and lethal conditions. In Brazil, the most frequently reported diseases in this group are dengue, chikungunya, yellow fever, and Zika. These viruses are predominantly transmitted by mosquitoes (Diptera: Culicidae). **OBJECTIVE** The objective of this study is to investigate the presence of arboviruses in mosquitoes (Diptera: Culicidae) collected from two areas with distinct environmental characteristics in the state of São Paulo, Brazil: (1) a forest edge area, located in the Serra do Mar State Park, and (2) an urban park in the municipality of São Paulo. **METHODS:** In each sampled area, culicids were collected at different times throughout the day. Multiple sampling methods were employed, such as Shannon and CDC traps and active capture. The mosquitoes were pooled into groups of 5 specimens of the same species. For viral RNA detection, RT-qPCR assays were performed for: Dengue, Zika, Chikungunya, Mayaro, Yellow Fever, and West Nile. **RESULTS:** A total of 860 specimens were collected across both sampled areas. In area (1), 436 individuals (50.7%) distributed among 19 species were recorded. In area (2), 424 specimens (49.3%) belonging to 9 species were collected. Of the 860 mosquitoes tested (184 pools), all yielded negative results for the analyzed pathogens. **CONCLUSION:** The lack of viral RNA detection in the analyzed specimens suggests low circulation of these pathogens in the sampled areas. Although all results were negative, continuous monitoring remains highly relevant for epidemiological surveillance. **FINANCIAL SUPPORT:** São Paulo Research Foundation (FAPESP).

PT.391

Non-invasive monitoring of porcine viruses and genomic characterization of porcine circovirus type 2 in family pig farms in Uruguay

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Within the framework of a participatory research project aimed at strengthening the sustainability of family pig production in Uruguay, a non-invasive population monitoring strategy was implemented to detect porcine viruses of sanitary relevance and to deepen the genomic characterization of porcine circovirus type 2 (PCV2). The study was conducted on four farms in southern Uruguay and included the collection of oral fluids using chew ropes suspended in pens, together with fecal samples from pigs of different ages. Samples were analyzed by PCR, RT-PCR, and nested PCR for porcine reproductive and respiratory syndrome virus, swine influenza virus (SIV), PCV2, PCV3, porcine coronaviruses, norovirus, sapovirus, and group A porcine rotavirus (PRVA). PCV2 and PRVA were detected on three farms, SIV on two, and PCV3 on one, whereas the remaining viruses were not identified. Because PCV2 is a major pathogen associated with clinical and subclinical disease and substantial production losses, positive oral fluid and fecal samples were further analyzed through amplification of overlapping genomic fragments, sequencing, assembly, and phylogenetic analysis. Complete and nearly complete PCV2 genomes were successfully recovered from both sample types, confirming the suitability of non-invasive matrices for molecular and genomic surveillance. Phylogenetic analysis identified the circulation of PCV2a and PCV2d in the studied farms. These findings provide relevant information on PCV2 genetic diversity in family production systems and support genomic surveillance as a tool to improve herd health management, monitor circulating variants, and assess their relationship with strains detected in larger commercial pig operations at the national and regional levels.

FINANCIAL SUPPORT: INIA-FPTA 379

PT.392

TRACKING RESPIRATORY VIRUS IN CHILDREN AND ELDERLY PATIENTS DURING 2025 IN THE CAMPINAS METROPOLITAN REGION, SÃO PAULO, BRAZIL

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Respiratory viruses are among the leading causes of human infectious diseases worldwide, particularly affecting young children and older adults, resulting in substantial morbidity, mortality, and economic burden. Because these infections share similar clinical manifestations, such as cough, fever, runny nose, sneezing, sore throat, and fatigue, laboratory diagnosis is essential for their differentiation. This study aimed to monitor the circulation of major respiratory viruses in children and elderly people from the Campinas metropolitan region (São Paulo, Brazil) during 2025. Nasopharyngeal-oropharyngeal swab samples, collected from 294 symptomatic patients aged 0–6 or over 60 years old, were analyzed by probe-based RT-qPCR for the detection of SARS-CoV-2, Influenza A/B (IAV/IBV), HRSV and HMPV, with the molecular and socioeconomic results data analyzed in RStudio environment. Our results demonstrated that HRSV and IAV co-circulated during the same period of the year. HRSV was detected from March to September, peaking in April, and IAV circulated from April to October, with most cases occurring between April and June. Interestingly, the distribution of these viruses differed between age groups: HRSV was predominantly detected in children, whereas IAV was more frequently detected in elderly people. Furthermore, none of the groups were positive for IBV, and all HMPV-positive cases were detected in children during the last months of the year. Similar to IAV, SARS-CoV-2 was predominantly detected in elderly people but showed no clear seasonal peak. These findings provide an overview of the seasonal circulation of major respiratory viruses in the Campinas region during 2025.

Financial Support: CAPES

PT.393

REPORT ON VIROLOGICAL ANALYSES OF FOODS FROM A FOOD AND NUTRITION UNIT (FNU)

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This study reports a virological monitoring of 86 food samples from a Food and Nutrition Unit conducted at the Laboratory of Applied Virology over a 10-month period. Viral concentration of vegetables was performed based on viral elution followed by polyethylene glycol (PEG) precipitation. For animal-derived samples, the protocol was based on viral elution with glycine buffer, followed by concentration via PEG precipitation. Murine Norovirus 1 was used as an internal recovery control. Viral nucleic acids were extracted by a magnetic bead-based methodology, and detection of Norovirus genogroups I and II, Rotavirus A (RVA), Human Adenovirus (HAdV), and Hepatitis A and E viruses (HAV/HEV) was performed in duplicate by StepOnePlus real-time PCR. HAdV was the most prevalent, detected in 34.9% of all samples, with a high prevalence of 53.3% in water/vegetables. RVA was detected in 27.9% of samples. Sporadic detections included NoV GI and HEV in rice samples and NoV GII in three vegetable samples. HAV was not detected. The data suggest a higher occurrence of enteric viruses at the beginning and end of academic semesters, coinciding with higher temperatures and increased tourist influx in Florianópolis, overburdening the sanitation systems. The monitoring demonstrated that enteric viruses can be recurrent in different food matrices and reinforced that virological surveillance constitutes a relevant predictive indicator for failures along the food production chain.

Keywords: food monitoring; qPCR; Rotavirus; Adenovirus; Norovirus.
Financial Support: FAPESC grant number 2024TR002259; CNPq grant number 408073/2023-3; FAPESC grant number 57/2025.

PT.394

MOLECULAR INVESTIGATION OF AVIAN POLYOMAVIRUS AND BEAK AND FEATHER DISEASE VIRUS IN WILD BIRDS FROM THE ATLANTIC FOREST, BRAZIL

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In Brazil, few studies have investigated the occurrence of avian polyomavirus (APV) and beak and feather disease virus (BFDV) in free-ranging wild birds, with most reports involving captive symptomatic psittacines. Parrots are primary hosts of these viruses, although both have also been detected in other avian orders, including birds of prey and passerines. Therefore, this study aimed to assess the viral circulation of APV and BFDV in wild birds from Parque Estadual da Serra do Mar (Núcleo Curucutu), São Paulo State, Brazil. A total of 286 fecal samples of wild birds were collected between February 2022 and August 2023. Nucleic acids were extracted from fecal samples using the commercial IndiMag Pathogen Kit, followed by qPCR targeting the VP1 and Rep genes for APV and BFDV, respectively. A positive control plasmid containing the target sequence and nuclease-free water were used as positive and negative controls, respectively. All fecal samples were negative for both viruses in qPCR assays. The lack of molecular detection of the pathogens in this study is consistent with the limited evidence of these viruses in free-ranging wild birds in Brazil, suggesting that they pose a limited health threat to Brazilian wild birds. Although no evidence of viral circulation was detected in wild birds during the study period, continued surveillance of APV and BFDV remains important considering that no specific treatment is available, and infections caused by these viruses may pose a threat to the conservation of susceptible wild avian species.

Key-words: surveillance, wild-birds, PCR.
Financial Support: FAPESP (2025/00448-9).

PT.395

Tracking Influenza A Across Flyways: Epidemiological Surveillance in Wild Birds on the Brazilian Coast

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Influenza A viruses (IAVs; family *Orthomyxoviridae*) are a major One Health concern due to their genetic diversity and zoonotic and pandemic potential. Wild aquatic birds of the orders Anseriformes and Charadriiformes are recognized as natural reservoirs, while migratory species contribute to viral maintenance and dissemination across flyways. This study investigates IAV circulation in migratory and resident wild birds along the Brazilian coast. Birds are captured with mist nets at feeding and resting sites in Rio de Janeiro and Santa Catarina states, while nestlings are manually captured at coastal nesting colonies in Rio de Janeiro state. Cloacal swabs and/or feces are collected. Cloacal swabs from debilitated seabirds admitted to the Albatroz Institute (Beach Monitoring Project of Campos and Espírito Santo Basins) are also included. Samples will be screened by RT-qPCR, and positive samples will undergo Illumina next-generation sequencing (NGS). To date, 586 birds have been sampled: 564 (96.2%) were alive and 22 (3.8%) were found dead due mainly to trauma. Most birds (89.24%, n= 523) were apparently healthy and captured in the field, while 63 of them (10.75%) originated from the Albatroz Institute presenting mostly exhaustion or trauma during admission. Overall, 526 (89.8%) were migratory and 60 (10.2%) were residents. Regarding developmental stage, 339 (57.84%) were nestlings, 25 (4.26%) juveniles, and 222 (37.9%) adults. The findings are expected to improve knowledge of IAV circulation and genetic diversity in Brazilian coastal ecosystems, supporting One Health surveillance strategies.
Financial support: CAPES and FAPERJ

PT.396

MOLECULAR DIAGNOSIS OF DENGUE USING INFRARED SPECTROSCOPY AND MACHINE LEARNING

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Dengue remains one of the most relevant arboviral diseases worldwide, where rapid and accurate diagnosis is essential for epidemiological surveillance and clinical management. Conventional serological and molecular assays present important limitations, especially during the first five days after symptom onset, when antibody-based tests often fail to provide reliable results and molecular techniques such as PCR become mandatory, increasing costs and diagnostic complexity. In this study, we evaluated the potential of Fourier Transform Infrared (FTIR) and Near-Infrared (NIR) spectroscopy combined with machine learning for rapid, reagent-free dengue detection using serum samples collected within the first five days of symptoms. A total of 80 serum samples were analyzed, including 40 dengue-positive samples confirmed by RT-PCR and 40 negative controls. FTIR spectra were acquired in the 4000–400 cm^{-1} range and preprocessed using SNV normalization. NIR spectra were acquired in the 1100–2700 nm region and preprocessed using second-derivative transformation. Principal component analysis (PCA) was used for dimensionality reduction, while support vector machine (SVM) classifiers were employed for sample discrimination. The spectral profiles revealed biochemical alterations associated with proteins, lipids, and immune-response-related biomolecules. PCA demonstrated a clear clustering tendency between dengue-positive and control samples. The optimized SVM model achieved accuracy values above 90%, demonstrating the feasibility of infrared spectroscopy as a complementary diagnostic strategy for dengue detection. The proposed approach is rapid, low-cost, minimally invasive, highlighting its potential for screening and point-of-care applications in resource-limited regions.

PT.397

TUSAVIRUS DETECTED IN SERUM SAMPLES FROM SHEEP IN SOUTHERN BRAZIL

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Tusavirus (TuV) belongs to the genus *Protoparvovirus* (*Parvoviridae*) with a broad host range, including humans and domestic ruminants, raising concerns regarding its zoonotic potential. Direct virus and IgG detection remain rare. Here, we report the full-length genomic sequence and phylogenetic characterization of a novel TuV isolate (TuV 80364) detected in serum samples from sheep in southern Brazil via high-throughput sequencing. Phylogenetic analysis based on the NS1 protein and whole-genome sequences classified the isolate within the species *Protoparvovirus incertum1*. The novel strain occupied an intermediate phylogenetic position, bridging human- and ruminant-derived clades. Comparative mutation analysis revealed 55 amino acid substitutions among TuV strains, with 45 shared with human-hosted prototypes, 6 with ovine-derived strains, and 4 unique mutations. These substitutions were primarily localized in solvent-exposed variable regions (VRs) of the capsid, specifically between VR6 and VR8. Although these mutations suggest significant evolutionary pressure, electrostatic surface potential analysis indicated that the local charge distribution is strictly conserved, suggesting that the observed changes facilitate subtle refinements in capsid surface morphology rather than drastic alterations in fundamental binding properties. The identification of this TuV isolate in sheep serum confirms its capacity to cause viremia in asymptomatic animals and highlights its evolutionary mosaicism. The concentration of unique mutations within the VR8 loop—a known determinant of host tropism and immune interaction—underscores the potential for ongoing adaptation at the host interface. These findings contribute to the understanding of the TuV host range and emphasize the relevance of continuous genomic monitoring within a One Health framework.

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PT.398

ENVIRONMENTAL SURVEILLANCE OF NOROVIRUS GENOGROUPS I AND II IN THE GUAÍBA WATER SYSTEM, SOUTHERN BRAZIL

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Noroviruses are the leading causes of acute viral gastroenteritis worldwide and are commonly introduced into aquatic environments through sewage contamination. Monitoring their occurrence in urban surface waters provides valuable information on populational viral circulation and environmental contamination, particularly because norovirus infections are frequently underdiagnosed. The Guaíba water system receives water from 251 municipalities from Rio Grande do Sul state (Brazil) and undergoes substantial hydrological changes during seasonal flooding, making it an important target for environmental surveillance. This study investigated the occurrence of Norovirus genogroups I (NoV-GI) and II (NoV-GII) in surface water samples from Guaíba. Water samples were collected quarterly at 17 monitoring sites distributed along the Guaíba shoreline. After concentration by ultracentrifugation, RNA extraction was performed using the Maxwell® automated system. Molecular detection of both viruses was performed using a TaqMan RT-qPCR assay targeting ORF2/ORF3. During the first sampling campaign (March 2026), NoV-GI RNA was detected in 4/17 samples, whereas no samples were positive for NoV-GII. In samples from the second campaign (May 2026), genomes from neither Norovirus GI nor GII were detected. These findings demonstrate the presence of Norovirus GI in the monitored watershed and reinforce the value of environmental surveillance as a complementary approach for monitoring viral gastroenteritis agents. Continued monitoring throughout the project will improve the understanding of temporal patterns of viral occurrence and their relationship with environmental and hydrological conditions. Effective superficial water surveillance is critical for safeguarding recreational and drinking water resources and informing public health interventions in advance of and following extreme weather events.
Financial Support: CNPq; FAPERGS (25/2551-0002524-9)

PT.399

SCREENING FOR HIV AND VIRAL HEPATITIS B AND C IN PREGNANT WOMEN IN THE MUNICIPALITY OF BREVES, MARAJÓ ARCHIPELAGO, PARÁ

Vale RC[1], Freitas CES[2], Cardoso LC[2], Vilhena EM[1], Rodrigues RS[3], Pinho ECC[1], Santos JDB[4], Freitas FB[5], Machado LFA[6] - [1]Universidade Federal do Pará - Programa de pós graduação em biologia dos agentes infecciosos e parasitários, [2]Laboratório Municipal de Breves, [3]Laboratório de Virologia, [4]Universidade Federal do Pará - Laboratório de Virologia, [5]Instituto Evandro Chagas, [6]Universidade Federal do Pará - Instituto de Ciências Biológicas - Laboratório de Virologia

Infection by human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV) during pregnancy represents a major public health problem due to the risk of vertical transmission. Screening for these infections during prenatal care allows for early diagnosis and measures to prevent mother-to-child transmission. However, information on the occurrence of these infections in pregnant women residing in the Marajó archipelago remains limited. This study aimed to evaluate screening results for HIV, HBV, and HCV in pregnant women treated in the public health network of Breves, Pará. A cross-sectional study was conducted with 91 pregnant women. Screening was performed using rapid tests, and HIV samples were additionally subjected to a fourth-generation immunoassay (ELISA HIV-1/2 Ag/Ab). Sociodemographic and behavioral data were also collected using a standardized questionnaire. No pregnant woman presented a reactive result for HIV, HBV, or HCV. The mean age of participants was 24.3 ± 6.9 years; 53.8% had not completed elementary school; 65.9% had a family income below one minimum wage; 45.6% reported sexual initiation between 13 and 15 years of age; and 85.7% reported not using condoms during sexual intercourse. Although no HIV, HBV, or HCV infections were identified in the evaluated population, a high frequency of vulnerability characteristics was observed. These findings reinforce the importance of maintaining universal prenatal screening and strengthening vertical transmission prevention actions in Breves.
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PT.400

Monitoring Enteric Viruses in Cultivated Oysters (*Magallana gigas* and *Crassostrea gasar*) as Bioindicators of Coastal Sanitary Quality in Florianópolis, Brazil

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Oysters are economically important bivalve mollusks used as sentinels for environmental monitoring. As non-selective filter-feeding, they bioaccumulate microorganisms, including enteric viruses, making them indicators of fecal contamination and coastal sanitary quality. Previous studies published between 2003 and 2018 reported enteric viruses in shellfish-growing areas of Santa Catarina. In the early 2000s, hepatitis A virus (HAV) and human adenovirus (HAdV) were detected in 22% and 90% of oyster samples, respectively, highlighting the historical circulation of enteric viruses in coastal cultivation areas. This study monitored the occurrence of enteric viruses in oysters collected in March 2026 from Florianópolis, Santa Catarina, Southern Brazil. Ten specimens each of *Magallana gigas* and *Crassostrea gasar* were collected. Tissue samples from each species were pooled, concentrated by polyethylene glycol (PEG) precipitation, and analyzed by PCR/RT-qPCR for HAdV, norovirus genogroups I and II, HAV, and hepatitis E virus. No viral genetic material was detected. The absence of enteric viruses differs from previous reports in Santa Catarina, suggesting favorable sanitary conditions during the sampling period and lower fecal contamination of the coastal environment. These findings highlight the importance of continuous environmental surveillance and demonstrate the potential of M. gigas and C. gasar as sentinel species for early detection and long-term monitoring of pathogen circulation in coastal ecosystems.

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PT.401

Serological survey of western equine encephalitis virus in domestic animals from indigenous communities of the Pantanal, west-central Brazil

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Western equine encephalitis virus (Alphavirus western or WEEV) is a zoonotic arbovirus believed to be transmitted in Brazil by *Culex* and *Aedes* mosquitoes. Wild species of birds and mammals are considered amplifying hosts. Between 2023 and 2024, a new lineage C of WEEV caused an important outbreak of neurological disorder in equids and humans in Uruguay and Argentina. Although exposure to WEEV has been reported in Brazilian equids for decades, its circulation in other vertebrate hosts and the true extent of the virus's circulation in the country remains unknown. Indigenous communities are susceptible to zoonotic viruses due to greater contact with non-human animals and limited access to healthcare services. However, little is known about WEEV circulation in these territories. In this context, serum samples from 36 domestic birds, 29 dogs, seven equids, six pigs, two cattle and one domestic cat sampled in 2025 from the communities of Guató and Bororo in the Pantanal of Mato Grosso were tested for WEEV antibodies. All sera were seronegative by plaque reduction neutralization test ($PRNT_{90}$) for WEEV suggesting no exposure of the animals tested. The continuous monitoring of zoonotic viruses in indigenous communities contributes to arbovirus surveillance in Brazil and it should be encouraged as part of a one health approach. Financial Support: FAPEMIG, CAPES, CNPq e Funarbe.

Keywords: equids; Mato Grosso; zoonotic; surveillance; arbovirus.

Thematic Area: Veterinary Virology; Viral Epidemiology and Public Health

PT.402

INVESTIGATION OF ILHEUS VIRUS EXPOSURE IN BROWN BOOBIES (*Sula leucogaster*) AND FRIGATEBIRDS (*Fregata magnificens*) IN THE NATURAL MONUMENT OF THE CAGARRAS ISLANDS ARCHIPELAGO, RIO DE JANEIRO, SOUTHEAST BRAZIL

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The Ilheus virus (*Orthoflavivirus ilheusense,* ILHV), classified to the *Flaviviridae* family, is an arbovirus that circulate in tropical regions of the Americas, including Brazil. ILHV has medical importance, as it can cause febrile and neurological disorders in humans. In Brazil, ILHV is believed to be maintained between species of mosquitoes (*Psorophora ferox*, *Aedes scapularis* and *Aedes serratus*) and wild passerine birds, such as double-collared seedeater (*Sporophila caerulescens*) and shiny cowbird (*Molothrus bonariensis*). The exposure to ILHV of other groups of birds in Brazil, such as marine species of the Fregatidae and Sulidae families, has been even less explored. In this scenario, this study aimed to investigate the exposure to ILHV in brown boobies (*Sula leucogaster*) and frigatebirds (*Fregata magnificens*) from the Natural Monument of the Cagarras Islands Archipelago, Rio de Janeiro, Brazil. A total of 54 brown boobies and 38 frigatebirds were captured during February/2022 and April/2024 and had blood sample collected. Sera were tested by plaque reduction neutralization test (PRNT) for ILHV (GenBank: OP947886.1). All sera presented $PRNT_{90}$ titer <20 for specific neutralizing antibodies for ILHV and were then considered seronegative. These findings suggest that the subset of brown boobies and frigatebirds tested has not been exposed to ILHV. The continuous monitoring of zoonotic viruses in seabird populations in sites of interest as Natural Monument of the Cagarras Islands Archipelago contribute to arbovirus surveillance along the Brazilian coast and it should be encouraged as part of a one health approach. Financial Support: FAPEMIG, CAPES, CNPq, Funarbe and ICMBio.

PT.403

Comparative nucleotide and amino acid sequence analyses reveal distinct phylogenetic patterns in BVDV-2 and a putative novel antigenic cluster

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Bovine viral diarrhea viruses (BVDVs) exhibit high genetic variability, comprising two viral species (BVDV-1, BVDV-2) and several subtypes. These classifications are mainly based on nucleotide (nt) sequence analysis and may not fully reflect the antigenic diversity of BVDVs, which is particularly relevant for serological assays and vaccine formulation. Herein, we evaluated the concordance between BVDV-2 phylogenetic classifications based on nt and amino acid (aa) sequences, exploring how potential discrepancies would improve our understanding of antigenic relationships among different BVDVs. We collected 258 complete BVDV-2 coding region (CDS) sequences (GenBank). After curation, alignment and trimming, four datasets were generated: nt and aa datasets for the CDS, as well as corresponding nt and aa datasets for the E2 gene/protein, the primary target of neutralizing antibodies. Maximum-likelihood phylogenetic trees and pairwise distance matrices were generated for each dataset. The greatest differences were observed in the phylogenetic trees and distance matrices based on the E2 aa sequences, in which a novel BVDV-2 cluster, termed BVDV-2f, was identified. Furthermore, several BVDV-2 sequences that could not be consistently clustered based on CDS nt and aa analyses were confidently assigned using the E2 aa analysis, whereas others were redistributed into different groups in the E2 aa phylogeny. Overall, these findings support that E2 aa-based analyses may yield classifications that differ from the nt-based analyses commonly used for BVDV classification. These findings warrant further investigation to determine whether they reflect the circulation of distinct BVDV-2 serotypes, which would have implications for vaccinology and serological diagnosis.

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PT.404

EXPLORING THE MIDGUT VIROME OF THE BANANA WEEVIL (*Cosmopolites sordidus*) FROM BRAZIL

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The banana weevil (*Cosmopolites sordidus*) is the most important insect pest of banana crops worldwide. This study aimed to explore the virome associated with the midgut of this curculionid. Thirty adult weevils were collected from a banana plantation in Pariquera-Açu, São Paulo. After 72h of fasting, midgut tissues were dissected and pooled in groups of ten. Nucleic acids were extracted, and six libraries (Total RNA and mRNA) were prepared and sequenced on NextSeq 500. Raw data were preprocessed, assembled with SPAdes, and classified using the Read Annotation Tool (RAT), followed by BLASTx and phylogenetic analysis. A total of 23 viral contigs with an average coverage ≥20× were assigned to four DNA virus families, including six contigs of *Adintoviridae* (483–1,252 bp), three of *Parvoviridae* (3,828–6,470 bp), two of *Polydnaviriformidae* (389 and 463 bp), and six of *Poxviridae* (968–1,868 bp). Two additional contigs were assigned only to the closest species-level reference (Yalta virus), whereas four showed no RAT support but were similar to *Caudoviricetes* spp. by BLASTx. Although no RNA viruses were detected, the exclusive detection of DNA viruses may indicate transcriptional activity during the sampling period. However, the presence of residual viral DNA that was not completely removed during sample processing cannot be excluded. This study provides the first overview of the midgut virome of *Cosmopolites sordidus*, expanding current knowledge of the viral diversity associated with this economically important agricultural pest and supporting the discovery of viral biocontrol agents. Financial support: EMBRAPA

PT.405

Characterization of a Tupanvirus TylF-like O-methyltransferase supports a broader viral repertoire of antibiotic-associated tailoring functions

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Giant viruses belonging to the phylum *Nucleocytoviricota* possess remarkably complex gene repertoires; however, their capacity to encode enzymes involved in small-molecule modification remains largely unexplored. In this study, we identified and characterized a conserved TylF-like O-methyltransferase encoded by the genomes of *Tupanvirus salinus* and *Tupanvirus altamarinense*. This enzyme family is primarily recognized in bacteria for its role in the late-stage tailoring of antibiotics and other specialized metabolites. Sequence analyses, domain prediction, structural comparisons, and molecular docking support the classification of the viral proteins as TylF-like sugar O-methyltransferases, preserving key catalytic and cofactor-binding features compatible with a structural role in deoxysugar tailoring reactions. Phylogenetic analyses place the tupanvirus enzymes within the broader TylF-like family while revealing a distinct viral lineage, suggesting acquisition from a cellular ancestor followed by diversification in giant viruses. Motivated by these findings, we further examined both tupanvirus genomes for additional enzyme families commonly associated with small-molecule tailoring and identified a broader enzymatic repertoire, including nonribosomal peptide synthetase-related proteins, cytochrome P450 monooxygenases, glycosyltransferases, NAD-dependent epimerase/dehydratase-like enzymes, and ABC-type transport-associated proteins. Although these genes do not constitute a canonical antibiotic biosynthetic pathway, their combined occurrence is consistent with a viral enzymatic repertoire potentially involved in oxidation, glycosylation, sugar modification, and other tailoring reactions. Overall, these findings broaden the currently recognized functional potential of giant virus genomes and provide evidence that tupanviruses encode enzyme classes plausibly linked to small-molecule tailoring.

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PT.406

BEYOND THE BASIC STRUCTURE: THE EVOLUTIONARY COMPLEXITY OF THE VP54 CAPSID PROTEIN IN CHLOROVIRUSES

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With pseudo-icosahedral symmetry and a structure composed of 30 distinct proteins, the chlorovirus capsid exhibits a structural complexity that is unique and fascinating in the world of viruses. In addition to the characteristic spike located at the structure's single vertex and the numerous secondary proteins that ensure the stability and integrity of the outer layer, the major capsid protein (MCP Vp54) itself has demonstrated increasing genetic and structural diversity as new viral species are described. Previous analyses, based on genetic sequence alignment, identified that certain alphachlorovirus isolates appeared to harbor additional copies of Vp54. To thoroughly investigate the evolutionary history of this essential gene, we performed a phylogenetic reconstruction using the maximum-likelihood method using IQTree3 software. The dataset included Vp54 sequences from all known chloroviruses, encompassing previously identified paralogs, using Ostreococcus tauri viruses as outgroups for tree rooting. Visualization and aesthetic refinement of the resulting topology were conducted on the online server iTol. The results of the phylogenetic reconstruction indicated that Vp54-like paralogs do not share a single origin, but arose through independent and relatively recent gene duplication events within restricted clades. This study highlights the critical importance of analyzing multiple viral isolates to reveal the true extent of genetic diversity. It is concluded that the evolution of the viral capsid is a significantly more dynamic and complex process than previously assumed, shaped by frequent events of genomic variation.

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PT.407

DIVERSITY OF VIRGA-LIKE VIRUSES IN WILD-CAUGHT CULICIDS

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Viral metagenomics of culicid hosts is an widely applied approach for arbovirus surveillance and general viral discovery. This study investigated viral genomes from wild-caught culicids collected in the Marajo archipelago, municipality of Breves – PA, Brazil, in 2025. The specimens were pooled according to the species, collection site and date. Total RNA was purified following filtering and nuclease digestion-based viral enrichment. Samples were sequenced in the NextSeq 500 platform (Illumina). Raw sequencing data were filtered for low quality and rRNA reads and then assembled into contigs, which were taxonomically classified. Three complete Virga-like viral genomes were identified in three different host species: Uranotaenia lowii Virga-like virus 1 (9,130 nt), Anopheles intermedius Virga-like virus 1 (10,211 nt) and Limatus durhamii Virga-like virus 1 (8,785 nt). Genomes were annotated based on the closest reference and conserved protein domains were predicted. The highest nucleotide and amino acid identity with known viruses ranged from 45.2 - 74.27% and 34.85 - 78.53%, respectively. A phylogenetic inference was performed based on the predicted sequence of the non-structural polyprotein with high support of the maximum-likelihood mapping test (96.0%). The isolation of the sequences in different clades and their relatively high nucleotide and amino acid divergence suggests the existence of far more diversity among viruses related to the plant-associated *Virgaviridae* family than previously thought. In addition to our findings, entire new branches of insect-associated Virga-like viruses have been described and may contribute to the official taxonomic classification of these agents.

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PT.408

MULTIPLE MYCOVIRUS INFECTION AND PUTATIVE HORIZONTAL GENE TRANSFER BETWEEN dsRNA AND ssRNA(-) VIRUSES IN *Pochonia chlamydosporia*

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Mycoviruses are viruses that infect fungi and are often associated with latent infections or subtle phenotypic effects, including hypovirulence and hypervirulence. Most mycoviruses possess RNA genomes (dsRNA, +ssRNA, and –ssRNA). In this study, we investigated the presence of mycoviruses infecting *Pochonia chlamydosporia*. Those micoviruses were obtained by total nucleic acid extraction followed by treatment with S1 and RQ1 nucleases. Sequencing was performed in-house using the Illumina sequencing platform. Raw reads were quality-filtered with Trimmomatic, and the assembly was performed with rnaSPAdes. Using BLASTp searches revealed a co-infection of two mycoviruses. The first virus, named Pochonia chlamydosporia chrysovirus 1 (PcCV1), was identified as a dsRNA virus with four genomic segments and classified within the genus *Alphacrysovirus*. The second virus, named Pochonia chlamydosporia negative-stranded RNA virus 1 (PcNSRV1), has a ssRNA(-) genome with a single segment of 11,212 bp and was classified within the genus *Mymonavirus*. Comparative genomic analyses revealed that ORF V of PcNSRV1 shares 99% coverage and 99.73% amino acid identity with a hypothetical protein encoded by the third genomic segment of PcCV1. BLASTp also identified a similarity to the homologous protein encoded by the third segment of Ilyonectria pseudodestructans chrysovirus 1, with 99% coverage and 58.94% identity. Additional analyses are required to further investigate the evolutionary origin of this shared genomic region. These results reveal a multiple mycovirus infection in *Pochonia chlamydosporia* and provide evidence of a potential horizontal gene transfer event between mycoviruses distinct genomic structure (dsRNA and ssRNA).

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PT.409

MOLECULAR CHARACTERIZATION OF AVIAN ADENOVIRUSES IN SOUTHERN BRAZIL

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Avian adenoviruses (*Aviadenovirus sp.*) affect poultry production as secondary pathogens or primary agents of emerging diseases. Five species are relevant to broilers: *A. ventriculi*, *A. quintum*, *A. hydropericardii*, *A. gallinae*, and *A. hepatitidis* (formerly Fowl aviadenovirus species A–E, respectively). This study characterized 20 Aviadenovirus isolates by partial sequencing. Samples, received for diagnostic investigation, were collected between 2022 and 2025 in the South (n=15), Southeast (n=3), and Central-West (n=2) regions of Brazil. Sixteen samples were obtained from broilers and comprised liver (n=5), bursa (n=3), trachea (n=2), heart (n=2), kidney (n=1), spleen (n=1), intestine (n=1) and joint (n=1). Four additional samples consisted of water collected from poultry establishments. Viral DNA was extracted using an automated system. Samples were isolated into embryonated chicken eggs or cell cultures, confirmed by molecular detection, and stored at −70 °C. A 959-bp fragment of the *hexon* gene was amplified by PCR, purified, and sequenced. Sequences were aligned with GenBank references representing five FAdV species and 12 serotypes, followed by phylogenetic analysis using MEGA 12. All water isolates were identified as *A. ventriculi* (serotype 1). Among avian isolates, 10 belonged to *A. hepatitidis* (serotypes 8a and 8b), four to *A. ventriculi* (serotype 1), and two to *A. gallinae* (serotype 11). *A. ventriculi* is associated with adenoviral gizzard erosion, whereas *A. gallinae* and *A. hepatitidis* are associated with inclusion body hepatitis. The detection of diverse Aviadenovirus species highlights the importance of molecular surveillance for disease monitoring, prevention, and control.

Financial support: Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina (FAPESC).

PT.410

DISSEMINATION PATTERNS OF ANTIRETROVIRAL RESISTANCE MUTATIONS IN HIV-1 SUBTYPES B, C AND F1 IN BRAZIL BETWEEN 2008 AND 2017

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The co-circulation of human immunodeficiency virus type 1 (HIV-1) subtypes B, C, and F1 creates a complex resistance surveillance landscape in Brazil. This study investigated the evolutionary and geographic dynamics of drug resistance mutations among these subtypes between 2008 and 2017. We analyzed 55,165 HIV-1 pol sequences combining national genotyping data with international sequences, performed maximum-likelihood phylogenetic reconstruction, and identified transmission clusters using bootstrap support ≥70% and genetic distance ≤2.5%. Drug resistance was classified according to World Health Organization criteria. We characterized 1,163 transmission clusters comprising 2,418 sequences, with a highly fragmented network dominated by pairs of connected sequences (over 90%). Subtype B showed the highest resistance prevalence in clusters (66.9%), followed by F1 (58.1%) and C (49.9%). The Northeast and North regions emerged as high-risk areas for resistance transmission, while São Paulo functioned as the main national hub, accounting for 45% of interstate dissemination events. Temporal analysis revealed a marked shift in nucleoside-analogue resistance mechanisms: legacy mutations declined substantially, while the K65R mutation, associated with tenofovir resistance, increased from absence in 2008 to 8.4% in 2017, becoming responsible for 48.1% of tenofovir resistance cases by that year. Most K65R-carrying sequences harbored compensatory mutations, indicating circulation of highly fit resistant lineages. These findings reveal a substantial reconfiguration of Brazil's resistance landscape, challenging current tenofovir-based first-line regimens and reinforcing the need for regionalized molecular surveillance and pre-treatment genotyping in high-risk areas.

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PT.411

A solid workflow to detect recombination in Enterovirus C

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Recombination is a major evolutionary mechanism in Enterovirus C, but its detection remains challenging due to frequent false-positive signals. This study investigated recombination in Enterovirus C using a multi-step validation workflow. Complete Enterovirus C genomes were retrieved from NCBI Virus. Maximum-likelihood phylogenies were inferred with IQ-TREE v2.0.7. Recombination events were initially identified using RDP5. Candidate events were retained when supported by at least three detection methods from two independent statistical approaches, exhibited a minimum recombinant fragment of 50 bp. Events involving recombinant major parents were re-evaluated excluding the parental sequence to eliminate secondary recombination signals. Remaining candidates were validated through maximum-likelihood phylogenetic analyses of recombinant and non-recombinant genomic regions and by bootscanning in SimPlot. Events were classified as confirmed, highly probable, probable, or improbable according to the concordance between phylogenetic and bootscanning evidence. RDP5 identified 36 recombination events. After validation, 7 were confirmed, 8 highly probable, 4 probable, and 17 improbable, indicating that nearly half of the initial candidates lacked independent phylogenetic or bootscanning support. Supported events occurred throughout the genome, with breakpoint clustering mainly near the 5′UTR/VP4 and P3 regions. Fewer breakpoints were detected across the central genome. Recombinant fragments nevertheless spanned extensive genomic regions. Nearly half of the RDP5 candidates lacked independent support, highlighting the importance of complementary validation. Although recombinant fragments frequently encompassed structural genes, breakpoint clustering was concentrated mostly outside of the P1 region, suggesting that purifying selection may eliminate recombination events that disrupt structural proteins while preserving exchanges involving intact genomic regions

PT.412

Effects of micropollutants on HAdV replication: from acute infection at 37 °C to environmental persistence in aquatic microcosms

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The interaction between contaminants of emerging concern and enteric viruses is a relevant interface within the One Health framework. This study evaluated how five micropollutants - carbamazepine, fluoxetine, amoxicillin, sulfamethoxazole, and plastic microfibers - affect human adenovirus (HAdV) replication under two complementary experimental scenarios. The first simulated acute host exposure, incubating virus and pollutant together for 2 min at 37 °C before infecting host cells. The second assessed viral persistence in aquatic microcosms maintained at 22–25 °C for 10 days using environmentally relevant pollutant concentrations (0.01 µg/L). Viral replication was quantified by RT-qPCR targeting HAdV mRNA 24 h after infection. Under acute exposure, all five pollutants significantly increased viral replication by up to 2 logs, suggesting that, at physiological temperature, these compounds can enhance viral infectivity. In contrast, under environmentally realistic conditions, none of the treatments altered viral decay over the 10-day period, with treatment curves overlapping the control, indicating that this effect does not persist in the environment. These findings highlight the importance of distinguishing experimental scales: acute assays at host temperature are suitable for investigating mechanisms of virus–pollutant interactions during infection, whereas microcosm assays are essential for evaluating environmental persistence and ecological risk. Although these micropollutants enhanced HAdV replication under physiological conditions, their influence appears negligible under environmentally relevant exposure, reinforcing the need for multi-scale approaches when assessing the public health implications of virus–pollutant interactions in water systems.

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PT.413

DISCOVERY OF pTJK, A NOVEL *MAMMALIICOCCUS* LENTUS BACTERIOPHAGE WITH BROAD HOST RANGE, ANTIBIOFILM ACTIVITY, AND SYNERGISTIC INTERACTIONS WITH ANTIMICROBIALS AGAINST RESISTANT *STAPHYLOCOCCACEAE*

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Antimicrobial resistance among *Staphylococcaceae* represents a growing threat to human and animal health, highlighting the need for alternative antimicrobial strategies. This study aimed to isolate and characterize a bacteriophage targeting *Mammaliicoccus* lentus and evaluate its therapeutic potential against resistant *Staphylococcaceae* . Phage pTJK and its host strain, M. lentus TJK24, were isolated from sewage-contaminated stream water and characterized by host-range analysis, transmission electron microscopy, stability assays, antibiofilm and phage antibiotic synergy tests, whole-genome sequencing, and comparative genomic analyses. pTJK is a novel lytic bacteriophage with a broad host range, infecting multiple *Mammaliicoccus* and *Staphylococcus* species. The phage significantly inhibited biofilm formation and disrupted established biofilms, including those produced by non-host strains and demonstrated synergistic antibacterial activity when combined with gentamicin and erythromycin. pTJK remained stable under moderate temperature and pH variations, ultraviolet exposure and chloroform treatment, supporting its robustness for potential therapeutic applications. Genome sequencing revealed a 135.8 kb double-stranded DNA genome containing 231 predicted open reading frames, including multiple endolysin- and depolymerase-associated genes that may contribute to its antibiofilm activity, while no antimicrobial resistance or bacterial virulence genes were detected. Comparative genomic analysis classified pTJK within the genus Sciuriunavirus, representing a previously undescribed species. These findings identify pTJK as one of the first reported bacteriophages infecting M. lentus and demonstrate its genomic uniqueness, broad antibacterial spectrum, antibiofilm capacity, and phage-antibiotic synergy, supporting its potential as a safe and promising biotechnological tool for combating antimicrobial-resistant *Staphylococcaceae*

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PT.414

LARGEST BRAZILIAN DATASET OF HTLV-1 COMPLETE GENOMES REVEALS MULTIPLE INTRODUCTIONS AND APOBEC-ASSOCIATED DEFECTIVE VARIANTS

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Historically, HTLV-1 has been considered to exhibit limited genomic diversity. However, few studies have used full-length HTLV-1 genome sequences to compare phylogenetic patterns across clinical presentations or to reassess the virus's transmission history through phylodynamic analyses. Expanding the number of available complete genomes and conducting more comprehensive phylogenetic and phylodynamic analyses are therefore essential to test this prevailing assumption and improve our understanding of HTLV-1 evolution and transmission. This study aimed to characterize complete HTLV-1 genomes obtained from individuals, including subjects with and without HAM/TSP. A total of 85 HTLV-1-positive samples were subjected to whole-genome sequencing using NGS. High-quality complete genomes were successfully obtained from 73 samples (85.9%). Phylogenetic analyses showed that all sequences belonged to the Cosmopolitan subtype, Transcontinental subgroup. Brazilian genomes were broadly dispersed throughout the phylogenetic tree, supporting multiple independent introductions of HTLV-1 into the studied population. In addition, four consensus genomes presented premature stop codons associated with GG→AG substitutions, a mutational signature consistent with APOBEC-mediated editing. However, read-level inspection of these genomic regions revealed the coexistence with non-mutated variants within the samples, indicating that the truncating mutations were not fixed in the viral population and may represent subpopulations generated during ongoing editing events. The mutational profiles associated with different symptoms are currently being analyzed. To our knowledge, this study constitutes the largest Brazilian collection of HTLV-1 complete genomes reported to date and provides new insights into the evolutionary history and intrahost diversity of HTLV-1.
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PT.415

GENOMIC SURVEILLANCE REVEALS NOVEL H3N2 INFLUENZA A VIRUS CLADES IN BRAZILIAN SWINE

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Influenza A virus H3N2 exhibits extensive genetic diversity and plays an important role in viral evolution through reassortment and interspecies transmission. However, the diversity of H3N2 circulating in Brazilian swine remains incompletely understood. This study investigated the evolutionary relationships of the HA gene of H3N2 viruses isolated from nasal swabs and lung tissues of swine from six Brazilian states. Twenty-one H3N2 viruses collected between 2022 and 2025 were sequenced and analyzed with publicly available swine and human H3N2 HA sequences from South America. Maximum-likelihood phylogenetic reconstruction and time-scaled analyses characterized viral lineages and estimated their evolutionary history. Phylogenetic analyses showed that most Brazilian swine viruses (n = 52) clustered within the previously described 1990.5 clade. In addition, seven sequences formed two well-supported monophyletic groups, indicating previously undocumented introductions of human-origin viruses into pigs. One lineage, designated 2010.4, was detected in southern Brazil and had an estimated time to the most recent common ancestor in late 2012. The second lineage, provisionally designated Other-Human-2020, was detected exclusively in Minas Gerais and is estimated to have emerged in 2022. The distinct temporal and geographic patterns observed for these lineages suggest different evolutionary histories and circulation dynamics within swine populations. These findings expand current knowledge of H3N2 genetic diversity in Brazil, demonstrate that previously unrecognized viral clades are circulating in swine, and reinforce the importance of continuous genomic surveillance to monitor viral evolution and identify emerging influenza lineages with potential implications for animal and public health.
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PT.416

TEMPORAL CHANGES IN RESPIRATORY VIRUS CIRCULATION BEFORE, DURING AND AFTER THE COVID-19 PANDEMIC IN BRAZIL

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The COVID-19 pandemic profoundly disrupted the circulation of respiratory viruses worldwide, yet its long-term effects on epidemic timing and viral interactions remain incompletely understood. This study investigated changes in the temporal dynamics of respiratory viruses circulating in Brazil before, during and after the COVID-19 pandemic using national surveillance data. Weekly laboratory-confirmed cases of influenza A(H1N1)pdm09, A(H3N2), influenza B (IBV) and respiratory syncytial virus (RSV) were retrieved from the SIVEP-Gripe surveillance system. Epidemic waves were identified using a four-week growth algorithm, and inter-epidemic intervals were compared among pre-pandemic, pandemic and post-pandemic periods using Gaussian linear models. Additional analyses evaluated the temporal relationship between epidemic onset and peak timing among co-circulating viruses before the pandemic and during the post-pandemic resurgence. During the COVID-19 pandemic, epidemic intervals increased compared with the pre-pandemic period, with the strongest effect observed for influenza A(H3N2). However, no significant differences were detected between the pre- and post-pandemic periods, indicating restoration of historical circulation patterns. Pairwise analyses showed that, before the pandemic, A(H3N2) epidemics consistently occurred after RSV nationwide and in the Tropical Brazil Central climate, and after IBV in the same climate. During post-pandemic resurgence, RSV epidemic onset and peaks consistently preceded those of all influenza viruses. Although the COVID-19 pandemic temporarily altered respiratory virus epidemic periodicity, historical circulation patterns rapidly re-emerged after the relaxation of control measures. The persistent temporal precedence of RSV over influenza suggests stable ecological relationships among respiratory viruses and highlights the value of national surveillance data for monitoring changes in viral circulation dynamics.
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PT.417

BAYESIAN PHYLOGEOGRAPHY OF THE SOUTH AMERICAN AND ANTARCTIC LINEAGE OF H5N1 CLADE 2.3.4.4b HEMAGGLUTININ

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Highly pathogenic avian influenza (HPAI) H5N1 clade 2.3.4.4b became established in South America and expanded to Antarctica, but the country, host, and flyway pathways remain unquantified. We applied Bayesian discrete-trait phylogeography to the hemagglutinin (HA) segment. Hemagglutinin sequences from the Americas (2022-2026) were compiled from GenBank and GISAID; BLAST restricted North American sequences to the closest relatives of each South American introduction, yielding 1,585 HA sequences (688 South American, 897 North American). Maximum-likelihood analysis of the whole dataset identified a major South American and Antarctic lineage (576 sequences) containing seven clades of multiple Brazilian introductions. Datasets were downsampled with Augur to five and ten sequences per country-year, yielding four downsampled HA alignments (whole-dataset: 206 and 316 sequences; major lineage: 129 and 192 sequences); the 192-sequence alignment is reported here. Isolates were annotated by country, host order-type, and flyway. In BEAST, time-scaled trees (general time-reversible model, relaxed clock, constant-size coalescent) carried joint asymmetric reconstructions with Markov-jump counts (500 million generations) and Bayes-factor support. Diffusion followed a Pacific-coast axis (Peru-Chile-Argentina) feeding an Argentina-centred radiation to Brazil, Uruguay, and Bolivia, and a southern corridor into the Antarctic (South Georgia-Antarctica-Chile; Antarctic-Pacific flyway), decisively supported (Bayes factor >100). Seabirds were a consistent source. Sampling bias limits interpretation: the strongest host transitions (mammal-to-seabird, poultry-to-waterfowl) track intensively sequenced poultry and mammal die-offs, and sparse North American sampling prevents resolving individual introductions. Seabirds constitute the Pacific and Antarctic reservoir of this lineage, while continental circulation is waterfowl-driven.

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PT.418

HOST ECOLOGY STRUCTURES THE FLYWAY DISTRIBUTION OF H5N1 CLADE 2.3.4.4b IN THE AMERICAS: SEABIRDS AS THE PACIFIC AND ANTARCTIC RESERVOIR

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The panzootic expansion of highly pathogenic avian influenza (HPAI) H5N1 clade 2.3.4.4b has been shaped by wild-bird migration, but sequence records rarely link viral isolates to host ecology. We enriched American H5N1 sequences with host-ecology metadata and tested how host groups and flyways structure viral distribution. We compiled 11,955 H5N1 sequences (2022-2026) from GenBank and GISAID, with North American sequences restricted by BLAST to the closest relatives of each South American introduction, and annotated host order-type and migratory flyway from AVONET, BIRDBASE, eBird Status and Trends, Birds of the World, and United States shorebird flyways across 150 host species. Host was resolved to species for 6,473 sequences, but flyway was assigned to 7,408, including 935 placed by order or family despite lacking species-level identification. Mammals, poultry, and domestic birds (2,567 sequences) fall outside migratory-flyway categories. Per-species flyway percentages combined eBird seasonal ranges with Pacific, Central, and Atlantic boundaries; all sequences were georeferenced (2,788, 23%, only to country level) and mapped to flyway zones. Host groups differed sharply: seabirds showed the highest Pacific (32%) and the only Antarctic (4%) range affiliation, whereas waterfowl and raptors were continental. This mapped onto sampling: seabirds were the leading host in the Pacific zone (1,163, 48%), whereas waterfowl dominated the Central zone (1,666, 45%; seabirds 378, 10%), and seabirds carried nearly all Antarctic samples. Seabirds constitute the Pacific and Antarctic reservoir of H5N1 clade 2.3.4.4b, while continental circulation is waterfowl-dominated, identifying pelagic seabird surveillance as critical for southward spread.

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PT.419

Replication cycle of Condorvirus: a novel mimivirus-like particle isolated in São Paulo

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Giant Viruses (GVs) have been challenging classical concepts in virology due to their large particle sizes, high genomic complexity, and the ability to encode functions previously considered exclusive to cellular organisms. In addition to this, GVs play central ecological roles on their host population dynamics, as well as in the maintenance of global biogeochemical cycles, even though much of their morphological, functional and host diversity remains unexplored. In a previous survey performed in Lagoa do Taquaral (Campinas, São Paulo), four giant virus isolates, named Condor, Flandres, Schiavo and Salvator, were recovered using *Acanthamoeba castelanii* as an isolation platform. Initial characteization by negative staining transmission electro microscopy (TEM) revealed that all isolates displayed morphologies compatible to mimivirus-like particles. In the present work, we advances the characterization of one representative isolate, Condorvirus, by surveying its replication cycle in *A. castelanii* using TEM. Key points were observed: 15 min post-infection (p.i.), particle internalization and uncoating; at 2 h.p.i., host nuclei was still present among many virions; 4 h.p.i the viral factory was present, no host nuclei, and viral morphogenesis was ongoing; 6 and 8 h.p.i, particle maturation and host deterioration; at 24 h.p.i, virion liberation by host lysis and crescents left behind. These observations provide a detailed morphological description of the Condorvirus replication cycle and support its classification as a mimivirus-like GV. Together, our findings contribute to advancing knowledge of giant virus' diversity and ecology in Brazil and contribute to understand their morphological diversity, host interaction and replication strategies in amoeba.

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PT.420

Yellow Fever Virus Genetic Diversity Across Three Neotropical Primate Genera During the 2017–2018 Outbreak in Minas Gerais

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Yellow fever virus (YFV) is an arbovirus maintained in sylvatic transmission cycles involving non-human primates (NHPs) and mosquito vectors, responsible for recurrent epizootics in Brazil. The large outbreak that occurred between 2017 and 2018 in the Southeast region highlighted the state of Minas Gerais as one of the main areas of viral circulation outside the Amazon Basin. In this study, we investigated two complementary aspects related to YFV dynamics and NHP mortality in the state. First, we assessed intra- and inter-host genetic variability of YFV using 65 near complete genomes obtained from NHP samples of three different genera (*Callithrix*, *Callicebus* and *Alouatta*) collected during the 2017–2018 epizootics in MG. Single nucleotide variant (SNV) analysis revealed a predominance of synonymous mutations and a heterogeneous distribution across the viral genome, consistent with strong purifying selection acting on the virus. Comparisons among hosts showed similar patterns of genetic diversity across different NHP genera, suggesting no evidence of host-specific viral adaptation during the outbreak. Given the continued reports of YFV infections in Brazil, particularly in Minas Gerais, we further conducted molecular surveillance on samples collected between 2023 and 2025. No viral genome was detected in the analyzed samples. This may indicate the absence of active viral circulation in the investigated areas or limitations associated with RNA preservation, as samples originated from carcasses at different stages of decomposition.

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PT.421

Widespread Circulation of Avian Astroviruses in Free-Living Wild Birds from Southeastern Brazil, 2022-2025

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Avian astroviruses (AAstVs) are non-enveloped, single-stranded RNA viruses belonging to the family *Astroviridae* that infect domestic and wild birds worldwide, causing enteric, renal and hepatic diseases, mainly in young poultry. Despite their high genetic diversity and broad host range, data on AAstVs circulating in wild birds in Brazil remain scarce, limiting understanding of their ecology, circulation and zoonotic potential. This study investigated the occurrence of AAstVs in fecal samples from free-living wild birds at seven sites in Sao Paulo and Espirito Santo states, Brazil. Between 2022 and 2025, 954 specimens collected from 95 bird species, comprising resident and migratory birds, were screened using hemi-nested RT-PCR targeting a 422-bp fragment of the RNA-dependent RNA polymerase (RdRp) gene. AAstVs were detected in 6.7% (64/954) of samples. The highest positivity rate was observed at Nucleo Curucutu-SP (12.0%, 20/167), followed by Clube de Campo de Sao Paulo-SP (6.6%, 33/501), Estrada da Barra/Iguape-SP (7.8%, 8/103) and Linhares-ES (1.8%, 3/163). Positive samples spanned 23 bird species, with the highest numbers of detection in *Dendrocygna autumnal*is (13), *Dendrocygna viduata* (10) and *Columba livia* (7), in addition to sporadic detections in multiple Passeriformes, demonstrating viral circulation among diverse hosts. Notably, *Conopophaga lineata* was the only species in which AAstVs were detected at two sampling sites (Nucleo Curucutu and Iguape). These findings represent the largest surveillance study of AAstV in Brazilian wild birds to date, revealing the widespread circulation of AAstV in Brazil and reinforce the need for continued monitoring to better understand their diversity and epidemiology.
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PT.422

STUDY OF THE SPATIOTEMPORAL DISSEMINATION DYNAMICS OF DENV-2 GENOTYPE II IN BRAZIL.

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Genotype II (GII) has been the prevalent variant of Dengue virus (DENV) serotype 2 (DENV-2) in Brazil since 2022. Previous studies showed that a novel lineage of GII, designated II.F.1.1.2, was associated with the increasing circulation of this genotype in Brazil. This study aims to elucidate the spatiotemporal dynamics of the II.F.1.1.2 lineage in Brazil by analyzing 3,796 complete genome sequences collected worldwide between 2021 and 2025, including 2,974 from Brazil, retrieved from the GISAID database. Phylogeographic reconstructions were performed using discrete models implemented within both Maximum Likelihood (ML) and Bayesian frameworks. The ML analysis identified at least 19 introductions of the lineage II.F.1.1.2 from Bolivia or Peru into Brazil. Most introductions resulted in clades of small (63%, $n < 10$) or medium (26%, $10 < n < 100$) size, indicating limited spread within the country. Two introductions, however, generated large clades, BR-I (n = 1,686) and BR-II (n = 1,077). Bayesian reconstructions traced the origin of clade BR-I to São Paulo (posterior state probability [*PSP*] = 0.67) or Paraná (*PSP* = 0.29) states at 2020-07-02 (95% HPD: 2019-10-19 - 2020-11-25) and of clade BR-II to Sao Paulo (*PSP* = 0.71) or Acre (*PSP* = 0.24) states at 2020-03-14 (95% HPD: 2019-07-02 - 2020-08-07). We conclude that most (>90%) DENV-2 cases of GII detected in Brazil originated from only two viral introductions, most likely in São Paulo state in early 2020, which subsequently spread throughout Brazil.

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PT.423

Genomic Mosaicism and Recombination Drive the Emergence of a Novel *Autographivirales* Lineage Infecting *Xanthomonas*

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The extreme diversity and rapid expansion of the class *Caudoviricetes* challenge traditional morphology-based classification, driving genome-based taxonomic restructuring, such as the elevation of *Autographiviridae* to the order *Autographivirales*, which introduced new genome-based criteria for higher-level classification within the class. We describe five bacteriophages infecting *Xanthomonas axonopodis*, all belonging to the proposed species *Pindoramavirus bahia*. Comparative analyses revealed that these isolates form a deep-branching lineage with ten related unclassified Pindoramavirus-like viruses, supported by both distance-based and maximum-likelihood phylogenetic methods. However, order-level HMM profiles, proposed for order-level classification, failed to detect 4 of 11 core genes in any genome within this lineage. To resolve this inconsistency, genomes were reannotated, and encoded proteins clustered by sequence identity and coverage. Comparative analyses identified homologous proteins with equivalent predicted functions despite extensive sequence divergence. Phylogenetic analyses based on these conserved proteins demonstrated that the Pindoramavirus and Pindoramavirus-like viruses form a distinct and well-supported monophyletic lineage within *Autographivirales*, showing the closest relationship to members of *Autonatataviridae*. However, genomic comparisons revealed that nearly half of the genomes, particularly regions encoding structural proteins, are highly divergent and more similar to viruses classified within *Hendrixvirinae*, an orphan subfamily within the class *Caudoviricetes*. Together, these findings suggest that recombination between distantly related viral lineages played a central role in the emergence and diversification of the Pindoramavirus-like lineage, while also highlighting the challenges of taxonomic classification for highly complex, mosaic genomes.

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PT.424

Comparative Biological Characterization of DENV-2 Genotypes II (Cosmopolitan) and III (Asian-American) in C6/36 cells

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Dengue is an arboviral disease caused by *Orthoflavivirus denguei* (DENV), which comprises four serotypes (DENV-1 to DENV-4), each further divided into genotypes and lineages. DENV-2 includes five genotypes, of which genotype III (GIII, Asian-American) circulated in Brazil for more than three decades. Between 2020 and 2024, however, GIII was largely replaced by genotype II (GII, Cosmopolitan), suggesting enhanced transmission potential. To investigate potential biological differences between these genotypes, three viral isolates from each genotype were recovered from acute-phase serum samples of dengue patients in Paraná State, Brazil. The viral isolates were serially passaged four times in mosquito C6/36 cells (MOI = 0.01), to generate viral work stock. The resulting viral stocks were subsequently titrated and subjected to whole-genome sequencing using the Illumina MiSeq platform. Sequencing confirmed the genetic stability of the viral isolates following serial cell culture passage relative to the sequences from the clinical sample. Phylogenetic analysis revealed that the viral isolates clustered into two distinct clades within each genotype, defined by unique amino acid mutations. Viral titration demonstrated that GII produced significantly higher viral titers than GIII, with an approximately two-log increase. These findings provide initial evidence of phenotypic differences between the two genotypes. Comparative infectivity assays are ongoing, and preliminary analyses also suggest differences in infectivity not only between genotypes but also among the distinct clades within each genotype. This study may contribute to a better understanding of the biological differences among circulating DENV-2 genotypes and their potential epidemiological implications.

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PT.425

FIRST REPORT AND CHARACTERIZATION OF A GIANT VIRUS INFECTING THE MICROALGAE *Chlorella heliozoae* IN BRAZIL.

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Chloroviruses are among the first giant viruses to be discovered, remarkable for their size and genetic complexity. These DNA viruses belong to the family *Phycodnaviridae* and are classified into three different subgenera of genus *Chlorovirus*. They infect Chlorella-like microalgae and are ubiquitous in freshwater environments. These viruses have been isolated mainly in the USA and Europe, with few viruses recovered from samples in the Southern hemisphere. Given this scenario, and considering that Brazil is a biodiversity hotspot, this study aims to characterize the first *Gammachlorovirus* isolate in the country. *Chlorella heliozoae* cultures inoculated with a river water sample from Itabira city, Minas Gerais, showed cytopathic effects, characterized by cell lysis after 2 days. Electron microscopy analyses revealed icosahedral particles approximately 180 nm in size. Analysis of the replication cycle showed a 2,200-fold increase in viral titer at 24 hpi. In addition, the virus was subjected to UV-C radiation and temperature resistance tests, demonstrating good thermotolerance ranging from -80°C to 56°C; however, it showed low tolerance to radiation, being inactivated after 10 minutes of exposure. Finally, plaque assays revealed homogeneous plaques of approximately 3 mm in diameter. DNA sequencing revealed a genome of 301 kbp, containing 376 CDSs. Using the DNA polymerase B gene, phylogenetic reconstruction showed that the isolate belongs to the species *Chlorovirus novaeterrae*, subgenus *Gammachlorovirus*. Altogether, our results provide new insights into the diversity of chloroviruses in Brazil. Additionally, comparative analyses with new isolates from the subgenus are ongoing, expanding knowledge about these viruses in the country.

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PT.426

BACTERIOPHAGES IN GINGIVAL BIOFILMS FROM PERIODONTITIS PATIENTS AND HEALTHY INDIVIDUALS: AN EXPLORATORY METAGENOMIC ANALYSIS

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Abstract: Bacteriophages associated with oral biofilms remain poorly studied, particularly their diversity across different microbial groups. This study characterizes bacteriophages in gingival biofilm samples from patients with periodontitis and healthy individuals using an exploratory metagenomic approach. Samples were organized into three pools, each composed of 17 individuals: two pools from patients with periodontitis and one from healthy controls. Direct sequencing of the clinical samples yielded insufficient reads for adequate characterization. Therefore, the samples were cultured in Brain Heart Infusion (BHI) medium under agitation (150 rpm) for 16 hours to favor the recovery of metabolically active aerobic and facultative bacteria and their associated bacteriophages. Sequencing was performed using the MinION platform (Oxford Nanopore Technologies), and taxonomic classification was conducted with Kraken2 using the NCBI RefSeq Viral and Actinobacteriophage databases. Following metagenome assembly, bacteriophage genomes were searched for and reassembled, yielding 16 bacteriophages of sufficient quality for further analysis. Of these, 14 (87.5%) were classified as *Caudoviricetes* sp., with genome completeness ranging from 26% to 100%. Most of the identified bacteriophages (14/16; 87.5%) exhibited lysogenic characteristics, suggesting potential integration into their bacterial hosts' genomes and more stable host interactions. Further studies are needed to confirm these interactions and elucidate the role of these bacteriophages in the structure and dynamics of gingival biofilms.

Keywords: Caudoviricetes; lysogenic; nanopore sequencing.

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PT.427

DIVERSITY AND ABUNDANCE OF BACTERIOPHAGES IN CLINICAL SAMPLES: COMPARISON BETWEEN NUCLEIC ACID EXTRACTION METHODS

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Abstract: Characterization of the bacterial virome in clinical samples is a critical step in the bioprospecting of phages with therapeutic potential, and the choice of extraction method directly influences the viral profile obtained through metagenomic sequencing. This study compared the ZymoBIOMICS 96 MagBead DNA Kit (Beads) with heat-based genetic material extraction in terms of the diversity and relative abundance of bacteriophages in samples from patients with periodontal disease and healthy individuals. Seven sample pairs (diseased n = 5; healthy n = 2) were processed in parallel and sequenced on the Oxford Nanopore MinION platform. Reads were quality-filtered using Chopper (minimum average quality Q≥8, minimum length ≥500 bp), and diversity metrics were used to compare the extraction methods. The beads method showed superior performance, yielding on average 25 times more sequencing reads across sample pairs. It also recovered greater taxonomic richness and consistently higher Shannon diversity values than the heat-based method. The beads method also recovered a substantially larger number of exclusive taxa, including phages associated with clinically relevant pathogens. Overall, the ZymoBIOMICS 96 MagBead DNA Kit outperformed the heat-based extraction method, supporting its use for phage metagenomics. These findings highlight the impact of DNA extraction protocols on metagenomic outcomes and the importance of selecting methods according to study objectives.
Keywords: oral microbiome; viral DNA extraction; metagenomics.

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PT.428

GENETIC CHARACTERIZATION OF CHIKUNGUNYA VIRUS CIRCULATING IN THE STATE OF RIO GRANDE DO NORTE, BRAZIL, FROM 2022 TO 2025: EVOLUTIONARY, STRUCTURAL, AND IMMUNOINFORMATIC ANALYSES

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Chikungunya virus (CHIKV) has circulated in Rio Grande do Norte (RN), Brazil, since 2014, although recent molecular data remain limited. This study characterized CHIKV circulating in RN between 2022 and 2025 through evolutionary, structural, and immunoinformatic approaches. The dataset comprised 154 E1 gene sequences, including 61 RN sequences collected between 2016 and 2025 and 93 genotype references, and 51 RN structural polyprotein (E3–E2–6K–E1) sequences from 2022–2025. Sequences retrieved from GISAID and GenBank were curated and analyzed. Maximum-likelihood phylogenetic analysis showed that all RN sequences clustered within the East/Central/South African (ECSA) genotype, while the 2022–2025 sequences formed a well-supported monophyletic group consistent with sustained local circulation. The 2025 sequences formed a cohesive subclade with 87% bootstrap support. Ten amino acid substitutions were identified, mainly in E1 and E2. E1-A98T occurred in 2022–2023, E1-K211T was detected in 2022, 2023, and 2025, E1-I317T occurred in all six 2025 sequences, and E2-N214S was restricted to 2022. MHC class I predictions showed quantitative stability, with loss of two reference epitopes and acquisition of two new predicted epitopes, whereas MHC class II remained largely conserved, except for one epitope loss in the 2023 consensus. Linear B-cell epitope prediction identified conserved potentially antigenic regions in E2. Structural mapping localized substitutions in three-dimensional models of E1 and E2. Overall, the findings indicate continued ECSA circulation in RN, limited structural-protein diversification, and no broad remodeling of the predicted immunogenic repertoire, reinforcing continuous genomic surveillance. Financial support: FAPERN and CAPES.

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DESCRIPTION OF BACTERIOPHAGES ISOLATED INFECTING *Ralstonia solanacearum*

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Bacterial wilt is a disease affecting crops, caused by phytopathogenic bacteria belonging to the *Ralstonia solanacearum* species complex (RSSC). In this study, five bacteriophages that infect RSSC were isolated from soil samples collected in different regions of Bahia. Phage genomic DNA was extracted using the phenol–chloroform method and sequenced on the Illumina MiSeq. Raw reads were quality-filtered using Trimmomatic and Fastqc. PhaBOX was used for lifestyle analysis, and taxonomic classification was performed using VIPTREE. Phage 1 showed a low concentration, consequently, it did not proceed to next steps. In the BLASTN, it was not possible to observe hits for phage 2, given that it is a giant virus. In phage 3 yielded a hit for *Ralstonia* phage RS-PII-1, with 84% coverage and 79.21% identity. In phage 4 three distinct nodes were analyzed; the hit for *Ralstonia pseudosolanacearum* strain RS 476 chromosome, with 100% coverage and identity, the hit for *Ralstonia* phage RSB3 DNA, with 99% coverage and 94.84% identity. Finally, the hit for *Ralstonia syzygii* strain BDBR229 plasmid pmega, with 91% coverage and 88.60% identity. In phage, the hit was *Ralstonia* phage RSB3 DNA, with 91% coverage and 78.58% identity. Phage 2 exhibits a temperate lifestyle, the phages 3 and 5 are virulent, and the phage 4 exhibits both lifestyles, though the virulent form predominates. Comparative genomic analyses indicated that the bacteriophages represent new viral species belonging to different genera.

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PT.430

Social media as a tool for scientific dissemination in virology and public health in the Jequitinhonha Valley, Minas Gerais, Brazil

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Scientific dissemination through social media has emerged as a strategic tool for strengthening communication between academic institutions and the general population, particularly in socially vulnerable regions such as the Jequitinhonha Valley, Minas Gerais, Brazil, where the Núcleo de Ações e Pesquisa em Apoio Diagnóstico (NUPED-VALE) operates. This project aimed to expand public access to reliable scientific information in virology, immunology, and public health, while promoting the Center's research activities, laboratory services, and extension initiatives. To achieve these objectives, Instagram was adopted as a digital platform for scientific communication, enabling the dissemination of content derived from peer-reviewed scientific literature and epidemiological surveillance data translated into accessible language for the lay public. The published materials addressed viral diseases investigated by the laboratory, including arboviruses, COVID-19, influenza, mpox, vaccinia, and varicella, as well as broader topics related to disease prevention, health education, scientific outreach, and professional training activities. Between April 13 and May 12, 2026, the profile demonstrated substantial growth in digital engagement, with approximately 3,700 additional views, 137 additional interactions, and 107 new followers compared with the preceding period. At present, the profile hosts 112 scientific and educational posts. These findings demonstrate that the strategic use of social media represents an accessible, dynamic, and innovative approach to democratizing scientific knowledge, contributing to the dissemination of evidence-based health information, strengthening the relationship between universities and society, and supporting efforts to combat health-related misinformation.

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PT.431

Making Viruses Tangible: Challenges in Teaching Virology in K–12 Education in Brazil

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Understanding virology is essential for promoting preventive behaviors, supporting public health measures, and combating misinformation, especially during outbreaks. However, in school settings, teaching virology remains challenging due to its conceptual complexity and the lack of accessible and engaging teaching resources. Aiming to identify gaps, difficulties, and demands related to virology teaching in basic education, ann online questionnaire, was applied to k12 teachers. Of 139 responders, 123 reported teaching virology-related content. The most frequently covered topics were viral diseases (28.5%) and prevention and vaccination (27.2%). In contrast, subjects such as viral mutations and variants, although less frequently addressed, were identified as topics of interest by students (41.8%). The main challenges reported were the lack of adequate resources (19.1%) and the abstract nature of the concepts (15.1%), while teachers expressed interest in using physical models (36.6%) Aand games or playful activities (28.7%). Based on these findings, a collection of three short booklets, freely available online (https://sites.google.com/view/arboteam-ufmg/ebooks?authuser=0), was developed using playful and accessible language for children. The materials address how cells are organized and function, what viruses are and how they interact with cells, how the immune system protects us against viral infections, and how infections can be prevented through vaccination. The study provided a foundation for the development of targeted materials that can make virology more understandable, engaging, and relevant to students, while strengthening science education, health literacy, and preparedness for future viral outbreaks.

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PT.432

Viroclube: Building an Open Community for Virology Education

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The dissemination of scientific knowledge and the creation of collaborative networks are key drivers of progress in virology. "Viroclube" emerged as an innovative educational and outreach initiative designed to democratize access to high-quality scientific discussions and foster a united community among students, researchers, and professionals. Viroclube operates as a weekly, 100% free, online lecture club. The platform provides a dynamic environment for discussing a wide range of subjects, including fundamental and applied virology, career development, and laboratory techniques. By leveraging digital platforms and live interactions, the project breaks geographical barriers and promotes continuous learning. Up to June 2026, Viroclube has successfully hosted 52 online lectures, featuring renowned researchers not only from Brazil but from all around the world. The initiative has achieved significant digital engagement, recording an average of 40,000 monthly accesses on its Instagram page (@viroclube) and building a community of over 3,000 followers across its social media platforms. Additionally, the project's YouTube channel acts as a rich, open-access video library, currently featuring 47 published videos and 432 active subscribers. Far beyond merely transmitting information, Viroclube has successfully established an active and united community for scientific discussion. The initiative not only enhances virology education and outreach but also provides a valuable, continuously growing repository of knowledge that is freely accessible to anyone interested in the field.

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#ADOPT A VIRUS: From the Research Bench to the Classroom

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Scientific literacy regarding viruses and vaccination remains a major challenge in secondary education, particularly in the context of widespread misinformation about vaccines. As part of the extension initiative **AEX 0018-10: Project #ADOPT**, the **ADOPT A VIRUS** project was developed within the Laboratory of Emerging Virus Research to bring high school students closer to the biological concepts underlying Influenza A virus and vaccine production. The activity consisted of a simulation of viral inoculation into embryonated eggs using commercially available eggs and adapted educational materials, combined with an interactive lecture and illustrated teaching resources. Learning outcomes were assessed through pre- and post-activity questionnaires comprising multiple-choice questions, open-ended questions, and a self-perceived knowledge scale. A total of three outreach events were conducted in public state and federal schools, involving approximately 150 students. More than 95.0% of participants correctly identified the proper sequence of the influenza vaccine production process, regardless of their initial self-perceived level of knowledge. In the open-ended responses explaining why the influenza vaccine must be updated annually, over 90% of students spontaneously used scientific terms such as *mutation*, in addition to *virus*, *vaccine*, *update*, and *protection*, demonstrating an understanding of the relationship between viral evolution and vaccine reformulation. Collectively, these findings indicate that hands-on outreach activities enhance the understanding of fundamental concepts in virology, encourage the appropriate use of scientific terminology, and reinforce the value of integrating universities and schools to promote scientific literacy.

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PT.434

DIAGNOSTIC PERFORMANCE OF CLINICAL MANIFESTATIONS FOR IDENTIFYING RT-PCR–CONFIRMED ZIKA VIRUS INFECTION AMONG ADULTS WITH ACUTE EXANTHEMA IN THE BRAZILIAN AMAZON

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Background: Zika virus (ZIKV) infection remains a diagnostic challenge in regions with co-circulation of arboviruses, particularly where access to molecular testing is limited. This study aimed to evaluate the diagnostic performance of clinical manifestations associated with RT-PCR–confirmed ZIKV infection among adults presenting with acute exanthema in the Brazilian Amazon. **Methods:** We conducted a cross-sectional study including 1,124 adults attending a reference center in Manaus between 2017 and 2022. Clinical, demographic, and hematological data were collected at presentation, and ZIKV infection was confirmed using RT-qPCR. Sensitivity, specificity, predictive values, and accuracy were calculated for individual symptoms and their combinations. Multivariable logistic regression and ROC curve analysis were performed to assess discriminatory capacity. **Results:** Among participants, 319 (28.4%) had confirmed ZIKV infection. Pruritus (94%) and conjunctivitis (73%) were significantly more frequent in confirmed cases. Pruritus showed the highest sensitivity (94%) and negative predictive value (95.8%), while conjunctivitis demonstrated the best balance between sensitivity and specificity, resulting in the highest diagnostic accuracy (73.3%). Symptom combinations improved specificity at the expense of sensitivity. In multivariable analysis, pruritus (OR 10.7; 95% CI: 2.69–54.3) and conjunctivitis (OR 4.88; 95% CI: 1.73–14.8) remained independently associated with infection, whereas thrombocytopenia and myalgia were negatively associated. The predictive model showed high apparent discrimination (AUC = 0.94; 95% CI: 0.90–0.98), although without external validation. **Conclusions:** These findings support a recognizable clinical pattern of ZIKV infection in adults with acute rash. However, the overlap with other arboviruses limits the specificity of clinical diagnosis. A syndromic approach may aid early suspicion, but laboratory confirmation remains essential.

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PULMONARY INFLAMMATORY RESPONSE INDUCED BY EMERGING OROPOUCHE VIRUS INFECTION IN MICE

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Oropouche virus (OROV), an arbovirus of the genus Orthobunyavirus (Peribunyaviridae family), causes an acute febrile illness in humans, typically associated with headache, myalgia, arthralgia, anorexia, dizziness, chills, and photophobia. Although most cases are mild, severe complications such as meningoencephalitis and acute hepatitis may occur. However, the pulmonary effects of OROV infection remain poorly understood. This study aimed to characterize lung alterations in BALB/c mice experimentally infected with OROV. Five-week-old BALB/c mice (CEUA 3113140325) were divided into two groups: a control group (C), which received 100µL of culture medium, and an infected group (I), which received 100µL of medium containing 10^6 plaque-forming units (PFU) of OROV (strain AM088, isolated from a human patient in 2024 in Coari, PE, Brazil). Injections were administered subcutaneously in the dorsal region. Animals were euthanized at 1, 3, 5, 7, 9, 11, 13, and 15 days post-infection (dpi), and bronchoalveolar lavage (BAL) and lung tissues were collected. Total leukocyte counts in BAL, analyzed by two-way ANOVA, showed a significant increase in infected animals between 3 and 11 dpi, with a peak at 5 dpi (9×10^5/mL). Histological and stereological analyses performed at 5 dpi revealed no significant differences between groups in the volume density of septa (0.34–0.36) or alveoli (0.65–0.68). These findings indicate that infection with the OROV AM088 strain induces a marked inflammatory response in the alveolar compartment, evidenced by leukocyte recruitment, without detectable acute structural damage to the lung parenchyma, contributing to the understanding of the pulmonary impact of OROV infection.

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PT.436

Accessible detection of dengue using a markerless electrochemical biosensor with a proprietary monoclonal antibody.

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Dengue is one of the most important mosquito-borne viral diseases worldwide and a major public health challenge in tropical and subtropical regions. Early detection of infection is essential to reduce complications and improve patient management. The Dengue NS1 protein is widely recognized as a key biomarker for early diagnosis during the acute phase of infection.

Traditional diagnostic methods such as polymerase chain reaction (PCR) and enzyme-linked immunosorbent assays (ELISA) provide reliable results but require specialized laboratory infrastructure and trained personnel. In recent years, biosensor technologies have emerged as promising alternatives for rapid and portable diagnostics.

Among these technologies, electrochemical biosensors have attracted considerable attention due to their high sensitivity, low cost, miniaturization capability, and compatibility with portable instrumentation.

In this work, we propose the development of a label-free electrochemical biosensor based on screen-printed carbon electrodes for the detection of dengue virus NS1 protein using a monoclonal(Mab) anti-NS1 antibody.

The sensing mechanism relies on monitoring electrochemical signal variations generated by antigen–antibody interactions occurring at the electrode interface. These changes are analyzed using cyclic voltammetry (CV), differential pulse voltammetry (DPV), and square wave voltammetry (SWV). The label-free detection strategy eliminates the need for additional labeling reagents during measurement, simplifying the analytical procedure.

The proposed biosensor aims to achieve high analytical performance in terms of sensitivity, selectivity, and limit of detection while maintaining a portable and cost-effective design suitable for point-of-care applications. This work highlights the potential of integrating locally produced Mab with electrochemical biosensor technology to develop accessible dengue diagnostic tools.

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PT.437

MOLECULAR DETECTION OF RESPIRATORY VIRUSES AND CLINICAL PROFILE OF PATIENTS WITH ACUTE RESPIRATORY SYNDROME ATTENDING A MIXED PUBLIC–PRIVATE HOSPITAL IN SOUTHEASTERN BRAZIL

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Viral acute respiratory infections are a leading cause of morbidity and mortality worldwide and impose a substantial burden on healthcare systems.These infections often present overlapping clinical manifestations, making differential diagnosis based solely on symptoms challenging. This study aimed to describe the molecular detection of respiratory viruses and the clinical profile of patients presenting with acute respiratory symptoms at a hospital in southeastern Brazil. A descriptive observational study was conducted using clinical and laboratory data from patients sampled between January and April 2026. Samples from 66 patients were tested by real-time PCR for SARS-CoV-2, Influenza A/B, rhinovirus, respiratory syncytial virus, metapneumovirus, and adenovirus. Among the 66 patients, 30 (45.5%) tested positive for at least one virus: HRV (33.3%), Influenza A (30.0%), SARS-CoV-2 (20.0%), human metapneumovirus (10.0%), and 3.3% each for Influenza B and adenovirus. No significant differences were observed in age or sex between virus-positive and virus-negative patients. However, age distributions differed significantly among the viral groups (Kruskal–Wallis, $p = 0.009$). Pairwise comparisons showed that patients infected with other viruses were significantly older than those with HRV ($p = 0.041$) and Influenza A ($p = 0.019$). The most frequent symptoms among positive cases were cough (80.0%), headache (76.7%), rhinorrhea (73.3%), fever (70.0%), and myalgia (63.3%). These findings highlight the co-circulation of respiratory viruses and reinforce the importance of hospital-based surveillance. Financial Support: FAPEMIG

PT.438

EVALUATION OF A SIMPLIFIED EXTRACTION METHOD COMBINED WITH LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) FOR ORTHOPOXVIRUS DETECTION

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Diseases caused by viruses of the genus Orthopoxvirus (OPXV) are emerging zoonoses, with outbreaks reported worldwide. These viruses affect both humans and animals, causing significant impacts on public health and the economy. In addition to the Variola virus, important members include Vaccinia virus (VACV) and Monkeypox virus (MPXV), both of which are associated with systemic disease. Although infections are often mild, severe cases may require specialized medical care. Due to their high transmissibility, rapid diagnosis is essential for outbreak control. This study aimed to evaluate a simplified extraction method combined with loop-mediated isothermal amplification (LAMP) for rapid OPXV diagnosis in clinical samples. VACV positive scab samples were macerated in lysis solution, heated at 99°C for 15 min, and centrifuged at 5,000 rpm for 10 min. The extracted DNA was quantified and subsequently diluted 10X, 50X, and 10X fold and used as input for LAMP reactions. Reactions were performed at 65°C for 40 min using primers described in the literature, with visual detection based on color change. Amplification was confirmed by agarose gel electrophoresis. Positive results were observed with all four primer sets tested, across all dilutions and samples. These findings support the development of a sensitive and accessible point-of-care test for OPXV detection. The proposed method is a simple, low-cost alternative with great potential for use in remote and hard-to-reach areas and can be performed in settings with minimal laboratory infrastructure.
Financial support: FAPEMIG, CAPES, CNPQ, INCTPOX, Instituto René Rachou-Fiocruz Minas, FINEP, Rede vírus.

PT.439

POXVIRUS INFECTION IN TWO HAIRY DWARF PORCUPINES (*COENDOU SPINOSUS*)

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Poxvirus infections in porcupines have been increasingly investigated, with the Brazilian porcupine poxvirus (BPoPV) proposed as a novel species. However, epidemiological data on BPoPV remain limited. This study describes two cases of suspected poxvirus infection in hairy dwarf porcupines (*Coendou spinosus*) at the Bauru Zoo, Brazil. Both animals were found dead six days apart and underwent necropsy and histopathological evaluation. Gross lesions in Animal 1 included periorbital edema, nasal myiasis, eyelid and genital hyperkeratosis, a distended abdomen, and cerebral edema. Histologically, cutaneous lesions featured acanthosis, parakeratotic hyperkeratosis, and necrotizing lymphoplasmacytic and histiocytic dermatitis with mucinosis and folliculitis, alongside large (10–15 µm) eosinophilic intracytoplasmic inclusion bodies characteristic of poxviruses. Additionally, moderate random fibrinohemorrhagic hepatic necrosis and mild neutrophilic interstitial pneumonia were noted. Animal 2 grossly presented a tail lesion and anasarca; microscopically, it showed severe cutaneous necrosis with mucinosis and secondary intralesional bacteria and fungi. Systemic findings included mild centrilobular hepatic lipidosis, neutrophilic pneumonia with atelectasis and emphysema, and moderate neutrophilic lymphadenitis. While Animal 1 was morphologically diagnosed with poxvirus-compatible viral dermatitis, Animal 2 lacked visible inclusion bodies. Nevertheless, their common origin, narrow temporal window, and cutaneous lesions strongly suggest poxvirus in both cases, with molecular confirmation pending. The characterization of this infection in captive *C. spinosus* contributes to conservation medicine, given the potential impact on the preservation of this species and the risk of dissemination in zoos and wild populations.

Financial Support: CAPES, CNPq, FAPEMIG.

PT.440

OUTBREAK REPORT: ANATOMOPATHOLOGICAL AND MOLECULAR DIAGNOSIS OF MYXOMATOSIS IN DOMESTIC RABBITS (*ORYCTOLAGUS CUNICULUS*) AT A ZOO IN BRAZIL

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Myxomatosis is a highly contagious disease affecting lagomorphs, caused by Leporipoxvirus myxoma (*Myxoma virus*), a double-stranded DNA virus of the Poxviridae family. Although it is a disease reportable to WOAH (World Organisation for Animal Health), there are few reports of its occurrence in Brazil. In domestic rabbits (*Oryctolagus cuniculus*), the classic presentation includes immunosuppression, dermatitis, and respiratory disorders, being frequently fatal. This study describes the characterization of a myxomatosis outbreak in domestic rabbits from the feeder animal facility of the Belo Horizonte Zoo, Brazil. In March 2025, 12 animals (10 adults and 2 juveniles) presented varying degrees of clinical involvement, with signs including periocular and nasal edema and pododermatitis, culminating in death. All animals underwent necropsy, histopathological processing, and molecular diagnosis by DNA extraction and PCR. Grossly, eyelid and nasolabial planum edema, blepharoconjunctivitis, ulcerative dermatitis, pulmonary hemorrhage and edema were variably noted. Histologically, main findings included heterophilic necrotizing blepharitis and conjunctivitis, with myxoid matrix and eosinophilic intracytoplasmic inclusion bodies. Heterophilic necrotizing dermatitis, at times vesicular, was also noted, with myxoid matrix and occasional pseudocarcinomatous hyperplasia in the nares, pinnae, footpads, vulva and mammary glands. Necrotizing hepatitis, myocarditis, and lymphadenitis were also identified, indicating systemic viral dissemination. On PCR, *Poxviridae* DNA was amplified from the 10 adult animals. The outbreak was reported to the official veterinary service, leading to total depopulation of the facility and a 90-day sanitary downtime. The investigation of these cases contributes to disease monitoring in Brazil and the implementation of control measures to prevent future events.
Financial Support: CAPES, CNPq, FAPEMIG.

PT.441

SALIVARY TORQUE TENO VIRUS LOAD IN HIV PRE-EXPOSURE PROPHYLAXIS USERS: COMPARISON BETWEEN PRE-INITIATION AND FOLLOW-UP

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Torque teno virus (TTV) is a ubiquitous, non-pathogenic anellovirus whose viral load mirrors host immune status and is increasingly explored as a biomarker of immune competence. Its behavior in the saliva of individuals receiving HIV pre-exposure prophylaxis (PrEP) is unknown. We compared salivary TTV detection and viral load before PrEP initiation and during PrEP follow-up. Saliva samples were collected using the Salivette device (Sarstedt GmbH). DNA was extracted using the Extracta Kit DNA e RNA Patógenos in the EXTRACTA 96 extractor (Loccus do Brasil Ltda.). Real-time PCR was performed on the QuantStudio™ 6 Flex System (Applied Biosystems, USA), and data were analyzed using Design & Analysis Software v2.6 (Thermo Fisher Scientific, USA). Detection frequency was compared using Fisher's exact test, and viral load (log10 copies/mL) using the Mann–Whitney test. Analyses were performed in Jamovi. A total of 275 saliva samples were included: 178 at baseline and 97 at the follow-up visit. TTV DNA was detected in 91.6% (163/178) at baseline and 95.9% (93/97) at follow-up, with no significant difference ($p = 0.219$). The follow-up group presented a slight but significant increase in mean log10 viral load compared to the pre-PrEP group (6.64 vs. 6.41; $p = 0.048$). In conclusion, TTV is a near-universal component of the salivary virome in PrEP users, and its load was significantly higher during PrEP follow-up than at pre-initiation. Funding Information: Sao Paulo Research Foundation (FAPESP) according to grant number 2025/05019-9.

PT.442

LONGITUDINAL DETECTION OF ORAL HUMAN PAPILLOMAVIRUS IN HIV PRE-EXPOSURE PROPHYLAXIS USERS: A PILOT SALIVA-BASED STUDY

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Human papillomavirus (HPV) is the main etiological agent of HPV-related oropharyngeal cancer. However, longitudinal data on oral HPV acquisition, persistence, and clearance among HIV pre-exposure prophylaxis (PrEP) users remain scarce. This pilot study investigated oral HPV detection during the first year of PrEP. Individuals initiating PrEP completed a structured questionnaire on sociodemographics, sexual behavior, and HPV vaccination, and saliva samples were collected at follow-up visits using Salivette® devices. DNA was extracted with the Extracta®96 platform (Loccus) and analyzed by real-time PCR using the Allplex HPV28 Detection® assay (Seegene), detecting 28 HPV genotypes. Oral HPV was not detected at baseline (0/15). During follow-up, one participant (6.7%) had incident detection of high-risk HPV-58 at the third collection, followed by viral clearance at the subsequent visit. No persistent infection, recurrent detection, or multiple-genotype infection was observed. The participant with incident HPV detection was ≤30 years old, reported more than five sexual partners per month, had a recent bacterial sexually transmitted infection, and had completed HPV vaccination. No significant associations were identified between HPV detection and demographic or behavioral variables (all $p>0.05$). These findings indicate that oral HPV detection in this pilot cohort was uncommon and transient, providing preliminary evidence that oral HPV infection among PrEP users may be predominantly transient. Future studies should investigate the contribution of HPV vaccination and other behavioral factors to this epidemiological profile. This study also demonstrates the feasibility of saliva-based molecular surveillance for longitudinal monitoring of oral HPV infection. Financial support: São Paulo Research Foundation (FAPESP 2025/05019-9).

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Primary infection with a Brazilian chikungunya virus isolate induces sterilizing immunity and protects immunocompetent mice from reinfection

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Chikungunya virus (CHIKV) is an arthropod-borne virus that causes an acute febrile disease associated with severe polyarthralgia, which may progress to chronic manifestations and significantly impair quality of life. The development of experimental models is essential for understanding viral pathogenesis and evaluating antiviral therapies and vaccine candidates. In this study, an experimental CHIKV infection model was established using immunocompetent C57BL/6 mice. Animals were infected with CHIKV and monitored for clinical signs, viremia, viral dissemination, leukocyte counts, and susceptibility to reinfection. Infected mice developed significant viremia and presented detectable viral loads in the popliteal lymph node, femoral bone marrow, and muscle tissue, demonstrating efficient viral dissemination to target organs. In addition, infected animals exhibited a reduction in total leukocyte counts during infection. Reinfection assays showed that previously infected mice were resistant to a second viral challenge, indicating the establishment of a protective immune response associated with the presence of neutralizing antibodies. These findings demonstrate that the experimental model reproduces important aspects of CHIKV infection and may serve as a useful platform for studies on viral pathogenesis, immune responses, and the evaluation of new antiviral and vaccine strategies.

Keywords: chikungunya virus; murine model; viremia; neutralizing antibodies; viral dissemination.
Financial Support: CAPES, FINEP, FAPESB

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MOLECULAR OPTICAL SPECTROSCOPY APPLIED TO YELLOW FEVER DIAGNOSIS

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Yellow fever is an acute febrile disease endemic to tropical regions, where recurrent outbreaks represent a major public health concern. Rapid and accurate diagnosis is essential for reducing mortality and improving epidemiological surveillance; however, conventional diagnostic methods remain dependent on reagents, specialized infrastructure, and time-consuming laboratory procedures. In this study, we evaluated the potential of infrared spectroscopy combined with artificial intelligence for rapid and reagent-free photodiagnosis of yellow fever using human serum samples. Fourier-transform infrared (FTIR) spectra were acquired in the 4000–400 cm^{-1} spectral range and preprocessed using standard normal variate normalization, while near-infrared (NIR) spectra were obtained in the 1000–2700 nm range and preprocessed using second-derivative transformation. Principal component analysis (PCA) was applied for exploratory analysis and dimensionality reduction, followed by support vector machine (SVM) algorithms for classification. Spectral analysis revealed biochemical alterations associated with proteins, lipids, and immune-response-related molecular components, particularly within the amide I and II regions. PCA demonstrated a tendency toward separation between infected and control groups, supporting the development of predictive models. The optimized machine learning model achieved classification accuracies above 95% during external validation, demonstrating the feasibility of infrared spectroscopy as a complementary diagnostic strategy for yellow fever. The proposed methodology offers rapid analysis, low operational cost, minimal sample preparation, and independence from specific molecular reagents. These findings highlight the potential of vibrational spectroscopy and artificial intelligence for point-of-care screening and epidemiological monitoring in endemic and resource-limited settings. Financial support: CAPES, CNPq, and FUNDECT.

PT.445

MAYARO VIRUS INFECTION CAUSES HISTOPATHOLOGICAL DAMAGE IN WILD-TYPE MOUSE KIDNEY MODEL

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Alphavirus Mayaro (MAYV) is the etiological agent of Mayaro fever, an arboviral disease characterized by an acute febrile illness accompanied by arthritis, arthralgia, and myalgia, which may become chronic and result in long-term sequelae in some patients. Studies involving other alphaviruses, particularly Chikungunya virus (CHIKV), suggest that infection can impair kidney function, especially in severe or fatal cases. As the kidneys play a crucial role in maintaining fluid and electrolyte homeostasis and excreting metabolic waste, renal injury may lead to severe systemic consequences and contribute to disease severity and mortality. This study aims to investigate the pathophysiological mechanisms underlying kidney involvement during MAYV infection. Hematoxylin and eosin (H&E) staining revealed progressive renal injury over a 14-day period, affecting both the cortex and medulla. At 1 day post-infection (dpi), early interstitial edema and initial cellular injury were observed in the renal cortex. By 7 dpi, tubular degeneration and epithelial desquamation reached their peak and were accompanied by alterations in glomerular morphology. At 14 dpi, interstitial inflammatory cell infiltration and remodeling of the tubular epithelium became evident. Additional pathological features, including tubular collapse and glomerulonephritis, were also observed. In the renal medulla, marked interstitial edema was evident at 7 dpi, followed by pronounced structural disorganization at 14 dpi. These preliminary findings demonstrate that the kidneys are affected during MAYV infection. Further experiments are underway to elucidate the mechanisms responsible for renal pathology and to determine the contribution of kidney injury to disease severity.

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Betacoronavirus muris Infection Impairs Neutrophil Antifungal Responses to *Candida albicans* via Type I Interferon Signaling

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COVID-19, caused by *Betacoronavirus pandemicum* (SARS-CoV-2), remains associated with high morbidity and mortality, especially in unvaccinated individuals. *Candida albicans* is a pathobiont yeast frequently isolated from hospitalized COVID-19 patients, and candidemia is linked to high lethality. Neutrophil recruitment is essential for fungal control; however, SARS-CoV-2 infection alters neutrophil function. This study aimed to investigate how prior coronavirus infection affects the host response to *C. albicans*, focusing on neutrophil function and the role of IFN-I signaling. To address this, we used a murine model in which C57BL/6 mice were infected with *Betacoronavirus muris* (MHV-3) and, three days later, challenged intratracheally with *C. albican*s. Survival, lung injury, pathogen burden, pulmonary function, and immune responses were evaluated. Neutrophil recruitment, activation, phagocytosis, and killing capacity were assessed in vivo and in vitro. IFNAR-deficient mice were used to determine the contribution of IFN-I signaling. Coinfected mice exhibited increased mortality, higher viral and fungal loads, and exacerbated lung injury compared to single infections. Pulmonary function was significantly impaired, consistent with a restrictive pattern. Despite heightened inflammation, neutrophil recruitment to the bronchoalveolar space and activation (MPO activity) were reduced. In vitro, prior MHV-3 infection impaired neutrophil phagocytosis and fungal killing. Notably, IFNAR deficiency restored neutrophil migration, improved fungal clearance, and attenuated lung injury. In conclusion, prior betacoronavirus infection disrupts neutrophil-mediated antifungal immunity in an IFN-I-dependent manner, increasing disease severity. These findings highlight neutrophils and IFN-I signaling as potential therapeutic targets to prevent secondary fungal infections in coronavirus-associated diseases. This work was supported by FAPEMIG, CAPES, and CNPQ.

PT.447

Detection of Canine Parvovirus (CPV-2) in Domestic Cats: Implications for Viral Circulation and Interspecies Transmission Risk

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Canine parvovirus (CPV-2) is a highly contagious pathogen and one of the leading causes of fatal viral gastroenteritis in dogs. However, due to its close similarity to feline panleukopenia virus (FPV), CPV-2 is also capable of infecting felines. This study aimed to investigate the presence of CPV-2 in canine and feline hosts using CPV-2 immunochromatographic assays. Ten samples (eight felines and two canines) collected from necropsied animals with clinical signs consistent with parvoviral infection were analyzed. Among the felines, 37.5% (3/8) tested positive for CPV-2, and all positive animals originated from shelters with unknown vaccination histories. None of the canine samples (0/2) tested positive. In parallel, questionnaires were administered to 42 participants, revealing that 57.1% were aware of parvoviral disease, whereas only 35% reported adopting core vaccination protocols. Identified risk factors included unrestricted outdoor access (16.7%), multi-species cohabitation, and high population density. Laboratory findings confirmed the presence of CPV-2 in felines, while the questionnaire data identified epidemiological factors supporting viral circulation. This integrated approach demonstrates not only the ability of CPV-2 to infect alternative hosts by crossing interspecies barriers but also the epidemiological mechanisms that facilitate its persistence. These findings are consistent with previous reports of CPV-2a, CPV-2b, and CPV-2c variants infecting felines through mutations in the VP2 gene, highlighting the potential for interspecies transmission. Overall, this study demonstrates that CPV-2 circulates in feline populations with inadequate vaccination coverage, reinforcing the need for integrated surveillance and expanded diagnostic protocols encompassing both canine and feline species.

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Standardization of Primary Chicken Embryo Fibroblast (CEF) Culture for Applications in Avian Virology

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Primary Chicken Embryo Fibroblast (CEF) culture is widely used for vaccine production and for the isolation of viruses of veterinary importance, especially those affecting poultry, such as avian influenza virus, Newcastle disease virus, and Marek's disease virus. This study aimed to standardize a protocol for the isolation and cultivation of CEF as a model for avian virology studies. Ten-day-old embryonated chicken eggs were used (Animal Ethics Committee of the Federal Rural University of Pernambuco, Protocol No. 7949080424). After removal of the head, limbs, and viscera, the remaining tissue was subjected to 0.25% trypsin digestion in three five-minute cycles under magnetic stirring. Following filtration and centrifugation, the cells were cultured in RPMI medium supplemented with 20% fetal bovine serum and maintained at 37°C in a humidified atmosphere containing 5% CO_2. Cell adhesion was observed within the first 24 hours, with morphology consistent with fibroblasts and the ability to proliferate through serial passaging, resulting in six 25cm² culture flasks and one 75cm² culture flask. After seven days, cell counting was performed using a Neubauer hemocytometer with Trypan Blue staining. A total of 13.2×10^6 cells was obtained, with a viability of 93.18%. These results demonstrate that this standardized protocol enables the generation of viable, reproducible, and proliferative CEF cultures, providing a promising platform for future studies involving the isolation of avian viruses and the production of antigens for diagnostic applications in Veterinary Medicine.

PT.449

FTIR AND NIR SPECTROSCOPY AS A NOVEL APPROACH FOR CHIKUNGUNYA DIAGNOSIS

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Chikungunya virus infection represents a major public health concern in tropical and subtropical regions due to its rapid dissemination and the persistence of chronic arthralgia in infected patients. Early and accurate diagnosis is essential for appropriate clinical management and epidemiological surveillance; however, conventional serological and molecular assays present important limitations related to reagent dependency, elevated costs, time-consuming protocols, and potential cross-reactivity with other co-circulating arboviruses. In this study, Fourier-transform infrared (FTIR) and near-infrared (NIR) spectroscopy associated with machine learning algorithms were investigated as rapid and reagent-free diagnostic strategies for chikungunya infection using human serum samples. A total of 80 serum samples from infected and control groups were analyzed in the mid-infrared (4000-400 cm^{-1}) and near-infrared (1100-2700 nm) spectral regions. FTIR spectra were preprocessed using standard normal variate (SNV) normalization to improve spectral reproducibility and minimize experimental variability, while second-derivative transformation was applied to NIR spectra to remove baseline. Principal component analysis (PCA) was employed for exploratory analysis and dimensionality reduction, whereas support vector machine (SVM) models were developed for sample classification. Spectroscopic analyses revealed biochemical alterations associated with proteins, lipids, and immune-response-related biomolecules, particularly in the amide I and II spectral regions. PCA demonstrated a tendency toward discrimination between infected and control groups. Optimized SVM models achieved validation accuracies above 90%, demonstrating the potential of FTIR and NIR spectroscopy as rapid, low-cost, and reagent-free diagnostic tools for chikungunya infection, with promising applicability for point-of-care testing and epidemiological surveillance in endemic and resource-limited settings.

PT.450

IDENTIFICATION AND CHARACTERIZATION OF INFLUENZA A VIRUS IN PEDIATRIC SAMPLES BY MOLECULAR METHOD AND SEQUENCING

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Influenza A virus (IAV) is responsible for epidemics and pandemics throughout history, causing a high number of infections and deaths worldwide. Due to its high mutation rate, continuous genomic monitoring of circulating variants is essential for public health. In this project, we aimed to evaluate IAV circulation between 2024 and 2025 in pediatric patients from public hospitals in São Paulo state. Out of 50 influenza A/B positive cases detected by one-step real-time RT-qPCR, 22 were identified as IAV. To amplify the complete target genes, conventional PCR was performed using eight primer sets designed to cover overlapping fragments (431 to 585 base pairs) spanning full-length hemagglutinin (HA1 and HA3) and neuraminidase (NA1 and NA2). Agarose gel electrophoresis confirmed complete gene amplification in 6 H1N1 and 9 H3N2 positive samples. Subsequently, eight samples were sequenced using the BigDye Terminator v3.1 Kit on an ABI Prism 3130xl platform. Sequence assembly and consensus generation were performed using Geneious Prime®, and coding regions were compared against the GenBank database for subtype identification. Seven samples were identified as H1N1 and one as H3N2, showing high similarity (>90% nucleotide identity) to Influenza A viruses from the United States. Furthermore, Maximum-Likelihood phylogenetic trees constructed with IQ-TREE 2 revealed that all seven H1N1 samples clustered within North American clades from 2024 and 2025. These results demonstrate that the developed Sanger sequencing protocol effectively recovers full-length HA and NA gene sequences, proving to be a robust, reliable, and low-cost alternative for genomic surveillance of circulating influenza A variants. Financial Support: FAPESP Process: 2024/09139-6 Keywords: Influenza A; Sequencing; PCR

PT.451

MOLECULAR INVESTIGATION AND CHARACTERIZATION OF *FELINE MORBILLIVIRUS* IN DOMESTIC CATS FROM RIO GRANDE DO SUL, BRAZIL

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Feline morbillivirus (FeMV), a member of the *Paramyxoviridae* family, was first described in 2012 and has been associated with chronic kidney disease (CKD), although its role in disease pathogenesis remains controversial. In Brazil, information on FeMV circulation remains scarce, and no cases had previously been reported in Rio Grande do Sul, Southern Brazil. This study describes the first molecular detection of FeMV in cats from this region, expanding current knowledge of the virus epidemiology in southern Brazil. Moreover, the work evaluated different collection matrices in order to define the best sample source for FeMV detection. Samples were collected from 53 healthy, household cats, whose samples had previously been used in a study conducted in 2021. Urine, serum and rectal swabs were analyzed using a semi-nested RT-PCR, amplifying a 486-bp fragment of the *L gene* of FeMV. FeMV was detected in 28/53 cats (52.8%), and positive samples were submitted for DNA and phylogenetic analysis where all sequences were classified as FeMV-1. Positive samples included urine (32.1%), serum (21.6%), and rectal swabs (33.3%). Statistical analysis showed no significant difference in FeMV detection rates among the different sample sources ($p > 0.05$). Considering that rectal swabs are less invasive, faster, and more practical to collect compared with urine samples, rectal swabs may represent a suitable alternative for molecular detection of FeMV. These findings provide the first evidence of FeMV circulation in cats from Rio Grande do Sul state and contribute to a better understanding of FeMV epidemiology.

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PT.452

Cannabinoid Type 1 Receptor (CB1R) modulation during wildtype rabies virus infection: possible implications for synaptic and neuroimmune homeostasis

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The cannabinoid type 1 receptor (CB1R) is an inhibitory G protein-coupled receptor (GPCR) involved in neurogenesis, neuroprotection, and neuroimmune modulation. Because the rabies virus (RABV) disrupts synaptic function while preserving neural architecture, we investigated CB1R expression in the brains of RABV-infected mice to explore its potential role in rabies pathogenesis. BALB/c mice (CEUA IEC no. 17/2021) were inoculated intramuscularly with 0.02 mL of AgV-3 viral suspension ($10^{6\cdot10}$ LD_{50}/mL) or vehicle and evaluated over time. CB1R expression was quantified by immunohistochemistry using ImageJ Fiji in whole-slide scanned sections (CaseViewer 2.4). Cellular localization was further characterized by double-label immunofluorescence and confocal microscopy (Leica SP8). Differences between groups were analyzed using the Mann–Whitney test. RABV was detected in the brain from day 3 post-infection and progressively reduced CB1R expression in the hippocampus (control: 0.004 ± 0.001, n = 9; infected: 0.002 ± 0.001, n = 14; p = 0.008). Moreover, CB1R was coexpressed with NeuN+, GFAP+, and Iba1+ resident cells, as well as infiltrating CD3+ T cells. These findings suggest that CB1R modulation may contribute to RABV-induced synaptic dyshomeostasis and neuroimmune regulation, supporting a potential role for the endocannabinoid system in rabies pathogenesis. This study was funded by CAPES, the Ministry of Health, and the Institute of Science and Technology in Emerging Viral Diseases.

PT.453

OROV infection induces a durable and year-long neutralizing antibody response in mice

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Oropouche virus (OROV) is an arthropod-borne virus endemic to Latin America and the Caribbean that causes Oropouche fever, a nonspecific and self-limiting disease. During the 2023-2024 outbreak, severe manifestations were reported, including neurological and pregnancy complications and death. Recently, studies in mouse models have indicated a central role for neutralizing antibodies and marginal zone B cells in controlling neuroinvasion and death induced by OROV. However, the mechanism and duration of the antibody neutralizing response in controlling OROV pathogenesis remains unexplored. Here, we investigated neutralizing antibodies kinetics in mice infected with prototype and contemporary OROV strains. Neutralizing antibody titers were determined by $FRNT_{50}$ in serum collected from C57BL/6 mice at 28, 90, 180, and 360 days post-infection with OROV BeAn19991 or AM0088 isolates. Median $FRNT_{50}$ were 640 (IQR: 640-640), 640 (IQR: 640-640), 480 (IQR: 320-640), and 120 (IQR: 35-520) in mice infected with OROV BeAn19991 at 28, 90, 180 and 360 dpi, respectively. For OROV AM0088 isolate, median $FRNT_{50}$ titers were 320 (IQR: 140-560), 160 (IQR: 80-640), 320 (IQR: 120-640) and 80 (IQR: 10-120) at 28, 90, 180 and 360 dpi, respectively. Neutralizing antibody titers were maintained from 28 to 360 dpi for both OROV isolates (AM0088: Kruskal–Wallis, $p = 0.090$; BeAn 19991: $p = 0.183$), with no significant differences between them at any sampling time (Wilcoxon test, all adjusted $p > 0.05$). These findings demonstrate a durable neutralizing antibody response that persists for at least one year after OROV infection, suggesting a sustained humoral immune response in mice.

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PT.454

Human metapneumovirus reprograms the transcriptional and functional states of macrophages during infection.

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Respiratory viruses modulate host cell function through transcriptional reprogramming and cellular processes that promote viral persistence and dissemination. Human metapneumovirus (hMPV) is a major respiratory pathogen causing bronchiolitis, bronchitis, and pneumonia, particularly in children, older adults, and immunocompromised individuals. Although hMPV remodels the cytoskeleton of airway epithelial cells to facilitate cell-to-cell spread, its effects on macrophage reprogramming remain poorly understood. Bone marrow-derived macrophages (BMDMs) from C57BL/6 mice were differentiated with M-CSF for seven days and infected with hMPV (MOI 10) or maintained under mock conditions. Transcriptomic profiling was performed by RNA sequencing using three biological replicates per condition, followed by differential expression, pathway enrichment, and DoRothEA master regulator analyses. Phenotypic and functional changes were evaluated by flow cytometry, assessing CD86, CD206, and intracellular cytokine expression. hMPV infected only a small subset of macrophages, coexisting with a majority population of uninfected bystander cells. Transcriptomic analyses revealed enrichment of interferon-dependent antiviral pathways, reduced expression of M2-associated genes, and suppression of extracellular matrix organization, epithelial–mesenchymal transition, glycolysis, and TNFα/NF-κB signaling. Functionally, infected macrophages displayed a predominantly $CD86^{+}CD206^{+}$ phenotype and produced the highest levels of both pro-inflammatory and immunoregulatory cytokines, whereas bystander cells remained mainly $CD206^{+}$. Together, these findings demonstrate that hMPV selectively reprograms macrophages toward a distinct antiviral functional state while suppressing extracellular matrix-associated programs, highlighting their potential contribution to immune regulation during hMPV infection.

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PT.455

HUMAN METAPNEUMOVIRUS INDUCES A REMODELING THAT IS DEPENDENT ON TIME AND THE SUBSET OF MYELOID-DERIVED SUPREME CELLS.

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Human metapneumovirus (hMPV) infects lung epithelial cells and alters antiviral immunity, but whether it directly or indirectly remodels granulocytic and monocytic myeloid-derived suppressor cells (MDSCs) remains unknown. This study investigated how the secretome of hMPV-infected lung epithelial cells and direct viral exposure differentially modulate both MDSC subsets. LET-1 cells were infected with hMPV or mock-treated for 24 hours, and their secretomes were used to stimulate mouse bone marrow-derived MDSCs for 24 or 48 hours. In parallel, MDSCs were directly exposed to hMPV or mock conditions. Viral antigen and markers of signaling activation, antigen presentation, and immunoregulatory function were analyzed by flow cytometry. The secretome of hMPV-infected epithelial cells preferentially remodeled monocytic MDSCs, increasing CD126, inducible nitric oxide synthase (iNOS), and major histocompatibility complex class II (MHC-II) expression at 24 hours (all $P<0.0001$), whereas granulocytic MDSCs exhibited a more heterogeneous and time-dependent response. Direct hMPV exposure resulted in only 1.1–2.2% viral-antigen-positive MDSCs but significantly increased phosphorylated STAT3, iNOS, CD317, MHC-II, and programmed death-ligand 1 (PD-L1) in both subsets. Together, these findings provide the first evidence that hMPV remodels MDSCs through complementary epithelial secretome-mediated and direct viral mechanisms, revealing a previously unrecognized pathway that may contribute to antiviral immune regulation during hMPV infection.

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PT.456

ZIKA VIRUS INFECTION MODULATES APOPTOSIS AND MITOPHAGY-RELATED MARKERS IN TROPHOBLAST CELLS

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Zika fever is an arboviral disease transmitted mainly by mosquitoes of the *Aedes* genus, but it can also be transmitted from mother to fetus. During the 2015 outbreak in Brazil, Zika virus (ZIKV) became a major public health concern due to its association with congenital Zika syndrome. Although ZIKV can cross the placental barrier and infect the fetus, the mechanisms underlying placental injury remain poorly understood. In our recently published study, we demonstrated that ZIKV infection induces mitochondrial dysfunction and oxidative stress in trophoblast cells. Based on these findings, this preliminary study investigated apoptosis and mitophagy-related markers in ZIKV-infected trophoblast cells. HTR-8/SVneo cells were infected with ZIKV (MOI 1, 24 h) and immunofluorescence was performed to evaluate the expression of PINK1, EEA1, RAB7, BAX, and BCL2, while *park2* (Parkin) mRNA expression was assessed by RT-qPCR. Our results suggest that PINK1/Parkin-dependent mitophagy is not activated in infected cells, as indicated by reduced Parkin expression and the absence of PINK1 accumulation. In contrast, ZIKV infection significantly increased EEA1, RAB7, and BAX expression, whereas BCL2 expression was significantly reduced compared with mock-infected cells. Together, these preliminary findings suggest activation of cellular pathways associated with cell death and/or autophagy during ZIKV infection. Further studies are needed to clarify the underlying mechanisms and their contribution to placental damage. Funding: IOC-FIOCRUZ, UERJ, CNPq and FAPERJ.

PT.457

MICROBIAL NETWORK ORGANIZATION AND HUMAN PAPILLOMAVIRUS PERSISTENCE INFECTIONS IN PARAGUAYAN WOMEN: A 16S rRNA GENE-BASED STUDY

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Persistent high-risk human papillomavirus (hr-HPV) infection drives cervical cancer risk, with persistence/lesion linked more to community connectivity than to diversity shifts or single-genus abundance. The frequency of protective Lactobacillus-dominated communities (CST I, II, V) varies by ethnicity. We conducted the first 16S rRNA gene-based study of the cervical microbiota to analyses potential differences between 3 groups of Paraguayan women (50 women with hr-HPV-negative results, 33 with hr-HPV-positive non-persistent results, and 33 with hr-HPV-positive persistent results at 18 months). Sequencing of the V4 region (Illumina MiSeq) revealed Lactobacillus dominance, with CST III (Lactobacillus iners) most frequent across groups. This contrasts with European populations, where CST I and II are more common. The predominance of CST III, enriched in anaerobes, suggests increased susceptibility to dysbiosis and a higher risk of cervical lesion development. However, diversity metrics did not distinguish groups. In the hr-HPV-positive persistent group, network analysis revealed a marked shift in microbial connectivity. Lactobacillus lost centrality, showing negative correlations with anaerobes such as Prevotella ($\rho = -0.786$) and Dialister ($\rho = -0.745$). In contrast, strong positive associations were observed among opportunistic genera, including Pandoraea–Staphylococcus ($\rho = 0.897$), Corynebacterium–Staphylococcus ($\rho = 0.896$), and Dialister–Prevotella ($\rho = 0.799$). These patterns indicate an increase in anaerobic and opportunistic connectivity, consistent with a less protective and more persistent microbial environment. This study provides the first network-based characterization of cervical microbiota in Paraguayan women, and underscore the need for regional studies integrating microbial ecology and HPV persistence to inform prevention strategies across Latin America.

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Comparison of Two Distinct Strategies to Expand Phage Host Range

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The global rise of antimicrobial resistance has renewed interest in lytic bacteriophages as alternatives for treating multidrug-resistant bacterial infections. However, the narrow host range of most phages remains a major limitation for their therapeutic application. This study compared two strategies for obtaining lytic phages with expanded host range against clinical isolates of *Pseudomonas aeruginosa*: traditional environmental isolation and laboratory phage training. Using conventional isolation methods, ten phages were recovered against five genetically distinct clinical isolates of *P. aeruginosa*. Morphological and genomic analyses revealed that all isolates belonged to the genus *Pbunavirus* and exhibited a myovirus morphotype. Although only five phages were genetically distinct, they displayed different infection efficiencies across the tested bacterial panel. For the second approach, a phage training protocol based on the Appelmans method was established. A cocktail composed of three phages (ZC01, ZC03, and LAFX) was serially propagated for 30 rounds against ten clinical *P. aeruginosa* isolates: two initially susceptible and eight resistant strains. By the twentieth round, all previously resistant isolates became susceptible to the evolved phage cocktail. However, whole-genome sequencing revealed that the final populations contained a mixture of lytic phages and induced prophages, highlighting a potential limitation of this strategy. Overall, both approaches successfully generated phages with distinct host-range profiles. While environmental isolation yielded naturally diverse phages, phage training effectively expanded bacterial susceptibility but introduced complex phage populations. These findings contribute to a better understanding of phage–host interactions and provide insights for a rational development of phage therapy. Funding support: FAPESP and CNPQ.

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INOCULATION ROUTE SHAPES VIRAL PERSISTENCE, PATHOLOGY AND HOST IMMUNE RESPONSES IN A MURINE MODEL OF CHIKUNGUNYA VIRUS INFECTION

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Despite the widespread use of murine models to investigate chikungunya virus (CHIKV) infection, the influence of the inoculation route on disease progression and host responses remains poorly understood. This study compared the course of CHIKV infection in C57BL/6 mice following inoculation with 10^5 PFU via the intraperitoneal (IP), intraplantar (IPL) or intra-articular (IA) route. Clinical parameters, tissue viral RNA loads, histopathological changes, serum cytokine profiles and splenic Ly6C+ monocyte frequencies were evaluated up to 30 days post-infection (dpi). IPL inoculation resulted in the intense disease phenotype, characterized by increased paw edema and prolonged viral persistence in joints, kidneys and liver. Histopathological damage was characterized by synovial hyperplasia, chondrocyte injury, meniscal inflammation, skeletal muscle degeneration, perimysial inflammation, adipose infiltration and renal inflammatory lesions. In contrast, IP inoculation elicited a stronger antiviral immune response, with increased IFN-γ and IFN-β production associated with progressive viral clearance. Regardless of the inoculation route, infected animals exhibited increased frequencies of splenic Ly6C+ monocytes at 10 dpi compared with their respective controls. Collectively, these findings demonstrate that the inoculation route is a critical determinant of CHIKV pathogenesis by modulating viral dissemination, tissue injury, persistence and host immune responses. Our findings provide a rationale for selecting experimental CHIKV infection models according to specific research objectives and advance the understanding of the mechanisms underlying viral persistence and CHIKV immunopathogenesis.

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PT.460

CHARACTERIZATION OF THE WNT/β-CATENIN PATHWAY DURING DENGUE AND CHIKUNGUNYA VIRUS INFECTION

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Arboviral diseases, including dengue (DENV) and chikungunya (CHIKV), pose a global public health challenge, characterized by high morbidity, lack of specific antiviral treatments, and the socioeconomic impact from hospitalizations and chronic conditions. Despite their relevance, some aspects of the modulation of cellular pathways by these viruses remain unexplored. The Wnt/β-catenin pathway regulates cell proliferation, differentiation, and survival, regulating gene transcription associated with development, tissue homeostasis, and cell cycle progression. Mainly described in oncogenesis processes, its involvement in viral infections remains poorly understood, especially in arboviruses. This study aims to characterize possible modulations Wnt/β-catenin pathway undergoes during CHIKV and DENV infections. For this study, viral vectors containing a Wnt pathway reporter gene were generated to transduce HeLa cells, creating a reporter cell line for monitoring transcriptional activity via a luciferase assay. Viral infection will be conducted with and without pharmacological activation of the pathway, with the effects evaluated by plaque assays; viral and cellular protein content; and luciferase expression. Preliminary results include the validation of reporter cells by detecting the baseline luciferase activity in HeLa cells, but also a 2-log difference in light signal emission compared to cells treated with the pathway activator. Further work aims to complete the initial characterization and, onward, investigate whether the pathway profile varies depending on cell type by generating additional reporter cell lines. In conclusion, it is expected that the data obtained will contribute to characterizing new mechanisms by which DENV and CHIKV interact with proteins and cellular signaling pathways in host cells.

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PT.461

Genetic divergence of SARS-CoV-2 JN.1 variant leads to immune escape after Spikevax XBB.1.5 booster: a prospective cohort and bioinformatics study

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SARS-CoV-2 evolution has been marked by an accumulation of mutations, driven particularly by genetic recombination events, that appear to challenge broad-spectrum immune protection against the virus's variants. Here, a prospective cohort study was conducted to evaluate the neutralizing antibody response induced by a booster dose of the Spikevax XBB.1.5 vaccine in Brazilian adults. Serum samples were collected prior to and 15 days after vaccination. Live-virus neutralization assays were used to test the wild-type SARS-CoV-2 strain (WT-SC2), the Omicron B.1.1.529 lineage, and the XBB.1.5 and JN.1 subvariants. Additionally, bioinformatic analyses were performed to assess the genetic distance of circulating variants and identify mutations that might influence the observed serological results. The booster dose significantly increased neutralizing antibody titers against WT-SC2, B.1.1.529, and XBB.1.5 ($p<0.0001$). Conversely, neutralizing activity against JN.1 remained near the lower limit of detection (Log2 titer ~3). *In silico* analysis reveals 88 mutations identified across the Spike RBD. Phylogenetic clustering confirmed a marked antigenic divergence of JN.1 relative to XBB.1.5, driven by some substitutions like I350V, K374T, R421K, N468D, L473S, N499K that provide a structural basis for the near-complete loss of neutralization. Taken together, these findings indicate that circulating and emerging SARS-CoV-2 variants can partially or completely evade neutralizing antibodies induced by monovalent vaccines, especially those based on XBB that is a variant displaced in current epidemiological dynamics. Our results highlight the growing disparity between vaccine antigens and currently circulating viruses, underscoring the need for updated or broad-protection vaccination strategies capable of keeping pace with ongoing viral evolution.

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COMPARATIVE ANALYSES AMONG MAYARO VIRUS GENOTYPES REVEALS DIFFERENCES IN VIRAL FITNESS AND INDUCED ARTHRITOGENIC DISEASE IN MURINE MODEL

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Mayaro virus (MAYV) is an emerging arbovirus belonging to the family Togaviridae, genus Alphavirus, and is associated with persistent musculoskeletal manifestations and arthralgia. MAYV is classified into three genetically distinct genotypes based on their geographic distribution: Disseminated (D), Limited (L), and New (N). Distinct geographic distribution of MAYV genotypes may be associated with differences in replication fitness in both human and invertebrate hosts, although these differences remain poorly understood. Here we compare infection of three strains of MAYV of two genotypes, where ATCC (1954) and ACRE (2004) belong to D, and SINOP (2011) belong to L. We observed differences of size plaques among three strains such ACRE presents smaller plaque size compared to the others. In viral replication in arthropod cells demonstrated similar replication kinetics among all strains. However, in murine muscle cells, ACRE exhibited lower replication with no cytopathic effect up to 72 hpi and greater activation of the antiviral response leading to increased expression of interferon-stimulated genes (ISGs) in myoblasts. Surprisingly, in the young murine model, ACRE infection induced a severe gastrointestinal disease with 100% death of mice after 5 dpi. Nevertheless, the SINOP resulted in greater viral load, especially in muscles and paws, culminating in death until 4 dpi. In the adult murine model, all the strains of infection demonstrated similar disease and survival until 30 dpi. These findings highlight the importance of considering MAYV genotypic diversity in pathogenesis and surveillance studies, as genomic variations, including mutations in key entry and virulence proteins, may influence disease severity.

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CHARACTERIZATION OF REPLICATION AND PATHOGENESIS PROPERTIES OF CHIKUNGUNYA VIRUS ISOLATES FROM RIO DE JANEIRO IN SV129 MICE

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Chikungunya virus (CHIKV) is one of the most prevalent arboviruses in Brazil. Previous work from our group characterized two isolates from the RJ 2016 outbreak, RJ-IB1 and RJ-IB5, with specific and uncharacterized aminoacid substitutions. Since genomic variability can alter viral phenotype, we investigated the in vitro and in vivo (WT SV129 mice) replication patterns of RJ isolates and compared with a parental CHIKV strain, (BHI3745). C2C12, SH-SY5Y, MEF, and HEK cells were infected at MOIs of 0.1 and 0.01. In vitro results indicate that RJ-IB1 produced higher viral progeny across all cell lines at 36 and 48 hpi and RJ-IB5 showed the lowest production in SH-SY5Y and HEK. Binding and entry assays showed no increased efficiency of RJ isolates compared with BHI3745 in SH-SY5Y and MEF. For the in vivo assays, 12-day and 21-day WT SV129 mice were inoculated with 10^6 PFU at the left hind paw. All viruses caused significant paw edema, whereas RJ-IB1 induced larger edema compared to RJ-IB5. In 12-day mice, viremia peaked at 2 dpi, with RJ-IB5 showing higher viremia and more severe clinical outcome, as well as infectious virus detected in the brain at 6 dpi. 12-day mice developed cutaneous erythema and alopecia, and showed persistent viral RNA in left hind paw at 60 and 90 dpi. Therefore, RJ isolates display distinct kinetics and manifestations relative to each other and the parental strain, and all three can persist in WT SV129. Ongoing studies will identify which substitutions underlie these phenotypes. Financial support: CAPES, CNPq and FAPERJ.

PT.464

HUMAN MICROGLIAL CELL RESPONSES TO OROPOUCHE VIRUS INFECTION

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Neurotropic viruses can cause central nervous system diseases, ranging from severe acute effects to late manifestations. Studies on the emerging arbovirus *Orthobunyavirus oropoucheense* (OROV) have reported neurological manifestations such as meningoencephalitis in infected individuals. Our group recently showed that OROV can infect microglia and trigger a pro-inflammatory response in ex vivo human brain slices. However, the role of microglia in OROV neuropathogenesis remains to be determined. We aimed to characterize the susceptibility and cellular response of human microglial cells either in 2D cultures (T0251) or in human brain slices to OROV, comparing the historical BeAn19991 and emerging AM0088 strains. Microglial cells were infected at MOI 1 and viral antigens were detected by immunofluorescence at 48h post-infection with similar infectivity for both strains (≥ 95%), indicating that both strains efficiently infect microglial cells *in vitro*. Infection with AM008 strain led to a 26% higher mean fluorescence intensity per cell than with BeAn19991. Morphological alterations were more pronounced in AM0088- compared to BeAn19991-infected cells at 24 and 48hpi. Quantification of cell viability and inflammatory response triggered by OROV infection, including IL-1β release and activated microglial markers, are ongoing. In order to also compare the virulence of AM0088 and BeAn19991 in a more complex model, we determined the levels of IL-1β in infected human brain slices. Both strains induced higher IL-1β levels than controls. Our findings corroborate human microglial susceptibility to AM0088 and BeAn19991 infection and establish a relevant experimental platform for understanding the role of microglia cells in OROV neuropathogenesis.

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PT.465

REDUCED in vitro MAYARO VIRUS REPLICATION DURING COINFECTION WITH OROPOUCHE

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The recent expansion of Oropouche virus in Brazil, together with the circulation of the neglected Mayaro virus, raises the possibility of coinfections whose virological and clinical consequences remain poorly understood. To address this gap, we investigated how MAYV and OROV affect each other's replication during in vitro coinfection. A GFP-expressing MAYV strain was used to visually monitor MAYV infection in coinfected cultures. Vero cells were infected with GFP-expressing MAYV or OROV alone or simultaneously coinfected with both viruses at multiplicities of infection of 1.5 or 3. Supernatants collected at 24 hours post-infection were analyzed by reverse transcription quantitative PCR, and infectious MAYV titers were determined by the 50% tissue culture infectious dose assay using GFP fluorescence as the readout. Coinfection reduced MAYV RNA levels relative to MAYV monoinfection. At a multiplicity of infection of 3, the infectious MAYV titer decreased from 10^4.7 $TCID_{50}$/mL in monoinfection to 10^2.3 $TCID_{50}$/mL during coinfection with OROV, corresponding to a 2.4-log_{10} reduction, accompanied by markedly reduced GFP fluorescence. These preliminary findings indicate antagonistic interference during MAYV–OROV coinfection, with OROV suppressing MAYV replication under the tested conditions. This interaction highlights the importance of considering coinfection dynamics when interpreting the epidemiology and biological impact of cocirculating arboviruses.

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PT.466

OROPOUCHE VIRUS INFECTS BONE MARROW CELLS FROM IFNAR KNOCKOUT MICE EX VIVO

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Oropouche virus (OROV) is an arbovirus that has caused multiple outbreaks of febrile illness in the Amazon region. Over the last three years, a newly emerged OROV reassortant (OROV-AM0088) has spread to other regions of Brazil beyond the Amazon, and even to other countries. OROV-AM0088 causes more severe disease and maternal-fetal transmission, with teratogenesis. We have previously demonstrated that OROV BeAn19991 was detected in peripheral blood PBMCs from acute-phase patients and replicated modestly in human PBMCs ex vivo, causing apoptosis. PBMCs lifespan in peripheral blood is shorter than the OROV replication cycle, prompting this study to investigate whether OROV infects bone marrow progenitor cells. Bone marrow and peripheral blood samples from 6-week-old WT and IFNAR knockout C57BL/6 mice were infected ex vivo with 10^6 PFU of OROV BeAn19991 or AM0088 for 24 or 48 h. Viral RNA was quantified by RT-qPCR, and cells were examined by immunofluorescence for OROV antigen. OROV RNA replication was detected in bone marrow from IFNAR knockout mice infected with OROV BeAn19991, but not with OROV-AM0088. Viral RNA was undetectable in peripheral blood cultures. Immunofluorescence confirmed intracellular OROV BeAn19991 antigen in bone marrow cells, more abundantly at 48 hpi in IFNAR knockout mice. Therefore, bone marrow cells are potential hosts of OROV BeAn19991, supporting future investigations into their role in OROV persistence and systemic dissemination. Absence of bone marrow tropism in OROV-AM0088 in this model suggests distinct pathogenic phenotypes between these strains.

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PT.467

IMMUNE RESPONSE DYNAMICS IN EXPERIMENTALLY YELLOW FEVER VIRUS-INFECTED CALLITHRIX SPP.: AN IMMUNOHISTOCHEMICAL ANALYSIS OF MULTIORGAN CYTOKINE EXPRESSION

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Introduction: Yellow fever (YF) is a highly lethal arboviral disease that remains a significant public health concern in tropical regions. Although the liver is the primary target organ, organ-specific immune responses across multiple tissues remain poorly understood. Objective: To characterize the tissue immune response in non-human primates (NHPs) of the genus Callithrix experimentally infected with YFV. Samples of liver, kidney, spleen, and brain from three infected NHPs and one negative control were analyzed. The animals were infected via subcutaneous inoculation or the bite of experimentally infected Aedes albopictus mosquitoes. Viral antigen and the cytokines IFN-γ, TNF-α, IL-4, IL-10, and TGF-β were evaluated via immunohistochemistry and quantified by counting immunolabeled cells. Results: Viral antigen was detected in all evaluated organs, confirming the systemic dissemination of YFV. Distinct cytokine expression profiles were observed across the tissues. IFN-γ and TNF-α predominated during inflammatory responses and showed more intense expression in the animal that succumbed to the infection, whereas IL-4, IL-10, and TGF-β showed increased expression during the later stages of infection, suggesting the activation of immunoregulatory mechanisms. The liver exhibited the highest intensity of immunolabeling, with simultaneous expression of pro-inflammatory and anti-inflammatory cytokines. Conclusion: These findings demonstrate that Callithrix spp. reproduce key immunopathological characteristics of YF and support their use as a translational experimental model for investigating YFV pathogenesis and host immune responses.

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PT.468

The histidine triad nucleotide-binding protein 1 (HINT1) as a cellular factor in different Zika virus lineages

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HINT1 is a highly conserved and ubiquitously expressed cellular protein with tumor suppressor functions mediated through the regulation of p53, Bax, and Bcl-2, as well as inhibition of transcription factors involved in the WNT/β-catenin and MITF pathways. Its phosphoramidase activity, which hydrolyzes lysyl-adenylate, can be pharmacologically inhibited by tryptamine guanosine carbamate (TrpGc). Although several viral families modulate these signaling pathways, no previous studies have investigated the relationship between HINT1 and viral infection. HUH7 cells infected with the Brazilian Zika virus (ZIKV) strain BR-PE243 and treated with TrpGc or related derivatives exhibited increased production of infectious viral progeny. Similar effects were observed in SH-SY5Y and Vero E6 cells, suggesting that HINT1 functions as a restriction factor against orthoflaviviruses. To further investigate this role, HINT1 was overexpressed in HEK293T and HUH7 cells before ZIKV infection. Overexpression significantly reduced infectious particle production and viral RNA levels at 24 and 48 hours post-infection in both cell lines. However, treatment of HUH7 cells infected with distinct ZIKV lineages did not increase viral replication, indicating that HINT1-mediated restriction may be lineage-dependent. DENV-infected HUH7 cells displayed increased infectious particle production following HINT1 inhibition. In contrast, Chikungunya-infected SH-SY5Y cells showed only a transient increase at 6 hours post- infection, while HINT1 overexpression had no detectable effect on CHIKV or Mayaro virus replication. These findings identify HINT1 as a selective antiviral restriction factor, and ongoing studies are aimed at elucidating its underlying mechanisms.

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PT.469

TRANSLATIONAL PLASTICITY DISTINGUISHES ZIKA FROM DENGUE VIRUS REPLICATION IN HUMAN NEUROBLASTOMA AND HEPATOMA CELLS

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Zika virus (ZIKV) and dengue virus (DENV) have a positive-sense single-stranded RNA genome bearing a 5' cap structure that mimics eukaryotic mRNAs but lacks a poly(A) tail. In eukaryotic cells, canonical mRNA translation is cap-dependent, via the eIF4F complex (eIF4E, eIF4G, eIF4A), in which eIF4E recognizes the 5' cap. Although previous studies indicate that orthoflaviviruses modulate host translation, direct comparisons of DENV and ZIKV dependence on canonical translation components across cellular contexts remain limited. Here, we compared these factors' contribution to ZIKV and DENV2 replication in neuronal (SH-SY5Y) and hepatic (Huh-7) cell lines. ZIKV showed translational dependence that inverts between tissues: in Huh-7, eIF4E inhibition reduced replication ($p<0.0001$) while eIF4G1 silencing had no effect, indicating dependence on eIF4E but not eIF4G1; in SH-SY5Y the opposite occurred, with eIF4G1 silencing reducing replication ($p<0.05$) and eIF4E inhibition producing an increase ($p<0.05$), consistent with context-specific initiation modes. For DENV2, eIF4E inhibition increased replication in both lines (Huh-7, $p<0.05$; SH-SY5Y, $p<0.0001$), whereas MNK1 inhibition reduced it only in Huh-7 ($p<0.001$), with no effect in SH-SY5Y, indicating independence from the eIF4E pathway and dependence on the MNK1 axis only in Huh-7. Together, DENV2 shows lower dependence on canonical translation, whereas ZIKV exhibits translational plasticity, adjusting its use of the host machinery according to cell type. A feature that may sustain its broad tropism, relevant given its neurotropism, and shows that host translation factors, though attractive antiviral targets, are context-dependent.
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PT.470

EVALUATION OF THE VECTOR POTENTIAL OF *AEDES AEGYPTI* FOR OROPOUCHE VIRUS

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Introduction: Oropouche virus (OROV) is an emerging arbovirus in Brazil with the potential to spread to urban areas. Although *Culicoides paraensis* is its primary vector, the role of *Aedes aegypti* in OROV transmission remains unclear. This study evaluated the susceptibility of *A. aegypti* to OROV infection under laboratory conditions. Materials and Methods: Laboratory-reared female *A. aegypti* mosquitoes were orally challenged using two infection models: a single infectious blood meal or a double blood-feeding regimen consisting of a non-infectious blood meal followed by an OROV-infected blood meal. Each experiment included 30 females. The single blood-feeding protocol was performed three times, whereas the double blood-feeding protocol was performed six times. Mosquitoes were collected at 5, 10, and 15 days post-infection (dpi), processed in pools of five, dissected into thorax/abdomen and peripheral tissues, and analyzed by RT-qPCR. Results: OROV RNA was not detected in mosquitoes exposed to a single infectious blood meal. In contrast, viral RNA was detected in the thorax/abdomen and peripheral tissues of mosquitoes subjected to the double blood-feeding regimen at all evaluated time points, with Ct values ranging from 34 to 37. Detection in peripheral tissues suggests viral dissemination beyond the midgut. Conclusion: These findings indicate that a second blood meal may facilitate OROV infection and dissemination in *A. aegypti*. The detection of viral RNA in peripheral tissues supports the possibility of subsequent salivary gland infection, reinforcing the need for further studies to determine the vector competence of *A. aegypti* for OROV.

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PT.471

RESTRICTED CIRCULATION OF tomato severe rugose virus IN *Bemisia tabaci* MEDITERRANEAN IS ASSOCIATED WITH ABSENCE OF TRANSMISSION TO TOMATO PLANTS

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Tomato severe rugose virus (ToSRV) is one of the main begomoviruses affecting tomato production in Brazil and is transmitted by whiteflies of the *Bemisia tabaci* cryptic species complex in a persistent-circulative manner. *B. tabaci* Middle East-Asia Minor 1 (MEAM1) has been considered the predominant species and main vector of ToSRV. Previous studies showed that *B. tabaci* Mediterranean (MED) populations from São Paulo State can acquire ToSRV but do not transmit it to tomato plants. This study investigated whether the absence of ToSRV transmission by MED is associated with tissue-specific restriction of viral circulation within the vector. Aviruliferous MEAM1 and MED adults were allowed a 24-h acquisition access period on ToSRV-infected tomato plants and then transferred to virus-free cabbage or sweet pepper plants, respectively, for an additional 24 h to minimize non-internalized viral particles in the gut lumen. Whole insects, dissected guts, and head regions containing salivary glands were collected in pools of five insects. ToSRV was detected by TaqMan-based qPCR. In MEAM1, ToSRV was detected in whole bodies, guts, and head region. In MED, ToSRV was detected at lower levels in whole insects, whereas dissected guts showed low or absent amplification, and no amplification was detected in the head region. These preliminar results suggest that the absence of ToSRV transmission by MED can be associated with restricted virus circulation and limited access to the salivary gland region. The increasing prevalence of MED may alter the epidemiological scenario of ToSRV in areas where this cryptic species becomes predominant. **Financial support:** FAPESP, grant numbers 2025/11312-0 and 2024/22251-0.

PT.472

GENOMIC DIVERSIFICATION OF OROPOUCHE VIRUS DURING RECENT BRAZILIAN OUTBREAKS REVEALS ACCUMULATION OF NON-CONSERVATIVE POLYMORPHISMS

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Oropouche virus (OROV) has re-emerged in Brazil in recent years, being associated with outbreaks in regions previously considered non-endemic and raising concerns about genetically distinct viral variants. Despite this scenario, the evolutionary changes accumulated by contemporary viral lineages remain poorly understood. This study investigated the molecular evolution of OROV over time, with emphasis on the contemporary AM0088 variant associated with recent Brazilian outbreaks. Protein sequences coded by S, M, and L genomic segments deposited in public databases between 1955 and 2024 were analyzed. Sequences were compared to identify non-conservative amino acid substitutions accumulated throughout viral evolution. A comparative analysis was also performed between the prototype strain BeAn19991, the AM0088 variant, and Brazilian sequences obtained between 2023 and 2024. A total of 53 non-conservative substitutions were identified in the S segment, 331 in the M segment, and 148 in the L segment, demonstrating genetic diversification across all viral genomic segments. The M segment showed the highest variability, particularly in proteins involved in virus–host interactions. Comparison among BeAn19991, AM0088, and recent Brazilian sequences revealed high conservation in the S segment, whereas genetic differences were concentrated in the M and L segments. These findings demonstrate OROV diversification over the past decades and indicate that contemporary variants, including AM0088, differ from the prototype strain primarily in the M and L segments. Together, these results improve understanding of OROV molecular evolution and support future studies on the biological impact of genetic changes observed in recently circulating variants.

Keywords: Oropouche virus; molecular evolution; genomic diversification; viral outbreaks.
Financial Support: FINEP, FAPESB and CAPES.

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BLOOD SERUM INFRARED MOLECULAR SIGNATURES FOR DIFFERENTIAL DIAGNOSIS: Investigating the Biochemical Differences Between Dengue and Chagas Disease

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Dengue and Chagas disease are relevant tropical infections caused by biologically distinct pathogens, respectively an arbovirus and a protozoan parasite. Although they differ in transmission and immunopathogenesis, both induce systemic biochemical changes that modify the molecular composition of biofluids. This study investigated whether infrared spectroscopy combined with machine learning could distinguish dengue from Chagas disease by capturing global spectral alterations, rather than relying on a single disease-specific biomarker. Human serum samples previously classified by conventional methods were analyzed by Fourier-transform infrared (FTIR) and near-infrared (NIR) spectroscopy. FTIR spectra (4000 - 400 cm^{-1}) were preprocessed using standard normal variate normalization to minimize experimental variability. NIR spectra (1000 - 2700 nm) were preprocessed with a second-derivative transformation. Principal component analysis explored sample distribution and discriminant regions, while support vector machine models performed supervised classification. The comparison revealed that class separation was not driven by isolated or visually evident spectral changes. Instead, relevant loadings were concentrated in regions associated with amide vibrations (around 1600 cm^{-1}) and lipid bands (near 2800 cm^{-1}). These features reflect immune-response proteins, inflammatory mediators, lipid metabolism, and broader serum matrix remodeling. Therefore, discrimination relies on a multivariate biochemical signature distributed across the sample matrix, rather than a single marker. This supports the hypothesis that infrared photodiagnosis is antigen- and biomarker independent, using systemic molecular fingerprints shaped by host-pathogen interactions. The approach reinforces the potential of spectroscopy and machine learning for the differential diagnosis of tropical diseases. Financial Support: CAPES, CNPq, and FUNDECT.

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DEVELOPMENT OF FULL-LENGTH INFECTIOUS CLONES OF PASSION FRUIT GREEN SPOT VIRUS (*CILEVIRUS PASSIFLORAE*)

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Passion fruit green spot virus (PfGSV; *Cilevirus passiflorae,* family *Kitaviridae*) possesses a bipartite genome with positive-sense single-stranded RNA molecules of 8,740 and 4,783 nts in RNA1 and RNA2, respectively. PfGSV is transmitted by *Brevipalpus yothersi* mites and causes chlorotic and necrotic spots in passionfruit (*Passiflora edulis*). In addition, the virus can also affect plants of at least nine families. Here, we describe the development and the initial validation of full-length infectious clones of PfGSV. The complete RNA1 and RNA2 molecules were amplified and cloned into the binary vector pJL89 using the NEBuilder® HiFi DNA Assembly system. Recombinant plasmids were introduced into *Agrobacterium tumefaciens* strain GV3101 and agroinfiltrated into passionfruit plants and transgenic *Nicotiana benthamiana* expressing HC-Pro. Leaf extracts from symptomatic agroinfiltrated plants were prepared in 0.05 M phosphate buffer (pH 7.0) and mechanically inoculated onto HC-Pro *N. benthamiana* plants. Between 14 and 21 days after inoculation, scattered necrotic spots developed on two of the five inoculated leaves. RT-PCR confirmed the presence of PfGSV in symptomatic tissues, using primers targeting the *p29* and *p24* genes of RNA1 and RNA2, respectively, whereas *Agrobacterium*-specific primers were negative, indicating that viral detection resulted from virus infection rather than residual bacterial contamination. The development of these full-length infectious clones represents an important step toward establishing a standardized system for PfGSV studies. Ongoing analyses aim to quantify viral loads and evaluate cell ultrastructure, while these clones will support future investigations on viral replication, protein function, pathogenicity, and PfGSV-host interactions.

keyword: *Kitaviridae*, *Passiflora edulis*; virus infectious clones; *Brevipalpus*-transmitted viruses

Financial Support: Fapesp (processos 2019/25078-9 e 2024/23284-9)

PT.475

SARS-CoV-2 Gamma and Omicron Variants of Concern Display Distinct Pathogenic Profiles and Immune Responses in Human Lung Epithelial Cells

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The emergence of infectious diseases poses a growing challenge to global public health. The COVID-19 pandemic highlighted that most human pathogens have zoonotic origins, with RNA viruses being prominent. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), which caused the 2019 pandemic, led to unprecedented global health and socio-economic crises. SARS-CoV-2 is an enveloped, positive-polarity single-stranded RNA virus of the *Coronaviridae* family enclosed in a protein capsid. This study investigated the *in vitro* pathogenicity of three Variants of Concern (VOCs), Gamma (P.1), Omicron (BQ.1.1), and Omicron (JN.1) in Calu-3 human lung epithelial cells. Our results demonstrated that among the VOCs Gamma (P.1), Omicron (BQ.1.1), and Omicron (JN.1), there were significant differences in cellular morphology, viral load, cellular receptor expression levels, and immune responses. Regarding viral detection, infection with Gamma (P.1), Omicron (BQ.1.1), and Omicron (JN.1) did not impact the expression of the intracellular sensors RIG-I and MDA5, nor the cytokines TNF-α and IL-8. However, the gene expression of the pro-inflammatory cytokine IL-6 induced by Omicron JN.1 in the first 24 hours, compared to Gamma, demonstrated a significant capacity to induce inflammatory responses. Furthermore, the main inflammatory pathways, such as INF-β production, showed significant differences, particularly in VOC Omicron BQ.1.1. Finally, this study demonstrated early infection kinetics, with syncytia formation and specific inflammatory mediators unique to different SARS-CoV-2 VOCs.

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PT.476

EXPRESSION PROFILE OF HUMAN ENDOGENOUS RETROVIRUS AND THEIR ASSOCIATION WITH CLINICAL PROFILES AND INFLAMMATORY MARKERS IN HTLV INFECTED INDIVIDUALS

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Introduction: Human endogenous retroviruses (HERVs) are viral sequences integrated into the human genome that modulate immunological responses and inflammation. The HERV-H, HERV-K and HERV-W families are associated with cytokine regulation, justifying the analysis of HERV-H, HERV-K, HERV-W transcripts in individuals infected with HTLV-1. **Methodology:** RNA was isolated from buffy coat of eighteen (symptomatic n=6, asymptomatic n=6, and negative control n=6) patient samples (Care Service for People Living with HTLV-SAPEVH-UFPA) using trizol extraction and reverse transcribed as previoulsly described. HERV expression was performed by real-time PCR (qPCR) using HERV-H, HERV-K and HERV-W (pol and ENV) primers set. Data were analyzed via descriptive statistics, the Kruskal-Wallis test, Dunn's post-test, and Spearman's correlation. The cytokine concentration data used in this study were retrieved from the Laboratory database. **Results:** The analysis showed high expression variability among individuals, without significant differences for HERV-H, HERV-K, and HERV-W-ENV ($p>0.05$). For HERV-W-pol, a significant difference was observed among the groups ($p=0.0363$), with lower expression in symptomatic individuals when compared to negative ones ($p<0.05$). In the symptomatic group, HERV-K displayed a strong positive correlation with TNF-α and IFN ($rs=0.90$; $p<0.05$). **Conclusion:** These findings suggest that HERV-W-pol and the correlation between HERV-K and pro-inflammatory cytokines may be involved in the immune response associated with HTLV, indicating potential relevance as biomarkers of infection.

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PT.477

CHARACTERIZING CANNABINOID TYPE II RECEPTOR IN RABIES: A POTENTIAL PHARMACOLOGICAL TARGET IN THE NEUROIMMUNE INTERFACE

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The rabies virus (RABV) induces severe neuronal dysfunction while preserving tissue architecture by modulating neuroimmune interactions, and the type 2 cannabinoid receptor (CB2R) may play a role in this duality. Therefore, this study investigated CB2R expression throughout the course of RABV AgV-3 infection. BALB/c mice were allocated to infected (n = 8) or control (n = 8) groups and inoculated intramuscularly with AgV-3 (6.16 LD50/0.02 mL). Animals were euthanised at different time points during RABV infection (CEUA/IEC nº. 17/2022), and locomotor activity was assessed using the Open Field test. In addition, immunohistochemistry and double-label immunofluorescence were performed to evaluate RABV antigen distribution, CB2R expression, and its cellular localisation. An unpaired *t*-test and two-way ANOVA were used to compare groups. Viral antigen was detected from 3 days post-infection (dpi) onward. Infected mice exhibited reduced locomotor activity compared with controls (9.32 vs. 16.20 m; $p < 0.001$), with the greatest reduction observed at 15 dpi (4.8 vs. 17.12 m; $p = 0.02$). Mild-to-moderate neuronal degeneration, inflammatory infiltrates, and neuronal swelling peaked at 15 dpi. Hippocampal CB2R expression was significantly increased in infected animals overall ($p = 0.0002$) and at 15, 19, and 21 dpi ($p = 0.002$, 0.003, and 0.0005, respectively), but not at 3 dpi. Confocal microscopy demonstrated CB2R expression in neurons, astrocytes, and lymphocytes. In summary, RABV infection increases CB2R expression in neuronal, glial, and immune cells, supporting a potential role for CB2R in neuroprotection and immune regulation. Financial support: INCT em viroses emergentes e reemergentes (INCT-Ver).

PT.478

AI-DRIVEN EVALUATION OF POST-COVID-19 EFFECTS ON MALE REPRODUCTIVE HEALTH

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Viral infections, including COVID-19, cause adverse effects on the male reproductive system and may lead to fertility problems, even in patients who have already recovered from the disease. However, the methods for analyzing human semen recommended by the World Health Organization (WHO) are usually not capable of identifying significant differences in sperm parameters, such as morphology, motility, and sperm count, between patients who have recovered from COVID-19 and those who have never contracted the disease. Therefore, we propose an innovative methodology that enables the distinction between seminal samples from individuals who have never contracted COVID-19 and those who have recovered from the disease. To achieve this, an approach involving the application of machine learning was developed. Spectroscopy measurements, more specifically, ellipsometry, were used to characterize the same ejaculate samples. The machine learning tool was employed to classify a total of 42 samples based on the analysis of the measured data. Different classification algorithms were trained, optimized, and validated to perform this task. The approach achieved excellent performance, with 90% accuracy in sample classification. In this sense, it is noteworthy that the methodology presented here is effective, being capable of distinguishing and accurately classifying the analyzed samples. Therefore, this study demonstrates the potential of this tool as an additional resource for urologists and assisted reproduction centers to investigate fertility problems associated with the effects of COVID-19 on the male reproductive system. In addition, it opens the door to new research aimed at further advancing and deepening this still underexplored field.

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PT.479

Monkeypox virus induces neutrophil extracellular trap formation in human neutrophil

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Monkeypox virus (MPXV) causes a systemic infection characterized by cutaneous lesions and, in severe cases, pulmonary involvement and multiorgan dysfunction. Neutrophil extracellular traps (NETs) may contribute to tissue injury during mpox, but the mechanisms responsible for their induction remain unclear. This study assessed NET deposition in lung tissues from fatal MPXV cases, and investigated whether MPXV directly induces NET formation in human neutrophils in vitro. Briefly, paraffin-embedded lung tissues from three fatal cases showed colocalization of extracellular DNA, myeloperoxidase, and neutrophil elastase within consolidated lung parenchyma, as assessed by confocal immunofluorescence microscopy. Later, neutrophils were isolated from peripheral blood of three healthy donors by Percoll density-gradient centrifugation and infected with West African clade MPXV at multiplicities of infection of 0.1, 0.5, and 1.0 in a biosafety level 3 laboratory. NET formation was evaluated by quantification of myeloperoxidase–DNA complexes using an enzyme-linked immunosorbent assay combined with PicoGreen, confocal microscopy, and immunofluorescence staining for myeloperoxidase, neutrophil elastase, and citrullinated histone H3. In summary, MPXV induced robust NET formation within 4 hours, with a dose-dependent increase in myeloperoxidase–DNA complexes compared with mock-infected controls ($p<0.0001$). Confocal microscopy revealed characteristic extracellular web-like structures containing myeloperoxidase and neutrophil elastase. Sivelestat or the peptidylarginine deiminase 4 inhibitor Cl-amidine treatment did not significantly affect MPXV-induced NET formation ($p = 0.9002$), suggesting neutrophil elastase and PAD4 are not essential for response under the experimental conditions tested. These findings demonstrate that MPXV directly induces NET formation and further experiments will clarify the molecular mechanisms underlying MPXV-induced NET formation.

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PT.480

Distinct NLRP3 Inflammasome Signatures in Monocytes and Neutrophils from COVID-19 and HIV-1 Patients Are Associated with miR-223 Downregulation

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Background: The NLRP3 inflammasome is a key mediator of innate immune responses during viral infections. However, the mechanisms regulating inflammasome activity in circulating monocytes and neutrophils during COVID-19 and chronic HIV-1 infection remain poorly understood. **Methods:** We investigated basal and stimulus-induced NLRP3 inflammasome activation in monocytes and neutrophils from unvaccinated COVID-19 patients (mild and severe) and aviremic HIV-1 individuals receiving long-term ART. Flow cytometry, immunoblotting, ELISA, qPCR, and confocal microscopy were used to assess inflammasome activation, cytokine production, and miRNA expression. **Results:** Both patient groups displayed basal NLRP3 inflammasome hyperactivation in circulating monocytes, characterized by increased caspase-1 activity, IL-1β and IL-18 release, and NLRP3 oligomerization. Monocytes exhibited impaired responses to canonical NLRP3 stimulation (LPS + ATP), suggesting functional exhaustion. This phenotype was associated with reduced miR-223 expression, particularly in enriched monocytes from HIV-1 patients. Neutrophils also displayed basal inflammasome dysregulation and reduced miR-223 expression. However, unlike monocytes, neutrophils remained highly responsive to LPS + ATP stimulation, showing enhanced NLRP3 activation. **Conclusion:** Acute (COVID-19) and chronic (HIV-1) viral infections induce a shared profile of innate immune dysregulation characterized by basal NLRP3 hyperactivation and miR-223 downregulation. While monocytes develop an exhausted inflammasome phenotype, neutrophils retain responsiveness to secondary stimulation, revealing cell-specific mechanisms regulating inflammasome activity. These findings identify miR-223 as a potential regulator of innate immune dysfunction and a candidate therapeutic target in viral inflammatory diseases.

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PT.481

Plasma levels of the anti-inflammatory cytokines IL-4 and IL-10 in COVID-19 convalescent patients randomized to Bacillus Calmette-Guérin (BCG) vaccine or Placebo

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Long COVID is characterized by the persistence of symptoms after the acute phase of COVID-19 and may be associated with the host's immune response and cytokine imbalance, including IL-4 and IL-10. In this sense, the Bacillus Calmette-Guérin (BCG) vaccine has been investigated as a therapeutic alternative due to its immunomodulatory properties. Thus, plasma levels of IL-4 and IL-10 in COVID-19 convalescent individuals randomized to receive BCG or placebo at different follow-up periods were evaluated. Participants were 58 COVID-19 convalescent individuals randomized into two groups: BCG (n=29), who received an intradermal vaccine booster, and placebo (n=29). Blood samples were collected at three time points: T0 (study adherence), T45D (45 days post-vaccines) and T6M (6 months after vaccination), with plasma separation by centrifugation. IL-4 and IL-10 levels were measured by flow cytometry. Statistical analyses used the Mann–Whitney and Friedman tests. In the BCG group, IL-4 levels remained stable over time, while the placebo group showed an increase at T45D and T6M in relation to T0 ($p = 0.0001$ and $p = 0.0007$, respectively). For IL-10, a decrease from T45D to T6M was observed in the BCG group ($p = 0.0403$), with no differences in the placebo group. In the comparison between groups, IL-4 and IL-10 levels were lower in the BCG group compared to placebo at T6M ($p = 0.0097$ and $p = 0.0072$, respectively). It is likely that revaccination with BCG promotes modulation of anti-inflammatory cytokines, with reduced levels after six months, suggesting a potential immunomodulatory effect in COVID-19 convalescents.

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PT.482

EFFECTS OF IRON SUPPLEMENTATION ON RESPIRATORY HUMAN ADENOVIRUS INFECTIVITY AND VIRULENCE *IN VITRO*

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Although the influence of metals on viral behavior has been described in some environmental studies, the effect of iron on clinical infections caused by Human Adenovirus (HAdV) has not yet been addressed in the literature. This study experimentally evaluated the effect of ferrous bisglycinate supplementation on HAdV-5 replication, cytopathic effect, and cell death in an *in vitro* model using A549 cells. To this end, the Sulforhodamine B assay assessed iron cytotoxicity in the tested cells, while the influence of iron supplementation was evaluated via ICC-RT-qPCR (integrated cell culture with reverse transcription and quantitative PCR), cell viability assays, flow cytometry, and quantification of lysis areas using inverted light microscopy. Using three distinct methods of iron-cell-virus exposure, cells were treated with varying iron concentrations (750, 218.8, and 109.4 µg/mL) and infected with different HAdV-5 viral titers for method comparison and standardization. Samples exposed to high iron concentrations showed increased cytopathic effects and cell death, although no significant changes in viral genome copy numbers were observed. Thus, the results suggest that the interaction between iron and host cells led to increased infectivity and virulence, without apparent changes in viral replication. Therefore, the findings indicate that iron may promote HAdV-5 infection progression, opening avenues for investigating the underlying molecular mechanisms, such as oxidative stress induction, ferroptosis, and modulation of the antiviral immune response.

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PT.483

IRON SUPPLEMENTATION MODULATES ROTAVIRUS A REPLICATION AND INFECTIVITY *IN VITRO*

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Rotavirus A (RVA) is a major etiological agent of severe acute gastroenteritis in pediatric populations, and the host's nutritional status can influence its pathogenesis. This study investigated the role of iron as a modulator of RVA replication, virulence, and infectivity *in vitro*. To this end, RVA-permissive MA-104 cells and ferrous bisglycinate were used. Initially, the mean cytotoxic concentration (CC50) of iron supplementation for the tested cells was determined (218.8 µg/mL) to establish comparative pre- and post-treatment protocols with iron in RVA-infected cells. Viral load was monitored via ICC-RT-qPCR (integrated cell culture with reverse transcription and quantitative PCR) for mRNA quantification, while cell monolayer integrity was assessed through automated counting and digital image analysis of the cytopathic effect. The results revealed that iron pre-treatment promoted a significant, dose-dependent increase in viral replication, demonstrating that iron availability at the time of infection is a potential determinant of viral replication. However, this increase in genomic load did not result in significant reductions in cell viability or the area of cell detachment caused by the cytopathic effect, suggesting that iron may prolong the non-lytic phase of infection by attenuating early cellular damage. Therefore, these findings highlight the importance of nutritional immunity and suggest that iron balance may modulate not only viral transmission and persistence but possibly also the efficacy of live-attenuated virus vaccines in endemic regions where iron supplementation programs are implemented.

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PT.484

CHARACTERIZATION OF THE ORGANIZATION AND BIOLOGICAL FUNCTION OF AGGREGATES OF VIRAL PARTICLES OF EMERGING ARBOVIRUSES

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Arboviruses are infections caused by viruses transmitted by arthropod vectors. These viruses can produce symptoms ranging from asymptomatic to severe neurological or hemorrhagic manifestations, potentially resulting in fatal outcomes. Despite their clinical importance, fundamental aspects of arbovirus biology remain poorly understood, particularly regarding viral particle organization in biological fluids—essential for comprehending infection and replication processes. The literature indicates that arboviruses form aggregates, yet the mechanisms, reasons, and impacts on viral infectivity remain unclear. This project aimed to characterize aggregate formation in Mayaro and Yellow Fever viruses, two genera of significant public health importance, and to elucidate possible biological functionalities of these structures. The techniques used were dynamic light scattering (DLS), zeta potential analysis, nanoparticle tracking analysis (NTA), and transmission electron microscopy (TEM). The DLS and NTA assays for MAYV and YFV showed a wider size distribution range in the YFV data, indicating the presence of larger aggregates. We tested different disaggregation methods, with sonication proving to be the most promising technique. Through tests using different dilutions, intensities, and durations, we achieved a reduction in the average particle size of YFV. This result was also confirmed in in vitro assays conducted under the same conditions, which showed an increase in viral titer, while no significant differences were observed for MAYV under the same conditions. This research characterizes viral aggregates and their interaction mechanisms with host organisms. These insights will facilitate development of novel prevention and treatment strategies for emerging arboviruses, contributing to improved public health outcomes against these important pathogens.

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PT.485

INACTIVATION OF *Pseudomonas coronafaciens* pv. garcae, THE CAUSATIVE AGENT OF COFFEE PLANT BACTERIAL HALO BLIGHT, BY A FOUR-PHAGE LYTIC COCKTAIL: *IN PLANTA* STUDIES

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This study evaluated a four-phage cocktail, delivered either free or encapsulated, for the biocontrol of *Pseudomonas coronafaciens* pv. *garcae* (Pcg), the causal agent of coffee plant bacterial halo blight. Two encapsulation strategies were tested: a water-in-oil (W/O) microemulsion system with phage virions in the aqueous core, and chitosan-coated calcium alginate nanoparticles integrating the phage virions. Physicochemical analyses revealed that phage encapsulation in the W/O system reduced droplet size and slightly altered surface charge, while FTIR confirmed molecular interactions between virions and the droplet interface. Abiotic stress tests identified PCG-09T as the most UV- and temperature-resistant phage, whereas phage PCG-05T was the most susceptible. While the microemulsion system provided *in vitro* protection for the phages, it showed potential phytotoxicity, linked to mineral oil-induced leaf cuticle disruption. Nanoparticle formulations offered pH-triggered release and preserved phage infectivity under UV exposure. Greenhouse *in planta* trials demonstrated superior disease suppression with nanoparticle-encapsulated phage virions, significantly reducing AUDPC (area under disease progression curve) values across all Pcg strains compared to free phages, microemulsions, and chemical controls. Field trials were inconclusive due to low natural infection pressure. Overall, nanoparticle-based delivery emerged as the most effective strategy for phage biocontrol in the coffee plant phyllosphere, combining environmental stability with efficient release at infection sites, offering a promising alternative to conventional chemical treatments.
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PT.486

ISOLATION AND PRELIMINARY CHARACTERIZATION OF A LYTIC BACTERIOPHAGE FOR A VIRULENT *Ralstonia solanacearum* STRAIN WITH POTENTIAL FOR CONTROL OF TOMATO PLANT BACTERIAL WILT

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Among the phytopathogens of major agricultural relevance, the *Ralstonia solanacearum species complex* (RSSC) stands out as one of the most important bacterial groups affecting crop production worldwide. The RSSC comprises a group of soilborne Gram-negative bacteria capable of infecting a wide range of plant species and is recognized as the causal agent of bacterial wilt in solanaceous crops. Current management strategies rely mainly on agronomic practices, including crop rotation, grafting onto resistant rootstocks and sanitary measures. However, these approaches still present limited effectiveness due to the wide host range and long-term persistence of the pathogen in soil, reinforcing the need for alternative and more sustainable control strategies, reinforcing the need for alternative and more sustainable control approaches. Among the most promising alternatives is the use of bacteriophages, viruses capable of specifically infecting and lysing their bacterial hosts, providing a more targeted and environmentally sustainable strategy for disease control. A bacteriophage infecting *R. solanacearum* IBSBF-309, obtained from the Instituto Biologico Culture Collection of Phytobacteria, was isolated from water samples collected at the University of Sorocaba (UNISO) and was subsequently purified, amplified and titered. Initial characterization of the isolated phage initially encompassed TEM analyses and UV-Vis spectral scans for determination of its molar extinction coefficient, together with host range assessment and efficiency of plating analyses. In addition, the process of phage particle adsorption onto its target bacterial host cells was evaluated through adsorption curve assays, and phage growth parameters were determined using one-step growth curve experiments.
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PT.487

Prediction and experimental evidence of post-translational modifications in the dengue virus capsid protein

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The dengue virus capsid protein (DENVC) packages the viral genomic RNA into the nucleocapsid (NC) and performs multiple functions during infection, indicating that its activities require tight regulation. Post-translational modifications (PTMs) are key regulators of protein function and have been reported in several viral proteins, including capsid proteins, where they regulate viral replication, RNA binding, and subcellular localization. However, their role in DENVC remains largely unexplored. DENVC is a small protein (~100 amino acids) composed of four α-helices and an intrinsically disordered N-terminal region (IDR), with a highly positively charged surface. Its flexible IDR and high lysine and arginine content make DENVC a potential target for PTMs. We hypothesize that DENVC undergoes PTMs that contribute to the regulation of its functions during infection. To investigate PTMs in DENVC, we combined *in silico* predictions with experimental analyses. Sequence-based prediction tools identified putative PTM sites, several of which are conserved among orthoflavivirus capsid proteins. Western blotting analysis of DENVC immunoprecipitated from transfected HEK293T cells and probed with an anti-acetyl-lysine antibody suggested lysine acetylation. Conversely, immunoprecipitation with anti-acetyl-lysine or anti-SUMO antibodies, followed by western blotting detection of DENVC, provided evidence supporting DENVC acetylation and SUMOylation. These preliminary findings will be validated by mass spectrometry, followed by functional assays to determine how these modifications affect DENVC localization, RNA binding, and NC assembly. This study may provide new insights into the molecular mechanisms underlying DENV replication and assembly, with potential implications for antiviral strategies.
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PT.488

COMPARATIVE REPLICATION DYNAMICS OF CONTEMPORARY AND HISTORICAL OROPOUCHE VIRUS LINEAGES IN HUMAN PROSTATE EPITHELIAL CELLS PNT1A

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From 2024-2025 Brazil experienced a marked increase in Oropouche virus (OROV) cases. Genetic reassortment between lineages circulating in the eastern Amazon (2009-2018) and lineages detected in Peru, Colombia, and Ecuador (2008-2021) may have generated the contemporary lineage driving this outbreak. Given the high transmissibility of this new variant, several hypotheses have emerged, including unusual transmission routes for arboviruses — particularly after OROV detection in semen of infected patients, raising concern about possible sexual transmission. This study compares the replicative capacity of the contemporary (OROV43) and historical (BeAn19991) variants in a human prostate epithelial cell line (PNT1A). We first assessed susceptibility, permissiveness, and viral fitness through replication kinetics assays. Both variants established infection in PNT1A. In multiple-cycle kinetics (MOI 0.01), OROV43 showed significantly lower and delayed replication and virion release compared to BeAn19991. In single-cycle kinetics (MOI 5), the higher initial viral load allowed OROV43 to replicate more efficiently and produce more virions than BeAn19991 up to 24h post-infection, but this was significantly reversed by 96h. Next, to identify factors driving these differences, we will use RT-qPCR and ELISA to assess differential interferon-gamma expression and translation during both infections, examining whether viral immune-escape strategies compensate for lower replicative capacity and support persistence in new tissues (reproductive) and hosts (vectors and vertebrates). Investigating OROV persistence in reproductive cells may thus help assess the risk of sexual transmission amid geographic expansion, reinforcing surveillance and the need to understand this infection route's consequences.

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PT.489

INTER AND INTRAHOST INFECTION CYCLES DRIVE MICROEVOLUTION AND E2 PROTEIN VARIANT SELECTION IN MAYARO VIRUS: IN VITRO AND IN SILICO CHARACTERIZATION

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Genetic diversity in arboviruses is essential for maintaining viral cycles, mainly because the virus circulates among such distinct hosts. RNA viruses, such as Mayaro virus (MAYV), have high genetic diversity and consequently the potential for mutations to arise that can modulate tropism and replication efficiency in new vectors and hosts. This study investigated genetic and adaptive variability of MAYV between mosquitoes and humans using *in vitro* models. Successive and alternating passages in mammalian cells (HFF-1) and vector cells (Aag2) were performed and submitted to next-generation sequencing (NGS). Two mutations in envelope proteins were selected: E2:G95S and E2:E162K, near the viral receptor-binding interface. The E2:WT, E2:G95S, and E2:E162K variants were modeled from FASTA sequences using AlphaFold2 and evaluated by molecular dynamics simulations. The E2:G95S mutation favored interaction with the cellular receptor MXRA8 by preserving structural stability and increasing binding affinity, whereas E2:E162K compromised the interaction interface, resulting in greater flexibility and reduced receptor affinity. Through site-directed mutagenesis, the substitutions were introduced into infectious MAYV clones containing reporter genes (luciferase/Zs-Green). The viral clones containing the mutations were transfected into BHK-21 cells for viral expansion and will be used for biological characterization in different cell lines to evaluate the impact of these mutations. Our findings demonstrate that selective pressures from different host cells rapidly shaped MAYV populations. Ongoing additional *in silico* studies and *in vitro* assays will explore vector competence across species, enhancing our understanding of virus-host interactions and guiding future control strategies.

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PT.490

PURIFICATION STRATEGIES OF A NEGLECTED ORTHOFLAVIVIRUS, BUSSUQUARA VIRUS

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Arboviruses are arthropod-borne agents with considerable public health concern, especially the *Flaviviridae* family. Bussuquara virus, *Orthoflavivirus aroaense*, was first isolated in the Brazilian Amazon region in 1956. The first patient identified with the infection was in Panama, in 1964, presenting acute febrile illness and seroconversion. Sporadic detection of the virus suggests transmission by a wide range of unidentified vectors and hosts. However, the available information on this virus remains scarce. This work aims to elucidate the structure of the emerging Bussuquara virus by cryo-electron microscopy of purified viral samples. Here, we evaluated viral propagation in the established cell lines Vero, C6/36, BHK-21, SH-SY5Y and HuH 7.0 to test viral load for purification by centrifugation and density-based gradient separation. The results indicate Bussuquara virus causes cytopathic effects and forms discernible viral plaques in all tested cell lines. The virus exhibits better fitness replication to Vero cells that peak 10^5 PFU/mL at the third day post-infection. The purification process achieved a final titer of 10^6 PFU/mL. We also observed that the virus is sensitive to freeze–thaw cycles, declining almost 1 log per freeze–thaw. The next steps include improving the purification strategy, performing negative-staining and cryo-electron microscopy analyses, as well as trying to validate the virus adaptation in other cell lines. Until now, this work concludes that the Bussuquara virus infection in Vero cells yields a viral progeny with higher titer. The purification strategy is currently being optimized for future experiments on cryo-electron microscopy.
Financial Support: CAPES.

PT.491

Isolation and characterization of bacteriophages with potential to inactivate *Pseudomonas coronafaciens* pv. *garcae*, the causative agent of bacterial halo blight in coffee plants: *in vitro* studies

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Bacterial blight of coffee caused by *Pseudomonas coronafaciens* pv. *garcae* (Pcg) poses a significant threat to coffee production. This study reports the isolation and characterization of four lytic bacteriophages - PCG-05T, PCG-06T, PCG-07T, and PCG-09T - as potential biocontrol agents against Pcg. Isolated using multiple Pcg strains, the phages exhibited differential host specificity, plaque morphology, and lytic efficiency. Adsorption kinetics and one-step growth analyses revealed fast binding (PCG-06T and PCG-07T) and high burst sizes (PCG-05T and PCG-09T). In addition, phages PCG-06T and PCG-09T also displayed broad host range and strong lytic activity, with low frequencies of emergence of bacterial resistant mutants ($\leq 10^{-3}$), with all phages positioning themselves as promising candidates for phage therapy. Electrostatic and diffusion analyses suggested that adsorption efficiency was influenced by surface charge interactions, with PCG-06T showing enhanced binding due to a higher (less negative) Zeta potential. *In vitro* inactivation assays demonstrated MOI-dependent suppression of Pcg populations, with phage cocktails yielding superior reductions - up to 6.17 log CFU/mL - especially at MOI 10000. *Ex planta* assays on artificially contaminated coffee plant leaves further confirmed significant bacterial inactivation, achieving up to 4.08 log CFU/mL reduction after 36 h. Despite slight regrowth at later time points, phage persistence remained stable, highlighting the potential of high-MOI applications for effective short-term control. Genomic screening confirmed the absence of undesirable genes, reinforcing safety for agricultural use. While results are promising, further studies on formulation, environmental stability, and field efficacy are needed.

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PT.492

Characterization of a lytic bacteriophage for an extensively drug-resistant (XDR) *Pseudomonas aeruginosa* isolated from human urinary tract infections in Brazil: *in vitro* preliminary studies

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The *Pseudomonadaceae* family harbors opportunistic pathogens of great clinical relevance, with *Pseudomonas aeruginosa* standing out for causing complex urinary tract infections (UTI) and exhibiting a high capacity for biofilm formation. The ST357 clone of this bacterium acts as an international high-risk lineage, characterized by an extensively drug-resistant (XDR) phenotype that renders conventional antimicrobial therapies ineffective. The study reported herein aimed to isolate, characterize, and evaluate the inactivation efficacy of a strictly lytic bacteriophage against the XDR *P. aeruginosa* ST357 clone. Lytic bacteriophages represent a strategic, eco-friendly, and highly specific alternative for inhibiting bacterial growth and degrading biofilms without compromising the host. A search for viral agents was conducted in environmental stream water samples from the Sorocaba/SP region, resulting in the successful isolation of a bacteriophage designated PaeXDR-01. This phage was characterized via UV-vis spectral scans to determine its molar extinction coefficient and by generating its adsorption curve to evaluate cell binding kinetics. Additionally, host-range assays were performed on 15 bacterial strains along with determination of the efficiency of plating (EOP) on susceptible bacteria to confirm the phage's infection specificity. The results demonstrated that PaeXDR-01, obtained at a high phage titer, is highly specific to the pathogen, showing rapid binding to the bacteria in approximately 15 min and an extremely low desorption rate constant. Subsequent assays will validate the phage growth parameters and bacterial inactivation in *in vitro* models using simulated human urine and *ex vivo* using porcine bladder tissue.

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PT.493

INTEGRATED GENOMIC CHARACTERIZATION OF BACTERIOPHAGE pMEF REVEALS FUNCTIONAL TRAITS ASSOCIATED WITH PHAGE–HOST INTERACTIONS

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Antimicrobial resistance is one of the major global public health challenges, driving the search for bacteriophages as an alternative strategy to control pathogenic members of the *Staphylococcaceae* family. Genomic characterization of bacteriophages provides essential insights into their evolutionary diversity, functional organization, infection strategies, and taxonomic classification while supporting future biotechnological applications. Bacteriophage pMEF was sequenced using the Illumina MiSeq platform, followed by de novo genome assembly, functional annotation, and comparative analyses for taxonomic classification, lifestyle prediction, and the identification of genes associated with phage–host interactions. The genome comprises 140,994 bp, encoding 241 predicted coding sequences and two tRNAs. Phylogenetic reconstruction and intergenomic similarity analyses placed pMEF within a previously established genus while supporting its classification as a novel species according to current taxonomic criteria. Functional annotation revealed no antimicrobial resistance genes, virulence factors, or mobile genetic elements. In contrast, homologs putatively associated with bacterial defense systems and recently described anti-defense mechanisms were identified, suggesting a genetic repertoire potentially involved in adaptation to bacterial immune responses. Collectively, these findings expand our understanding of the genomic diversity of bacteriophages infecting members of the *Staphylococcaceae* family and demonstrate how integrated genomic approaches can uncover evolutionary and functional traits shaping phage–host interactions, providing a foundation for future studies on phage biology and biotechnological applications.

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PT.494

BACTERIOPHAGE pSS8: BROAD MULTISPECIES LYTIC ACTIVITY, ANTIBIOFILM POTENTIAL, AND GENOMIC EVIDENCE FOR A NOVEL *BAOSHANVIRUS* SPECIES

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Staphylococcus cohnii is a coagulase-negative *staphylococcus* found as a commensal in humans and animals that may act as an opportunistic pathogen and potential reservoir of antimicrobial resistance genes. Bacteriophage pSS8 was isolated from swine wastewater using *S. cohnii* SS8 as the host and characterized through host range, adsorption, physicochemical stability, antibiofilm, and phage–antibiotic interaction assays, transmission electron microscopy, and comparative genomics. pSS8 formed clear plaques, displayed a tailed morphology, and reached approximately 99.8% adsorption within five minutes. It produced lysis in 17 of 40 strains representing eight species within *Staphylococcaceae*. The phage remained infective at pH 3–11 and at −20, 23, and 37 °C and tolerated chloroform exposure. pSS8 reduced biofilm formation by up to 75.5% and established biofilms by up to 63.3%. Synergy was observed with tetracycline and erythromycin, with fractional inhibitory concentration indices of 0.116 and 0.225, respectively. Its 145,736-bp genome contains 258 predicted coding sequences, and no antimicrobial resistance genes, bacterial virulence genes, or canonical lysogeny markers were detected. Comparative genomic analyses placed pSS8 within the family *Herelleviridae* and genus *Baoshanvirus*, with 67–73% intergenomic similarity to the closest related phages, below the 95% species demarcation threshold. These combined biological, functional, and genomic features support further evaluation of pSS8 for the control of *S. cohnii* and other members of *Staphylococcaceae*.

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PT.495

EVALUATION OF ANTIVIRAL COMPOUNDS FROM PIPER HISPIDUM SW. ESSENTIAL OILS AGAINST DENGUE SEROTYPE-2, ZIKA, AND CHIKUNGUNYA VIRUSES

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Infections caused by Dengue serotype 2 (DENV-2), Zika (ZIKV), and Chikungunya (CHIKV) viruses represent a significant public health challenge, with most patients relying strictly on palliative care due to the lack of specific antiviral treatments. To address this, compounds from the medicinal plant *Piper hispidum Sw.* were evaluated for their antiviral potential. Essential oils were isolated via hydrodistillation, and cytotoxicity assays were performed on Vero-76 and C6/36 cell lines. Results demonstrated that a 1/5000 dilution of the essential oil did not induce cytotoxic damage in Vero-76 cells. Viral titration using plaque assays in Vero-76 cells yielded concentrations of 7.24×10^6 PFU/mL for ZIKV, 1.2×10^6 PFU/mL for DENV-2, and 1.8×10^8 PFU/mL for CHIKV. Plaque reduction assays evaluating the efficacy of the *Piper hispidum* essential oil revealed a 34.4% reduction of ZIKV at a concentration of 44.7 µg/mL. Furthermore, the oil exhibited an inhibitory effect against DENV-2 at concentrations ranging from 122.3 to 164.6 µg/mL, achieving reduction percentages of 59.32% and 61.91%, respectively. Finally, a concentration of 48 µg/mL resulted in a 56.4% inhibition of CHIKV. These findings propose P. hispidum essential oil as a promising candidate for antiviral therapies. Financial support: PCONFIGI 2025, project N° B25100891.

PT.496

A NUCLEOPROTEIN-BASED mRNA VACCINE PROTECTS MICE AGAINST H1N1 AND THE HIGHLY VIRULENT H5N1 (2.3.4.4b) INFLUENZA ISOLATES

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Viral surface glycoproteins such as hemagglutinin and neuraminidase are widely studied as antigen targets for vaccines. However, these proteins undergo constant mutations, reducing vaccine efficacy. In contrast, the nucleoprotein (NP) is highly conserved among different influenza virus strains and is immunogenic for T lymphocytes, making it an attractive target for vaccine development. mRNA vaccines are a promising platform due to the ease of updating to new antigens. This study aimed to develop an NP-based mRNA vaccine against influenza viruses using the NP sequence from the A/PR/8/34 (H1N1) isolate. The NP coding sequence was cloned into a pcDNA plasmid containing optimized 5′ and 3′ UTRs and a poly(A) tail. In vitro-transcribed mRNA was synthesized using N1-methyl-pseudouridine and a cap analog. NP expression was confirmed by immunofluorescence and western blot. C57BL/6 mice were immunized with two doses of NP mRNA encapsulated in lipid nanoparticles (LNP-NP) 21 days apart. Humoral and cellular immune responses were evaluated, followed by challenge with H1N1 or H5N1 (2.3.4.4b clade). Plasmid construction was confirmed by restriction digestion, and transfected cells successfully expressed NP. Immunized mice developed robust antibody (total IgG, IgG1 and, IgG2c) and memory T-cell responses. Immunized animals challenged with H1N1 or H5N1 maintained body weight, survived the infection, exhibited reduced viral loads and, showed preserved lung architecture compared with controls. These findings demonstrate that the LNP-NP vaccine induces protective immunity against distinct influenza A viruses and represents a promising candidate for broad protection against future seasonal and pandemic influenza outbreaks.

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PT.497

Integrated Bioinformatics and Immunoproteomics Screening for *Orthopoxvirus* Vaccines and Point-of-Care Testing

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The cessation of mass vaccination after smallpox eradication in 1980 has left the global population susceptible to other Orthopoxviruses (OPXV). This has driven the emergence of zoonotic diseases, including the 2022–2024 Mpox outbreak and the endemic circulation of *Orthopoxvirus vaccinia* (VACV) in Brazil. Current tools face critical limitations: serological methods lack specificity due to cross-reactivity, while existing vaccines are either contraindicated for vulnerable groups or show reduced neutralizing antibody levels. This study aims to identify OPXV antigens for a "Pan-Orthopox" vaccine and differential *point-of-care* diagnostics using integrated bioinformatics and immunoproteomics. Comparative *in silico* analysis of *Orthopoxvirus monkeypox*, *Orthopoxvirus vaccinia*, *Orthopoxvirus cowpox*, and *Orthopoxvirus variola* proteomes via *OrthoFinder* identified 155 conserved orthogroups containing 661 shared proteins. Functional annotation of 483 proteins revealed an enrichment in immune evasion and host manipulation. Further analysis identified 641 viral proteins with molecular mimicry to the human proteome, while topological characterization revealed 24 proteins with signal peptides. Linear B-cell epitope prediction identified 40 epitopes across 28 proteins, where 27 are conserved, while 11 are species-specific, highlighting their diagnostic potential. Additionally, T-cell epitope analysis confirmed that all 191 evaluated proteins possess immunogenic potential. These results provide a robust molecular foundation for designing next-generation immunotherapeutics and specific diagnostic tools that may help mitigate the public health impact of emerging and endemic orthopoxviruses.

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PT.498

BEYOND SUBTYPE: EVOLUTIONARY DIVERSITY OF CONTEMPORARY SWINE INFLUENZA A VIRUSES CIRCULATING IN BRAZIL

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Swine influenza A virus (swIAV) in Brazil has evolved through repeated introductions of human-origin viruses followed by long-term circulation in pigs, generating genetically diverse lineages whose current composition remains incompletely understood. This study characterized the genetic diversity of hemagglutinin (HA) and neuraminidase (NA) genes from 115 influenza A viruses isolated from pigs with respiratory disease on Brazilian commercial farms during 2025–2026. Viruses were sequenced and classified by phylogenetic analysis using representative Brazilian and international reference strains. Among the viruses analyzed, 52 (45.2%) possessed pre-2009 human seasonal H1 lineages established in Brazilian swine, 45 (39.1%) belonged to H3 lineages, 16 (13.9%) were H1N1pdm09-derived, and two viruses remained unclassified. The Brazilian H1 1B.2.3 and 1B.2.4 lineages predominated among seasonal H1 viruses and exhibited multiple associations with distinct N2 genetic groups. The H3 1990.5.1 lineage was the predominant H3 lineage and was associated with three genetically distinct N2 groups, including one not represented in current Brazilian classification schemes. Additional H3 viruses closely related to human seasonal viruses circulating in 2010–2011, 2016–2017 and a contemporary human virus detected in the United States in 2025 were also identified. Overall, 24 distinct HA–NA genetic constellations were detected among 111 fully characterized viruses. These findings demonstrate extensive genetic diversity, persistence of established lineages, continuous evolution at the human–swine interface, and the emergence of genetic groups beyond current classification frameworks, reinforcing the importance of continuous molecular surveillance to support evolutionary studies and rational selection of autogenous vaccine candidates.

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PT.499

SEEKING THE MECHANISM FOR THE POTENT ANTI-MAYV ACTION OF PHYLLANTHOSTATIN A AND TUBERCULATIN MOLECULES THROUGH AN IN SILICO APPROACH

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Transmitted by mosquitoes, Mayaro virus (MAYV) is emerging as a threat with the potential for urbanization in the Americas. The lack of licensed vaccines or specific antiviral therapies reinforces the urgency of developing effective therapeutic strategies. Preliminary data from our research group demonstrated strong antiviral activity of two natural compounds, phyllanthostatin A and tuberculatin, against MAYV. However, the molecular mechanisms underlying their antiviral effects remain unclear. This study aimed to investigate the potential molecular targets of these compounds, focusing on their interactions with viral proteins. In order, to identify possible targets, the chemical structures were submitted to the Braunschweig Enzyme Database (BRENDA) to identify structurally similar inhibitors and their associated protein targets. These targets were classified according to enzymatic function and compared with the MAYV proteome. Selected viral proteins were then subjected to molecular docking analyses with the compounds. The target search revealed hydrolases enzymes as possible targets for the compounds. Capsid protein, a viral structural protein, is classified as a hydrolase enzyme. The docking studies with capsid protein of MAYV, previously modeled by our group, showed that both compounds occupy the hydrophobic pocket in a region that is believed interact the viral glycoprotein E2. Interestingly, the binding energy of tuberculatin (−7.5 kcal/mol) was found to be less than that of the phyllanthostatin A (−6.3 kcal/mol), in agreement with bioassays, that showed greater antiviral activity for tuberculatin. The compounds represent two natural products that could be potential candidates for anti-Mayaro by interfering with key steps of the viral life cycle.

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PT.500

FUNGAL BIOSSURFACTANTS FROM *TRICHODERMA HARZIANUM* UELTH011 IN THE CONTROL OF POTYVIRUS VIGNAE (COWPEA APHID-BORNE MOSAIC VIRUS - CABMV) IN PASSION FRUIT AND RESISTANCE INDUCTION

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Brazil, the world's largest producer and consumer of yellow passion fruit (*Passiflora edulis* Sims), faces productivity limits due to passion fruit hardening disease caused by *Potyvirus vignae* (Cowpea aphid-borne mosaic virus - CABMV; Potyvirus), which is transmitted by aphids in a non-persistent, non-circulative manner. Given the lack of effective management, alternative control strategies are needed. Biosurfactants from *Trichoderma harzianum* UELTH011 (cultivated in a medium containing peptone and oil waste), and obtained by paper filtration (PF) and acid precipitation (PP), were evaluated against CABMV. The experiment was conducted in a greenhouse using 102 seedlings in a completely randomized design (6 plants per treatment group), distributed into control, prophylactic, and therapeutic regimes. Manual sprays were applied weekly (1 and 2 mg/mL) for four weeks, and viral incidence and typical symptoms (mosaic, chlorosis, leaf curling, and blistering) were evaluated for an additional two weeks post-treatment. Binary data (0/1) were transformed $\sqrt{(Y+0.05)}$ and analyzed by Tukey's test ($p < 0.05$). In the therapeutic regime, mean incidences matched the positive control, showing no curative effect. In the prophylactic regime, PP treatment at 2 mg/mL (PP2) significantly reduced the number of symptomatic plants compared to the viral control starting from the second week (means of 0.97 and 1.22, respectively). Control groups remained healthy. These results suggest that PP2 acts as a resistance elicitor, activating plant defenses prior to viral infection.

Financial support: CAPES, CNPq, FAP-PR

PT.501

ANTIVIRAL POTENTIAL OF AMAZON PLANT EXTRACTS AGAINST CHIKUNGUNYA VIRUS REPLICATION

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Chikungunya virus (CHIKV) is the responsible agent of chikungunya fever, a disease characterized by an acute febrile disease accompanied by arthralgia and skin rash, while chronic symptoms such as polyarthritis can also persist and become disabling. The illness is a public health problem, with 119 countries reporting autochthonous transmission of the virus. The treatment is currently limited to supportive care, highlighting a need for specific antivirals against CHIKV. The present work aimed to evaluate the antiviral potential of natural compounds isolated from the amazon plant *Eugenia punicifolia* (Mm4, Mm5 and Mm8) on the replication stage of CHIKV replicative cycle. Cytotoxicity and antiviral assays were carried out using BHK-21 cells expressing the subgenomic replicon of CHIKV (BHK-CHIKV-NCT), and the reporter gene Renilla luciferase. Cell viability of the compounds at 50, 10 and 2 μM was assessed through MTT assays for 24 h. Then, cells were treated with the highest non-cytotoxic concentrations of each compound for 24 h, when the inhibitory activity was measured by luminescence quantification. Out of the tested compounds, Mm4 and Mm8 demonstrated no inhibition of CHIKV replication, while Mm5 showed significant activity, reducing replication by 62% ($p < 0.0001$). These findings demonstrate the antiviral potential of amazon compound Mm5 against CHIKV. Thus, further studies into its inhibition potential and mechanism of action are in progress to further investigate the potential of this compound to the development of new antiviral therapies.
Financial Support: FAPEMIG, CAPES, CNPq.

PT.502

NAPHTHOQUINONE DERIVATIVES AS PROMISING ANTIVIRAL CANDIDATES AGAINST MAYARO VIRUS INFECTION

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Mayaro virus (MAYV), a member of the Togaviridae family and the Alphavirus genus, is an emerging arbovirus associated with acute febrile illness and persistent arthralgia. Since no specific antiviral therapies are available for MAYV infection, identifying compounds capable of inhibiting viral replication is essential to reduce disease-associated morbidity. This study aimed to evaluate the cytotoxicity and antiviral activity of naphthoquinone derivatives against MAYV. Twenty-one modified naphthoquinone derivatives were screened to identify compounds with anti-MAYV activity. Cytotoxicity was assessed in Vero cells cultured in 96-well plates using the MTT assay to determine the 50% cytotoxic concentration (CC_{50}). Antiviral activity was evaluated by plaque reduction, viral adsorption inhibition, and virucidal assays. The CC_{50} values were 466 µM for Naphto 16 and 695 µM for Naphto 17. At 20 µM, Naphto 16 and 17 inhibited viral infection by 95% and 99%, respectively, with EC_{50} values of 1.9 µM and 1.1 µM. Viral adsorption inhibition assays were performed at different concentrations to investigate antiviral activity. At 10 µM, Naphto 16 and 17 inhibited viral adsorption by 72% and 78%, respectively. In the virucidal assay, inhibition at the same concentration was 8% and 6%, respectively. These findings demonstrate that the evaluated naphthoquinone derivatives exhibit potent antiviral activity against MAYV. Among them, Naphto 17 showed the greatest antiviral potency, highest selectivity index, and lowest cytotoxicity, highlighting its potential as a candidate for antiviral therapy development against Mayaro virus infection.

Keywords: Mayaro; Naphthoquinone; Antiviral

Support: FAPERJ, CAPES, CNPq, PROPPI

PT.503

CURCUMIN, A MULTIFACTORIAL INHIBITOR OF IN VITRO MULTIPLICATION OF ARENAVIRUS JUNIN

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Curcumin (CUR) is a bioactive polyphenol found in *Curcuma longa*. In this study, we investigated the *in vitro* inhibitory effect of CUR on Junín virus (JUNV), the causative agent of Argentine Hemorrhagic Fever. The cytotoxic concentration (CC50) was determined by MTT on Vero and A549 cells treated with CUR for 48 h, yielding CC50 values of 56µM and 114µM, respectively. The inhibitory concentration (IC50) was determined by a viral yield assay with an IC50 of 8µM for Vero and 18µM for A549. CUR inhibits JUNV when present from 0-6h p.i. and from 6-24h p.i., affecting early and late stages of multiplication. An adsorption assay and an infection center assay, showed a decrease in adsorbed or internalized virus in the presence of CUR. Because both processes require an intact cytoskeleton, the effect of CUR on the integrity of actin microfilaments was assessed using phalloidin-FITC staining, revealing destabilization upon treatment with CUR. Furthermore, the effect of CUR on Akt phosphorylation, a signaling pathway involved in the recycling of transferrin receptor 1 (TfR1) used by JUNV for adsorption, was analyzed by WB. CUR was able to inhibit the positive modulation of Akt phosphorylation by JUNV, accompanied by a decrease TfR1 in cell membrane. To evaluate the compound's effect in later stages, cultures were treated with CUR at 8 or 12h p.i., revealing a decrease in viral yield. These results allowed for the optimization of protocols and yielded an SI >372. In conclusion, curcumin is a polyfunctional inhibitor of JUNV with potential antiviral applications.

Financial Support: CONICET, UBA.

PT.504

ANTIVIRAL AND VIRUCIDAL ACTIVITY OF BIOSURFACTANTS PRODUCED BY BACTERIAL ISOLATION FROM AMAZONIAN REGION AGAINST ARBOVIRUSES

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Biosurfactants are structurally and chemically diverse molecules with surface-active properties and broad industrial applications, including pharmaceuticals. Interest in these compounds is growing primarily due to their low toxicity, biodegradability, and production from renewable sources. In this work, the antiviral and virucidal capacity of biosurfactants produced by bacterial isolated from the Brazilian Amtazon region, using commercial glycerin as a substrate, was evaluated against the arboviruses dengue (DENV), Zika (ZIKV), yellow fever (YFV), and chikungunya (CHIKV). The bacterial BM02 strain was isolated from the soil of a mining area at Paragominas, Pará, and the AMSD-02 and AMSO-04 strains from copper tailings at the Salobo mine, Marabá, Pará. In Vero cells, the cytotoxic concentration 50 (CC50) for biosurfactants was determined to be greater than 50 µg/ml using the MTT method. When its antiviral activity was evaluated on Vero cells at 24 h using a pre-treatment followed by a post-treatment, a 60% inhibition of ZIKV and YFV was observed with 25 µg/ml, and a 90% inhibition of CHIKV with 50 µg/ml of biosurfactant from BM02. The activity of the biosurfactants from AMSD-02 and AMSO-04, which inhibited the replication of DENV-2, ZIKV, and CHIKV, was also evaluated. Furthermore, the biosurfactants from BM02 and AMSO-04 were found to have virucidal properties against CHIKV. These results demonstrate the importance of characterizing industrially important compounds, such as biosurfactants, constituting a promising and sustainable alternative for the development of new antiviral and virucidal products, such as additives in repellents or disinfectants.

Keywords: Dengue, Zika, Yellow fever, Chikungunya.
Financial Support: Fapespa, CNPq, CAPES, and Amazônia+10 Initiative.

PT.505

CONSTRUCTION OF AN EXPRESSION VECTOR FOR CHIMERIC PROTEINS DERIVED FROM HPV 16 IN *PICHIA PASTORIS*

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Human papillomavirus (HPV) prolonged infection is associated with many types of cancers, especially cervical cancer, with HPV 16 being the most common oncogenic type in Brazil. At present, six prophylactic HPV vaccines are currently available globally, but they have limited therapeutic effects against established HPV infections and HPV-associated lesions. Therefore, investment in prophylactic-therapeutic vaccination strategies is essential to enhance the breadth of antiviral protection, alongside with the development of alternative vaccine platforms to reduce vaccine production and implementation costs. Thus, the present study aimed to generate vectors for heterologous expression in Pichia pastoris containing the HPV16 L1 gene, for the prophylactic properties of the vaccine antigen, and chimeric L1E5 gene, in which the h4 loop region of L1 has been replaced by epitopes from the E5 oncoprotein for therapeutic action. The recombinant constructs were generated from shuttle vectors and subsequently subcloned into the pPGKΔ3 vector, using Escherichia coli Top 10 as the host cell. The colonies were screened for the presence of recombinant vectors by culturing them in medium supplement with Zeocina. Following the successful generation of L1wt and L1E5(-h4) clones in the pPGKΔ3 vector, it will be possible to proceed to the generation of recombinant yeast strain, witch will serve as biofabrictories for the production of prophylactic-therapeutic antigen.
This work was supported by the National Council for Scientidic and Technological Developmento (CNPq), Brazil.

PT.506

POLY(I:C)-ADJUVANTED BSA NANOPARTICLES ENHANCE THE EFFICACY OF LOW-DOSE MVA VACCINATION

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Orthopoxvirus infections remain a global public health concern and Modified Vaccinia Ankara (MVA) is a safe and effective vaccine platform that can induce cross-protective immunity. In response to emergencies and the limited vaccine availability, World Health Organization has proposed dose-sparing strategies. However, vaccination with low doses may impair the magnitude and quality of the immune response. In this case, the use of adjuvants could act as a promising tool to enhance immune responses. Nanoparticles can act as adjuvants and enhance the immunogenicity and stability of a given antigen. Therefore, this study evaluated the adjuvant effect of bovine serum albumin (BSA) nanoparticles loaded with poly(I:C) (NPPI) combined with low doses of MVA in a murine model of *Vaccinia virus* infection. NPPI did not affect viral infectivity and exhibited appropriate physicochemical properties. While total IgG levels were similar among immunized groups, NPPI significantly enhanced neutralizing antibody activity after low-dose vaccination. Mice immunized with MVA in the presence of NPPI showed improved clinical outcomes, including faster weight recovery, reduced clinical scores, and decreased lung injury after viral challenge. These findings suggest that NPPI enhances the functional quality of the immune response and may contribute to improved protection, even with a low-dose immunization regimen. This approach represents a promising strategy to optimize MVA-based vaccination under dose-reduction scenarios.

Financial support: CAPES (Finance code 001), FAPEMIG, CNPQ and INCT-Pox.

PT.507

EXPRESSION OF ESCHERICHIA COLI OF RECOMBINANT PROTEINS OF THE ORTHOPOXVIRUS MONKEYPOX AND A CHIMERA OF THESE PROTEINS FOR THE DEVELOPMENT OF DIAGNOSTIC TESTS AND VACCINES

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Monkeypox virus (MPXV), a member of the *Poxviridae* family, has emerged as a global public health concern due to its zoonotic transmission and potential for human-to-human spread. The 2022 outbreak resulted in over 87,000 cases and 140 deaths across 111 countries. Transmission occurs mainly through direct contact with lesions, bodily fluids, or prolonged physical interaction. Despite the availability of vaccines such as MVA-BN (Jynneos) and LC16m8, challenges related to accessibility and rapid response persist, highlighting the need for improved diagnostic tools and alternative vaccine strategies. In this study, three recombinant MPXV proteins (A29L, H3L, and the chimeric A29H3L) were expressed in *Escherichia coli* and purified by affinity chromatography, achieving yields of 25–40 mg/L and purity above 95%, confirmed by SDS-PAGE and Western blot. The chimeric protein was designed using immunogenic regions of A29L and H3L and evaluated as a vaccine candidate in BALB/c mice (n = 8/group). It induced a strong humoral response, with IgG titers reaching 1:625,000, and a predominantly Th1-biased cellular response. For diagnostics, monoclonal antibodies were produced via hybridoma technology and used to develop a rapid antigen-capture assay, evaluated with 65 samples using PCR as reference. The test showed 100% specificity and 75% sensitivity. These results demonstrate the potential of MPXV recombinant proteins for diagnostic and immunoprophylactic applications, although further optimization and validation are required.

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PT.508

RECOMBINANT PRODUCTION OF THE OROPOUCHE VIRUS N PROTEIN AND ITS APPLICATION IN THE DEVELOPMENT OF AN ELISA TEST FOR IGM ANTIBODY DETECTION

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Oropouche virus (OROV), an arbovirus of the genus *Orthobunyavirus*, is the causative agent of Oropouche fever, an acute disease with symptoms similar to other arboviral infections, such as dengue and chikungunya. Endemic to the Amazon region, its clinical diagnosis is complicated by overlapping symptoms, making the development of specific laboratory methods—such as ELISA-based serological tests for IgM detection—essential. In this study, the OROV nucleocapsid (N) protein was produced using recombinant technology in *Escherichia coli*. The gene was cloned and expressed in the BL21(DE3) strain, and the protein was purified via nickel-affinity chromatography. Expression was confirmed by SDS-PAGE (~39.7 kDa), and identity was verified by Western blot. The final yield after purification and dialysis was approximately 2.8 mg. The purified protein was used to standardize an indirect ELISA for IgM antibody detection, utilizing both positive and negative samples. In the assays, optimal antigen concentrations ranged from 100 to 250 ng, yielding positive-to-negative ratios greater than 2.5. Secondary antibody dilutions between 1:20,000 and 1:40,000 showed the best performance, with ratios exceeding 4. The results demonstrated a good ability to discriminate between samples, although some overlap at low concentrations indicates a need for further optimization. It is concluded that the recombinant N protein shows potential for application in ELISA tests for OROV diagnosis, contributing to the development of more specific tools. Further studies are required for clinical validation and to enhance test performance.

Financial Support: CAPES, CNPq

PT.509

Antiviral Effect of Echinatin Against Human Respiratory Syncytial Virus in A549 Cell Culture

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Human respiratory syncytial virus (hRSV) is one of the leading causes of severe acute respiratory infections, especially in children, the elderly, and individuals with comorbidities. Despite advances in prophylaxis, including vaccines and monoclonal antibodies, there remains a shortage of effective antiviral therapies for treating the infection. In this context, natural compounds have been investigated as potential antiviral agents. Thus, this study aimed to evaluate the antiviral mechanism of action of echinatin against hRSV in A549 cells. Cytotoxicity was determined by the MTT assay, while antiviral activity was investigated using a recombinant hRSV expressing the GFP protein at an MOI of 0.01, through virucidal, pretreatment, post-treatment, and time-of-addition assays. Echinatin exhibited CC_{50} values of 186.7 µM and 137.5 µM after 24 and 48 hours. In assays targeting the early stages of infection, the compound had no virucidal effect and exhibited limited activity when administered prior to cellular infection. However, concentration-dependent antiviral activity was observed in the post-infection assay, with a reduction of up to 68% in viral replication. The IC_{50} values were 23.6 µM and 21.8 µM for 24 and 48 hours, respectively, resulting in selectivity indices of 7.8 and 6.2. Furthermore, echinatin maintained antiviral activity when administered after infection had been established, achieving inhibition of over 70% at a concentration of 50 µM. The results suggest that echinatin acts predominantly during post-entry stages of the hRSV replication cycle, highlighting its potential as a candidate for the development of new antivirals.

PT.510

IN SILICO PREDICTION OF INTERACTIONS BETWEEN COMPOUNDS PRESENT IN THE ESSENTIAL OILS OF *MELALEUCA LEUCADENDRA* AND *CYMBOPOGON DENSIFLORUS* AND TARGET PROTEINS OF THE DENGUE VIRUS AND HOST CELLS.

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Dengue is an arboviral disease with high morbidity and mortality, and the genotypic variability of the dengue virus (DENV) limits the development of specific antivirals. In this context, *Melaleuca leucadendra* and *Cymbopogon densiflorus* stand out as potential sources of bioactive metabolites with described antimicrobial activity. Thus, the antiviral potential of constituents from the essential oils (EOs) of these species was evaluated using an *in silico* approach against viral and host molecular targets. The essential oils of *Melaleuca leucadendra* (OEML) and *Cymbopogon densiflorus* (OECD) were characterized by gas chromatography coupled to mass spectrometry, enabling the identification and quantification of volatile metabolites. The compounds were subjected to PASS screening, adopting a bioactivity significance threshold of $Pa - Pi \geq 0.5$. OEML exhibited monoterpenoid constituents, including veridiflorol (17.17%), 1,8-cineole (16.58%), limonene (14.37%), α-pinene (13.28%), and δ-terpineol (11.95%). OECD presented oxygenated monoterpenes, including trans-p-mentha-1(7),8-dien-2-ol (16.61%), cis-p-mentha-1(7),8-dien-2-ol (11.37%), trans-p-mentha-2,8-dien-1-ol (9.93%), and piperitols (13.5%). The *in silico* analysis indicated that, except for limonene, all constituents of OEML showed potential inhibition of matrix metalloproteinase-9 (MMP-9), which is associated with plasma leakage in severe dengue cases, as well as modulation of 27-hydroxycholesterol, a metabolite involved in DENV replication. In OECD, prediction of β-glucosidase inhibition was observed, enzymes related to glycosidic bond hydrolysis and viral structuring, except for cis-p-mentha-1(7),8-dien-2-ol. In conclusion, although there is no direct inhibition of DENV, the compounds may attenuate viral replication and disease severity through modulation of host targets. Financial Support: FAPEMIG.

PT.511

DO ANCESTRY COMPONENTS CONTRIBUTE TO POST-CHIKUNGUNYA CHRONIC ARTHRALGIA?

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The chronic post-infection manifestations caused by the Chikungunya virus represent a challenge for public health due to their clinical variability and impact on the patients' quality of life. Although evidence suggests that host genetic factors may influence the predisposition to persistent forms of the disease, this area remains largely unexplored. This study aims to evaluate the association between clinical and genetic (SNPs and ancestral components) factors identified in whole-exome sequencing (WES) and chronic post-infection arthralgia using a machine learning method. Initially, 67 samples of patients diagnosed with CHIKV and classified by the presence or absence of chronic symptoms were whole-exome sequenced. Individuals' whole exomes were produced following GATK best practices. Filtering of MAF > 5% and a call-rate of 95% was applied. Principal Component Analysis was executed with the PLINK tool. We applied an embedded feature selection method that assigned rankings to the clinical and genetic variables based on their relation to the case–control variable. The top 20 were selected to compose the Histogram-based Gradient Boosting Regression Tree algorithm. Ancestral components did not achieve statistical significance to be included in the final model. The model achieved a mean AUC of 0.680 (95% CI: 0.523-0.830) with a mean sensitivity of 79% (95% CI: 54.5-100%). This study contributes to the comprehension of biomarkers associated with Chikungunya chronic manifestations, providing a basis for the development of personalized medicine strategies and for early identification of chronic post-infection high-risk individuals.

PT.512

ESTABLISHMENT OF AN AG129 MOUSE MODEL FOR CHIKUNGUNYA VIRUS PATHOGENESIS AND PRECLINICAL ANTIVIRAL EVALUATION

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Chikungunya virus (CHIKV) infection leads to diverse clinical manifestations, ranging from mild self-limiting illness to severe fatal outcomes. Although animal models are essential for investigating disease pathogenesis and evaluating therapeutic candidates, well-characterized models assessing dose-specific impacts in AG129 mice remain limited. This study established an in vivo model using male AG129 mice to evaluate how different viral concentrations (10, 100, or 1000 PFU/mL) of a Brazilian strain from the East/Central/South African (ECSA) lineage affect disease progression following intraperitoneal inoculation. Clinical signs, body weight, survival, viral dissemination, and histopathological alterations were evaluated. Viral RNA levels in serum, spleen, liver, kidneys, and hind-limb muscles were quantified by RT-qPCR, and tissue pathology was assessed by histopathological analysis. Inoculation with 10 PFU/mL induced a controllable infection characterized by delayed viral replication, limited tissue dissemination, minimal histopathological alterations, and a 78.95% survival rate. Conversely, higher doses (≥100 PFU/mL) triggered accelerated systemic infection, resulting in severe lesions in the spleen, liver, and kidneys, resulting in 100% mortality within four days. Maximum viral loads converged across all experimental groups, indicating that disease severity was associated with early viral replication kinetics rather than maximum viral load. Viral RNA was detected in skeletal muscle without corresponding histopathological alterations, suggesting that viral presence alone is insufficient to induce tissue injury. These findings establish a reproducible CHIKV infection model and support the use of the 10 PFU/mL inoculum as a suitable platform for preclinical evaluation of antiviral candidates.

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PT.513

A BUNYAMWERA VIRUS-BASED SCREENING PLATFORM TO IDENTIFY POTENTIAL ANTIVIRALS AGAINST OROPOUCHE VIRUS

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Arboviral infections continue to represent a significant public health threat in tropical and subtropical zones, where environmental and socioeconomic factors drive the circulation of numerous RNA viruses, including more than 350 members of the reclassified Peribunyaviridae family. Notably, Oropouche virus (OROV) has recently triggered major outbreaks across the Amazon and expanded its reach within Brazil, with 2024 marking a critical turning point due to confirmed neurological complications and fatalities. Given the total lack of approved antiviral treatments, identifying effective anti-OROV agents is a matter of clinical urgency. In this study, we screened the Pandemic Response Box and Global Health Priority Box libraries from Medicines for Malaria Venture (MMV) to identify potential inhibitors. To facilitate high-throughput analysis, we used a well-characterized Bunyamwera virus (BUNV) system, the prototype for the Peribunyaviridae family, engineered to express an eGFP reporter gene. This allowed for a screening protocol based on real-time fluorescent detection via live-cell imaging. Our primary screen identified nine compounds with significant inhibitory activity against BUNV-eGFP, from which three: MMV-1, MMV-2, and MMV-3, were selected as the most promising candidates. Subsequent validation against OROV revealed that MMV-2 possessed the lowest EC50 and the most favourable selectivity index. These findings highlight MMV-2 as a candidate for future pharmacokinetic and efficacy studies. Furthermore, this research demonstrates that employing BUNV-eGFP as a surrogate model is a robust and valid strategy for discovering novel therapeutics to combat Oropouche fever.

Financial support: FAPEMIG, CNPq, CAPES, Wellcome Trust.

PT.514

An experimental multiplex RT-PCR assay for the differential diagnosis of dengue, Zika, chikungunya and Oropouche virus infections

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Dengue virus (DENV), Zika virus (ZIKV), chikungunya virus (CHIKV) and Oropouche virus (OROV) represent a major public health burden, particularly in South America and the Caribbean, where their cocirculation and overlapping clinical presentations make diagnosis challenging. Consequently, molecular diagnostics represent a valuable tool for the investigation of arboviral infections, offering high sensitivity and specificity while overcoming the cross-reactivity frequently observed in serological assays. Given the diagnostic challenges posed by cocirculating arboviruses and the advantages of molecular methods, we developed a multiplex RT-PCR assay for the simultaneous detection of DENV, ZIKV, CHIKV and OROV. Herein, we designed specific primers for the amplification of all target viruses, including a single primer set for all four DENV serotypes. All primers were evaluated *in silico* for specificity to avoid cross-amplification with other related arboviruses, including yellow fever virus (YFV), West Nile virus (WNV) and Mayaro virus (MAYV). Furthermore, each primer set was experimentally evaluated for nonspecific amplification against the other targets included in the multiplex assay. The multiplex reaction conditions were optimized for the simultaneous amplification of CHIKV (379bp), ZIKV (238bp), DENV (155-161bp) and OROV (84bp), with no nonspecific amplification or detectable primer-dimer formation. The assay will be further evaluated for its analytical sensitivity, and its performance will be assessed using clinical samples positive and negative for the target viruses. Overall, our multiplex assay represents a promising tool for the diagnosis and surveillance of arboviral infections in regions where DENV, ZIKV, CHIKV and OROV cocirculate.
Financial Support: FAPERGS

PT.515

Silymarin Mitigates Yellow Fever Virus–Induced Oxidative Stress and Inflammation in HepG2 Cells

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Yellow fever virus (YFV) infection exhibits variable clinical severity in humans, from mild febrile illness to life-threatening systemic disease. Despite the availability of an effective vaccine, the molecular mechanisms underlying YFV-induced hepatic pathogenesis remain poorly understood. Our prior studies demonstrated that oxidative stress contributes to YFV pathogenesis and identified silymarin as a potential antioxidant and hepatoprotective agent. In this study, we evaluated the antioxidant, hepatoprotective and anti-inflammatory properties of silymarin against YFV infection using *in vitro* (HepG2). To evaluate the therapeutic potential of silymarin against YFV infection, we infected HepG2 cells with YFV at an MOI of 1 and treated with silymarin at 25 μg/uL. Preliminary results revealed a significant decrease in Reactive Oxygen Species levels at 4 days post-infection (dpi) in silymarin-treated cells. When measuring oxidative parameters at 4 dpi, treatment with silymarin resulted in a decrease in lipid peroxidation and carbonyl protein. Furthermore, there was a significant increase in the activity of antioxidant enzymes, superoxide dismutase and glutathione peroxidase, indicating an antioxidant environment. Also, silymarin showed a significant decrease in viral titre of YFV and modulation of IL-6, IL-1β, TNF and INF-β. Therefore, our results show that silymarin restores redox homeostasis, in addition to having antiviral and anti-inflammatory action against YFV infection, making this natural product a promising therapeutic agent against YFV infection.

Keywords: Yellow fever virus, Silymarin, Oxidative stress, Hepatoprotection.
Financial Support: FAPEMIG, CNPq, CAPES and UFOP

PT.516

CIPOVIRUS POLYHEDRA (*THIRINTEINA ARNOBIA*) AS A MATRIX FOR FUNCTIONAL IMMOBILIZATION OF DENGUE NS1 PROTEIN.

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Preserving antigens in biological matrices requires an efficient encapsulation system that ensures their stable, durable incorporation while maintaining their native biological and functional properties. The dengue virus a pathogen of critical global public health importance, for which early and accurate diagnosis is paramount. To determine whether the immobilization of dengue antigens within cypovirus polyhedra can preserve their stability and antigenicity against antibodies from dengue patients, we expressed the Wing domain of the NS1 protein from all four dengue serotypes, both as a multi-epitope construct and as individual serotype-specific epitopes, each fused to the H1 alpha-helix incorporation domain from TharCPV-14 polyhedrin, and expressed them in insect cells using a baculovirus expression system. The recombinant proteins were confirmed by SDS-PAGE and western blot. Light microscopy and scanning electron microscopy revealed the presence of polyhedral crystals in the cytoplasm of insect cells. Serological analysis by ELISA demonstrated that sera from patients previously diagnosed with dengue reacted significantly with the NS1 antigens, revealing that NS1 antigens immobilized in TharCPV polyhedra preserve the functional stability and antigenicity of the dengue NS1 proteins. This work constitutes a proof-of-concept study, aiming to establish the technical and functional viability of this methodology for potential applications in serological testing and beyond.

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PT.517

Next-Generation Molecular Diagnostics: A novel LAMP-CRISPR approach for Viral Detection

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The effects of globalization, such as the increasing population mobility, have contributed to the establishment of infectious diseases as threats to public health and the world economy, making the development of accurate diagnostic methods essential for effective epidemiological surveillance. Among current molecular methods, Loop-Mediated Isothermal Amplification (LAMP) stands out, not only for its similarities with the well-established Polymerase Chain Reaction (PCR) but mainly for its portability, making it suitable for point-of-care testing (PoCT) diagnostics, essential for remote regions. However, due to high rates of false-positives, the integration of LAMP with sequence-specific detection systems, such as CRISPR/Cas, has become fundamental to overcome technical limitations and ensure greater diagnostic specificity. The present study aimed to develop a molecular platform for Zika Virus (ZIKV) detection by integrating LAMP-CRISPR/Cas12. Nucleotide sequences were evaluated for the synthesis of primers, guide RNAs (gRNAs), and synthetic genomes of ZIKV, DENV1, DENV2, DENV3 and DENV4. Different probes were synthesized for efficiency evaluation. CRISPR assays were conducted by combining the Cas12-gRNA ribonucleoprotein complex with LAMP amplification products. *Trans*-cleavage occurred by adding probes (Poly-A, Poly-C and FAM-BHQ), and the supernatant fluorescence was measured. Consequently, this diagnostic platform allowed the detection of the target with fluorescent signals up to seventy times higher than negative controls, demonstrated no cross-reactivity with the four serotypes of Dengue Virus (DENV), and achieved a limit of detection of 120 femtomolar. Thus, this innovative diagnostic platform demonstrated robust performance and presents a promising solution for next-generation molecular diagnostics.

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PT.518

IN VITRO IMMUNOMODULATORY EFFECT OF PICEATANNOL (Passiflora edulis) IN HTLV-1-CARRIERS.

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Introduction: The HTLV-1/2 retrovirus can cause serious diseases such as HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) and adult T-cell leukemia/lymphoma by infecting immune cells. As no effective therapy is available, bioactive plant compounds may be promising therapeutic candidates in the Amazon region. **Objectives:** To evaluate the immunomodulatory potential of piceatannol (Passiflora edulis) in the peripheral blood mononuclear cells (PBMCs) from HTLV-1-infected individuals. **Methodology:** PBMCs from three individuals (asymptomatic HTLV-1 carrier, HAM/TSP patient, and uninfected control) were isolated using Histopaque® 1077 g/L. Cells were treated with piceatannol (12.5–400 μM), and viability was assessed by MTT after 24, 48, and 72 h. After 48 h of treatment with 12.5 μM piceatannol, culture supernatants were analyzed for cytokines profile (BD® Cytometric Bead Array-CBA). Data were analyzed using descriptive statistics and expressed as mean ± SD. **Results:** Piceatannol maintained PBMC viability in all tested concentrations. Cytokine analysis showed an immunomodulatory effect, when considering HTLV-1 carriers. IFN-γ, TNF-α, IL-6, and IL-17 from HAM patients levels decreased after treatment, whereas IL-4 and IL-2 exhibited no changes. The higher reduction in IFN-γ occurred in the HAM/TSP patient, while IL-6 decreased more markedly in the asymptomatic carrier. Minimal changes were observed in the uninfected control. **Conclusions:** Piceatannol reduced key pro-inflammatory cytokines associated with HTLV-1 and demonstrates that natural compounds can be an alternative to treat HTLV-1/2 chronic inflammation-related disease but more comprehensive studies are required to validate the hole of bioextracts on HTLV-1 diseases.

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PT.519

ANTIVIRAL, ANTIOXIDANT, AND ANTI-INFLAMMATORY PROPERTIES OF AN ISOLATED LIGNAN FROM *PHYLLANTHUS BRASILIENSIS* AGAINST CHIKUNGUNYA VIRUS

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Chikungunya virus (CHIKV) is the etiological agent of Chikungunya Fever (CF), a disease characterized by fever, joint and muscle pain, headache, fatigue, nausea and rash. However, no clinically approved virus-specific drugs are available for CF. Then, the investigation of natural compounds with antiviral potential represents an alternative due to their low toxicity and chemical diversity. Previous studies demonstrated that the hydroalcoholic leaf extract of *Phyllanthus brasiliensis* exhibits antiviral activity against several arboviruses. So, this study aimed to evaluate the antiviral, antioxidant, and anti-inflammatory effects of an Isolated Lignan (IL) from *P. brasiliensis* against CHIKV and to elucidate its mechanism of action. Cytotoxicity and antiviral assays were performed in Vero cells, including analyses of different stages of the viral replication. The IL showed anti-CHIKV activity with a selectivity index of 2.912. No significant effects were observed during viral adsorption or virucidal stages. However, the compound significantly inhibited viral replication during early intracellular stages, up to 3 hours post-infection, reducing viral load by 4 log units. CHIKV infection increased superoxide dismutase (SOD) and catalase (CAT) activities compared to uninfected controls, while treatment with the IL reduced both enzyme activities relative to the infected group. Lipid peroxidation was also elevated in CHIKV-infected cells and decreased upon treatment. Furthermore, pro-inflammatory cytokines TNF, CCL2, and IL-17 were increased during infection and significantly reduced in treated cells. These findings highlight the antiviral and immunomodulatory potential of IL and support further investigation as a candidate for CF treatment.
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PT.520

A SYNTHETIC 7-CLOROQUINOLINE DERIVATIVE AS A POTENTIAL ANTIVIRAL AGAINST ARBOVIRUSES

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Arboviruses transmitted by hematophagous arthropods represent a major global public health concern, particularly in tropical regions. Among the most relevant are *Alphavirus chikungunya* (CHIKV), *Alphavirus mayaro* (MAYV) and *Orthoflavivirus zikaense* (ZIKV), for which effective antiviral therapies remain unavailable. In this context, chloroquinoline derivatives have emerged as promising scaffolds for antiviral drug development. This study evaluated the in vitro antiviral activity of a synthetic 7-chloroquinoline derivative against CHIKV, MAYV, and ZIKV. Cytotoxicity was assessed in Vero cells using the MTT assay (CC_{50}), and antiviral activity was determined by EC_{50}, selectivity index (SI), and relative potency (RP). Antiviral effects were further confirmed by cytopathic effect (CPE) inhibition assays, viral titration, RT-qPCR, and virucidal assays. The compound exhibited antiviral activity against all three arboviruses, with the most pronounced effect against MAYV (EC_{50} = 14.77 ± 3.13 µM; SI = 4.47; RP = 29.20). CPE inhibition assays revealed a significant reduction in viral titers, reaching up to 5 $\log_{10}$ PFU·mL^{-1} at 48 h post-infection. RT-qPCR analysis confirmed a marked decrease in MAYV replication, while no direct virucidal activity was observed, suggesting inhibition of viral replication. These findings highlight this compound as a promising lead for anti-MAYV drug development and support further studies to elucidate its mechanism of action and optimize its antiviral efficacy. Financial support: FAPMIG, CNPq, UFOP, Rede Mineira de Antivirais.

PT.521

VALIDATION OF THE ANTIVIRAL ACTIVITY OF HITS COMPOUNDS AGAINST *ORTHOFLAVIVIRUS LOUISENSE* (SLEV) *IN VITRO*

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Orthoflavivirus louisense (SLEV), a member of the Flaviviridae family, is a neglected arbovirus circulating in the Americas. Although infection is generally asymptomatic, the virus can affect the nervous system and cause encephalitis, particularly in the elderly. To date, there are no commercially available vaccines or treatments for SLEV infection. In this context, high-throughput screening (HTS) has emerged as a powerful strategy for the rapid identification of compounds with antiviral potential from large compound libraries. We previously performed HTS of the TargetMol library (7,784 compounds) in 384-well plates to evaluate cytoprotective activity in SLEV-infected Huh-7.0 cells and identified anisindione as a hit compound. We aimed to validate and characterize the antiviral activity of anisindione against SLEV. Antiviral assays were performed in 24-well plates using Vero and Huh-7.0 cells infected with SLEV at a multiplicity of infection (MOI) of 0.1, with virus and compound added simultaneously. Anisindione was tested at 5, 10, and 20 µM, and DMSO was used as the vehicle control. After 72 h, supernatants were collected, and viral titers were determined by plaque assay (PFU/mL) using Vero cells. Cell viability was assessed by the MTT assay in non-infected cells treated with anisindione. Although anisindione was not cytotoxic, no significant reduction in viral load was observed. Therefore, additional concentrations and compounds will be evaluated to identify candidates with significant antiviral activity against SLEV. Collectively, these studies are expected to support *in vivo* studies and the development of therapeutics for SLEV infection.

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PT.522

EVALUATION OF THE HUMORAL RESPONSE OF TWO VACCINE CANDIDATES AGAINST OROPOUCHE VIRUS

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Oropouche fever is an emerging arboviral disease of significant public health relevance, with no specific treatment or vaccine currently available. In Brazil, it is estimated that 5.5 million people have already been infected with Oropouche vírus (OROV) between 1960 and 2025, with a significant increase in the number of cases in the last 3 years, as well as unprecedented reports of deaths, transplacental transmission, and possible associations with congenital anomalies. In this context, vaccine development is essential. This study, conducted in partnership with Fiocruz Bio-Manguinhos, we evaluated two mRNA vaccine candidates based on the M segment in 4-week-old BALB/c mice. Animals were immunized with two doses of each vaccine candidate at a 21-day interval and challenged with OROV (strain AM0088) 14 days after the second dose. Control groups received vehicle alone, with or without viral challenge. Animals were euthanized on days 1, 3, 5, 7, 9, 11, 13, and 15 post-infection. Preliminary ELISA results, using the N protein as antigen for IgG and IgM detection in serum, demonstrated differences between the two vaccine candidates. The first candidate induced lower levels of anti-N protein antibodies (IgM and IgG). In contrast, the second vaccine candidate elicited a stronger anti-N protein response (IgM and IgG) compared to the first candidate, but still lower than the titers observed in the non-immunized and challenged group. Further analyses, including viral load assessment, are ongoing to investigate the potential protective effects of the vaccine candidates and their ability to control viral replication.

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PT.523

A 100-fold and 1000-fold reduced dose of a live-attenuated MAYV/IRES vaccine protects against Mayaro virus challenge in C57BL/6J mice without the need for adjuvants

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Mayaro virus (MAYV) is an emerging mosquito-borne alphavirus in South America, where it is responsible for sporadic outbreaks of acute febrile illness, often followed by debilitating and chronic arthralgia. Although there are currently no approved vaccines, the live-attenuated vaccine candidate MAYV/IRES has been developed as a promising option in pre-clinical research. In this study, we tested whether 100-fold and 1000-fold dose reductions of MAYV/IRES could induce protection in C57BL/6 mice, and whether bovine serum albumin nanoparticles containing poly(I:C) (NPPI) or Alhydrogel could enhance the immunogenicity of reduced doses and protect animals following challenge. The animals were vaccinated with 1×10^4 PFU/mouse (standard dose), 1×10^2 PFU/mouse, 1×10^2 PFU/mouse + NPPI, 1×10^1 PFU/mouse, 1×10^1 PFU/mouse + NPPI, or 1×10^1 PFU/mouse + Alhydrogel. Vaccination did not affect body weight or footpad swelling. Furthermore, we demonstrated that the vaccine, at both standard and reduced doses, induced anti-MAYV CH IgG antibodies, with most samples yielding neutralizing PRNT80 titers of 1:320. Both reduced doses also conferred protection against challenge with MAYV CH, preventing footpad swelling and reducing viremia and viral load at the site of infection (footpad) to below the limit of detection. The inclusion of NPPI or Alhydrogel did not substantially improve immune responses or protection induced by the reduced vaccine doses. Our data suggest that 100-fold and 1000-fold dose reductions of MAYV/IRES provide protection comparable to the standard dose, and that the tested adjuvants did not further enhance vaccine immunogenicity in this model. Dose reduction may expand vaccination coverage during emergencies without compromising immunogenicity.

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PT.524

NOVEL ANTI-HPV ACTIVITY OF *TARAXACUM OFFICINALE* ROOT EXTRACT: E6/E7 DOWNREGULATION AND CANCER CELL DEATH

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Cervical cancer caused by high-risk human papillomavirus (HPV) remains a major global health concern, driven largely by the viral E6 and E7 oncoproteins. Chemotherapy's side effects and resistance issues keep the search for new therapies open. Taraxacum officinale (dandelion) has traditional use against HPV-associated warts and known antitumor activity, but its effect on HPV oncogenes had not been studied. This work evaluated an ethanolic root extract of T. officinale on viability, proliferation, migration, and death of cervical cancer cells, and on HPV16/18 E6/E7 expression. Three cervical cancer cell lines (HPV16-positive, HPV18-positive, HPV-negative) and one non-cancerous line were treated with the extract; cytotoxicity, survival, migration, apoptosis, autophagy, and E6/E7 expression were assessed at two concentrations, and chemical composition was profiled by high-resolution LC-MS. The extract was selectively toxic to cancer cells with minimal effect on healthy cells, and reduced HPV16/18 E6/E7 expression dose-dependently; the first report of a dandelion extract directly inhibiting these oncoproteins. It also induced cell death via two simultaneous pathways, apoptosis and autophagy, potentially helping overcome treatment resistance, and strongly suppressed clonogenic proliferation and migration. Chemical analysis identified phenolic acids and sesquiterpene lactones, suggesting a possible synergistic effect. These findings position T. officinale root extract as a promising source of compounds warranting further investigation, supporting future work to isolate and characterize them for HPV-associated cervical cancer.

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PT.525

EVALUATION OF THE ANTIVIRAL ACTIVITY OF TYRPHOSTIN AG879 AGAINST MADARIAGA VIRUS USING REPLICON AND IN VITRO INFECTION MODELS

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Arbovirus surveillance in Brazil is challenged by the country's continental dimensions and the cryptic circulation of zoonotic viruses. Madariaga virus (MADV), an emerging alphavirus in Latin America, represents a growing public health concern due to its epidemic potential and the absence of specific antiviral therapies. Given the previously reported activity of tyrphostin AG879 against Chikungunya virus, this study evaluated its preliminary activity against MADV using a replicon system and infectious virus assays. Initially, MADV replicon expressing luciferase were treated with 10 µM AG879, reducing reporter activity from 311.000 to 41.250 relative light units (RLU), corresponding to approximately 87% inhibition of viral replication. Antiviral activity was validated in Vero CCL-81 cells infected with MADV at multiplicities of infection (MOI) of 0.1 and 1 and treated with AG879 (2.5, 5.0, and 10 µM) for 24 h. Virus titers were quantified by TCID 50 . At MOI 0.1, AG879 reduced viral titers by 4.12 log 10 (10 µM; p=0.0020) and 2.87log 10 (5 µM; p=0.0076). At MOI 1, reductions reached 3.00og 10 (10 µM; p=0.0004) and 1.62log 10 (5 µM; p=0.0020). The highest tested concentration tested maintained 72% cell viability. These findings demonstrate promising in vitro antiviral activity and strong agreement between replicon and infectious virus assays. Ongoing studies aim will determine the EC 50 , CC 50 , and selectivity index to further assess AG879 as a potential antiviral candidate against MADV.

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PT.526

MODULATION OF ENDOGENOUS MICRORNAS VIA DRUG REPURPOSING: A NOVEL STRATEGY AGAINST ZIKA VIRUS INFECTION

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The persistent global threat of emerging RNA viruses highlights the urgent need to new therapeutic approaches. In this scenario, Zika virus (ZIKV) remains a major public health concern due to congenital Zika syndrome and the complete lack of approved antiviral treatments. As pivotal regulators of post-transcriptional gene expression, host microRNAs (miRNAs) represent an appealing therapeutic axis to directly target viral RNA genomes. Here, we implemented a dual computational and experimental pipeline aimed at identifying and pharmacologically inducing endogenous human miRNAs with anti-ZIKV activity. Initial bioinformatic algorithms screened human miRNA databases for high-affinity binding sites across the genome of a epidemic Brazilian ZIKV strain. Functional validation in modified VERO cell cultures revealed that 12 selected miRNAs candidates attenuated virus effect by more than 50%. To translate these insights into viable therapeutics without requiring synthetic RNA delivery, we utilized an in silico drug screening framework to identify host- directed small molecules capable of boosting these specific antiviral miRNAs. Subsequent cellular assays confirmed that candidate compound treatment achieved robust anti-ZIKV efficacy, suppressing viral activity by over 90%. Overall, this study establishes a novel therapeutic paradigm that bridges computational RNA target discovery with drug repurposing. By leveraging host-encoded miRNA machinery via small molecules, this strategy provides an immediate framework to counteract ZIKV infection and could be rapidly deployed against other emerging positive-sense single-stranded RNA pathogens.

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PT.527

DEVELOPMENT AND STANDARDIZATION OF A LAMP ASSAY FOR THE DETECTION OF INFECTIOUS LARYNGOTRACHEITIS VIRUS (ILTV)

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Infectious laryngotracheitis, caused by ILTV, is a respiratory disease of high economic impact in poultry farming due to severe mortality and its capacity for viral latency. Outbreak control is hindered by the reversion to virulence of vaccine strains and the urgent need for rapid diagnosis. Currently, gold-standard methods (such as qPCR) require robust laboratory infrastructure, prolonged processing times, and highly trained personnel, which delays decision-making in the field. Loop-mediated isothermal amplification (LAMP) methodology emerges as a rapid, sensitive, and viable alternative for point-of-care diagnosis. Therefore, the objective of this study was to design and optimize a LAMP assay for ILTV detection. To achieve this, a novel primer set (F3, B3, FIP, BIP, LB) was designed targeting the *gB* gene (UL27) using GLAPD system. *In silico* specificity and thermodynamic analyses for hairpin formation were performed, and the selected set proceeded to benchwork evaluation. Standardization assays for temperature, time, and primer concentration were conducted, as well as sensitivity and specificity tests against 12 distinct avian viruses of sanitary importance. As a result, the primers showed strictly specific amplification for ILTV, with no hairpin formation. The assay reached its fluorescence peak at the temperature of 65 °C in less than 15 minutes, exhibiting an analytical sensitivity of 100% for 100 viral DNA copies and 60% for 10 copies. In terms of future perspectives and practical implications, this assay will serve as a foundation for developing lateral flow strips (point-of-care) and for the crucial molecular differentiation between field and vaccine strains in epidemiological surveillance.
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PT.528

Analytical validation of immunoinformatics-predict *Orthoflavivirus zikaense* structural peptides identifies a capsid-derived epitope with high IgG specificity

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Identifying antigenic targets of Orthoflavivirus zikaense (ZIKV) remains challenging due to high degree structural conservation and antibody cross-reactivity with other co-circulating arboviruses. In this context, immunoinformatics-guided approaches offer a rational strategy for prioritizing conserved yet structurally specific viral epitopes that can overcome the limitations imposed by antigenic similarity. n this study, five conserved, ZIKV-exclusive synthetic peptides, previously predicted by immunoinformatics, were experimentally validated as a proof-of-concept for an in silico antigen discovery pipeline These peptides were derived from structural proteins of ZIKV and peptides reactivity was assessed by indirect ELISA using a panel of IgM- and IgG-positive human sera. IgM reactivity was characterized by high sensitivity for some peptides but limited specificity, consistent with the intrinsic features of early flavivirus immune responses. In contrast, IgG-based analyses revealed a more favorable and discriminatory profile, with one peptide derived from the ZIKV capsid protein (PEP03) exhibiting 80% sensitivity, 93.9% specificity, and strong discriminatory capacity between groups, with an area under the ROC curve (AUC) of 0.9183 (95% CI: 0.8301–1.000; $p < 0.0001$). Overall, these findings provide preliminary experimental evidence supporting the use of in silico-based rational antigen design, revealing distinct IgM and IgG recognition profiles in serological assays. Furthermore, this study identifies a capsid-derived epitope as a promising yet underexplored candidate for immunogen development and the improvement of ZIKV serological tools. However, further large-scale validation studies using a large set of human serum samples.
Financial support: FAPEMIG, CNPQ, CAPES

PT.529

High-Throughput Identification of Dengue Virus-Binding Antiviral Peptides Targeting E and NS1 Proteins via Phage Display

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Dengue virus (DENV) causes more than 390 million infections and over 9,000 deaths annually worldwide, and in the absence of na antiviral treatment, early clinical management remains essential to favorable outcomes. Among strategies to discover antiviral molecules, the identification of peptides capable of binding to key viral proteins, Envelope (E) and Non-structural protein 1 (NS1), may directly interfere with infection and multiplication and reveal candidates with therapeutic potential. To identify high-affinity peptide ligands for DENV, E and NS1 proteins were incubated with phages displaying peptides on their surface, and successive rounds of biopanning enriched specific clones that were sequenced to identify consensus motifs. In NS1-targeted assays, phage titers declined progressively from 10.000 colonies in the first round, to approximately 100 in the second, and 15 in the third. Forty colonies from rounds one and two, and all 15 from round three were sequenced, with one peptide sequence consistently identified in both rounds, suggesting selective enrichment. For the E protein, phage titers decreased from around 10.000 in the first round to 150 in the second and 23 in the last, with analysis ongoing. These preliminary findings demonstrate the feasibility of selecting peptides with specific affinity for DENV proteins. Future steps, including molecular docking, surface plasmon resonance, and AI-based predictions aim to determine potential hits, enhance interaction affinity and to predict other putative ligands. The development of panels composed by potential anti-DENV peptides represents an innovative strategy with the potential to address critical scientific gaps and fulfill pressing public health needs.

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PT.530

PROSPECTION OF FUNGI-DERIVED EXTRACTS WITH POTENTIAL VIRUCIDAL ACTIVITY AGAINST HUMAN RESPIRATORY SYNCYTIAL VIRUS (RSV)

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Respiratory syncytial virus (RSV) is a major pediatric pathogen that causes bronchiolitis and lower respiratory tract infections in infants and children under 2 years of age. Although prophylactic strategies, including palivizumab and maternal vaccination, are available, no specific antiviral therapy has been approved. In this context, fungal natural products represent a promising source of novel antiviral compounds. Fruit-associated fungi, in particular, remain largely unexplored despite their remarkable taxonomic and chemical diversity. This study evaluated the virucidal activity of extracts obtained from fruit-associated fungi against RSV. Cytotoxicity was assessed in HEp-2 cells using the Alamar Blue assay, and antiviral activity was determined by quantifying the reduction in RSV (genotype ON1) replication by qPCR. Thirteen fungal extracts were tested at four concentrations. One extract was cytotoxic only at 100 µg/mL, six showed no cytotoxicity at any concentration, three were cytotoxic at all concentrations except 1 µg/mL, and three were cytotoxic at 100 and 50 µg/mL. Nearly all non-cytotoxic extracts inhibited viral replication by more than 80% at all tested concentrations. Only one extract achieved reductions above 80% exclusively at 100 and 50 µg/mL. These findings suggest that the active compounds act by extracellularly inactivating viral particles before cell adsorption, highlighting their potential as candidates for RSV preventive formulations or disinfectants.

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PT.531

ANTIVIRAL POTENTIAL OF BRAZILIAN RED PROPOLIS CONSTITUENTS AGAINST CHIKUNGUNYA VIRUS: AN INTEGRATED IN VITRO AND IN SILICO STUDY

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Arboviral diseases represent a public health concern, particularly chikungunya virus (CHIKV) infection, which is associated with persistent polyarthralgia and lacks antiviral therapies. This study evaluated the antiviral potential of twelve Brazilian red propolis (BRP) constituents against CHIKV through integrated *in vitro* and *in silico* approaches, including antiviral assessment, molecular interaction, and pharmacokinetic and toxicological predictions. Cytotoxicity and antiviral screening were performed by MTT assay at 50 µM. Selected compounds were further evaluated from 3.12 to 400 µM to determine the maximum non-toxic concentration, CC_{50}, EC_{50}, and selectivity index (SI). For the most selective compounds, CHIKV-positive cells were assessed by intracellular flow cytometry, viral RNA levels were determined by absolute RT-qPCR, and antiviral activity at different replication stages was investigated. Docking, molecular dynamics simulations, and ADMET analyses were performed. Among the 12 compounds evaluated, six exhibited antiviral activity. Isoliquiritigenin and catechin showed the most promising profiles, with SI values of 4.87 and 18.31, respectively, and significantly reduced the percentage of infected cells. Viral RNA levels decreased from 3.2×10^6 copies/µL in untreated cells to 9.4×10^4 and 4×10^4 after treatment with isoliquiritigenin and catechin, respectively. Both compounds remained active when added up to 8 hours post-infection, indicating post-entry antiviral activity. Molecular docking identified nsP3 as a putative target, while molecular dynamics simulations supported complex stability. ADMET predictions suggested some favorable drug-like properties. These findings identify isoliquiritigenin and catechin as promising anti-CHIKV candidates and highlight BRP as a source of bioactive antiviral agents.
Financial Support: FAPEAL, CAPES, CNPq.

PT.532

ANTIVIRAL ACTIVITY OF CRUDE EXTRACT AND ISOLATED COMPOUNDS FROM BRAZILIAN *Copaifera langsdorffii* AGAINST OROPOUCHE VIRUS INFECTION

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Oropouche fever is caused by Oropouche virus (OROV), an emerging arbovirus that has recently received increasing attention from health authorities. OROV infection can cause significant morbidity and lead to severe complications, such as encephalitis and meningitis. Currently, there is no antiviral therapy against OROV, and treatment approaches are limited to symptomatic management. Thus, Brazilian natural products represent attractive candidates for developing novel therapeutic approaches. These include crude extracts and isolated compounds from *Copaifera* spp., such as *Copaifera langsdorffii*. This study aimed to investigate the antiviral activity of a crude extract and five isolated compounds from *Copaifera langsdorffii* against OROV. Vero E6 cells were used to determine the maximum non-toxic concentration by MTT assay after 5 days of treatment. Antiviral activity was evaluated using plaque reduction assays, where compounds and OROV (MOI 0.001) were co-incubated for 1 hour, followed by 5 days of incubation with carboxymethylcellulose (CMC)-supplemented media in the presence of compounds. Subsequently, cells were fixed with 4% formaldehyde, stained with crystal violet, and viral plaques were quantified and normalized to the 0.1% DMSO vehicle control. Results demonstrated that the crude extract of *Copaifera langsdorffii* strongly inhibited 99.5% of OROV replication, and its isolated compound polyalthic acid significantly reduced 83% of virus infection. These findings demonstrate the potent anti-OROV activity of extracts and isolated compounds from *Copaifera* on OROV infection. Further investigations are underway to elucidate the mode of action of these compounds.

Keywords: Antiviral, Oropouche virus, *Copaifera langsdorffii*, Natural compounds
Financial Support: FAPEMIG, CNPq, CAPES

PT.533

DISCOVERY OF ANTIVIRAL DRUGS AGAINST MAYARO, WEST NILE, AND OROPOUCHE FEVERS

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Mayaro (MAYV), West Nile (WNV), and Oropouche (OROV) are arboviruses causing febrile illnesses with nonspecific symptoms. Clinical similarities and the lack of approved antivirals hinder diagnosis and treatment, posing significant public health challenges. In this context, this study aims to identify natural and synthetic substances with antiviral activity against Mayaro, West Nile, and Oropouche viruses, as well as to investigate their respective mechanisms of action. The antiviral and cytotoxic activity of 64 substances, 8 essential oils, and 264 crude extracts of plants and fungi against the MAYV virus were screened; 54 substances and 81 plant extracts against the WNV virus; and 21 different substances against the OROV virus, using the MTT cell viability method. As a result, none of the substances and essential oils showed antiviral activity against the tested viroses. However, five crude fungal extracts were active against the MAYV virus, four of which belonged to fungi of the genus Talaromyces sp. The active extracts went through Ultra-High Performance Liquid Chromatography (UHPLC) coupled with high-resolution mass spectrometry fractionation. Two potentially novel anti-MAYV molecules were identified; The processes of confirmation and mechanism of action studies are underway. Our next steps are related to elucidating the antiviral mechanisms of action of the active molecules. The data obtained in this study reinforce the potential of natural products as sources of possible antivirals to be used in the future as treatment for the arboviruses studied.

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PT.534

LARVICIDAL EFFECT OF *MELALEUCA LEUCADENDRA* ESSENTIAL OIL ON C6/36 CELLS DERIVED FROM *AEDES ALBOPICTUS*

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Arboviral diseases represent a major global health challenge, with dengue, Zika, and chikungunya being highly prevalent in Brazil. Vector control is a crucial strategy to reduce viral spread; however, the resistance of Aedes mosquitoes to conventional larvicides needs the search for alternative agents. The essential oil of Melaleuca leucadendra (EOML) has shown promising potential as a bioinsecticidal agent. Thus, the present study aimed to characterize the chemical composition of EOML and evaluate its cytotoxic effects on Aedes albopictus-derived C6/36 cells. The chemical profile was determined by gas chromatography-mass spectrometry (GC-MS). C6/36 cells were cultured in supplemented L-15 medium at 28 °C with EOML at different concentrations (0.01, 0.1, 1, and 10 mg/mL) for 24 h. A control culture without EOML was produced. Cell viability was assessed via MTT assay. GC-MS identified δ-terpineol (11.95%), 1,8-cineole (16.58%), and viridiflorol (17.17%) as the major compounds. A significant reduction in cell viability was observed at high concentrations ($p < 0.05$). While low concentrations did not impact viability under the tested conditions, these results provide a preliminary indication of the oil's biological activity and establish a baseline for forthcoming bioassays involving Aedes larvae. These findings contribute to the development of novel strategies to disrupt the arbovirus transmission cycle by targeting the vector, although further studies are required to confirm EOML efficacy and safety, so as the main compound responsible for its action. CAPES, FAPEMIG PCE-00181-26 and APQ-04955-23.

PT.535

From the Amazon to antivirals: BretVir ® and BretMune ® as promising candidates against mpox

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The recent global resurgence of mpox (formerly monkeypox) has renewed global concern about the resurgence of neglected zoonotic diseases and the urgent need for effective treatments. In this scenario, BretVir® and BretMune®, solutions derived from Amazonian plant extracts, emerge as promising alternatives, as phytotherapy offers a natural, accessible, and potentially effective antiviral strategy. These commercial solutions were developed as adjuvants in viral respiratory infections and to strengthen the immune system. This study evaluated toxicity and antiviral activity against MPV infection. Dose-response assays, virucidal activity, and potential mechanisms of action of these solutions were also investigated. Cell viability analysis using Alamar Blue and Crystal Violet assays showed low toxicity in BSC-40 cells, indicating their suitability for antiviral testing under BSL-3. In antiviral assays, BretVir® and BretMune® were tested both undiluted and at dilutions up to 2560-fold. BretVir® reduced MPV viral load by 6 log units at dilutions up to 80-fold, while BretMune® achieved a 6-log reduction at dilutions up to 20-fold. Both solutions, BretVir® (IC_{50} = 19.79 µg/mL) and BretMune® (IC_{50} = 8.97 µg/mL), demonstrated antiviral activity even at dilutions up to 1280-fold. Preliminary mechanistic studies indicated that BretVir® exhibits virucidal activity, and that both solutions act during the early stages of the viral replication cycle, showing significant activity when added up to 6 hours post-infection (hpi). These findings highlight the Amazonian extract-based solutions BretVir® and BretMune® as promising antiviral alternatives against MPV, supporting their advancement as novel therapeutic candidates.

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PT.536

ANTIVIRAL ACTION OF GREEN AND RED PROPOLIS AGAINST THE ZIKA VIRUS (ZIKV)

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The Zika virus (ZIKV) poses a significant threat to public health due to the rise in cases of arboviral diseases associated with environments conducive to the proliferation of transmitting vectors. The infection is transmitted by the Aedes aegypti mosquito, causing symptoms such as conjunctivitis, low-grade fever, arthralgia, and body aches. Currently, there are no antivirals available for the treatment of the disease. In this context, alternatives such as the use of native flora have been investigated, particularly green and red propolis, whose diverse composition supports the presence of a wide range of bioactive compounds, such as flavonoids. This study evaluated the antiviral activity of these propolises against ZIKV through cytotoxicity assays and mechanism of action studies. The results indicated that green propolis exhibited a CC50 of 451.91±48.44 µg/mL, a EC50 of 24.82 ± 20.97 µg/mL, and an IS of 18.20, while red propolis recorded a CC50 of 500.00 ± 0.16 µg/mL, EC50 of 22.54 µg/mL, and an IS of 22.18. Furthermore, an anti-ZIKV effect was observed for both, with a 2 log units reduction in viral titers at a concentration of 250 µg/mL. In conclusion, the analyses indicate that green and red propolis exhibit antiviral potential against the zika virus. Additional studies are underway to understand the mechanisms involved, including virucidal, adsorption, and post-adsorption assays.

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PT.537

A novel and versatile primer set for detection of Oropouche virus by both real-time and endpoint RT-PCR

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Since its first isolation in 1955, Oropouche virus (OROV) has caused numerous outbreaks across South America and the Caribbean. However, in 2024, OROV re-emerged, expanded its geographic distribution and raised concerns regarding its potential for vertical transmission and teratogenicity, particularly in Brazil. This scenario highlights the importance of implementing appropriate laboratory diagnostic methods for OROV, particularly in regions with cocirculating arboviruses, such as dengue (DENV), Zika (ZIKV) and chikungunya (CHIKV) viruses, whose infections present with overlapping clinical manifestations, making clinical and epidemiological diagnosis challenging. To strengthen OROV surveillance and diagnosis, we designed a versatile primer set for OROV detection by both real-time (SYBR Green-based) and endpoint RT-PCR, enabling its implementation across diverse laboratory settings. Primers were designed based on complete OROV S-segment sequences available in GenBank. RT-PCR and RT-qPCR assays were optimized using a two-step workflow, with the limit of detection (LoD) determined for OROV and analytical specificity evaluated against DENV 1-4, ZIKV, CHIKV and yellow fever virus (YFV). Furthermore, the assays were evaluated using 12 OROV isolates. Both assays generated an 84bp amplicon, with a Tm ranged from 71.5 to 72.3 ◦C, no primer-dimer formation and high specificity, with no amplification of the arboviruses tested. The LoDs for RT-PCR and RT-qPCR were 1.5 and 0.15 PFU, respectively, and both assays successfully amplified all 12 OROV isolates tested. Finally, the performance of the assays will be evaluated using OROV-positive and OROV-negative clinical samples to validate their application for OROV diagnosis and surveillance.
Financial Support: FAPERGS

PT.538

BIOPROSPECTING FOR NATURAL PRODUCTS FROM THE PLANT SPECIES *Spiranthera odoratissima* AGAINST THE MAYARO VIRUS

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Mayaro virus (MAYV), the causative agent of Mayaro fever, causes symptoms such as fever, rash, and arthralgia. Despite Brazil reporting the highest number of cases, no specific vaccines or antiviral therapies are available, highlighting the need for novel therapeutic alternatives. In this context, natural products (NPs) are an important source of bioactive compounds, and *Spiranthera odoratissima*, a Brazilian medicinal plant with reported anti-inflammatory properties, remains unexplored for its antiviral potential. This study evaluated the antiviral activity of products obtained from *S. odoratissima* against *in vitro* MAYV replication. Cytotoxicity was assessed in Vero cells by MTT assay (10-400 µg/mL), and antiviral activity was evaluated against MAYV (MOI = 0.1) after 24 h of treatment at 50 µg/mL. The dichloromethane extract inhibited 70% of viral replication and exhibited low cytotoxicity (CC_{50} = 195.80 µg/mL). Fractionation yielded five fractions (**FM1-5**) with CC_{50} values ranging from 142.63 to >400 µg/mL. Among the tested fractions, only **FM1-3** blocked virus replication with inhibition rates of 93.75%, 36.67%, and 100%, respectively. Subsequently, five isolated products (**I1-I5**) were investigated. They showed CC_{50} values ranging from 69.36 to 103.7 µg/mL. Further *in silico* ADMET predictions indicated that the isolated compounds present favorable pharmacokinetic and toxicological properties, comparable to the broad-spectrum antiviral drug favipiravir. Their antiviral activity is currently under investigation. Overall, these findings highlight the potential of *S. odoratissima* NPs as promising candidates as anti-MAYV agents.

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PT.539

IMMUNOPHENOTYPIC AND FUNCTIONAL PROFILING OF DISTINCT MURINE MODELS UPON IMMUNIZATION WITH THE RECOMBINANT C-TERMINAL REGION OF HTLV-1 TAX PROTEIN

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Human T-cell lymphotropic virus type 1 (HTLV-1) is a retrovirus estimated to infect 10 million people worldwide and is etiologically related to adult T-cell leukemia/lymphoma and HTLV-1-associated myelopathy/tropical spastic paraparesis. Despite its heterogeneous distribution and high endemicity in Brazil, no prophylactic vaccine exists, and therapeutic options remain limited. Previous analyses showed that the C-terminal region of Tax viral protein could induce IFN-γ and IL-4 production, and that peptides derived from this region reacted to HLA-A*02 molecules. Thus, the present study aimed at characterizing the immunophenotypic and functional profiles induced by in vivo immunization with recombinant C-terminal Tax (C-TaxRec), followed by in vitro analysis of T-cell responses. C-TaxRec was first evaluated by immunizing A2-B6 transgenic mice, which express human HLA-A*02. After immunization, spleen and lymph node mononuclear cells were analyzed for IFN-γ production by ELISpot and for IFN-γ$^+$ CD8$^+$ and IL-4$^+$ CD4$^+$ T-cell responses by flow cytometry. In BALB/c mice, splenocytes were analyzed by flow cytometry after immunization to characterize T-cell subsets and effector-associated markers. Additionally, systemic soluble mediators were evaluated using a 23-plex Luminex assay. In A2-B6 mice, C-TaxRec induced strong lymph node IFN-γ and IL-4 production by CD8$^+$ and CD4$^+$ T cells, respectively. In BALB/c mice, C-TaxRec increased total CD4$^+$ T cells, effector memory CD4$^+$ T cells, augmented Tregs, and enhanced CD4$^+$ and CD8$^+$ T-cell expression of CD107a, granzyme B, IFN-γ, IL-2, and TNF, together with higher systemic soluble mediator levels. These findings indicate that C-TaxRec induces a broad protective immune profile, supporting future HTLV-1 challenge studies in vivo.

Financial support: FAPEMIG, CAPES, and CNPq.

PT.540

DEVELOPMENT, STANDARDIZATION, AND PRELIMINARY EVALUATION OF A DIRECTLY INFECTED C6/36 CELL-BASED ELISA FOR THE DETECTION OF ANTIBODIES AGAINST OROPOUCHE VIRUS

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Oropouche virus (OROV) is an emerging arbovirus of increasing public health importance, highlighting the need for specific, reproducible, and practical serological assays. This study aimed to develop and standardize an enzyme-linked immunosorbent assay (ELISA) based on the direct infection of C6/36 cells in 96-well microplates for the detection of anti-OROV IgM and IgG antibodies. Different cell concentrations, blocking solutions, multiplicities of infection (MOI 0.05 and 0.1), and infection times (24 and 48 h) were evaluated. The optimized protocol consisted of 5 × 10^4 cells per well, MOI 0.1, 24 h of infection, and blocking with 5% bovine serum albumin (BSA). The preliminary performance of the assay was assessed using OROV-positive serum samples confirmed by virus neutralization testing and panels of sera positive for other arboviruses. The anti-OROV IgM ELISA showed a specificity of 94.12% (32/34; 95% CI: 80.32–99.28%), a cross-reactivity rate of 5.88% (2/34), and a preliminary sensitivity of 100% (4/4; 95% CI: 39.76–100%). The anti-OROV IgG ELISA demonstrated a specificity of 88.24% (15/17; 95% CI: 63.56–98.54%), a cross-reactivity rate of 11.76% (2/17), and a preliminary sensitivity of 100% (8/8; 95% CI: 63.06–100%). The proposed strategy simplifies antigen preparation by enabling direct infection of C6/36 cells in microplates, thereby reducing experimental steps and cell handling without compromising assay performance. These findings demonstrate the potential of this methodology as an alternative approach for the serological diagnosis of OROV infection and support further validation using a larger panel of clinical samples.

Keywords: Oropouche virus; ELISA; Serological diagnosis; Arbovirus.
Funding support: CNPq, FAPEAL, CAPES, NEGLI-ONE.

PT.541

ANTIVIRAL ACTIVITY OF SELENOPYRAZOLE DERIVATIVES AGAINST ZIKA AND MAYARO VIRUSES IN HUMAN GLIOBLASTOMA (U87) AND OSTEOSARCOMA (MG-63) CELL MODELS

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Zika virus (ZIKV) and Mayaro virus (MAYV) are arboviruses of major public health concern in Brazil, and no specific antiviral therapy is currently available. Selenopyrazole derivatives have been investigated as promising antiviral agents due to their biological potential. This study evaluated the antiviral activity against ZIKV and MAYV in human cell models. U87 (glioblastoma) cells were infected with ZIKV, whereas MG-63 (osteosarcoma) cells were infected with MAYV and treated with the evaluated compounds at concentrations ranging from 0.65 to 10 µM. Viral replication inhibition was determined by titration of culture supernatants in Vero cells to quantify the reduction in the production of infectious viral particles. In assays performed with ZIKV, the compound tested in U87 cells exhibited dose-dependent antiviral activity, with an EC_{50} of 1.1 µM and a selectivity index of 405.2, achieving more than 90% inhibition from 5 µM and approximately 99% at higher concentrations. Similarly, in assays performed with MAYV, the compound tested in MG-63 cells exhibited an EC_{50} of 0.94 µM and a selectivity index of 237, with inhibition exceeding 90% from 5 µM and reaching approximately 99% at 10 µM. These results, consistent with those previously obtained in Vero cells, demonstrate that both compounds maintain high antiviral activity and high selectivity in clinically relevant human cell models, reinforcing their potential as antiviral candidates against arboviruses for which no specific treatment is currently available.

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PT.542

SCREENING FOR VIRUSES POTENTIALLY ONCOLYTIC FOR KIDNEY CANCER CELLS

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Kidney cancer corresponds to 70% of all renal cell carcinoma. Although several therapeutic strategies have been developed, high mortality rates and low overall survival indicate that new therapeutic strategies are necessary. Virotherapy is a potential strategy for treating solid tumors. The aim of our study was to evaluate the potential anti-kidney cancer activity for several viruses: Human Adenovirus (HAdV), Herpes Simplex Virus 1 (HSV-1), Vesicular Stomatitis Virus (VSV), and attenuated oral poliovirus vaccine (OPV). For that, we infected renal cancer cell lines (Caki-1, Caki-2, ACHN) and the control non-tumoral kidney cell line (HK-2) with those viruses. For this assay, 10.000 cells/well were seeded in 96-well plates and 24 hours later the cells were infected with different MOI values (1, 0.1, and 0.01). After 24, 48, and 72 hours, 0.5 mg/mL MTT solution was applied and read after 3 hours with MTT solubilization by DMSO to enable optical density quantification. The rates of cell lysis induced by VSV were 60% for Caki-1 and Caki-2, and 80% for ACHN; OPV killed 80% of Caki-1, Caki-2 and ACHN. HAdV killed 60% of Caki-2 and ACHN; and HSV-1 killed 80% of Caki-1 and 60% of Caki-2, albeit both at the highest MOI only. It is remarkable that HAdV, OPV and HSV-1, but not VSV, were specifically lytic for tumor cells, and not for non-tumor HK-2 cells. The data are promising for the further development of modified oncolytic virus candidates for kidney cancer.

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PT.543

Evaluation of the antiviral effect of synthetic licochalcones and their analogs on the *in vitro* replication cycle of Zika virus (ZIKV).

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Zika virus (ZIKV), a neglected tropical disease lacking approved antivirals or vaccines, is globally relevant due to its association with severe neurological disorders, including congenital Zika syndrome. The positive-sense single-stranded RNA virus (ssRNA+) depends on two nonstructural proteins for replication: the NS2B-NS3 protease and the NS3 helicase (NS3Hel). Because of their essential role, these proteins are attractive molecular targets for antiviral development. Chalcones are a promising class of bioactive compounds that inhibit viral proteases by binding to the catalytic site. This study evaluated the inhibitory activity of licochalcones (genus Glycyrrhiza) against NS2B-NS3Pro. Four compounds were tested: the prototype (LCH) and three closely related analogs with minor structural modifications (LCH-H, LCH-Me, and ECH). Antiviral activity was determined by EC50, and cytotoxicity by CC50. The selectivity index (SI = CC50/EC50) was calculated to assess compound safety and selectivity. Assays were performed in Vero ATCC CCL81 cells. LCH showed an EC50 of 97.57 μM, whereas LCH-H, LCH-Me, and ECH displayed EC50 values of 57.21, 54.80, and 145.2 μM, respectively. CC50 values were 50.7 μM for LCH, 61.85 μM for LCH-H, 20.74 μM for LCH-Me, and 30.55 μM for ECH. All compounds exhibited selectivity indices below 1, with LCH-H showing the highest SI (0.92). Additional experiments are underway to confirm these findings, and the final data will guide structural modifications to improve antiviral activity and reduce cytotoxicity.

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PT.544

STRATEGIC APPLICATION OF LONG AMPLICONS IN RT-qPCR FOR ENHANCED VERIFICATION OF RABIES VIRUS VACCINE INACTIVATION

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Global initiatives promote the 3R's (Reduce, Reuse, and Recycle) to replace animal-based safety tests in vaccine quality control. The current *in vivo* protocol for detecting residual live rabies virus (RABV) in the Rhabdocell vaccine, used by the Brazilian Ministry of Health in national vaccination campaigns for both dogs and cats, is a major ethical and logistical burden, utilizing 58 Swiss Webster mice and requiring a 21-day observation period. RABV is inactivated with β-propiolactone (BPL), which induces genomic damage via N-7 guanine alkylation, forming apurinic sites that convert into strand breaks during PCR thermal cycling. This study aimed to develop an RT-qPCR protocol to evaluate viral inactivation, thereby reducing animal usage and significantly shortening the assay duration. RABV vaccine strain was inactivated with BPL (1:4000). RNA was extracted and analyzed by SYBR Green RT-qPCR targeting seven genomic sites, ranging from 501 to 1152 base pairs (bp) and an 83 bp control (Gene N). Data were normalized using the ΔΔ*Ct* method to assess the absence or reduction in PCR amplification. Among the evaluated candidates, the 870 bp amplicon within Gene L showed the best results in reducing PCR amplification. ΔΔ*Ct* normalization showed a 9.52 cycle reduction in the inactivated samples, representing a 2.87 log reduction in template integrity. The 870 bp target provides a reliable metric for monitoring the chemical efficiency of RABV inactivation. However, for safety control, this molecular approach should be integrated into a hybrid protocol (RT-qPCR + 72-hour cell culture) to distinguish residual non-infectious RNA from viable particles.

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PT.545

BETTER DATA, BETTER TREES: GENBANK-GISAID DEDUPLICATION AND SOURCE-SPECIFIC ARTIFACT MASKING IN VIRAL GENOMICS

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GenBank and GISAID are the primary repositories for viral genomic data, but integrating records across them remains a challenge. The same sequence could be made available in both databases without any cross-reference linking the two entries. Consequently, there is no systematic way to identify this redundancy, which compromises the compilation of representative, non-redundant large-scale datasets. In parallel, the growth of viral genomic data has increased the risk of systematic technical artifacts introduced during sequencing or assembly. These artifacts can inflate substitution rate estimates and degrade temporal signal, biasing evolutionary rate estimates. To address both challenges, here we present a formal, reproducible workflow integrating two newly developed complementary tools: G2G matcher for cross-repository harmonization and Lab-Specific Bias FILTer (LSBFILT) for masking of laboratory-specific artifacts. Using the Eastern/Central/South African (ECSA) chikungunya virus lineage as a proof-of-concept, we demonstrate that our integrated workflow restores temporal signal and provides a robust, curated dataset for downstream phylodynamic analyses. Critically, restricting masking of homoplastic sites to specific sequences reduces the substitution rate estimate from an inflated 8.517×10^{-4} to 5.078×10^{-4} substitutions per site per year and increases the coefficient of determination (R^2) of the root-to-tip regression analysis from 0.353 to 0.677. By enabling systematic cross-repository harmonization and source-specific artifact masking, we provide the molecular epidemiological community with scalable tools to reconcile fragmented genomic data and reduce technical biases, fostering more accurate and reproducible phylogenetic analysis. G2G matcher is available at https://github.com/andrezaleite/G2G-Matcher, and LSBFILT at https://github.com/khourious/LSBFILT.

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PT.546

Metagenomic Profiling and Virome Diversity in Swine Wastewater: A One Health Approach Using Hybrid-Capture NGS

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Swine wastewater is a strategic matrix for environmental surveillance under a One Health framework, enabling the continuous monitoring of viral pathogens with veterinary and zoonotic significance. This study characterized the viral diversity in swine wastewater samples using a hybrid-capture Next-Generation Sequencing (NGS) approach with the Illumina VSPv2 panel, processed through the CZID platform. Sequence analysis yielded 266,702 annotated microbial reads, comprising 183,349 bacterial reads, 37,625 archaeal reads, 7,795 eukaryotic reads, and 37,819 viral reads. Within the total viral fraction, 12,010 reads were identified as bacteriophages. Virome profiling revealed a high frequency of swine enteric viruses, predominantly *Enterovirus G* (1,604 reads), *Astroviridae* (826 reads), *Bastrovirus* (802 reads), *Porcine torovirus* (233 reads), and *Aichivirus C* (*Kobuvirus*, 352 reads). Crucially, from a One Health perspective, pathogens of significant public and environmental health relevance were detected, including *Paslahepevirus balayani* (Hepatitis E virus, 20 reads), *Norovirus* (26 reads), *Sapovirus* (24 reads), *Rotavirus A* (6 reads), and *Mastadenovirus* (including Human mastadenovirus B). These findings demonstrate the efficiency of the VSPv2 target enrichment panel in recovering a broad spectrum of enteric and zoonotic viruses directly from complex swine wastewater matrices. This highlights the value of wastewater-based epidemiology as an indispensable One Health tool for genomic surveillance in swine production and public health interfaces. Financial Support: CAPES (PROSUC), FAPERGS, and institutional research funds.

PT.547

A LARGE-SCALE TRANSCRIPTOME SURVEY EXPANDS THE KNOWN DISTRIBUTION OF *RICE STRIPE NECROSIS VIRUS*

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Rice stripe necrosis virus (RSNV; family Benyviridae) is an economically important pathogen of rice associated with significant yield losses in affected fields. Despite its agricultural relevance, the global distribution and occurrence of RSNV remain poorly understood, largely due to the limited availability of surveillance data. In this study, we developed a bioinformatics pipeline to investigate the presence of RSNV in publicly available transcriptomic datasets. A total of 2,178 transcriptomes representing cultivated and wild *Oryza* species were retrieved from the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) and analyzed. The workflow included automated data retrieval, quality processing, alignment against RSNV reference genomes using Bowtie2 in very-sensitive-local mode, read processing with SAMtools, and genome coverage assessment. Candidate alignments were subsequently inspected using the Integrative Genomics Viewer (IGV) to confirm mapping quality and exclude spurious alignments. Among all transcriptomes analyzed so far (n = 2,178), only a single dataset showed consistent evidence of RSNV-derived transcripts, whereas all remaining datasets were negative. This positive sample originated from China, a geographic region where RSNV has not been previously reported, suggesting a potentially broader distribution than currently recognized.To our knowledge, this study represents the first large-scale transcriptome survey specifically designed to detect RSNV in public *Oryza* datasets, providing new insights into the distribution of this economically important pathogen and establishing a reproducible workflow for future surveillance, epidemiological investigations, and evolutionary studies of RSNV and other agriculturally relevant plant viruses.

PT.548

IN SILICO STRUCTURAL STUDIES OF BOVINE SERINE/THREONINE-PROTEIN KINASE PIM1 REVEAL A POTENTIAL THERAPEUTIC CANDIDATE FOR BOVINE PAPILLOMATOSIS

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Bovine papillomavirus (BPV) is the causative agent of papillomatosis in cattle, a disease with a wide global distribution associated with decreased productivity and economic losses. Given that Brazil is one of the world's largest producers of beef and dairy, this study sought to identify targets and molecules for the treatment of the disease. Therefore, we aimed to screen and design new inhibitors of bovine PIM1, a proto-oncogene with serine/threonine kinase activity, using structural computational approaches, including molecular docking and molecular dynamics simulations (MDS). For the molecular docking analysis, we used a ligand obtained through structural similarity among active compounds, along with the cross-inhibition function between the ChEMBL and DrugBank databases. Molecular modeling was performed, and the generated models were validated using PROCHECK and Verify 3D. The first 32 residues of the PIM1 protein were modeled using TrRosetta. Molecular docking was conducted using AutoDock Vina, and the docked complexes were ranked based on binding modes and Gibbs free energy. MD simulations of the top-ranked complexes were run for 100 ns to explore binding stability in explicit water. Docking analyses showed that the selected ligand had a high binding affinity for PIM1 and formed hydrogen bonds near the hydrophobic pocket and the nucleotide-binding region. MDS results supported the docking findings, as the ligand showed similar and stable interactions with amino acids at the protein's binding site. These results highlight the identification of a possible bovine PIM1 inhibitor; however, further studies are necessary to validate these findings experimentally.

Keywords: Bovine papillomavirus, PIM1, inhibitor, docking, MDS, treatment.
Financial Support: CAPES, CNPq and FAPITEC/SE

PT.549

Reactivation of SINE retrotransposons in regions near genes involved in multiple tumor hallmarks during cervical carcinogenesis

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Although short interspersed nuclear element-1 (SINEs) are normally silenced by genetic and epigenetic mechanisms, they are reactivated in the context of cervical cancer induced by HPV16, the most carcinogenic genotype, with the potential to impact mechanisms associated with uncontrolled cell proliferation. In this context, the present study aimed to characterize SINE expression using RNA-seq data (GEO accession: GSE147009) from HPV16-associated normal cervical epithelium (NC), HPV-positive high-grade squamous intraepithelial lesion (HSIL), and squamous cell carcinoma (SCC), focusing on three HPV/carcinoma integration hotspots: 3q28, 13q22.1, and 21p11.2. Reads were aligned to Human Reference Genome (hg38) with HISAT2, host gene quantification used HTSeq-Count, and SINE quantification was performed in R with the atena package (TEtranscripts method). Differential expression was analyzed with DESeq2, applying two filters: count sum > 50 and padj < 0.05, focusing on upregulated elements, and RepeatMasker coordinates were intersected with host genes within 10 kb using GENCODE annotation. In NC vs. SCC, upregulated elements included AluSp near MIR6724-4 (21p11.2), involved in stability and translation of mRNAs; AluSg4 near IL1RAP (3q28), related to the amplification of the immune response, and AluJo neighbored TPRG1 (3q28), linked with the genetic regulation of the P63 tumor protein. No functional genes were found in NC vs. HSIL comparison. In HSIL vs. SCC, AluSx1 neighbored LPP (3q28), linked to cell adhesion and motility. Those findings indicate localized SINE reactivation events spatially linked to cancer-relevant genes at HPV integration hotspots, suggesting a regulatory role for SINE activity in malignant transformation.

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PT.550

EVOLUTIONARY ANALYSIS OF DENGUE VIRUS REVEALS SEROTYPE- AND GENOTYPE-SPECIFIC CODON USAGE PATTERNS WITH VECTOR- AND HOST-ADAPTATIONS

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Dengue is an infectious disease caused by Dengue virus (*Orthoflavivirus denguei*, DENV), classified into four serotypes, DENV-1-4. DENV is maintained in sylvatic and urban transmission cycles. In recent decades, the spread of DENV across continents with frequently reported epidemics indicate a growing public health threat, highlighting the importance of understanding its genomic patterns linked to virus-host specificity. Here, we aimed to investigate the genomic features associated with virus-host specificity for one of the main arboviruses circulating in urban environments, DENV. The data analysis was conducted using complete genomic sequences recovered from GenBank, characterizing the evolutionary divergence within DENV. Codon usage profiles were characterized using Relative Synonymous Codon Usage (RSCU) and the Codon Adaptation Index (CAI) for its major urban host and vector in urban environments (*e.g.*, *Homo sapiens* and *Aedes aegypti*). The results indicate that different serotypes of DENV present different codon usage patterns, and within serotypes, the RSCU are distinct among genotypes. The distance between the different serotypes according to the codon usage clusters in Principal Component Analysis based on RSCU correlates with the genomic diversity of DENVs. For all serotypes, CAI values were higher for humans when compared to *Aedes aegypti*, suggesting that codon usage bias can be important to coordinate viral replication in both biological systems and highlighting codon adaptation as a complementary tool for investigating virus-host and -vector relationships. In conclusion, the results demonstrate different patterns of ecology and evolution of DENV, with possible impact on DENV epidemiology. **Financial Support**: FAPESP and FAEPEX.

PT.551

STRUCTURAL STABILITY AND BINDING AFFINITY ASSESSMENT OF CANDIDATE HLA–EPITOPE COMPLEXES FOR A PROPHYLACTIC VACCINE AGAINST ZIKA VIRUS

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Zika virus has become a public health problem due to its causal association with Congenital Zika Syndrome and Guillain–Barré syndrome. Despite the risk of re-emergence, no approved vaccines are currently available. Envelope and NS1 proteins stand out as important vaccine targets, enabling the identification of candidate epitopes through immunoinformatics, whose antigen presentation potential requires structural and energetic validation. This study aimed to evaluate the structural stability and binding affinity of HLA–epitope complexes derived from a multiepitope vaccine construct. Highly conserved epitopes from the Envelope and NS1 proteins, previously predicted for HLA class I and II molecules, were subjected to molecular docking evaluation with their associated HLA molecules. Subsequently, the complexes were subjected to molecular dynamics and evaluated in terms of RMSD, RMSF, hydrogen bonds, and binding free energy (MM/GBSA). Structural parameters were normalized and integrated into an overall stability score. Molecular docking generated 80 HLA–epitope complexes with binding scores between −10.6 to −6.2 kcal/mol, indicating favorable initial binding affinity between HLA molecules and the candidate epitopes. Molecular dynamics revealed stability differences among HLA–epitope complexes that were not detected by molecular docking. Seven complexes were classified as highly stable, highlighting the epitopes LVTCAKFAC (HLA-A*68:01 and HLA-B*53:01), KGPWHSEEL (HLA-B*44:03), and WRLKRAHLI (HLA-DRB1*15:01). MM/GBSA corroborated this classification, and most HLA alleles presented at least one epitope with moderate or high stability, indicating potential presentation by different HLA molecules. The results support the potential of the multiepitope vaccine construct for antigen presentation by HLA molecules

PT.552

GENOMIC AND BIOLOGICAL CHARACTERIZATION OF BRAZILIAN VACCINIA VIRUSES

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Vaccinia virus emerged in Brazil in 1999 as an occupational zoonosis affecting dairy cattle and rural workers, but important gaps remain in understanding the diversity and evolution of the viruses currently circulating in the country. This study combines biological assays and comparative genomics to investigate Brazilian vaccinia viruses, with emphasis on Group I field isolates. Plaque phenotype and replication assays showed that Group I viruses produce smaller plaques and display slower in vitro replication than Group II viruses, supporting marked phenotypic divergence between Brazilian lineages. Genomic analyses of 23 new Brazilian Group I genomes revealed strong conservation in the central region, increased variability in terminal regions, and a conserved core genome associated with a more plastic accessory fraction. Preliminary phylogenomic analyses placed these genomes within a cohesive Brazilian Group I clade and showed congruent whole-genome and concatenated core topologies, indicating that the internal structure of this lineage is not explained solely by terminal variation. A limited number of coding differences in core genes, together with greater structural heterogeneity in terminal genes linked to host interaction, suggest ongoing diversification on a highly conserved genomic backbone. Overall, these results indicate that Brazilian Group I vaccinia viruses have been circulating and diversifying over time, highlighting the importance of genomic surveillance to better understand their evolution and spread in Brazil.
Financial Support: CAPES, CNPQ, FAPEMIG, INCT-POX

PT.553

GENETIC DIVERSITY AND MAPPING OF CANDIDATE EPITOPES IN THE M SEGMENT POLYPROTEIN OF OROPOUCHE VIRUS CIRCULATING IN BRAZIL (2023–2025)

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Oropouche virus (OROV, Peribunyaviridae) caused a re-emergence outbreak in Brazil between 2023 and 2025, associated with a reassortant lineage. The M segment polyprotein (Gn–NSm–Gc) is the main target of the humoral immune response and a promising vaccine target, although its variability among circulating isolates remains poorly characterized. This study evaluated the genetic conservation of the M polyprotein and identified B- and T-cell epitopes in Brazilian OROV isolates. A total of 233 M polyprotein sequences (1,420 amino acids) retrieved from GenBank were analyzed using Python 3 (NumPy, SciPy, and Matplotlib). The M polyprotein showed an average conservation of 99.3%, with only 31 highly variable positions (<90% conservation), 20 located in the Gc glycoprotein. Twenty-eight candidate B-cell epitope regions (mostly ≥97% conserved) and 21 candidate T-cell epitope nonamers in nearly invariant regions were identified. Three amino acid positions (846, 1309, and 1342) showed complete linkage, defining two haplotypes: one exclusive to 2024 (n = 137) and another detected from 1960 to 2024 (n = 50). These findings indicate that the OROV M polyprotein is highly conserved and contains promising targets for vaccine development and diagnostic applications. Candidate epitopes require experimental validation and binding affinity analyses.

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AI Statement: Python analysis scripts and the initial organization of results were developed with the assistance of Claude (Anthropic). Research questions, biological interpretation, and final validation of the results were performed exclusively by the authors.

PT.554

Prediction and evaluation of epitopes for the design of a prophylactic vaccine against Human Papillomavirus genotypes 16 and 18 based on the L2 protein using bioinformatics approaches

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Persistent infection with high-risk oncogenic HPVs (hrHPVs) 16 and 18 is associated with 70% of cervical cancer cases. Available vaccination strategies are L1 based, which induces a type-specific immune response. Although it has been shown to induce broadly neutralizing antibodies that are highly conserved across different genotypes, the L2 protein remains unexplored. Thus, the aim of this study was to predict, analyze, and select epitopes from the L2 protein of hrHPVs 16 and 18 to compose the antigen for a prophylactic vaccine construct. To predict epitopes based on binding affinity and antigenic processing associated with the Major Histocompatibility Complex (MHC) class I, the algorithms NetMHCpan 4.1 EL, NetMHCpan 4.1 BA, and MHCFlurry 2.0 were used, and NetMHCIIpan 4.1 EL and MHCII-NP for MHC-II. Epitopes with similar sequences were grouped, and their representatives were analyzed for toxicity, conservation among the most prevalent genotypes, and murine and human allelic coverage. The final set underwent molecular docking analysis with HLAs and population coverage. Four MHC-I and three MHC-II epitopes were selected from each genotype, all non-toxic and with an average binding affinity >1, that fit into the appropriate cleft of the HLAs, in a set with 99.56% global population coverage. The results pave the way for the development of safe, universal vaccines. The molecular dynamics of the epitope-HLA complexes and analyses of the sequence and structure of the multi-epitope protein will be conducted to evaluate the stability of these interactions and the integrity of the construct in silico.

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PT.555

RAPID MULTICLASS DIAGNOSIS OF ARBOVIRAL INFECTIONS USING VIBRATIONAL SPECTROSCOPY AND MACHINE LEARNING

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Arboviral infections such as dengue, chikungunya, and yellow fever represent major public health challenges due to overlapping clinical manifestations that complicate differential diagnosis. Conventional serological and molecular assays are often limited by cross-reactivity, reagent dependence, time-consuming procedures, and laboratory infrastructure requirements. In this study, a multiclass diagnostic approach based on Fourier-transform infrared (FTIR) and near-infrared (NIR) spectroscopy combined with machine learning was developed for the discrimination of arboviral infections using human serum samples. FTIR spectra were acquired in the 4000?400 cm^{-1} range and preprocessed using standard normal variate normalization, while NIR spectra were collected in the 1000?2700 nm range and treated using second derivative transformation. Principal component analysis (PCA) was applied for exploratory analysis and dimensionality reduction, and support vector machine (SVM) models with cross-validation were employed for multiclass classification. Distinct spectroscopic profiles associated with proteins, lipids, nucleic acids, and immune-response-related biomolecules were observed among the evaluated arboviruses. PCA score plots demonstrated clustering trends according to infection type, and the optimized models achieved validation accuracies above 90%. The proposed platform demonstrated rapid, reagent-free, and low-cost diagnostic potential, with minimal sample preparation and possible application in point-of-care screening and epidemiological surveillance in endemic and resource-limited settings.
Financial Support: CAPES, CNPq and FUNDECT.

PT.556

Changes in Institutional Prevention Strategies Alter the Transmission Dynamics of Respiratory Viruses: A Longitudinal Within-Subject Analysis in a Long-Term Care Hospital

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The COVID-19 pandemic led to the implementation of enhanced strategies to prevent respiratory virus transmission, including universal masking, early isolation of suspected cases, contact tracing, active epidemiological surveillance, and molecular diagnosis. However, evidence regarding the impact of these strategies on respiratory virus transmission dynamics remains limited. To evaluate whether changes in institutional surveillance and infection prevention strategies modify the transmission dynamics of respiratory viruses. An observational study with a longitudinal within-subject analysis was conducted between 2020 and 2023 in a long-term care hospital. A total of 115 patients continuously hospitalized during two distinct periods were included: 2020–2021, when enhanced infection prevention strategies were maintained, and 2022–2023, following their progressive withdrawal. Respiratory viral infections were confirmed by multiplex RT-PCR, and paired comparisons between periods were performed using McNemar's test. The occurrence of respiratory viral infections increased significantly during the second period ($p<0.001$). During 2020–2021, infection occurrence was associated with patient mobility and functional dependence. Following the withdrawal of institutional prevention strategies, these differences in infection risk disappeared, leading to risk equalization across patients with distinct clinical characteristics. This finding suggests that the institutional environment became a stronger determinant of viral transmission than individual patient characteristics. Longitudinal analysis demonstrated that changes in institutional strategies altered the transmission dynamics of respiratory viruses. Withdrawal of enhanced preventive measures was associated with risk equalization, reinforcing the importance of maintaining these strategies to reduce respiratory virus transmission among vulnerable populations.

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Keywords: Viral Respiratory Infections; Epidemiological Surveillance; Infection Control; Long-Term Care Hospitals.

PT.557

OUTBREAK OF H5N1 AVIAN INFLUENZA IN COSCOROBA SWANS (*Coscoroba coscoroba*) AT THE TAIM ECOLOGICAL STATION

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The Taim Ecological Station (Southern Brazil) plays a critical role in the conservation of numerous species, including resident and migratory birds such as the coscoroba swan (*Coscoroba coscoroba*). These animals are vital to understanding Influenza A virus (FLUAV) dynamics, as an important susceptible hosts and due to their interaction with other animals, increasing the risk of significant spillover events. The present research investigated the presence of FLUAV in feces and tissue samples from *C. coscoroba* at the Taim Ecological Station in May 2026. Total RNA was extracted using a magnetic beads kit. Molecular screening was performed via RT-qPCR targeting the FLUAV matrix (M) gene. M-positive samples were further tested for the H5 subtype gene. Plasmid controls containing the M and H5 genes were used for assay validation and analytical sensitivity determination. For sample enrichment, whole-genome amplification was conducted prior to libraries construction to Ion Torrent GeneStudio S5 Plus next-generation sequencing (NGS). Raw reads were processed and assembled using the IRMA pipeline. Of the 15 samples tested, four were M-gene positive and three were confirmed as H5 subtype. One sample was confirmed as H5N1 by NGS. Combined with recent wildlife mortality reports, these findings demonstrate the presence of FLUAV in the Taim Ecological Station. The detection of the virus in this ecologically important protected area reinforces the need for ongoing surveillance, given the potential impacts on wildlife and the risk of spillover to local mammal populations.

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PT.558

MOLECULAR CHARACTERIZATION OF ORAL HPV INFECTION IN ARGENTINA: GENOTYPE, CO-INFECTIONS AND HPV16 GENETIC VARIABILITY

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Human papillomavirus (HPV) has emerged as a potential risk factor for oral carcinogenesis, although the contribution of other sexually transmitted pathogens remains unclear. This study investigated the prevalence of HPV, *Chlamydia trachomatis (C. trachomatis),* and Herpes Simplex Virus (HSV) in oral lesions and characterized clinically relevant HPV16 mutations.
Oral samples were collected from 318 patients with oral lesions, including benign lesions (B), oral potentially malignant disorders (OPMD) and oral squamous cell carcinoma (OSCC). HPV, *C. trachomatis* and HSV were detected by PCR, while HPV genotyping was performed by RFLP. HPV16-positive samples were further analyzed by sequencing the E6 and LCR genes to identify point mutations.
HPV was detected in 34% of samples, with prevalence increasing according to lesion severity (28% B, 35% OPMD and 42% OSCC). *C. trachomatis* and HSV were identified in 16% and 3% of samples, respectively. HPV–*C. trachomatis* co-infection was observed in 4% of cases, whereas HPV–HSV co-infection was detected in a single sample. Sequence analysis of HPV16 identified the E6 T350G (L83V) mutation in 18/23 samples and the LCR G7521A mutation in 7/8 samples. Additional E6 and LCR mutations not previously reported in oral lesions were also identified.
These findings provide a comprehensive molecular characterization of oral HPV infection, highlighting the predominance of HPV16, co-infections and the high frequency of mutations associated with viral persistence and oncogenic activity. This study represents the first report of HPV16 genetic variability in oral lesions from Argentina and supports further investigation of viral biomarkers involved in oral carcinogenesis.

PT.559

DETECTION OF YELLOW FEVER VIRUS IN DIDELPHIS SPP. DURING YELLOW FEVER EPIZOOTIC SURVEILLANCE IN THE STATE OF SÃO PAULO

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Yellow fever is an arboviral disease considered an important public health problem in tropical regions. In Brazil, the outbreak that occurred between 2016 and 2018, particularly in the Southeast region, resulted in hundreds of deaths among humans and non-human primates (NHPs). NHPs are the main sentinels of yellow fever virus (YFV) circulation; however, other wild mammals may also become naturally infected, although their role in the eco-epidemiology of YFV remains poorly understood. In this context, this study describes the occurrence of natural YFV infection in opossums (*Didelphis* spp.) detected during epizootic surveillance activities in the state of São Paulo. Between June 2025 and June 2026, 74 liver samples from *Didelphis* spp. were collected from areas with active YFV circulation in NHPs in the state of São Paulo. The samples were processed and analyzed using YFV-specific RT-qPCR. Five samples (6.7%) tested positive: three from Campinas, one from Ribeirão Preto, and one from São Paulo, detected between February 2025 and January 2026. All cases had Ct values above 30, suggesting a low viral load. These findings demonstrate the occurrence of natural infection in marsupials during yellow fever epizootic events, expand knowledge of YFV-susceptible hosts, and indicate that the role of *Didelphis* spp. in the eco-epidemiology of YFV should be further investigated. Financial Support: FESIMA/SES-SP No. 024.00115733/2025-27 CAF 063/2025

PT.560

SEROLOGICAL DETECTION OF DENGUE AND CHIKUNGUNYA VIRUS INFECTIONS IN PATIENTS WITH SUSPECTED ARBOVIRAL DISEASE IN MINAS GERAIS, BRAZIL

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Brazil is endemic for important arthropod-borne arboviruses, such as Dengue virus (*Orthoflavivirus denguei*) and Chikungunya virus (*Alphavirus chikungunya*), which cause recurrent outbreaks and substantial public health impacts. Considering the cocirculation of these pathogens and the influence of sociodemographic factors on viral exposure, this study investigated the presence of anti-Dengue and anti-Chikungunya IgM antibodies in patients with suspected arboviral infection treated at Santa Casa de Caridade de Diamantina, Minas Gerais, Brazil. A total of 264 serum samples were analyzed using enzyme-linked immunosorbent assay (ELISA), of which 89 (33.7%) were seropositive, including 83 (93.1%) positive for Dengue. Age-group analysis was performed in 63 individuals, and no association between age and seropositivity was observed (p=0.50); however, individuals aged 23–33 years showed the highest frequency of reactive results. Among anti-Dengue IgM-positive cases, 54 (65.1%) occurred in female patients (p=0.02). Among the 26 samples with recorded warning signs, 25 were positive for Dengue and 1 for Chikungunya. The most frequent clinical manifestations were orthostatic hypotension and/or lipothymia (36.8%), abdominal pain (26.3%), lethargy or irritability (15.7%), mucosal bleeding (10.5%), persistent vomiting (5.2%), and hepatomegaly >2 cm below the costal margin (5.2%). These findings demonstrate a higher circulation of *Orthoflavivirus denguei* in the analyzed population, particularly among women and young adults, highlighting the need to strengthen epidemiological and serological surveillance of arboviral diseases in the region.
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PT.561

THEORETICAL LOGICAL MODEL FOR THE HEALTHCARE PATHWAY OF VERTICAL TRANSMISSION OF HUMAN T-CELL LYMPHOTROPIC VIRUS (HTLV) IN PERNAMBUCO, BRAZIL

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Human T-cell Lymphotropic Virus (HTLV) remains a neglected public health issue despite its association with severe diseases such as adult T-cell leukemia/lymphoma and HTLV-1 associated myelopathy. In Brazil, particularly in the Northeast region, gaps in surveillance, diagnosis, and healthcare organization contribute to the persistence of vertical transmission. This study aimed to develop a theoretical logical model to support the implementation of a healthcare pathway for preventing vertical transmission in Pernambuco, Brazil. An applied qualitative study was conducted through a literature review, documentary analysis of national and state public health policies, epidemiological data, and technical guidelines related to HTLV. The logical model was developed using health evaluation frameworks, including problem identification, definition of structural components, organization of inputs, activities, outputs, and expected outcomes, followed by theoretical validation of internal coherence. The proposed model integrated epidemiological surveillance, universal prenatal screening, confirmatory diagnosis, multidisciplinary care, counseling, infant feeding guidance, laboratory monitoring, and long-term follow-up of people living with HTLV. The analysis identified fragmentation of healthcare services, limited access to early diagnosis, and insufficient integration among surveillance and care networks as major barriers to effective prevention of mother-to-child transmission. The resulting logical model established causal relationships between available resources, strategic interventions, and expected short-, medium-, and long-term outcomes, providing a structured framework for planning and evaluating healthcare actions. This study presents an innovative systems-based approach for organizing HTLV care in Pernambuco and offers a practical tool to strengthen public health policies, improve healthcare coordination, reduce vertical transmission, and support future implementation and evaluation of comprehensive HTLV healthcare pathways in endemic settings.

PT.562

MOLECULAR DIVERSITY AND CIRCULATION PATTERNS OF HUMAN ENTEROVIRUSES IN BRAZILIAN CHILDREN WITH ACUTE UNDIFFERENTIATED FEBRILE ILLNESS

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Human enteroviruses (HEVs) are underdiagnosed causes of acute undifferentiated febrile illness (AUFI) in children due to their nonspecific clinical presentation. This study evaluated the frequency and molecular diversity of HEVs in patients ≤14 years old with AUFI treated at a tertiary hospital in São José do Rio Preto, Brazil. A total of 405 oropharyngeal swab samples collected between 2021 and 2024 were analyzed by in-house RT-qPCR. Positive samples underwent VP1-targeted RT-PCR, Sanger sequencing, phylogenetic analysis and isolation in RD cells to assess infectivity. HEV was detected in 7.2% (29/405) of cases. Patients ranged from 0 to 10 years old (mean 3.3 years), with the highest frequency observed among children aged 2–4 years (62.1%; $p < 0.05$). Cytopathic effect was observed in 79.3% (23/29) of positive samples, and 37.9% (11/29) were successfully genotyped. Identified genotypes included Coxsackievirus (CV)-B5 (n=5), CV-B1 (n=1), CV-B2 (n=1), CV-A21 (n=1), CV-A5 (n=1), CV-A8 (n=1) and Echovirus (E)-3 (n=1). Phylogenetic analyses indicated distinct circulation patterns. CV-B5 clustered with Brazilian strains (98.4–100%), suggesting sustained endemic circulation. In contrast, CV-B1 (96-97.2%), CV-A8 (96–97%), E-3 (97.1-97.9%) and CV-A5 (96.9–98%) were closely related to Asian, European and North American strains, indicating possible introductions from abroad. CV-B2 was genetically distinct from Brazilian strains (77.6–79.7%), implying underexplored diversity. CV-A21 was closely related to global strains (92.7–98.8%) and represents its first report in Brazil. These findings highlight diverse HEV circulation in Brazil, including endemic lineages and potential international introductions, and support oropharyngeal swabs for pediatric AUFI surveillance.

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PT.563

Applicability of an NSP3-Targeted RT-qPCR Assay for Rotavirus A Screening in Animal Fecal Samples

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Rotavirus A (RVA) is an important cause of gastroenteritis in humans and animals. Mammalian hosts may contribute to the maintenance and dissemination of RVA and represent important sources of viral diversity, particularly in the context of widespread rotavirus vaccination in humans. This study evaluated a TaqMan® real-time RT-PCR assay, originally developed for human RVA detection, for screening animal fecal samples and determining RVA detection rates across host species. The assay targets an 87-bp fragment of the conserved NSP3 gene and was assessed using three characterized canine RVA strains and three RVA-positive human fecal samples tested in triplicate. All samples were successfully amplified, with sigmoidal curves and Ct values ranging from 22.39 to 27.45 (mean Ct: 23.15 for canine and 26.98 for human samples), supporting the assay's applicability for screening animal fecal specimens. A total of 1,131 fecal samples from dogs (n=517), cats (n=84), pigs (n=192) and capybaras (n=338), collected in the states of São Paulo and Paraná between 2012 and 2025, were tested by RT-qPCR in duplicate. RVA was detected in 7.9% of dogs (41/517; mean Ct, 32.75), 3.6% of cats (3/84; 32.99), and 16.1% of pigs (31/192; 32.93), while all capybaras were negative. To confirm RT-qPCR-positive results, all 75 samples underwent conventional RT-PCR targeting VP7 gene, with amplification in 27 (36.0%): 11 dogs, one cat and 15 pigs. The NSP3-targeted RT-qPCR assay proved suitable for RVA screening across mammalian hosts, supporting molecular surveillance of RVA circulation in companion and domestic animals within a One Health framework.

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PT.564

LARGEST MOLECULAR SURVEY OF BOVINE PESTIVIRUSES IN BRAZIL REVEALS THE BROAD GEOGRAPHIC DISTRIBUTION OF HoBi-LIKE PESTIVIRUS

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Bovine pestiviruses are responsible for major reproductive and economic losses worldwide. Despite Brazil harboring the world's largest cattle herd, the nationwide molecular epidemiology of bovine pestiviruses remains poorly characterized. We analyzed 37,409 bovine serum samples collected between 2014 and 2021 through Brazil's national Foot-and-Mouth Disease surveillance program coordinated by the Brazilian Ministry of Agriculture and Livestock (MAPA). Samples originated from eight Brazilian states: Rio Grande do Sul, Paraná, Acre, Amazonas, Rondônia, Roraima, Pará, and Amapá, and were analyzed by RT-qPCR, Sanger sequencing, and phylogenetic analysis. This study represents the most comprehensive molecular survey of bovine pestiviruses conducted in Brazil. Seventy-three pestiviruses were detected and characterized, including BVDV-1a, BVDV-1b, BVDV-1d, BVDV-2b, and HoBi-like pestivirus (HoBiPeV). Unexpectedly, HoBiPeV was the predominant pestivirus in Northern Brazil and Paraná state, accounting for 90.5% (19/21) of all detected pestiviruses, whereas Rio Grande do Sul exhibited a distinct epidemiological profile, with exclusive circulation of BVDV-1a (25/49; 51%), BVDV-1b (1/49; 2%), BVDV-1d (7/49; 14.3%), and BVDV-2b (16/49; 32.7%). The predominance of HoBiPeV in geographically distant regions of Brazil highlights the need to ensure that molecular diagnostic assays adequately detect this species and to reassess vaccine formulations used in Brazil, which currently do not include HoBiPeV strains. These findings redefine the current epidemiological scenario of bovine pestiviruses in Brazil and have direct implications for diagnosis, surveillance, and vaccine design.

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PT.565

ECOLOGICAL ASYNCHRONY AND POST-PANDEMIC SHIFTS IN RESPIRATORY VIRAL EPIDEMICS: A DECADE-LONG COMPARATIVE ANALYSIS OF BRAZIL AND GLOBAL TRENDS

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This study focused on Brazilian hospitalisations and used global sources only with explicit construct distinction. We analysed approximately 3.5 million severe acute respiratory infection hospitalisations from the Brazilian national surveillance system (2015-2025) for influenza, respiratory syncytial virus (RSV), and SARS-CoV-2. Weekly series were standardised by Z-score. Epidemic peaks were estimated by calendar-year and season-aligned detection. Post-pandemic proportional shifts were assessed with the Chow test and negative binomial models (pandemic 2020-2022 and recovery 2023-2025 versus 2015-2019). World Health Organization FluNet laboratory positivity was not treated as equivalent to hospitalisations. As a closer but still distinct comparator, World Health Organization FluID syndromic severe acute respiratory infection case counts (Brazil versus a global FluID aggregate) provided contextual influenza timing in endemic years (2015-2019). In Brazilian hospitalisations, calendar-year peak search introduced episodic displacement of up to about 16 weeks relative to season-aligned estimates. Influenza remained below pre-pandemic proportional levels in recovery (incidence rate ratio 0.70, 95% confidence interval 0.57-0.85), while RSV exceeded baseline (1.41, 1.18-1.68); both were strongly suppressed during the pandemic. FluID syndromic cases suggested a delayed influenza peak in Brazil versus the global FluID aggregate (median lag about +10 weeks), without equating this to matched national hospitalisation registries. These findings show pathogen-specific post-pandemic recovery in Brazilian hospitalisations and caution against Brazil-world comparisons across mismatched surveillance constructs. **Financial Support:** This study was supported by Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG).

PT.566

MOLECULAR CO-INFECTION MONITORING OF HEPATITIS A AND E VIRUSES IN PATIENTS FROM SOUTHERN BRAZIL

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Hepatitis A (HAV) and E (HEV) viruses represent global public health threats; however, investigations into co-infections by these pathogens are scarce. While HAV faces an epidemiological transition that, alongside its severity in children, has become severely elevated in adults, HEV has a markedly zoonotic character, with high prevalence in locations with inadequate basic sanitation infrastructure and increased severity in immunosuppressed patients. The simultaneous presence of both viruses can potentiate cellular damage and induce fulminant hepatic failure, making molecular monitoring relevant. To evaluate prevalence and co-infection risks, 50 serum samples from patients who attended a public hospital in Santa Catarina, Brazil, were analyzed via RT-qPCR, in conjunction with the analysis of clinical data from patients' electronic medical records. HEV prevalence was 4% (2/50). All samples were negative for HAV, indicating the absence of co-infection in this cohort. These findings hold epidemiological relevance by providing \ evidence of HEV circulation in the region. The detection of HEV infections, combined with the lack of data on HAV/HEV interactions, exposes a critical diagnostic gap. This study highlights the importance of implementing continuous molecular surveillance to mitigate severe clinical outcomes and redefine screening strategies in public health.
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PT.567

Trend in the Incidence of Viral Meningitis in the Municipality of Marituba, Pará: 2015–2025

LUANY CRUZ

Introduction: The pathophysiological basis of viral meningitis is characterized by an acute inflammatory process in the leptomeninges (the two delicate, innermost membranes lining and protecting the brain and spinal cord), manifesting as cerebrospinal fluid (CSF) pleocytosis (an elevated leukocyte count in the CSF). Commonly referred to as aseptic meningitis, it involves inflammation of the leptomeninges where conventional microbiological cultures of the CSF fail to detect bacteria. Clinical manifestations (headache, fever, nuchal rigidity, photophobia, etc.) can mimic the signs and symptoms characteristic of bacterial meningitis. Some infected individuals may experience temporary complications or long-term residual sequelae, even following cases considered mild. Material and Methods: This is an observational, analytical epidemiological study of the ecological type, employing a quantitative approach based on secondary data. Annual incidence rates of viral meningitis were obtained via the Tabnet system. Trend analysis was performed using the Prais-Winsten linear regression model (via StataMP 17 software), which allows for the correction of serial autocorrelation in time series. Results: A p-value of 0.191 was obtained, with a lower limit of -24.7 and an upper limit of 5.8. This non-significant value classified the trend as "stationary." Conclusion: Over the past 11 years, the incidence of viral meningitis in Marituba, Pará, has shown a stationary trend. However, its clinical resemblance to bacterial meningitis underscores the need for constant epidemiological surveillance and strengthened laboratory diagnostics to ensure appropriate clinical management and control of the disease within the municipality.
Keywords: viral epidemiology; leptomeninges; epidemiological surveillance
Financial Support: Amazon Foundation for Studies and Research Support (FAPESPA)

PT.568

Trend in Chikungunya Fever Incidence in the Municipality of Marituba, Pará: 2020–2025

LUANY CRUZ

Introduction: Chikungunya Fever (CF) manifests as a febrile illness associated with polyarthralgia and severe polyarthritis. This study is justified by the need to understand the disease's behavior in the municipality of Marituba (PA) using rigorous statistical methods, such as Prais-Winsten regression, which provides greater precision in time-series trend analysis. The objective of this abstract is to analyze the temporal trend of CF incidence from 2020 to 2025. Material and Methods: This is an observational, analytical, ecological epidemiological study employing a quantitative approach based on secondary data. Annual CF incidence data were obtained via the Tabnet system. Trend analysis was performed using the Prais-Winsten linear regression model (via StataMP 17 software), which allows for the correction of serial autocorrelation in time series. Results: Annual incidence figures from 2020 to 2025 were 4, 2, 2, 3, and 0, respectively. Application of Prais-Winsten regression yielded a p-value of 0.075, with a lower limit of -1.08 and an upper limit of 0.08. This non-significant result, combined with limits of opposite signs, classified the trend as "stationary." Conclusion: The results indicate a stationary trend in the incidence of Chikungunya Fever during the analyzed period. This scenario highlights the need for continued public prevention policies and constant monitoring to prevent potential outbreaks and new epidemiological variations in the region.
Keywords: viral epidemiology; arboviruses; epidemiological surveillance.
Financial Support: Amazon Foundation for Studies and Research Support (FAPESPA)

PT.569

CLINICAL PROFILE AND MOLECULAR DETECTION OF HEPATITIS E VIRUS INFECTION IN HBV/HCV-NEGATIVE NON-PREGNANT PATIENTS FROM SOUTHERN BRAZIL

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Despite its growing recognition as a public health concern, Hepatitis E Virus (HEV) infection remains poorly characterized in Brazil, where limited epidemiological data and the lack of routine molecular testing obscure its true circulation, thereby affecting Brazilian public health. To investigate the prevalence, clinical profiles, and hepatic function markers of HEV infection in HBV/HCV-negative non-pregnant patients, molecular screening via RT-qPCR was performed on 722 serum samples from patients attended at a reference hospital in Santa Catarina, Brazil, and was complemented by analysis of medical records. The prevalence in this subgroup was approximately 3.8% (29/722), and the predominant profile of infected individuals included adult, white, single females with no comorbidities, presenting mild manifestations and good clinical outcomes. Critical exceptions included two deaths associated with prior comorbidities (immunosuppression/cancer and renal disease) and three detections in sexual assault victims. Evaluation of biochemical markers (ALT, AST, total bilirubin, alkaline phosphatase, and lymphocytes) showed results mostly within normal ranges, revealing no differences in liver function between individuals with and without detectable HEV RNA. The absence of marked transaminase elevations demonstrates that the disease frequently follows a biochemically silent course. These findings reinforce the imperative need to implement molecular surveillance to uncover the true epidemiology of HEV and mitigate clinical underdiagnosis.

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PT.570

COXSACKIEVIRUS A16 (CLADE D) AS AN AGENT OF HAND, FOOT AND MOUTH DISEASE IN URUGUAY: EVIDENCE OF ITS CIRCULATION IN SOUTHERN SOUTH AMERICA

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Hand, foot, and mouth disease (HFMD) is a common pediatric illness caused by enteroviruses like Coxsackievirus A16 (CVA16). While CVA16 is well-studied in Asia-Pacific regions, its circulation and evolutionary history in South America remain poorly documented. This study characterized CVA16 strains from a 2019 HFMD outbreak in Uruguay to contextualize them within a regional evolutionary framework. Partial VP1 sequencing identified CVA16 in eight outbreak cases. Maximum likelihood phylogenetic analysis revealed that these Uruguayan strains belong to clade D, a genetically divergent lineage rarely reported globally. Bayesian phylodynamic analysis estimated an evolutionary rate consistent with enterovirus A and dated lineage D's most recent common ancestor to the mid-2000s. To explore its regional history, Bayesian analysis integrated reference sequences with 35 previously uncharacterized partial VP1 sequences from Uruguayan and Argentine wastewater (2011–2012). Results suggest lineage D diversified early in southern South America (late 2000s–early 2010s) before being detected worldwide between 2011 and 2021. The 2019 Uruguayan strains share a common ancestor with a 2016 Mozambique strain and are more closely related to globally distributed sequences than to South American environmental variants from 2011–2012, reflecting temporal shifts in local viral strains. Overall, this study provides new evidence on the evolutionary history and circulation of the CVA16 D lineage in Uruguay and highlights the contribution of environmental surveillance to understanding enterovirus diversity in understudied regions.

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PT.571

Detection of Rio Mamoré-like Hantavirus in non-classical Wild Mammal Hosts from the Brazilian Amazon

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Hantaviruses are zoonotic pathogens of substantial public health importance, associated with a spectrum of severe human diseases, including haemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS). South American hantaviruses are genetically diverse, with more than 38 New World hantaviruses described to date. In this study, we analysed 223 oral and anal swab samples collected from 112 wild animals between April 2024 and August 2025 in areas under distinct anthropogenic influence in Amapá state, Brazil. Three animals tested positive for hantavirus by RT-PCR: an oral swab from a marsupial opossum (*Marmosops pinheiroi*) and anal swabs from two lowland pacas (*Cuniculus paca*), sampled in two areas characterized by moderate-to-low anthropogenic influence: the Maracá Community, located in the municipality of Mazagão, and the AMCEL Reserve, in the municipality of Santana. Phylogenetic analyses revealed that the three samples from Amapá were closely related to the Rio Mamoré virus (RIOMV) isolated from a small-eared pygmy rice rat (*Oligoryzomys microtis*) captured in northeastern Peru in 1996. The detection of viral RNA in oral and anal swabs demonstrates that these wild mammals are infected and actively shedding RIOMV, suggesting that local communities could be exposed during forest-associated activities such as subsistence hunting. However, the role of lowland pacas and opossums in the RIOMV transmission cycle, whether acting as amplifying hosts, dead-end hosts, or experiencing incidental infections, remains to be elucidated.

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PT.572

DETECTION OF HUMAN PAPILLOMAVIRUS IN WASTEWATER FROM SÃO JOSÉ DO RIO PRETO CITY, BRAZIL

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Wastewater-Based Epidemiology (WBE) has emerged as a valuable tool for public health surveillance, using wastewater matrices as an indicator of presence and incidence of pathogens in a population approach. Among these pathogens, human papillomavirus (HPV) is of particular concern due to its strong association with cervical cancer, a disease characterized by high incidence and mortality rates worldwide. Despite its public health relevance, studies investigating HPV in wastewater remain limited. In this context, WBE represents a promising approach for monitoring HPV infections at the population level. Therefore, this study aimed to conduct the epidemiological and molecular surveillance of HPV in wastewater from the municipality of São José do Rio Preto, São Paulo State, Brazil. Viral particles were concentrated using PEG 8000, followed by nucleic acid extraction. HPV was detected using nested PCR, followed by Sanger sequencing of the resulting amplicons to identify the viral genotypes circulating in the region. In 2026, a total of 16 weekly wastewater samples, collected between March 12 and June 25, were analyzed. HPV was detected in 10 samples (62.5%), with the highest detection frequencies observed in April (30%) and May (40%). Sequence analysis revealed that six of the positive samples (60.0%) belonged to the HPV-18 genotype, a high-risk type strongly associated with cervical cancer. These findings demonstrate the circulation of a potentially oncogenic HPV in the study area and highlight the relevance of WBE as a complementary strategy for monitoring sexually transmitted infections and supporting public health interventions, including vaccination and awareness programs.
Financial support: CAPES.

PT.573

Predominance of DENV-3 in symptomatic patients from the municipality of Pitangueiras (SP) and genomic characterization by next-generation sequencing

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Dengue remains a major public health problem in Brazil, making molecular surveillance essential for monitoring viral circulation and identifying variants with potential epidemiological impact. This study, approved by the Research Ethics Committee (CAAE 94452225.3.0000.5403), investigated the circulation of dengue virus (DENV) serotypes among symptomatic patients in Pitangueiras (SP), comparing different diagnostic methods used in the public health system. Between April 22 and June 17, 2026, 491 blood samples were collected from febrile patients with suspected dengue. The study population had a mean age of 36.8 years (0.6–93 years), with a predominance of females (52.7%) and a mean of 3.8 days between symptom onset and sample collection. The most reported symptoms across cases were myalgia, fever, and headache. By RT-qPCR, 125 samples tested positive for DENV, with DENV-3 being the predominant serotype. Of the total samples, 178 were tested for NS1 using a rapid test in the local public health system, yielding 32 positive and 146 negative results. Concordance between RT-qPCR and the NS1 test was observed in 20 positive and 121 negative cases, whereas 25 samples were positive only by RT-qPCR and 12 only by NS1, with a mean symptom duration of 3.6 days (discordant cases). Among RT-qPCRs tested by Adolfo Lutz Institute, there were 10 positive correspondences and 9 discrepancies. For NGS, 21 samples with the lowest Ct values (20.08–30.65) were selected to characterize the circulating lineages and identify mutations of potential epidemiological interest.

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PT.574

SURVEILLANCE AND STANDARDIZATION OF MOLECULAR DIAGNOSTIC TECHNIQUES FOR THE DETECTION OF ARBOVIRUSES IN THE STATE OF SÃO PAULO, BRAZIL

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Arbovirus research is a priority due to its rising risk to animal and public health in regions with high vector diversity. This study aims to strengthen epidemiological surveillance in São Paulo State by standardizing RT-qPCR assays for Alphavirus, Flavivirus, and Orbivirus. Special emphasis is placed on Eastern, Western, and Venezuelan equine encephalitis viruses (EEEV, WEEV, VEEV), Rocio (ROCV), Saint Louis encephalitis (SLEV), West Nile (WNV), Bluetongue (BTV), Peruvian horse sickness (PHSV), and Epizootic hemorrhagic disease (EHDV) viruses. The project began in October 2025, with six sampling campaigns conducted during the 2025–2026 summer in Jaboticabal, Andradina, Pariquera-Açu, and Pirassununga. Sites were selected based on livestock production, historical viral circulation, and ecological factors. Mosquitoes (Culicidae) and biting midges (Culicoides) were collected, identified, and grouped into over 150 pools. To date, a subset of samples has undergone tissue disruption and nucleic acid extraction. RT-qPCR standardization achieved analytical detection limits of 10^1 copies for *Flavivirus,* 10^4 copies for *Alphavirus*, and 10^3 copies for *Orbivirus*. A second sampling phase is scheduled for the 2026–2027 summer. Positive detections will undergo whole-genome sequencing via MinION™ or Illumina® platforms, followed by phylogenetic analyses using Maximum Likelihood and Bayesian inference. Ultimately, this project expects to provide a comprehensive mapping of arboviral circulation in the state to support public health interventions.

PT.575

Molecular Epidemiology of Sapoviruses in Acute Gastroenteritis in Brazil: A Five-Year Surveillance Study, 2018 – 2022

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Sapovirus is a leading cause of acute gastroenteritis (AGE) in children younger than five years globally. This work examined the frequency and genetic variability of sapovirus in patients seeking medical care for AGE in Brazil's Southeastern, Southern, and Northeastern regions from 2018 to 2022. A set of 1,994 stool specimens was screened for the virus using TaqMan RT-qPCR, and positive samples were genotyped via Sanger sequencing. Sapovirus was detected in 6.5% (129 out of 1,994) of cases, with the highest positivity found among children under 60 months of age. A seasonal pattern emerged, with detection rates peaking in autumn ($p = 0.0048$). Partial sequencing of the VP1 gene revealed extensive genotypic diversity, spanning four genogroups and ten distinct genotypes. The most common were GI.1 and GII.1, representing 31.6% and 26.3% of the strains, respectively. Given the shortage of recent data on sapovirus in Brazil, this investigation fills a critical gap by providing updated prevalence and genotype distribution information over a five-year period. It also strengthens the surveillance of emerging viral causes of gastroenteritis across different Brazilian regions.

PT.576

HEPATITIS DELTA AND HEPATITIS C CO-INFECTION AMONG PREGNANT WOMEN IN BRAZIL: A NATIONWIDE ANALYSIS USING SINAN DATA (2007–2023)

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Hepatitis C (HCV) and Hepatitis Delta (HDV) viruses cause severe hepatitis, yet data on co-infection among pregnant women remain scarce. This retrospective study analyzed confirmed HDV cases from the Brazilian Notifiable Diseases Information System (SINAN) from 2007 to 2023. Through the analysis of SINAN, positive cases of HDV and HCV infection were considered when, for these cases, there was the presence of any serological or molecular marker for these infections. Data were analyzed using RStudio (v4.5.1) with IBGE population data. Among 3,770 HDV cases, 42% were female and 51.4% were aged 25–45. The Northern region accounted for 67.9% of notifications, centered in Amazonas state (Atalaia do Norte and Eirunepé; >600/100,000 inhabitants). Pregnant women represented 80.7% of female cases (N=1,275/1,580; 33.8% of the total cohort), likely reflecting routine prenatal screening rather than higher true prevalence. Overall, 552 cases (14.6%) had HCV co-infection, including 162 pregnant women (29.3%). HDV remains highly endemic in northern Brazil. The high detection in pregnant women underscores both the impact of mandatory prenatal testing and the necessity of universal screening to mitigate vertical transmission. Additionally, the substantial HDV/HCV co-infection rate reveals a complex syndemic profile requiring integrated, decentralized healthcare pathways. This study was supported by a research grant from FAPESP foundation (2025/06771-6).

PT.577

ETIOLOGIES OF ACUTE FEBRILE ILLNESS IN BRAZIL (2010–2023): HIGHLIGHTING THE ROLE OF PARVOVIRUS B19 AND ARBOVIRUSES

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Recent studies highlight Parvovirus B19 (B19V) detection in acute-febrile illness (AFI) globally. This study investigated AFI etiologies across Brazilian states, focusing on B19V, arboviruses, and bacterial co-factors. We conducted a descriptive cross-sectional study (2010-2023) analyzing 3,113 population-proportional AFI serum samples from Amapá, Alagoas, Maranhão, Mato Grosso do Sul, Piauí, Paraíba, Tocantins, and São Paulo. Initial screening occurred at state Central Public Health Laboratories (LACENs), including arboviruses, enteroviruses, and Leptospira, with data logged in the Laboratory Environment Manager (GAL). Samples were then sent to the Laboratory of Virology, School of Medicine, University of São Paulo (USP), for real-time PCR targeting B19V, Dengue, Zika, Chikungunya, Mayaro, Oropouche, Yellow fever, and Enterovirus. Overall, 934 individuals tested positive, yielding 1,009 pathogen detections. Out of 3,113 samples, prevalences were: Dengue (n=554; 17.8%), Chikungunya (n=245; 7.9%), Zika (n=95; 3.1%), B19V (n=85; 2.7%), Leptospira (n=7; 0.2%), Enterovirus (n=2; 0.06%), and Mayaro (n=1; 0.03%). Other etiologies were identified in 0.6% (n=20), including rubella, measles, and hepatitis A/B. Etiologic agents were detected exclusively at LACENs in 18.5% (n=575) of cases, exclusively at USP in 5.3% (n=165), and jointly in 6.2% (n=194). In conclusion, B19V represents a notable proportion of AFI diagnoses. Clinical recognition remains challenging because early symptoms closely mimic arboviral infections. Strengthening laboratory capacity, integrating differential diagnostics, and expanding surveillance are essential to guide targeted interventions. Our data represent only the investigated pathogens within tested samples.

PT.578

CULICID DIVERSITY AND YELLOW FEVER VIRUS DETECTION IN URBAN GREEN AREAS OF BELO HORIZONTE, 2023-2024

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Between late 2016 and mid-2018, the state of Minas Gerais experienced extensive yellow fever outbreaks, followed by the continued occurrence of human cases and epizootics in subsequent years. These observations suggest that sylvatic vectors may sustain viral circulation within urban green areas. Therefore, this study assessed culicid diversity in Belo Horizonte (BH) and investigated the presence of arboviruses in these mosquito populations. Three sampling campaigns, encompassing the intermediate, rainy, and dry seasons, were conducted between October 2023 and April 2024 across six urban parks in BH. A total of 10,523 mosquitoes were collected, including the arbovirus vectors *Aedes* spp. (26.2%), *Culex* spp. (56.8%), *Haemagogus* spp. (1.9%), *Psorophora* sp. (0.4%), and *Sabethes* spp. (4.3%). Notably, the yellow fever virus (YFV) vectors *Haemagogus* and *Sabethes* were detected in all sampled parks. Analyses of abundance, species richness, and community composition showed a significant association between *Haemagogus* spp. and the onset of the rainy season (October–December; $p = 0.001$). Among 1,054 mosquito pools tested by RT-qPCR, none was positive for Chikungunya virus, whereas one pool of *Haemagogus leucocelaenus* tested positive for YFV. These findings demonstrate the diversity of arbovirus sylvatic vectors and ongoing YFV circulation in urban parks of BH, suggesting sylvatic YFV transmission within the urban matrix. Together, these results highlight a persistent public health risk due to the close ecological overlap between humans and non-human primates in urban environments.

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PT.579

HIGH PREVALENCE OF HUMAITÁ-TUBIACANGA VIRUS IN MOSQUITOES FROM URBAN PARKS IN BELO HORIZONTE, BRAZIL, 2023-2024

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Insect-specific viruses (ISVs) infect mosquitoes and phlebotomines but do not replicate in vertebrates. Although ISVs may inhibit arbovirus infection through superinfection exclusion, some, such as Humaitá-Tubiacanga virus (HTV) and Phasi Charoen-like virus (PCLV), have been associated with enhanced transmission of Zika and dengue viruses by shortening the extrinsic incubation period, potentially increasing public health risk. This study investigated the occurrence of HTV in mosquitoes collected from urban parks in Belo Horizonte, Minas Gerais, Brazil. Sylvatic mosquitoes were collected and grouped into pools of up to 20 individuals. A total of 10,523 mosquitoes (1,054 pools) were screened for HTV by RT-qPCR using virus-specific primers. HTV RNA was detected in 2,181 mosquitoes (20.7%), including *Aedes albopictus* (0.9%), *Aedes scapularis* (3.8%), *Haemagogus leucocelaenus* (0.8%), *Psorophora ferox* (0.03%), and *Sabethes* spp. (1.2%). Additional analyses will include screening for PCLV and attempts to isolate both viruses. The detection of HTV in nearly 21% of the sampled mosquitoes is noteworthy, particularly because this is, to our knowledge, the first report of HTV in some of these mosquito genera. These findings expand the known host range of HTV and underscore the need for further studies to clarify the role of ISVs in shaping arbovirus transmission dynamics and their potential influence on the emergence and spread of Zika, dengue, and other arboviral diseases in Brazil.

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PT.580

One Health surveillance of emerging arboviruses: investigation of OROV, MAYV, SLEV, and WNV in northern and northeastern Minas Gerais, Brazil

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Arboviruses have expanded into new regions of Brazil in recent years, increasing the need for integrated surveillance strategies. In this study, we investigated the circulation of Oropouche (OROV), Mayaro (MAYV), Saint Louis encephalitis (SLEV), and West Nile (WNV) viruses in northern and northeastern Minas Gerais using a One Health approach. Between 2019 and 2025, we analyzed 824 serum samples from patients with acute febrile illness, 3,484 mosquitoes, and 113 nonhuman primates. All samples had previously tested negative for dengue, Zika, and chikungunya viruses. Viral detection was performed using duplex RT-qPCR assays targeting OROV/MAYV and SLEV/WNV. No molecular evidence of viral circulation was detected in mosquitoes or nonhuman primates. Among human samples, only one imported case of Oropouche fever was identified in January 2025, with no evidence of autochthonous transmission of the investigated arboviruses in the study area. Although potential vectors such as *Culex* spp. and *Haemagogus* spp. were collected, the primary OROV vector, *Culicoides paraensis*, was not sampled. Our findings suggest that OROV, MAYV, SLEV, and WNV are not currently established in the evaluated regions of Minas Gerais. These data provide an important epidemiologic baseline for future surveillance studies and underscore the importance of integrated, continuous surveillance for early detection of emerging pathogens and prevention of future outbreaks.

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PT.581

FIRST DETECTION OF HOBI-LIKE PESTIVIRUS ASSOCIATED WITH MUCOSAL DISEASE-LIKE SYNDROME IN CATTLE FROM GOIÁS STATE, BRAZIL

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The genus *Pestivirus* (family *Flaviviridae*) includes bovine viral diarrhea viruses (BVDV-1 and BVDV-2) and HoBi-like pestivirus (HoBiPeV). In Brazil, HoBiPeV distribution is heterogeneous, and reports from the Central-West region remain scarce. The aim of this study was to describe the first confirmed case of HoBiPev associated with mucosal disease-like syndrome in the Central-Western Brazilian region. During an outbreak on a beef cattle farm in Mozarlândia, Goiás, between May and June 2024, 28 15-month-old bulls died after presenting apathy, anorexia, dehydration, diarrhea, mucopurulent nasal discharge and cutaneous lesions. One affected animal was submitted for necropsy, and spleen and lymph node samples were collected for molecular diagnosis. Viral RNA was extracted using TRIzol™, followed by complementary DNA synthesis and RT-PCR amplification using primer sets targeting the 5'UTR, E2, and N^{pro} regions. The amplification products were visualized by electrophoresis. Positive samples were purified and subjected to Sanger sequencing. Sequences were assembled and edited in Geneious and identified using BLASTn. Homologous reference sequences from GenBank were aligned with MAFFT, and maximum-likelihood phylogenetic trees were inferred in MEGA11 using 1,000 bootstrap replicates. The spleen sample tested positive for pestivirus RNA. Sequence analysis confirmed the presence of HoBiPeV. BLASTn searches revealed high nucleotide identity with previously described strains from Brazil and Australia. These findings expand the known distribution of HoBiPeV in Brazil and emphasize the importance of continuous surveillance for pestiviruses, particularly in regions with limited epidemiological data. Support: CAPES, CNPq, FAPERGS, Propesq/UFRGS.

PT.582

EPIDEMIOLOGICAL PROFILE OF HUMAN PARAINFLUENZA VIRUS INFECTIONS IN HOSPITALIZED CHILDREN FROM SÃO PAULO: INCIDENCE, COINFECTIONS, AND CLINICAL SEVERITY

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Human parainfluenza virus (HPIV) is the second cause of respiratory diseases, being correlated to mild to severe clinical presentation, such as croup, bronchiolitis and pneumonia. When other risk factors are related, HPIV infections can even result in death. Thus, the present study evaluated the incidence and clinical aspects of HPIV among children treated at five public hospitals in São Paulo. To this end, 11.222 samples from pediatric patients with suspected viral respiratory infections were analyzed between January 1, 2025, and June 30, 2026. They were tested for a panel of 15 respiratory viruses with RT-qPCR. Of these, 887 were positive for one of the tested HPIV serotypes (1, 2, 3 and 4), where 298 of these cases involved co-infections with one or more respiratory viruses, in which human Rhinovirus and Bocavirus were the most frequent. The more predominant serotype was HPIV-3, consistent with what was reported in literature. Regarding sex, 55,26% samples were from males, with 7,95% positive for HPIV, while 44,74% were from females, with 7,85% positives. Five age groups were established: neonates (≤28 days); infants I (≥29 days to ≤6 months); infants II (≥7 months to ≤2 years), being the more expressive group representing 38,49% of the samples, and with the higher parcel of positives (10,51%); young children (≥3 to ≤5 years); and older children (≥6 years). Among the positive samples, the diagnosis of Severe Acute Respiratory Syndrome had the highest incidence, highlighting the severity of HPIV infection in this population.

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PT.583

CIRCULATION OF ARBOVIRUSES IN SOUTHERN IN 2025

Santos NG[1], Piton KA[2], Lemos LS[1], Souza LC[2], Ribeiro TS[2], Santos HPC[3], Dantas ES[3], Alves LPR[4], Mariano APM[5], Mello SRG[5] - [1]Universidade Estadual de Santa Cruz (UESC) - Mestrando/Programa de Pós-Graduação em Ciências da Saúde - (PPGCS), Laboratório de Farmacogenômica e Epidemiologia Molecular (LAFEM)., [2]Universidade Estadual de Santa Cruz (UESC) - Mestranda/Programa de Pós-Graduação em Ciências da Saúde - (PPGCS), Laboratório de Farmacogenômica e Epidemiologia Molecular (LAFEM)., [3]Universidade Estadual de Santa Cruz (UESC) - Graduanda/Departamento de Ciências Biológicas (DCB), Bacharelado em Biomedicina, Laboratório de Farmacogenômica e Epidemiologia Molecular (LAFEM)., [4]Universidade Estadual de Santa Cruz (UESC) - Graduando/Departamento de Ciências Biológicas (DCB), Bacharelado em Biomedicina, Laboratório de Farmacogenômica e Epidemiologia Molecular (LAFEM)., [5]Universidade Estadual de Santa Cruz (UESC) - Docente/Departamento de Ciências Biológicas (DCB), Laboratório de Farmacogenômica e Epidemiologia Molecular (LAFEM).

LAFEM works in partnership with LACEN-BA, providing support for the diagnosis of arboviral diseases in southern Bahia. The objective of this study was to describe the prevalence of Dengue, Chikungunya, and Zika, based on laboratory tests conducted by LAFEM. This is an observational, retrospective, and descriptive study that analyzed the laboratory's database, containing data from notification forms and test results. It was observed that, from January through December 2025, 400 serum samples were received and 586 tests were performed. Dengue was the most tested infection, particularly IgM (n=259) and NS1 (n=148) tests, depending on the duration of symptoms. In the Dengue IgM test, 48.6% of the samples were positive and 3% were inconclusive. For Dengue NS1, 15.5% were positive and 3% were inconclusive. Regarding Chikungunya, IgM testing showed a 5.3% positivity rate (5/94) and 5.3% inconclusive results, while 28 IgG tests were performed, with a 42.8% positivity rate (12/28). For Zika, IgM was also tested (n=38), with all results negative, and IgG presented a positivity rate of 42.1% (8/19) and 15.8% inconclusive. This study revealed significant circulation of Dengue in the region, but low circulation of the other viruses tested. Despite the small sample size, the high prevalence of IgG for Zika and Chikungunya indicated prior exposure of the population to both viruses. It is important to note that, as these infections are dynamic, the monitoring of these health conditions must be continuous and effective.

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PT.584

GAMMAHERPESVIRUS PREVALENCE, VIRAL LOAD, AND CLINICAL ASSOCIATIONS IN HEMATOLOGIC MALIGNANCIES

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Gammaherpesviruses have been associated with the pathogenesis of hematological malignancies, however their clinical significance remain incompletely understood. This study evaluated the prevalence and viral load of Epstein-Barr virus (EBV) and Human Herpesvirus 8 (HHV-8) and investigated their association with clinicopathological characteristics in patients with hematological neoplasms. Saliva samples were collected from 132 patients diagnosed with non-Hodgkin lymphoma (NHL), Hodgkin lymphoma (HL), myeloid and lymphoid leukemia. Additionally, saliva samples from 152 healthy individuals were analyzed as controls. Viral DNA was detected and quantified by real-time PCR (qPCR) and correlation with clinicopathological characteristics were investigated using Kendall's Tau-b test. EBV was detected in 39.4% of patients samples, whereas only 13.2% of samples from healthy controls were EBV-positive. NHL patients exhibited the highest EBV frequency (39.0%) and viral load (6.96×10^7 viral copies/mL). HHV-8 was detected in only two patients (2.5%), including one case of EBV/HHV-8 co-infection, while no control sample was positive for HHV-8. No significant associations were observed between EBV detection and age, treatment, relapse, or survival. However, EBV positivity was significantly associated with higher remission rates among NHL patients ($p = 0.006$). The association between salivary EBV detection and remission in NHL suggests a potential prognostic role for EBV and supports saliva as a promising non-invasive specimen for viral monitoring. Further studies are warranted to clarify the biological and clinical significance of gammaherpesvirus infections in hematological malignancies.

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PT.585

CANINE DISTEMPER VIRUS (CDV) AND A DIVERGENT POXVIRUS IN FREE-RANGING ANTEATERS AND ARMADILLOS SAMPLED OVER 14 YEARS IN BRAZIL

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Anteaters and armadillos face increasing pathogen exposure at the wildlife–domestic animals' interface. This study investigated corona-, paramyxo-, and poxviruses in anteaters and armadillos from Brazil, and performed phylogenetic analyses of detected viruses. From 2011 to 2024, 406 samples from 236 individuals were collected, including nasal, ocular, and oral swabs, blood, and skin lesions from live animals, and organs from road-killed individuals. DNA and RNA were extracted and screened by PCR targeting the coronavirus RdRp, paramyxovirus RNA polymerase, and poxvirus RNA polymerase and metalloprotease genes. Positive amplicons were sequenced and subjected to phylogenetic analyses conducted with IQ-TREE. Paramyxovirus was detected in four giant anteaters (blood and spleen), and one six-banded armadillo (blood). A poxvirus with 88% nucleotide identity to molluscum contagiosum virus was detected in one giant anteater (skin). No coronaviruses were detected. Paramyxovirus-positive anteaters showed no clinical signs at the time of sample collection, whereas hyperkeratosis was observed in the armadillo. The paramyxovirus positive samples showed 100% nucleotide identity to canine distemper virus (CDV). The poxvirus-positive anteater presented papular skin lesions (~0.5 cm) on the inner surface of the pelvic limbs. Based on ICTV criteria (>98% nucleotide identity for species demarcation), the poxvirus represents a divergent virus. These findings expand the known host range of CDV, reporting its first detection in free-ranging giant anteaters and a new host record in six-banded armadillo, and identify a divergent poxvirus in one giant anteater. Wildlife viral surveillance is essential for detecting emerging and divergent viruses at the human–domestic animals'–wildlife interface.

PT.586

TARGET-ENRICHED METAGENOMIC PROFILING OF VIRUS-RELATED SEQUENCES IN FREE-RANGING ARMADILLOS FROM THE BRAZILIAN PANTANAL

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Viral diversity in wildlife remains unexplored, particularly among understudied hosts such as armadillos. Armadillos' species coexist with livestock and may be hunted for human consumption, highlighting their relevance to One Health, conservation, and wildlife viral surveillance. We characterized virus-related sequences in fecal samples from free-ranging armadillos from the Pantanal. Fecal samples from 30 armadillos (2023-2024), including six-banded armadillos (*Euphractus sexcinctus*), southern naked-tailed armadillos (*Cabassous unicinctus*), and giant armadillos (*Priodontes maximus*), were collected. Total nucleic acids were extracted and grouped into four pools according to species and nucleic acid concentration. Libraries were prepared using the Twist Target Enrichment Standard Hybridization v2 kit and sequenced on an Illumina platform. Raw reads were quality-filtered, assembled using SPAdes, and taxonomically classified with Contig Annotation Tool. Viral sequences were further evaluated by BLASTx similarity searches and phylogenetic analyses using IQ-TREE. Sequencing generated 17,709,672 reads, resulting in 331,638 assembled contigs, of which 433 were classified as viral. Among these, 33 contigs (>200 bp and lineage score 1.00) corresponded to eukaryotic virus-related sequences assigned to seven viral families: *Anelloviridae*, *Circoviridae*, *Orthoherpesviridae*, *Parvoviridae*, *Picornaviridae*, *Picobirnaviridae*, and *Retroviridae*. These viral groups include members associated with diverse hosts, ecological interactions, and potential impacts on animal health. These findings expand knowledge of viral diversity in free-ranging armadillos and highlight the importance of wildlife metagenomic surveillance for uncovering viral lineages in threatened ecosystems.

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PT.587

l Length Article Detection of SARS-CoV-2 and coinfections in companion animals from COVID-19-positive owners in Southeast, Brazil

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While zoonotic pathogens are a primary focus of the One Health framework, the impact of SARS-CoV-2 on companion animals—especially regarding co-infections—remains poorly understood. To address this, the present study evaluated the prevalence of SARS-CoV-2, hemopathogens, and concurrent infections in household dogs and cats living with COVID-19-positive owners in Southeast Brazil.

From January 2021 to August 2022, samples were collected from 86 pets (48 dogs and 38 cats) residing in Belo Horizonte, Minas Gerais. Viral detection was performed via RT-qPCR, while hemopathogens were screened using nested and species-specific PCR assays. Overall, 9.3% (8/86) of the animals tested positive for SARS-CoV-2, comprising 14.6% (7/48) of the dogs and 2.6% (1/38) of the cats. Notably, one canine sample was identified as the Omicron BA.1 variant.

Among the dogs, hemopathogen prevalence was high at 85.4%, dominated by *Bartonella* spp. (70.8%) and *Anaplasma* sp. (58.3%), with co-infections being common. In the feline group, *Bartonella* spp. (15.8%) and hemotropic *Mycoplasma* spp. (21.1%) were the most prevalent. Tests for the order Piroplasmida and *A. phagocytophilum* were entirely negative across all subjects.

Although 75.0% of the SARS-CoV-2–positive animals had concurrent hemopathogen infections, statistical analysis revealed no significant correlation between the viral infection and the presence of these pathogens ($p > 0.05$). Ultimately, these results confirm that SARS-CoV-2 and hemopathogens are co-circulating among companion animals, underscoring the continued necessity for One Health surveillance.

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ASSESSING VIRAL CIRCULATION: AN EPIDEMIOLOGICAL STUDY OF WILD BATS IN SOUTHEASTERN BRAZIL

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Although bats are crucial for ecological balance, they are significant reservoirs of zoonotic pathogens. Besides being primary reservoirs for *Lyssavirus rabies*, the causative agent of rabies, they can harbor distinct forms of *Alphainfluenzavirus influenzae* (IAV), the causative agent of Influenza A. Data on these agents in asymptomatic, wild-caught bats and their relevance in disease transmission remain scarce. This study evaluated the prevalence of *L. rabies* and IAV using RT-qPCR in bats captured at the UFMG Pampulha campus. Bats were caught using mist nets, followed by oropharyngeal swab collection, banding, and release at the capture site. RT-qPCR analyses for viral detection used commercial kits and established protocols, with amplicons confirmed by Sanger sequencing. Between October 2022 and December 2023, 88 bats were captured. Two tested positive for *Lyssavirus* amplification (RT-qPCR Ct values of 15.990 and 15.500) with viral RNA confirmed by Sanger sequencing, indicating a 2.27% prevalence (2/88). Additionally, four bats amplified IAV (Ct values of 13.254, 38.650, 39.908, and 37.188), also confirmed by Sanger sequencing, representing a 4.55% prevalence (4/88). Clinically, no bats exhibited signs of disease. This study highlights the complex relationship between clinical manifestation and the maintenance of bat-borne viral agents in urban and peri-urban environments. It emphasizes the importance of implementing active surveillance in risk areas to estimate rabies and IAV prevalence in asymptomatic bats.

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PT.589

EPIDEMIOLOGICAL TRENDS OF HIV/AIDS IN ALAGOAS, BRAZIL: A 26 YEAR RETROSPECTIVE ANALYSIS (2000–2025)

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Human Immunodeficiency Virus (HIV) infection remains a major public health concern despite advances in antiretroviral therapy and prevention strategies. Regional epidemiological analyses are essential to identify transmission patterns and support evidence-based public health interventions. This study aimed to characterize the epidemiological profile of HIV/AIDS in the state of Alagoas, Brazil, over a 26-year period. A descriptive, retrospective, cross-sectional study was conducted using secondary data obtained from the Brazilian Ministry of Health through the Electronic System for Citizen Information (e-SIC). Official records from 15,316 individuals diagnosed with HIV/AIDS between 2000 and 2025 were analyzed according to year of diagnosis, sex, and age group using descriptive statistical methods. Between 2000 and 2014, 7,528 HIV/AIDS cases were recorded, corresponding to a mean of 289.5 cases/year. From 2015 to 2025, 7,788 HIV cases were reported, with a mean of 649 cases/year. Following the implementation of mandatory HIV notification in 2014, HIV diagnoses increased steadily, reaching their highest level in 2024, whereas AIDS cases showed stabilization followed by a declining trend. Men accounted for the majority of HIV cases (male-to-female ratio approximately 2.17:1), and the highest burden occurred among individuals aged 20–34 years. These findings demonstrate persistent HIV transmission among young adults in Alagoas despite reductions in AIDS incidence, suggesting that expanded testing and improved survival may be contributing to increased HIV detection. Continuous epidemiological surveillance and targeted prevention strategies focused on young adults and men remain essential to reduce HIV transmission and strengthen regional public health policies.

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PT.590

Surveillance and molecular characterization of avian influenza in wild animals and selection of antiviral candidate peptides by *phage display*.

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Avian influenza is an infectious disease caused by viruses of the genus *Influenza A*, *Orthomyxoviridae* family, which affects domestic and wild birds. The disease is associated with high mortality rates and significant economic losses. Transmission occurs through direct contact with infected birds, respiratory secretions, feces, or contaminated surfaces. Therefore, this study aims to investigate the circulation of avian influenza in birds and mammals on the coast of Parana, Brazil. For this, we used oral, anal, and brain swab samples from bird and mammal species. Most samples are from carcasses, but there are also samples from live animals that are taken for rehabilitation. Initially, we performed screening using Trizol extraction and RT-qPCR, using primers and probes specific to the M gene (viral matrix protein) of *Influenza A*, which includes the avian strain. To date, 922 animals have been tested, with 2 positive individuals of the species *Spheniscus magellanicus* (penguin). Both positive samples were anal swabs, collected in 2025, July and the animals were in the initial stage of decomposition. The next steps, based on RT-qPCR data, will be metagenomic analysis and viral isolates for peptide testing obtained by *Phage Display*. This study is expected to monitor viral variants and mutations, confirm the presence of *Influenza A* viruses, and potentially detect other viral species. The data generated will be of paramount importance for the early detection of *Influenza A*, allowing for the rapid identification of the introduction of highly pathogenic strains, reducing sanitary, economic, and environmental impacts.
Financial support: CAPES, FAPESP, Cnpq

PT.591

Detection of Betacoronaviruses of the subgenera Sarbecovirus and Merbecovirus (MERS-like) in *Molossus molossus* bats from an urban area of Campinas, São Paulo

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Urban expansion brings synanthropic bats into ever closer contact with people and domestic animals, widening the interface across which viruses may cross species barriers. To assess this risk, we screened rectal samples from 395 bats collected in the urban area of Campinas (São Paulo, Brazil) by pan-coronavirus RT-PCR with degenerate primers, followed by Sanger sequencing of the RNA-dependent RNA polymerase (RdRp) gene, BLASTn/BLASTx searches and maximum-likelihood phylogenetic reconstruction. Coronaviruses were detected in 18 animals (4.56%). Two detections, both from Molossus molossus found fallen in urban settings, stood out for their epidemiological relevance. The first sequence fell within the subgenus Merbecovirus, clustering with Brazilian MERS-CoV-related bat lineages (98.9% and 98.6% nucleotide identity) and sharing approximately 88% identity with the MERS-CoV prototype. This placement reinforces a pattern observed across the Americas, in which MERS-related viruses associate with molossids rather than with the vespertilionids that predominate in Asia and Europe. As for the second sequence, it grouped within the subgenus Sarbecovirus and was identical to SARS-CoV-2 across the fragment analyzed (100% nucleotide and amino acid identity), suggesting a possible spillback (human-to-bat transmission) event and the attendant risk of secondary spillover; extraction and no-template controls remained negative throughout. Because both assignments rest on a single RdRp amplicon, they should be regarded as preliminary and require confirmation by complete genome sequencing. Taken together, these findings highlight the value of sustained viral surveillance in urban bats as a One Health strategy for the early detection of coronaviruses with zoonotic potential.

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PT.592

EFFECT OF FOOD MATRICES ON VIRAL QUANTIFICATION BY qPCR: IMPLICATIONS FOR SURVEILLANCE OF FOODBORNE DISEASES

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Foodborne illnesses represent a public health concern, causing approximately 1.6 million cases daily worldwide. Viruses are among the main etiological agents, but their quantification in food remains challenging due to complex matrices, the presence of inhibitors, and low viral loads. This study evaluated the effect of food matrices (lettuce, tomato, and a mixture of rice, beans, and chicken) on viral quantification by qPCR, comparing standard curves prepared in ultrapure water and in extracts of blank matrices, linear regression, t-test, and ANOVA. Thirty-six standard curves from serial dilutions containing targets of mastadenovirus, norovirus GI and GII, rotavirus A, and mengovirus were analyzed; 18 were prepared in water and 18 in matrix extracts. Significant differences ($p < 0.05$) in slopes were observed in 27.8% (5/18) of the comparisons, indicating variations in reaction efficiency for norovirus GI in lettuce and for mengovirus, norovirus GII, and rotavirus A in tomato. In contrast, differences in intercepts were observed in 88.9% (16/18) of comparisons, demonstrating matrix effects on qPCR quantification. In matrix-based curves showed lower intercept values than water-based curves. Differences in Cq values associated with the calibration curves ranged from 0.1 to 4.4 cycles, indicating that matrix effects can influence the estimated viral concentration. These findings support the use of matrix-based standard curves to improve the accuracy of viral quantification in food samples. The application of this approach may contribute to outbreak investigations and quality monitoring, strengthening surveillance and risk management of foodborne diseases.

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PT.593

Clinical and Epidemiological Characterization of Acute Febrile Illness Cases Negative for Dengue, Chikungunya, and Zika Viruses in the Jequitinhonha Health Macroregion, Brazil

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Arboviruses represent a public health challenge in Brazil, particularly in endemic regions where dengue virus (DENV), chikungunya virus (CHIKV), and zika virus (ZIKV) circulate simultaneously and cause overlapping clinical manifestations. In the Jequitinhonha Valley, Minas Gerais, Brazil, the Center for Diagnostic Support Actions and Research (NUPED-Vale) plays a strategic role in epidemiological surveillance and molecular diagnosis, receiving biological samples from 32 municipalities for the detection of DENV, CHIKV, and ZIKV. These clinical specimens are obtained from patients who seek health services for diagnostic evaluation and present symptoms of acute viral infection, such as fever, headache, myalgia, nausea and lower back pain. This cross-sectional study analyzed sociodemographic, clinical, and geographic data from suspected arbovirus cases, focusing on patients with acute febrile illness who tested negative for DENV, CHIKV, and ZIKV by real-time reverse transcription polymerase chain reaction (RT-qPCR). A total of 5,440 suspected arbovirus cases recorded between March 2023 and February 2025 were evaluated. Female individuals accounted for 59.4% of cases, and the mean age was 34.0 ± 20.4 years. The most frequent symptoms were headache (78.63%), myalgia (78.23%), fever (72.31%), and nausea (48.05%). The municipalities with the highest numbers of reported cases were Diamantina (1,482), Capelinha (808), and Araçuaí (504). The findings demonstrate that negative RT-qPCR results do not exclude infection by other etiological agents, highlighting the need for complementary investigations and broader differential diagnosis in endemic regions. Furthermore, the study reinforces the importance of continuous epidemiological surveillance and integrated laboratory approaches for monitoring febrile illnesses of public health relevance.

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PT.594

Indigenous ecological knowledge and zoonotic surveillance associated with subsistence hunting in the Brazilian Amazon

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Indigenous populations in the Amazon are at the frontline of human–wildlife interfaces relevant to zoonotic viral spillover. We investigated how subsistence hunting and Indigenous ecological knowledge may contribute to community-based zoonotic surveillance within a One Health framework. We conducted a mixed-methods study in two Ticuna villages in the Upper Solimões region (Tabatinga, Amazonas, Brazil), near the tri-border area. Household census data from 55 households (270 individuals) were analyzed using descriptive statistics. Semi-structured interviews with Indigenous hunters were analyzed through reflexive thematic analysis to explore perceptions of wildlife health and zoonotic risk. Among surveyed households, 69% reported at least one hunter, and wild meat represented an important source of dietary protein. Nineteen male hunters, all self-identified as Ticuna, participated in the qualitative component. Three interrelated themes emerged: (i) hunting as a strategy for food security and transmission of traditional knowledge; (ii) Indigenous ecological observations of wildlife health, including behavioral changes, disease signs and abnormal mortality potentially associated with pathogen circulation; and (iii) hunting practices as an informal community-based surveillance system for zoonotic threats at the human–wildlife interface. Indigenous hunters function as frontline sentinels of wildlife health, generating ecological information that may support early detection of zoonotic spillover, particularly involving viral pathogens circulating in wildlife reservoirs. Integrating Indigenous ecological knowledge into One Health surveillance systems could strengthen virological surveillance in remote Amazonian regions. Such efforts should be accompanied by investments in territorial protection, food security, sanitation, and equitable access to healthcare.

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PT.595

EVALUATION OF THE STANDARD™ M10 ARBOVIRUS PANEL FOR RAPID MOLECULAR DETECTION OF CHIKUNGUNYA VIRUS DURING AN OUTBREAK IN ALAGOINHAS, BAHIA, BRAZIL

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Introduction: Arbovirus diagnosis is essential for clinical management, surveillance, and research. A challenge is distinguishing arboviral infections from other acute tropical febrile illnesses. Rapid point-of-care molecular assays are critical to provide timely results and support immediate clinical decision-making. Methods: We evaluated the performance of the STANDARD™ M10 Arbovirus Panel (ECO Diagnóstica/SD Biosensor Brazil) as a point-of-care test for arbovirus diagnosis. This integrated cartridge-based RT-qPCR assay detects Chikungunya virus (CHIKV), Dengue virus serotypes 1-4, Zika virus, Yellow fever virus, and West Nile virus. A total of 625 serum samples from patients with suspected arboviral infection during an outbreak in Alagoinhas (Bahia, Brazil) were analyzed. Results: Among the 625 samples, 333 (53.3%) tested positive for CHIKV, while 292 (46.7%) were negative for all arboviruses included in the panel. Of the 333 CHIKV RT-qPCR-positive samples, 55 (16.5%) were also evaluated for Chikungunya antibodies using the lateral flow test CHIKV IgG/IgM ECO Teste (ECO Diagnóstica/SD Biosensor Brazil). Among these, 10 (18.2%) were IgM-positive, 11 (20.0%) were positive for both IgM and IgG, and 34 (61.8%) were negative. Conclusions: The point-of-care molecular test STANDARD™ M10 Arbovirus Panel supported diagnosis during the CHIKV outbreak in Alagoinhas, Bahia, mainly within the first week of symptom onset, when anti-CHIKV antibodies were frequently undetectable by lateral flow tests. These findings demonstrate that the STANDARD™ M10 Arbovirus Panel is a rapid, reliable tool for point-of-care molecular diagnosis during arboviral outbreaks, supporting timely clinical management and epidemiological surveillance.

Financial Support: ECO Diagnóstica, SD Biosensor.

PT.596

SPATIAL RISK ANALYSIS IN HERBIVORE RABIES SURVEILLANCE IN MATO GROSSO DO SUL, BRAZIL

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Since the early 2000s, Mato Grosso do Sul State, Brazil, has undergone substantial diversification of its agricultural production systems, with areas previously dedicated to beef cattle progressively converted to soybean, maize, orange, and eucalyptus cultivation. These land-use changes have influenced the epidemiology of herbivore rabies, as the common vampire bat (*Desmodus rotundus*), the primary reservoir and transmitter of the disease, may shift toward regions with greater livestock availability, altering the spatial distribution of disease risk. In order to optimize prevention, improve public resource allocation, and reduce economic losses, the Agência de Defesa Sanitária Animal e Vegetal (IAGRO-MS) implemented a spatial risk analysis methodology in 2022. The approach integrates historical rabies occurrence, the distribution of registered *D. rotundus* shelters, topographic characteristics, and disease cyclicity to identify priority intervention areas. In October 2023, risk classification and preventive recommendations began to be disseminated through digital platforms. From 2024 onward, risk assessments have been updated annually, while epidemiological information has been shared with livestock producers every six months. Preventive actions, including epidemiological surveillance, health education, and vampire bat control, have been prioritized in high-risk areas. Consequently, herbivore rabies cases decreased by 58% in 2025, declining from an annual average of 67 outbreaks during the 2020–2024 period to 28 outbreaks after the implementation of the risk-based surveillance strategy. During the first half of 2026, cases remained 57% below the average for the same period, demonstrating the effectiveness of risk-based surveillance for guiding prevention, prioritizing interventions, and supporting rabies control in Mato Grosso do Sul.

Key words: Desmodus rotundus; Prevention; Topographic.

PT.597

PUBLIC RABIES DASHBOARD: A TOOL FOR TRANSPARENCY, EPIDEMIOLOGICAL SURVEILLANCE, AND DECISION-MAKING SUPPORT IN MATO GROSSO DO SUL

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Rabies in herbivores remains a major challenge for animal and public health due to its significant economic impact and zoonotic potential, highlighting the importance of real-time epidemiological information to support surveillance and disease control strategies. In this context, in 2022, the Agência de Defesa Sanitária Animal e Vegetal (IAGRO-MS) developed a spatial risk analysis methodology based on epidemiological and environmental criteria to identify priority areas for surveillance, prevention, and strategic vaccination. To improve transparency and facilitate access to epidemiological data for livestock producers, veterinarians, and public health authorities, the Public Rabies Dashboard was launched in September 2025. The platform provides continuously updated georeferenced information on the spatial distribution of rabies cases transmitted by hematophagous bats (*Desmodus rotundus*), annual risk classification, affected areas, epidemiological indicators, and other data used to support surveillance planning and decision-making. The implementation of this methodology was followed by a reduction in the occurrence of rabies cases in 2025, with this declining trend maintained throughout 2026, guiding disease control measures. Since its implementation, the dashboard has recorded more than 1,600 views, highlighting its value as a tool for epidemiological surveillance and public communication. Public access to these data strengthens the transparency of official surveillance activities, enhances the planning and implementation of prevention and control strategies, contributes to protecting the livestock industry and the state's animal health status, and reduces the risk of human exposure to rabies virus by reinforcing the integration of animal health, public health, and the One Health approach.

Key words: One Health; *Desmodus rotundus*; Geospatial analysis.

Financial support: CAPES - Finance Code 001, INCT-One.

PT.598

Molecular Surveillance of Avian Paramyxovirus 1 in Antarctic Birds Across Two Consecutive Austral Summers (2024–2025 and 2025–2026)

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Avian paramyxovirus 1 (APMV-1) is the causative agent of Newcastle Disease in poultry, and wild birds are an important reservoir of the virus. About 100 million birds breed in Antarctica, including migratory species that can become infected and, along their migratory routes, spread viruses to other wild birds in other continents, potentially affecting poultry production worldwide. The aim of this study was to monitor the presence of APMV-1 in the bird population of South Shetland Islands, Antarctica, during the austral summer of 2024-2025 and 2025-2026. Under the support of the Brazilian Antarctic Program during the 43rd and 44rd Antarctic Operation, and in collaboration with the Chilean Antarctic Institute, 564 samples from Antarctic seabirds were collected, including excreta and swabs (cloacal and oropharyngeal). Viral RNA were extracted using magnetic beads by an automatic extractor and a qPCR for the M gene of APMV-1 were performed. No samples were positive to molecular detection of M gene. These results suggest that APMV-1 was not circulating in birds in the Antarctic during the last two years, even though previous studies have demonstrated viral circulation, especially in penguins, by molecular and serological analysis. These results contribute to the epidemiological surveillance of APMV-1 among wild birds and strengths the knowledge on viral ecology.

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PT.599

MOLECULAR SURVEILLANCE OF AVIAN CORONAVIRUSES IN ANTARCTIC BIRDS FROM THE SOUTH SHETLAND ISLANDS, ANTARCTICA

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Avian coronaviruses are important pathogens of wild and domestic birds, with members within both *Deltacoranavirus* and *Gammacoronavirus* genera. These viruses are.associated with respiratory, enteric, and reproductive diseases in several avian species. Wild birds, particularly migratory species, may contribute to the maintenance and dissemination of these viruses across continents, representing a potential pathway for viral introduction into isolated ecosystems, such as Antarctica. This study investigated the occurrence of avian coronaviruses in Antarctic birds sampled during the austral summer of 2024–2025 in the South Shetland Islands, Antarctica. Under the support of the Brazilian Antarctic Program (PROANTAR) during the 43rd Antarctic Operation , and in collaboration with the Chilean Antarctic Institute, 269 biological samples, including fecal samples and cloacal and oropharyngeal swabs, were collected from Antarctic seabirds. Viral RNA was extracted using magnetic beads, followed by cDNA synthesis. Coronavirus detection was performed by nested PCR targeting the RNA-dependent RNA polymerase gene and analyzed by agarose gel electrophoresis. None of the analyzed samples showed detectable coronavirus RNA. Despite the negative findings, previous studies have demonstrated evidence of coronavirus circulation in Antarctic avifauna, including the identification of a deltacoronavirus in *Larus dominicanus* (kelp gull). These findings suggest the episodic nature of viral shedding in wildlife populations and reinforce the importance of continuous molecular surveillance to better understand coronavirus ecology, transmission dynamics, and potential dissemination among Antarctic and migratory bird populations. The increasing interaction between resident Antarctic species, migratory birds, and human activities emphasizes the need for integrated surveillance approaches to monitor emerging viral threats in remote ecosystems.

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PT.600

INVESTIGATION OF RABIES VIRUS IN BATS FROM THE PANTANAL BIOME

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Rabies is a zoonotic disease that affects all mammals, including humans, responsible for approximately 60,000 deaths per year. The *Rabies lyssavirus* (RABV) belongs to the order *Mononegavirales*, family *Rhabdoviridae*, and genus *Lyssavirus*. Although the urban cycle of rabies is under control in Brazil, the disease remains endemic in the sylvatic cycle, with bats representing the main reservoirs of RABV. The high diversity of bat species in the Pantanal biome favors viral circulation and reinforces the importance of epidemiological surveillance due to the risk of transmission to domestic animals, livestock, and humans. This study aimed to investigate the presence of RABV in central nervous system (CNS) samples from bats from the Pantanal biome, Mato Grosso do Sul, Brazil, contributing to epidemiological surveillance and understanding of RABV circulation in the region. A total of 71 CNS samples from *Artibeus lituratus*, *Artibeus planirostris*, *Diaemus youngi*, *Desmodus rotundus*, *Platyrrhinus lineatus*, and *Myotis lavali* were tested using the Direct Fluorescent Antibody Test (DFAT). None of the samples showed characteristic fluorescence indicative of RABV, and all were classified as negative. Although RABV antigen was not detected in the analyzed samples, these findings provide an epidemiological snapshot of the region. The absence of virus detection may be associated with low viral circulation during the sampling period and does not exclude the occurrence of RABV in the Pantanal. These findings reinforce the importance of continuous surveillance in bat populations and further studies to improve understanding of RABV circulation and support rabies prevention and control strategies.

Financial Support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

PT.601

Eco-epidemiological surveillance of Orthopoxviruses (Poxviridae) in wild mammals:

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The genus Orthopoxvirus (Poxviridae) comprises enveloped zoonotic viruses capable of infecting different vertebrate species, including humans. Among the main species are Vaccinia virus (VACV), Cowpox virus (CPXV), and Mpox virus (MPXV), whose circulation among wild animals, domestic animals, and humans represents an important public health challenge. This study aimed to investigate the circulation of Orthopoxvirus in wild mammals from the state of São Paulo. Liver tissue samples from animals found dead and submitted by the Wild Animal Screening Centers (CETAS), located in different regions of the state, including the municipalities of Barueri, Campinas, Ribeirão Preto, and São José do Rio Preto, were analyzed. After nucleic acid extraction, the samples were subjected to conventional Polymerase Chain Reaction (PCR). In parallel, the Plaque Reduction Neutralization Test ($PRNT_{50}$) was performed to detect anti-VACV neutralizing antibodies. By July 2026, 319 samples had been processed by PCR, all yielding negative results. In the $PRNT_{50}$ assay, 43 samples were evaluated, of which six (13.95%) showed neutralizing antibodies, with titers ranging from 1:20 to 1:60. The seropositive animals belonged to the genera Callithrix sp., Didelphis albiventris, and Cerdocyon, originating from Ribeirão Preto and Campinas. The presence of antibodies suggests previous circulation of Orthopoxvirus in the wild fauna of São Paulo, reinforcing the importance of eco-epidemiological surveillance to better understand the dynamics of these viruses and to support strategies for the prevention and control of zoonotic diseases.

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PT.602

FIRST POPULATION-BASED HUMAN PAPILLOMAVIRUS SCREENING RESULTS FROM AN ORGANIZED CERVICAL CANCER PREVENTION PROGRAM IN THE BRAZILIAN AMAZON: PREDOMINANCE OF HIGH-RISK INFECTIONS OTHER THAN TYPES 16 AND 18 IN PARÁ STATE.

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The organized cervical cancer screening strategy implemented by the Brazilian Ministry of Health in 2025 in the state of Pará included 2,195 women from 21 municipalities until now, providing an opportunity to assess the molecular test to HPV infection and the performance of reflex cytology and colposcopy within a population-based screening program. The overall mean age of the participants was 39.8 years, while women with detectable infection had a slightly lower mean age of 38.7 years. Human Papillomavirus infection was identified in 227 participants (10.3%). The most frequent finding was infection by high-risk HPV types other than 16 and 18, representing 178 cases (78.4%), whereas type 16 and 18 accounted for 38(16,7%) and 11(5,9%) cases, respectively. This distribution highlights the evidence of other oncogenic HPV genotypes to the burden of cervical cancer risk in our population. Among the municipalities with largest number of screened women, Marituba presented the highest prevalence (18.2%), followed by Breves (13.4%), Ananindeua (12.4%), Santarém (11.0%), São Sebastião da Boa Vista (11.0%), and Belém (9.9%). Muaná showed the lowest prevalence (3.0%). Reflex cytology demonstrated that many women with detectable high-risk infection, had normal cytological findings, while epithelial abnormalities such as atypical squamous cells of undetermined significance and low-grade or high-grade squamous intraepithelial lesions were identified in a smaller proportion. These findings reinforce the value of molecular testing for detecting clinically relevant infections that may or not associated with cytological abnormalities and highlight the importance of organized screening to identify local epidemiological differences and guide public health interventions.

PT.603

IMPLEMENTATION OF HTLV-1/2 SCREENING IN PRENATAL CARE FOR RIVERINE PREGNANT WOMEN IN THE MUNICIPALITY OF BREVES, MARAJÓ ARCHIPELAGO, PARÁ

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Human T-cell lymphotropic virus (HTLV-1/2) infection is a major public health problem due to the risk of vertical transmission, making prenatal diagnosis an essential strategy. The recent incorporation of HTLV-1/2 testing into prenatal care by the Unified Health System (SUS) highlights the need to evaluate its implementation in interior Amazonian municipalities. This study aimed to describe the implementation of HTLV-1/2 screening in riverine pregnant women treated in the public health network of Breves, Marajó archipelago, Pará. A descriptive cross-sectional study was conducted with 91 riverine pregnant women. Blood samples were collected in Breves and sent to a reference laboratory in Belém for anti-HTLV-1/2 antibody screening by enzyme-linked immunosorbent assay (ELISA), with Western blot confirmation for reactive cases. The workflow established between collection, processing, and result release was completed within seven days. Sociodemographic and behavioral data were also collected using a standardized questionnaire. The mean age was 24.3 ± 6.9 years; 87.9% self-identified as mixed-race, 53.8% had incomplete elementary education, 65.9% had a family income below one minimum wage, 45.6% reported sexual initiation between 13 and 15 years, and 85.7% reported inconsistent or no condom use. No pregnant woman presented a reactive result for HTLV-1/2. Screening implementation proved operationally viable in an Amazonian riverine population, with a laboratory workflow capable of providing results within seven days. Although no infection cases were identified, the experience demonstrates the feasibility of incorporating screening into routine prenatal care in the Amazon, supporting its expansion.
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PT.604

TEMPORAL TREND OF HIV INFECTION AMONG PREGNANT WOMEN IN THE MARAJÓ INTEGRATION REGION, PARÁ, BRAZIL

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Human immunodeficiency virus (HIV) infection during pregnancy remains a major public health issue due to the risk of vertical transmission and the challenges associated with diagnosis and treatment, particularly in areas of high social vulnerability, such as the Marajó archipelago in Pará, Brazil. This study aimed to analyze the temporal trend of reported HIV cases among pregnant women in the Marajó Integration Region. An ecological, descriptive, time-series study was conducted using secondary data from the HIV/AIDS Indicators and Basic Data provided by the Brazilian Ministry of Health, covering the 17 municipalities of the region. Reported cases between 2013 and 2024 were described, while the temporal trend analysis was performed for the 2013–2023 period using simple linear regression, due to the incompleteness of the 2024 data. During the analyzed period, 226 HIV cases were recorded among pregnant women. The annual number of notifications increased from nine cases in 2013 to 27 in 2023. Linear regression demonstrated a statistically significant increasing temporal trend (cases/year; ;), indicating an average annual increase of approximately two cases. The municipalities of Ponta de Pedras, Soure, and Salvaterra accounted for the highest absolute numbers of notifications. These findings demonstrate the expansion of HIV notifications among pregnant women in the Marajó Integration Region and underscore the need to strengthen prenatal screening actions, timely diagnosis, antiretroviral treatment, and the prevention of vertical transmission, particularly in municipalities with higher infection rates.

Financial support: National Council for Scientific and Technological Development (CNPq).

PT.605

THE PANTANAL AS A HOTSPOT OF HOST–VECTOR CONNECTIVITY AND POTENTIAL ARBOVIRUS SPILLOVER

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The Pantanal is the world's largest tropical wetland, harboring extensive vertebrate and mosquito diversity and undergoing pronounced seasonal flooding which drastically alters the landscape. Arbovirus emergence is shaped by ecological networks connecting vector-borne pathogens, hematophagous vectors, and vertebrate hosts in space and time. Identifying sources of mosquito blood meals can reveal critical pathways of viral maintenance, amplification, and spillover Here, total nucleic acid was extracted from 1224 engorged female mosquitoes collected in the northern Pantanal of Mato Grosso, Brazil, amplified via conventional PCR, and sent for sanger-sequencing. A subset of samples (n=176) were tested for *Orthoflavivirus* NS5 gene by RTqPCR. *Culex (Melanoconion)* spp. showed the broadest host range, feeding on reptiles, amphibians, birds, and mammals. *Culex (Culex)* spp. fed mainly on birds, whereas *Aedes, Psorophora,* and *Mansonia* predominantly targeted mammals, linking wildlife, livestock, and humans. Thirteen vertebrate taxa were shared among two or more mosquito genera, highlighting substantial overlap in feeding patterns. *Culex, Aedes,* and *Psorophora* shared blood meals from cattle (n=53), humans (n=19), horses (n=9), deer (n=7), tapirs (n=5), and rabbits (n=3). Although all tested samples were negative for *Orthoflavivirus* RNA, the blood-meal network revealed a highly interconnected mosquito and host community in the Pantanal, suggesting high potential for enzootic maintenance, cross-species transmission, and arbovirus spillover into humans and domestic animals. This study is among the first to investigate mosquito blood-feeding associations in the Pantanal, and the concentration of hosts and vectors in non-flooded ecological refuges deserves further investigation.
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PT.606

URBAN MOSQUITO FEEDING NETWORKS REVEAL POTENTIAL ARBOVIRUS BRIDGES IN BELO HORIZONTE

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Arboviruses are transmitted by blood-feeding arthropod vectors and can cause diseases in vertebrate hosts. The transmission dynamics of arboviruses can be investigated through blood meal analysis of engorged female mosquitoes. In urban parks, the coexistence of mosquitoes, wildlife, domestic animals, and humans may create ecological interfaces favoring viral circulation. In this study, DNA and RNA were extracted from engorged mosquito females (n=79) collected in Belo Horizonte and analyzed by DNA barcode and pan-orthoflavivirus RT-qPCR, respectively. Blood-meal analysis revealed distinct feeding patterns. *Aedes albopictus*, *Aedes aegypti*, *Aedes scapularis*, *Aedes fluviatilis*, *Limatus durhamii*, and *Wyeomyia* sp. fed predominantly on humans, whereas *Culex* spp. fed on humans, non-human primates, opossums, dogs, and several birds, highlighting their potential role as bridge vectors between humans and other vertebrate hosts. Engorged *Aedes aegypti* females (n = 4) tested positive for orthoflavivirus and partial NS5 gene sequencing revealed sequences with 98% of nucleotide identity with sequences of *Phlebotomus-associated flavivirus* isolates, suggesting the circulation of a closely related insect-associated orthoflavivirus in the study area. These samples will subsequently undergo complete genome sequencing to confirm viral species assignment and genomic characterization. These findings reveal mosquito feeding connections among humans, wildlife, and domestic animals in urban parks of Belo Horizonte. Such ecological links may contribute to the establishment of transmission bridges between sylvatic and urban environments. Integrated entomological, molecular, and ecological surveillance is essential to clarify the role of these interactions in arbovirus maintenance and emergence in urban settings.

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PT.607

IMMUNOVIROLOGICAL AND MOLECULAR CHARACTERIZATION OF HIV-1 IN PRISON SETTINGS: PUBLIC HEALTH CHALLENGES IN RIO DE JANEIRO, BRAZIL

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Brazil's national prison health policy seeks to integrate prison health into the SUS, yet overcrowding and discontinuity of care may compromise HIV control. We assessed immunovirological, molecular, and public health indicators among men living with HIV in prisons in Baixada Fluminense (RJ). This cross-sectional study included 343 participants. Plasma samples were collected between July/2023 and November/2025. An exploratory within person repeated measures analysis was performed in 130 participants with multiple collections. Viral load (VL), CD4+ T-cell counts, rapid tests, prison occupancy, and infectious disease records were evaluated. Samples with VL>1,000 copies/mL underwent Sanger sequencing of protease/reverse transcriptase (PR/RT) and integrase (IN) for drug resistance genotyping. The subtypes were defined with MEGA7, SimPlot and REGA softwares and resistance was interpreted with Stanford HIVdb. Overcrowding occurred in 22/30 prison-semester observations (73.3%), reaching 271.5% of capacity. At baseline measurement, median CD4 was 539.5 cells/mm³, 61.2% had viral suppression, and 32.4% had VL>1,000 copies/mL. Suppression was higher in Magé than Japeri (83.1% vs 55.5%; p<0.001). In the repeated measures, suppression increased from 72.3% to 87.7% and median CD4 from 507 to 611 cells/mm³ (p<0.001 for both). Among 43 valid sequences (19 PR/RT; 24 IN), subtype B predominated (47.4% and 70.8%, respectively). Predicted resistance was detected to NRTIs in 5.3%, NNRTIs in 21.1%, and INSTIs in 16.7%, including R263RK; no protease inhibitor resistance was observed. These findings show improved immunovirological indicators across repeated measurements alongside structural, inter-prison, and virological challenges, supporting integrated monitoring and continuity of HIV care in prison health services. Financial support: CNPq.

PT.608

Investigation of western equine encephalitis virus in the Zona da Mata region, Minas Gerais, southeast Brazil.

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Western equine encephalitis virus (*Alphavirus western* or WEEV) is zoonotic arbovirus maintained in cycles involving *Culex* and *Aedes* as vectors and birds and mammals as amplifying hosts. Between 2023 and 2024, a new lineage C of WEEV caused an outbreak of neurological disorder in equids and humans in Uruguai and Argentina. Recent data regarding WEEV transmission cycles in Brazil is scarce. The objective of this study is to investigate the circulation of WEEV in the Zona da Mata region of the state of Minas Gerais, southeast Brazil. Between 2023 and 2026, blood samples of equids and domestic birds, and specimens of hematophagous dipterans were collected from three municipalities of the Zona da Mata region. Vectors were collected by CDC and Shannon light traps and manual aspirators. Serum samples are being tested by plaque reduction neutralization testing ($PRNT_{90}$) for a reference isolate WEEV (GenBank: GQ287640.1). Vectors will be identified, pooled and tested by real-time reverse transcription-polymerase chain reaction (RT-qPCR) followed by nucleotide sequencing. Blood samples of 147 equids and 93 domestic birds, and over a thousand mosquitoes and midges have been collected so far. All sera have been tested and considered seronegative for WEEV. Dipteran specimens are yet to be identified and tested by RT-qPCR for WEEV. Preliminary results indicate, no exposure of equids and domestic birds to WEEV in the Zona da Mata region. Testing is still ongoing, but more studies should be encouraged to discard recent circulation of WEEV in the region.
Financial Support: CNPq, CAPES, FAPEMIG, and Funarbe.

PT.609

EVIDENCE OF OROPOUCHE VIRUS EXPOSURE IN DOMESTIC BIRDS FROM THE JEQUITINHONHA VALLEY, MINAS GERAIS, BRAZIL

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The circulation of Oropouche virus (OROV) has been demonstrated in several locations across South America. In Brazil, most reported cases were historically concentrated in the country's Northern region. In 2024, Brazil experienced a major Oropouche fever outbreak that originated in the North and subsequently spread to southeastern states. Despite its public health importance, studies investigating OROV circulation in vulnerable populations remain scarce. Regarding animals, studies conducted in the Amazon in previous decades demonstrated OROV circulation in several animal groups, including wild birds. Although recent evidence suggests exposure to OROV in domestic animals, studies involving birds remain limited. The aim of this study was to investigate exposure to OROV in domestic birds from the Jequitinhonha Valley, Minas Gerais, Brazil. Between December 2025 and May 2026, blood samples were collected from 75 domestic birds, including chickens, guinea fowl, turkeys, geese, and domestic ducks, from rural and Quilombola communities in the municipalities of Salinas, Virgem da Lapa, and Araçuaí. Serum samples were tested using the plaque reduction neutralization test ($PRNT_{90}$) against an OROV strain isolated in Rio de Janeiro in 2024. Preliminary results indicate that seven (9.33%) chicken samples had detectable neutralizing antibody titers against OROV, defined by $PRNT_{90}$ values ≥20. All positive samples will undergo confirmatory testing. These initial findings suggest potential exposure of domestic chickens to OROV and reinforce the importance of active surveillance as a tool for the early detection of viruses of public health significance.

Financial Support: CNPq, CAPES, FAPEMIG, Funarbe

PT.610

HIGH SEROPREVALENCE OF NEUTRALIZING ANTIBODIES AGAINST OROPOUCHE VIRUS IN INDIGENOUS COMMUNITIES OF THE BRAZILIAN AMAZON

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Oropouche virus (OROV) is an emerging arbovirus responsible for recurrent outbreaks across the Amazon Basin. Following the emergence of a novel reassortant OROV lineage in the western Amazon (2022–2024), we investigated the seroprevalence of neutralizing antibodies against OROV in residents aged 2–84 years from two indigenous communities (Nova Aparecida and São Sebastião) in the Alto Rio Solimões Indigenous Health District (DSEI), Amazonas, during outbreaks of acute febrile illness in 2024. Serum samples from 200 participants were analyzed by plaque reduction neutralization test (PRNT) using serial dilutions from 1:40 to 1:160. Neutralizing activity was defined as ≥90% plaque reduction (PRNT90). Fifty-three participants presented with acute febrile illness and had previously tested negative by RT-qPCR for dengue, Zika, chikungunya, Mayaro virus, and OROV. Overall, 143 of 200 participants (71%) were positive by PRNT90, increasing to 181 (90%) using a 50% neutralization threshold (PRNT50). Among the 53 symptomatic individuals, 45 (85%) were PRNT90-positive and 48 (91%) were PRNT50-positive. Positive PRNT90 results were detected across all age groups, including 59 individuals ≤18 years, 73 aged 19–57 years, and 8 aged ≥60 years, indicating widespread exposure throughout the communities. The remarkably high prevalence of neutralizing antibodies suggests intense and likely underrecognized circulation of OROV or antigenically related orthobunyaviruses in these indigenous populations. These findings highlight the importance of integrating serological and molecular approaches to better define transmission dynamics and support public health surveillance in the Amazon region.
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PT.611

ACTIVE SURVEILLANCE REVEALS ONGOING ARBOVIRUS CIRCULATION IN URBAN NON-HUMAN PRIMATES FROM BELO HORIZONTE, BRAZIL, 2023–2025

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Urban non-human primates (NHPs) are increasingly recognized as sentinels for arbovirus surveillance, providing early evidence of viral circulation at the human–wildlife interface. Here we investigated arbovirus circulation in free-ranging NHPs inhabiting 15 urban parks and other green areas of metropolitan region of Belo Horizonte between 2023 and 2025. A total of 317 samples were collected from 306 NHPs belonging to five genera, including *Callithrix* (n=280), *Sapajus* (n=18), *Alouatta* (n=5), *Callicebus* (n=2), and *Saimiri* (n=1). All samples were screened by RT-qPCR for yellow fever virus (YFV), Oropouche virus (OROV), chikungunya virus (CHIKV), and Zika virus. RT-qPCR detected YFV RNA in eight NHPs and OROV RNA in one, demonstrating active arbovirus circulation in urban NHPs populations. Among 302 animals tested by YFV $PRNT_{50}$, 57 (19%) had neutralizing antibodies, while five (1%) remained positive using the more stringent $PRNT_{80}$ criterion. Anti-YFV IgM was detected in 53/304 (17%) animals. The detection of IgM antibodies, seropositive juveniles and infants, and seroconversion in 11 recaptured NHPs collectively provides evidence of recent YFV transmission in urban green areas. Among 117 animals tested for CHIKV, five were $PRNT_{50}$-positive, indicating previous exposure. These findings reveal ongoing circulation of multiple arboviruses in free-ranging urban NHPs and demonstrate that urban green areas function as active human–wildlife interfaces where arboviruses continue to circulate. Active wildlife surveillance can provide early warning of viral emergence and strengthen public health preparedness in rapidly urbanizing landscapes.

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PT.612

MORBILLIVIRUS IN *SOTALIA GUIANENSIS* FOUND STRANDED ALONG THE PARANÁ COAST, SOUTHERN BRAZIL

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Cetacean morbillivirus (CeMV) is an infectious threat to cetaceans, including the Guiana dolphin (*Sotalia guianensis*), currently classified as Endangered (EN), and associated with severe disease, immunosuppression, and mortality. This study investigated CeMV occurrence in 61 tissue samples from 32 *S. guianensis* individuals found along the coast of Paraná State, Brazil, between 2016 and 2025, all of which died from undetermined causes. Samples were analyzed by reverse transcription quantitative polymerase chain reaction (RT-qPCR), using *GAPDH* as an endogenous control. *GAPDH* was amplified in 49/61 samples (80.32%), corresponding to 30 individuals subsequently analyzed for morbillivirus. CeMV was detected in 26.66% (8/30) of individuals. Positive amplicons were confirmed by Sanger sequencing and BLAST analysis, yielding four consensus sequences from three individuals. Two skin samples were also analyzed but excluded from statistical analyses due to their limited number. One originated from an individual positive for CeMV in brain and was also positive in skin, whereas the other was from an individual negative in brain and lung, with CeMV detected exclusively in skin. No statistically significant differences in positivity were observed ($p > 0.05$) according to sex, tissue, or age. Detection rates were 35.71% (5/14) in males, 17.64% (3/17) in females, 13.79% (4/29) in lung, 12.50% (4/32) in brain, 41.18% (7/17) in juveniles, and 12.50% (1/8) in adults. These findings provide molecular evidence of CeMV in *S. guianensis* and reinforce the importance of continued surveillance to better understand its occurrence and implications for species conservation.

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PT.613

COMPARISON OF CONCENTRATION AND GENETIC MATERIAL EXTRACTION METHODS FOR THE DETECTION OF SARS-COV-2 AND INFLUENZA B IN WASTEWATER FOR EPIDEMIOLOGICAL SURVEILLANCE

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Viral surveillance in wastewater is a promising tool for epidemiological surveillance, especially in regions with a high circulation of viral diseases. Due to the low concentrations of viral particles in raw sewage, a sample concentration step is essential to ensure sensitivity in detection. This study aimed to standardize and evaluate methods for monitoring and quantifying respiratory viruses in wastewater. Suspensions containing SARS-CoV-2 and Influenza B viruses, at final concentrations of 2×10^4 copies/mL and 1×10^2 copies/mL, respectively, were added to sewage samples previously collected in Diamantina. Combinations of genetic material concentration methods (filtration using electronegative membranes vs. polyethylene glycol (PEG) precipitation) and genetic material extraction methods (magnetic beads vs. silica column extraction) were analyzed using two-way ANOVA. Comparative analysis demonstrated that, for both viruses, concentration by electronegative membrane associated with magnetic bead extraction showed the best performance, with statistically significant differences for SARS-CoV-2 ($p = 0.0001$) and Influenza B ($p = 0.0001$). In contrast, when PEG concentration was used, silica column extraction was more efficient for both viruses, showing significant differences for SARS-CoV-2 ($p = 0.0106$) and Influenza B ($p = 0.0134$). No statistically significant difference was observed regarding which extraction methodology was overall more efficient. These findings reinforce the relevance of wastewater monitoring as an effective strategy for environmental surveillance, providing rapid and effective responses to public health emergencies. Financial Support: CAPES, FAPEMIG PCE-00181-26 and APQ-04955-23, SES-MG Agreement no. 007/2022.

PT.614

GENOMIC SURVEILLANCE OF WASTEWATER DURING LARGE-SCALE CULTURAL FESTIVALS IN SALVADOR, BRAZIL.

Strobel IM, Mendonça-Santos T, de Moraes LEP, Cruz NS, Tschoeke DA, Campos TL, Florentino PTV, Canedo DP, Aleluia FTF, Leonidio TO, Haddad R, Cucco MS, Pedrosa LR, Jesus Santos FM, Cardoso CW, Prates APPB, Vieira Lima HCA, Carvalho-Assef AP, Farias LP, Boaventura VS, Ramos PIP, Barral-Netto M, Rego FFA, Kafetzopoulou LE, Santos LA, Khouri R

Mass gathering events are high-risk settings for infectious disease amplification and spread because they bring together people from diverse locations, increasing opportunities for close contact and pathogen transmission. Surveillance strategies capable of capturing these dynamics in near real time remain limited. We describe a wastewater-based surveillance (WBS) framework to investigate pathogen dynamics during mass gatherings in Salvador, Brazil's fifth largest city and home to major festivals, including Carnival. We analyzed 233 samples from wastewater treatment plants and event-associated chemical toilets collected before, during, and after four events held between 2023 and 2025, estimated to have mobilized over 23.4 million people. Using targeted RT-qPCR, Next-Generation Sequencing with Hybrid Capture (NGS-HC), and amplicon sequencing, we characterized viral and bacterial communities and antimicrobial resistance (AMR) profiles. Viral communities were dominated by enteric and environmentally stable taxa, including norovirus, sapovirus, adenovirus, and JC polyomavirus, indicating a persistent background signal. Respiratory viruses displayed event- and time-dependent patterns, whereas arboviruses were rarely detected, likely reflecting the absence of major outbreaks, as suggested by available epidemiological surveillance data. Amplicon sequencing enabled SARS-CoV-2 lineage characterization and showed partial concordance with metagenomics for low-abundance targets. Bacterial communities showed shifts in stable core taxa rather than emergence of novel organisms, while resistome profiles remained largely consistent, with transient AMR fluctuations. Overall, WBS captured amplification of pathogens already circulating in the community, supporting its value for preparedness and early warning during mass gatherings.

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PT.615

WHOLE GENOTYPING CARACTERIZATION OF A RARE CANINE-LIKE ROTAVIRUS A G3P[3] STRAIN DETECTED IN A BRAZILIAN CHILD: EVIDENCE OF INTERSPECIES REASSORTMENT

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Rotavirus A (RVA) is one of the major causes of gastroenteritis in human and animals globally. Genotype G3P[3] is common in dogs, less frequent in cats, and rare in humans, occurring exclusively in children, suggesting increased susceptibility. The identification of a novel genotype constellation in Brazilian canine G3P[3] strains in 2017 prompted the full-genome characterization of the historical RVA/Human-wt/BRA/IAL-R451/2011/G3P[3] strain, which had previously been characterized only for the VP7 and VP4 genes, to determine its genomic constellation and relationship to canine strains. All 11 genome segments were characterized by RT-PCR, sequencing and phylogenetic analyses. The uncommon genotype-lineage constellation G3.III-P[3]-I2.XX-R3.II-C2.V-M3.II-A9-N2.XXIV-T3.II-E3.II-H6.I shared with Brazilian canine strains was identified suggesting a potential common origin. High nucleotide identity (93.2–99.0%) was observed with canine, feline and canine/feline-like human strains worldwide, with six genes (VP1, VP6, NSP2-NSP5) clustered closely with Brazilian 2017 canine strains (97.6–99.0%), supporting a canine origin. Phylogenetic analyses revealed reassortment events among domestic animals, wildlife, humans and environmental strains. The NSP2 segment grouped with bovine, rat and human strains, whereas VP7 and VP4 were related to bat, raccoon dog, sewage and river water, highlighting the role of environmental reservoirs in RVA transmission dynamics. In addition, novel lineages were proposed within VP1, VP3 and NSP2 genes, expanding the known diversity of AU-1-like RVA strains. These findings provide evidence of zoonotic transmission of a canine-like RVA strain to humans and highlight the importance of integrated genomic surveillance under a One Health approach to better understand RVA evolution and public health risks in Brazil.

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PT.616

RETROSPECTIVE MOLECULAR SURVEILLANCE REVEALS EARLY CIRCULATION OF TOTTORIVIRUS A1 IN BRAZILIAN SWINE

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Emerging and re-emerging viral diseases, particularly those of zoonotic origin, represent a major challenge to global public health. Swine are recognized as important reservoirs of pathogens with the potential to affect both animal and human health. Tottorivirus A1 (TotV-A1), a recently described virus classified within the species *Tottorivirus asuscro*, was first identified in domestic pigs in Japan in 2015. However, information regarding its epidemiology, geographic distribution and zoonotic potential remains scarce. This study investigated the occurrence of TotV-A1 in swine fecal samples from Brazil through retrospective molecular surveillance. A total of 179 fecal samples collected from domestic pigs in 2013 and 2014 on farms in the states of Paraná and São Paulo were analyzed. Viral RNA was screened by conventional RT-PCR using an adapted protocol. Positive samples were confirmed by Sanger and next-generation sequencings. TotV-A1 was detected in 29 samples (16.2%), representing the first report of this virus in Brazil. Notably, all positive samples were collected in 2014, demonstrating that TotV-A1 was circulating in the country before its first description. These findings expand current knowledge of the temporal distribution of TotV-A1 and underscore the value of retrospective molecular surveillance using historically stored samples to identify previously unrecognized viruses. Ongoing research will investigate the genetic evolutionary history of TotV-A1 and assess its potential implications for public health, food safety, and environmental monitoring.

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PT.617

NOROVIRUS AS A LEADING CAUSE OF SEVERE PEDIATRIC GASTROENTERITIS: EPIDEMIOLOGICAL AND MOLECULAR FINDINGS FROM BELÉM, PARÁ

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Introduction: Currently, norovirus is a leading viral agent associate with acute gastroenteritis in children in scenario of severe rotavirus cases reduction from vaccination. Genetic diversity and recombination events in norovírus Genome reinforce the need for continuous molecular surveillance. **Materials and Methods:** A cross-sectional study was conducted at a pediatric hospital in Belém, Pará, Brazil, where fecal samples were collected from children under 10 years of age hospitalized with acute gastroenteritis between November 2024 and May 2026. Clinical specimens were subjected to molecular testing for norovirus detection (RT-qPCR) and molecular characterization (convencional PCR and nucleotide sequencing). Epidemiological data were analyzed using descriptive statistics and association tests. **Results:** A total of 159 children hospitalized with acute gastroenteritis were included and norovirus were detected in 25.8% (41/159) of cases. Viral circulation occurred throughout the study period, with an increase in cases between May and September 2025. Children aged 7–24 months (56.0%; 23/41) and males (56.0%; 23/41) were the most affected by norovirus infection. Concerning clinical aspects, fever was the only symptom significantly associated with norovirus infection (p = 0.011). Genogroup GII was the most prevalent, detected in 92.7% (38/41) of positive cases, with circulation of genotypes GII.17 (46.4%), GII.4 (35.7%), GII.7 (7.1%), GII.1 (3.5%), GII.3 (3.5%), and GII.6 (3.5%). **Conclusion:** These findings reinforce the importance of norovirus as a major etiological agent of severe pediatric gastroenteritis and highlight the need for continuous epidemiological and molecular surveillance to monitor viral circulation and the emergence of variants with potential public health implications.

Key words: norovírus; acute gastroenteritis; molecular surveillance.

Financial Support: CNPq, FAPESPA.

PT.618

INVESTIGATION OF POXVIRUSES IN THE STATE OF CEARÁ, BRAZIL

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Orthopoxviruses have caused outbreaks with impact on human and animal health in Brazil. In 2025, an outbreak of an exanthematic disease was reported in the municipality of Cariré, in the state of Ceará, Brazil, affecting dairy cattle and rural workers. Infected cows presented lesions on the teats and udders, whereas affected rural workers presented hand lesions. 7 bovine sera, 3 lesions and 2 milk samples were sent to our group to investigate the cause of these symptoms. The samples were submitted to DNA extraction using a commercial kit and submitted to qPCR targeting the C11R, A56R and E9L genes. Group 1 brazilian vaccinia virus DNA was detected in the lesions and milk samples. No evidence of the targeted genes was found in the serum samples tested. Serum samples were also submitted to plaque reduction neutralization tests to detect neutralizing antibodies against orthopoxviruses. Samples were diluted in minimum essential medium and mixed with a suspension of vaccinia virus, resulting in a dilution of 1:40, and inoculated on monolayers of BSC-40 cells. Samples with reduction in the number of plaque forming units of 50% or higher compared to the virus control were considered positive. 2 samples were positive, indicating exposure to orthopoxviruses. Viral isolation and whole genome sequence assays are underway to characterize the causative agent of the reported outbreak. We will also submit bovine serum samples from a slaughterhouse in Sobral, Ceará, to serological and molecular assays, in order to better understand the circulation of orthopoxviruses in the state.
Financial support: FAPEMIG, CNPq, CAPES, INCT-POX, Câmara-POX

PT.619

BEYOND BACTERIAL INDICATORS: A BRAZILIAN-DEVELOPED MULTIPLEX RT-qPCR ASSAY REVEALS HUMAN ENTERIC VIRUS CONTAMINATION ACROSS ANTROPOGENIC IMPACTED AQUATIC ECOSYSTEMS

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The occurrence of human enteric viruses across distinct aquatic ecosystems was investigated using a Brazilian-developed multiplex RT-qPCR assay and compared with conventional bacterial indicators. Water samples (2 L) were collected from 21 sites within the Guanabara Bay watershed (southeastern Brazil) in February 2026, including urban rivers, canals, beaches, and coastal lagoons. *Escherichia coli* and enterococci were quantified by defined substrate methods. Urban freshwater samples were concentrated by ultracentrifugation, whereas recreational waters were processed by adsorption–elution followed by ultrafiltration. Viral nucleic acids were extracted, and rotavirus A (RVA), norovirus GI, and GII were quantified using the IBMP Biomol multiplex RT-qPCR assay. *E. coli* and enterococci were detected in 85.7% and 50.0% of samples, with mean concentrations of 5.71 and 3.26 $\log_{10}$ MPN L^{-1}, respectively. RVA, norovirus GI, and GII were detected in 80.9%, 57.1%, and 52.3% of samples, with mean concentrations of 5.69, 6.64, and 5.62 $\log_{10}$ GC L^{-1}, respectively. Significant differences among aquatic environments were observed for *E. coli* ($p = 0.0004$) and RVA ($p = 0.0036$). A moderate correlation was found between *E. coli* and RVA ($r = 0.64$, $p = 0.013$), whereas norovirus GI and GII showed no significant correlations with bacterial indicators. These findings demonstrate that bacterial indicators alone do not adequately reflect viral contamination, highlighting the Brazilian-developed multiplex assay as a valuable tool for environmental monitoring across diverse aquatic ecosystems.Financial Support: Association for the Management of Water Resources in the Paraíba do Sul River Basin (AGEVAP); Hydrographic Region Committee for Guanabara Bay and the Maricá and Jacarepaguá Lagoon Systems (CBH-BG).

PT.620

RESEARCH ON ASTROVIRUSES IN SAMPLES FROM CHILDREN HOSPITALIZED WITH GASTROENTERITIS IN A POST-COVID-19 PANDEMIC CONTEXT IN THE METROPOLITAN REGION OF BELÉM, PARÁ

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Introduction: Human astrovirus (HAstV) is a viral agent commonly associated with cases of acute gastroenteritis, particularly in children. Given the decline in childhood vaccination coverage against RVA in Brazil following the COVID-19 pandemic, implementing measures to prevent and control diarrheal disease—especially in children—is crucial. **Material and Methods:** This cross-sectional study was conducted at a pediatric hospital in Belém, Pará, using fecal samples from children under 10 years of age hospitalized with severe gastroenteritis between September 2025 and June 2026. Following collection, clinical specimens underwent molecular testing for astrovirus detection (RT-qPCR and conventional RT-PCR) and molecular characterization (nucleotide sequencing). Epidemiological data were analyzed using descriptive statistics and association tests. **Results:** A total of 58 stool samples were tested, yielding an astrovirus positivity rate of 5.17% (3/58) by RT-qPCR and 3.45% (2/58) by conventional RT-PCR. Children aged 1 year and 9 months (3.45% – 2/58) showed the highest rate of astrovirus infection. Clinically, the triad of symptoms—diarrhea, fever, and vomiting—was present in all positive cases, and a higher incidence of cases was observed during the year-end period. Genomic sequencing revealed that both samples testing positive via conventional RT-PCR were characterized as genotype 4 (HAstV-4). **Conclusion:** These findings reinforce the significance of HAstV as an etiological agent of severe pediatric gastroenteritis and highlight the importance of continuous epidemiological and molecular surveillance to monitor viral circulation and the emergence of variants with potential public health impact.

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PT.621

GENOME-WIDE INTRA- AND INTERPOPULATION DIVERSITY OF SPODOPTERA FRUGIPERDA GRANULOVIRUS ISOLATES FROM THE AMERICAS REVEALS CONTRASTING POPULATION STRUCTURES

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The fall armyworm, *Spodoptera frugiperda*, is a major agricultural pest for which baculoviruses are important biological control agents. Among these viruses, Spodoptera frugiperda granulovirus (SfGV) remains less characterized than Spodoptera frugiperda multiple nucleopolyhedrovirus (SfMNPV), particularly regarding population diversity. We investigated genome-wide intra- and interpopulation diversity in four Brazilian SfGV isolates and compared their consensus genomes with previously sequenced isolates from Colombia, Argentina, and Mexico. Complete genomes were assembled from Illumina reads and analyzed using whole-genome alignments, phylogenetic reconstruction, intrapopulation variant profiling, and ORF-level assessment of nonsynonymous variation. Brazilian genomes were highly collinear, ranged from 138,901 to 139,878 bp, and had 46.3% G + C content. Whole-genome comparisons revealed geographic structure, with Brazilian isolates forming a compact subgroup closely related to the Argentinean isolate, whereas Colombian and Mexican genomes were more divergent. The three Minas Gerais isolates were nearly identical at the consensus level, with SfGV-BJA and SfGV-C92 sharing 100.00% whole-genome nucleotide identity. Read-level analyses, however, revealed marked population differences. SfGV-BJA contained 46 polymorphic sites comprising 49 alternative nucleotide variants, whereas SfGV-C92, SfGV-C93, and SfGV-STNJ contained 812-856 polymorphic sites and 813-858 alternative variants. Nucleotide diversity was lowest in SfGV-BJA, intermediate in SfGV-C92, and highest in SfGV-C93 and SfGV-STNJ. Nonsynonymous variation was concentrated in discrete loci, including enhancin-1, enhancin-2, bro family members, and selected core genes such as vlf-1, whereas many other core genes remained conserved. These findings show that consensus-genome identity can conceal substantial differences in baculovirus population structure and highlight the importance of read-level analyses for accurately characterizing natural SfGV diversity.

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PT.622

MOLECULAR SURVEILLANCE OF EMERGING ARBOVIRUSES IN CULICIDAE MOSQUITOES IN THE STATE OF SÃO PAULO

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The epidemiological landscape in São Paulo is marked by the challenge of reemerging sylvatic arbovirus cycles, such as the *Yellow Fever virus*, necessitating robust surveillance strategies for early detection in both sylvatic and urban areas amid continuous viral expansion. This study processed 4,671 mosquitoes (*Culicidae*) collected in the municipalities of Analândia, Mogi-Guaçu, Rosana, São José do Barreiro, São Roque, and Ubatuba between October 2020 and March 2022. Specimens were organized into macerate pools for molecular analysis. The methodology relied on automated RNA extraction and screening via RT-qPCR (*Zika, Chikungunya*, pan-*flavivirus*, *Mayaro*, and *Oropouche viruses*), with subsequent characterization via Sanger sequencing. Although results did not show the circulation of medically important arboviruses, screening identified the presence of classic insect-specific flaviviruses (cISFs) in 14 of the 31 pools evaluated, specifically in *Aedes aegypti* (n = 11), *Aedes terrens* (n = 1), *Aedes* sp. (n = 1) and *Culex* sp. (n = 1). Detections occurred predominantly in the urban environment (n = 12), providing new insights into local viral ecology. Genomic analysis revealed *Xishuangbanna-like* lineages with 76% nucleotide identity compared to reference isolates, suggesting the circulation of new species or highly divergent variants in South America. We conclude that the detection of these cISFs is of ecological relevance, as their persistence may modulate vector competence and mosquito biology. These findings reinforce the need for continuous monitoring and the consolidation of the macerate biobank as an essential infrastructure for active entomological-molecular surveillance and state public health security.
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PT.623

Influenza virus genomic surveillance in Northern Brazil supports a unified vaccine composition despite distinct seasonal dynamics

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Northern Brazil exhibits a distinct influenza seasonality compared with the rest of the country, with peak viral activity occurring during the rainy season. This epidemiological pattern prompted the implementation of a region-specific vaccination schedule in 2025. It raised the question of whether a different vaccine formulation, such as that recommended for the Northern Hemisphere, would be more appropriate for this region. To address this hypothesis, we analyzed 5,985 influenza A(H1N1)pdm09, 5,693 A(H3N2), and 3,165 influenza B/Victoria virus genomes collected between January 2024 and January 2026 from all Brazilian macroregions and representative countries across five global influenza transmission zones. Monthly subclade frequencies, maximum-likelihood phylogenetic analyses, and discrete phylogeographic reconstruction were performed to characterize viral diversity and transmission patterns. The same major subclades predominated in Northern Brazil and the rest of the country throughout the study period, although some variants appeared to be introduced into the North with a slight temporal delay. Phylogenetic analyses showed no evidence of persistent regional lineages or a genetically distinct viral population. At the same time, phylogeographic inference indicated that viral introductions originated predominantly from other Brazilian regions rather than from international sources. These findings suggest that differences in influenza epidemiology across Brazil are primarily driven by the timing of seasonal transmission rather than by distinct viral evolutionary trajectories. Consequently, the current evidence does not support the use of a Northern Hemisphere vaccine formulation in Northern Brazil. Instead, optimizing vaccination timing appears to be a more appropriate strategy to improve regional protection while maintaining a unified vaccine composition across the country.

PT.624

MOLECULAR DETECTION AND GENETIC CHARACTERIZATION OF AVIAN HERPESVIRUSES CIRCULATING IN FREE-RANGING SEABIRDS FROM THE CAGARRAS ARCHIPELAGO, RIO DE JANEIRO, BRAZIL

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Seabirds are valuable sentinels for monitoring emerging infectious diseases because of their extensive flight ranges and diverse ecological traits. Among the pathogens affecting these species, herpesviruses are of particular concern due to their global distribution and capacity to cause morbidity and mortality while establishing subclinical latent infections. The Cagarras Archipelago, located approximately 5 km off metropolitan region of Rio de Janeiro, Brazil, hosts the largest magnificent frigatebirds (*Fregata magnificens*) colony in the Atlantic Ocean (~7,500 nests), and approximately 2,000 nests of brown boobies (*Sula leucogaster*). This study investigated herpesvirus occurrence in fecal and cloacal swab samples from these seabirds. In total, 217 birds (99 frigatebirds and 118 brown boobies) were screened by nested PCR targeting the DNA polymerase gene, and positive amplicons were Sanger sequenced. Overall, 2.8% (6/217) tested positive, including two frigatebirds and four brown boobies. Frigatebird sequences (2/6) presented 96% nucleotide identity with *Fregata magnificens herpesvirus*, previously associated with chick mortality in French Guiana. Among boobies sequences (4/6), one was highly similar (98% nucleotide identity) to *Sulid herpesvirus 1*, previously detected in asymptomatic birds from Bahia, Brazil. The remaining three sequences were identical to each other and shared <77% identity with GenBank references, suggesting a novel herpesvirus, tentatively named *Sulid herpesvirus 2*. These findings represent the first herpesvirus report in seabirds from the Cagarras Archipelago and reinforce the importance of long-term surveillance to better understand herpesvirus ecology and its implications for seabird conservation within a One Health framework. Financial support: JCNE FAPERJ 200.244/2023

PT.625

A GENOME-RESOLVED VIRAL CATALOGUE REVEALS EXTENSIVE TAXONOMIC NOVELTY IN SOUTH AMERICAN WASTEWATER

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Sewage metagenomics provides valuable insights into microbial communities and their associated viromes, yet wastewater viral diversity remains largely unexplored in South America. This study aimed to construct the first genome-resolved viral catalogue of South American sewage and characterize its taxonomic diversity, novelty, and predicted bacterial hosts. We reanalysed 24 publicly available wastewater metagenome datasets (SRA/ENA), representing 19 cities across nine countries, using a consensus workflow for viral identification (VirSorter2), quality assessment (CheckV), taxonomic classification (vConTACT3, taxmyphage), and host prediction (iPHoP). This approach recovered 681 viral operational taxonomic units (vOTUs), following MIUViG-recommended thresholds. Taxonomic analyses revealed extensive viral novelty: 100% of vOTUs lacked species-level matches and 99.1% lacked genus-level matches to ICTV reference genomes. Furthermore, only 21.0% of vOTUs could be assigned to a recognized ICTV family and the predominant families identified among samples were *Peduoviridae, Autographiviridae, Schitoviridae, Inoviridae* and *Fredfastierviridae*. *Peduoviridae* and *Inoviridae* were identified in at least one sample of all countries in the study, highlighting the substantial proportion of viral diversity that remains unclassified. Predicted bacterial hosts were assigned at the genus level for 28.6% of the catalogue and were predominantly associated with the clinically relevant genera *Pseudomonas* and *Acinetobacter*. These findings reveal that South American sewage harbours a vast and previously uncharacterized reservoir of bacteriophage diversity and establishes the first genome-resolved viral reference catalogue for the region, providing a valuable resource for future comparative, ecological, functional, and wastewater-based epidemiological surveillance studies. This resulting catalogue was deposited in the public database Genbank (NCBI).

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PT.626

ISOLATION AND CHARACTERIZATION OF TWO LYTIC BACTERIOPHAGES TARGETING CARBAPENEM-RESISTANT NOSOCOMIAL AND ENVIRONMENTAL *ENTEROBACTER KOBEI* STRAINS

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Enterobacter is a ubiquitous bacterial genus distributed across environmental niches and the intestinal microbiota of humans and other animals. Members of this genus belong to the ESKAPE pathogens and are important because they cause opportunistic infections and acquire antimicrobial resistance mechanisms, including β-lactamase production and carbapenem resistance. In this scenario, lytic bacteriophages represent alternatives for controlling multidrug-resistant bacteria. This study evaluated lytic bacteriophages against a β-lactam-resistant *Enterobacter kobei* strain. The clinical isolate was obtained from the University Hospital of Brasília and cultivated in LB agar and broth at 37 °C. Phages were recovered from filtered sewage samples collected at urban wastewater treatment plants. Enrichment was performed by incubating bacterial cultures with filtered sewage for 48 h. Lytic activity was determined by spot assay, and phages were purified through three rounds of double-layer agar. Viral DNA was extracted and examined by agarose gel electrophoresis. Morphology was characterized by transmission electron microscopy with negative staining. Phage stability was evaluated under different temperatures, pH values, and organic solvents. Host range was tested against 12 *Enterobacter* strains, and bacterial biofilm formation was also assessed. Two phage isolates with reproducible lytic activity against *E. kobei* were obtained. Electron microscopy revealed particles with icosahedral capsids and long tails. The phages remained stable at low temperatures and between pH 3 and 11, infected three environmental strains besides the hospital isolate, and maintained stability under different chemical conditions. The association between biofilm formation and phage susceptibility is under investigation, supporting their potential for sanitizers and wastewater treatment applications.
Financial Support: CAPES, UnB, Serrapilheira, CNPq and FAP-DF.

PT.627

A Glimpse of the Global Virome Associated to Aquatic Organisms

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Aquatic organisms play a fundamental role in maintaining marine ecosystem stability and are essential for the biogeochemical cycles of oxygen and carbon. However, viral infections affecting these organisms pose a significant threat to ecosystem balance, highlighting the need for virological studies aimed at understanding and mitigating their impacts. Furthermore, viruses associated with aquatic organisms are increasingly recognized within the One Health framework, as recent studies have reported potential spillover events involving shrimp-associated viruses and humans. In this context, the present study aimed to characterize the virome of aquatic organisms through the analysis of publicly available RNA-seq libraries. The datasets were selected and processed using FASTp for quality control. Subsequently, sequencing reads were aligned to reference genomes with Bowtie2, and unmapped reads were assembled into contigs using MEGAHIT. Viral identification was performed through the detection of conserved domains using Hidden Markov Models (HMMs) and similarity searches with BLAST. A high viral diversity was identified in crustaceans and fish. Among the detected viral families, *Hantaviridae*, *Togaviridae*, and *Hepeviridae* were the most prominent. Notably, members of the *Hantaviridae* and *Togaviridae* families were detected in potentially invasive host species. All of these viral families include pathogens capable of infecting humans and a wide range of animal hosts. These findings reveal the remarkable diversity of viruses associated with aquatic organisms and emphasize the need for further investigations to better understand their ecological roles and their potential circulation among different host species

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PT.628

TRANSMISSION OF *Potyvirus halapensis* TO FORAGE GRASSES BY TWO APHIDS SPECIES

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Viral diseases have been associated to symptoms and losses of forage production in tropical pasture grasses. The prevalent virus species found in *Megathyrsus maximus* (syn. *Panicum maximum*) and *Urochloa brizantha* (syn. *Brachiaria brizantha*) plants is the *Potyvirus halapensis* (Johnsongrass mosaic virus – JGMV). The objective of this work was to determine the transmission relationship between two aphid species found in pasture areas and JGMV. Three independent experiments were performed in greenhouse conditions. The aphids *Hysteroneura setariae* (Thomas) and *Melanaphis sorghi* (Theobald) (Hemiptera: Aphididae) were rearing in *U. brizantha* and *Sorghum bicolor* plants, respectively. A total of 100 seeds of *U. brizantha* cv. BRS Paiaguas and 100 seeds of *M. maximus* cv. Mombaça were germinated in individual pots. After 10 days of germination, 50 plants of each cultivar were mechanically inoculated with JGMV isolate CG04/22. After symptom development with 10 days post-inoculation, 10 aphids were individually transferred to each plant for 15 min or 48h to accession acquisition time (AT). After the AT, aphids were transferred to healthy plants of the same cultivar for the accession transmission time (TT). The TT tested were 15 min and 48h. For each acquisition and transmission times, 10 replicates (plants) were performed. Both aphid species transmitted JGMV to *U. brizantha* BRS Paiaguas and *M. maximus* cv. Mombaça with highest efficiency of 90%. Non-persistent transmission occurred for both aphid species, but the efficiency in transmission presented variation according to the treatment and experiment performed.
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PT.629

DISCOVERY AND CHARACTERIZATION OF CANDOMBEVIRUS, A NOVEL GIANT VIRUS OF THE GENUS COTONVIRUS

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Mimiviruses are giant viruses that, among other unusual features, can be observed by light microscopy because of their exceptionally large size. Before the discovery of *Acanthamoeba polyphaga mimivirus* as a viral agent in 2003, these microorganisms had been misidentified as Gram-positive cocci, a finding that substantially changed our understanding of the viral world. Systematic sampling of aquatic environments has since expanded the known diversity of the family *Mimiviridae* and led to the discovery of numerous new giant viruses. In South America, the first reported isolates were *Megavirus chiliensis* in Chile and Sambavirus in Brazil.

In Uruguay, our group isolated a giant virus for the first time from water collected in Arroyo Vizcaíno using co-culture with *Acanthamoeba castellanii*. Transmission electron microscopy confirmed viral replication by revealing cytoplasmic viral factories and particles with morphology typical of mimiviruses. A one-step growth curve showed a relatively prolonged replication cycle compared with those reported for other giant viruses.

Genome sequencing yielded a 1,195,391-bp genome. Phylogenetic and comparative genomic analyses showed that the isolate is most closely related to *Cotonvirus japonicus*, the first described member of the genus *Cotonvirus*, reported in Japan in 2021. Comparative gene-content analysis within the subfamily *Megamimivirinae* showed that Candombevirus shared the largest number of genes with *Cotonvirus japonicus*, while 93 genes were unique to Candombevirus. Together, these findings document the first isolation and characterization of a giant virus in Uruguay and support the classification of Candombevirus as a new species within the genus *Cotonvirus*.

PT.630

ONE SAMPLE, TWO VIRUSES: PROSPECTION AND ISOLATION OF TWO NOVEL BRAZILIAN CHLOROVIRUSES

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Chlorovirus is a genus of giant DNA viruses from the family *Phycodnaviridae* that infects unicellular Chlorella-like green microalgae. They are found in various freshwater environments, making Brazil, a country with a wide biodiversity, a promising place to isolate new viruses. However, the studies about these viruses are still scarce in the country. This study aims to characterize the biological and morphological features of two different new isolates obtained from the same water sample. A river sample from Conselheiro Lafaiete city, Minas Gerais, was collected in 2025 and filtered through a 0.45 µm membrane and then inoculated in different species of microalgae cultures. In a solid-medium plaque assay made with *Chlorella variabilis* NC64A and *Chlorella heliozoae* SAG 3.83 cultures showed lysis plaques, derived from the same sample. The isolates were named BR-MG-N09 and BR-MG-S03, respectively. Both viruses showed circular lysis plaques with similar sizes, measuring approximately 2,4 mm. Analysis of the replication of the isolate BR-MG-S03 showed 6,8 x 10^4 PFU/mL after 30 minutes of infection and 5,1 x 10^7 PFU/mL after 24 hours of infection, demonstrating a 750-fold increase in the virus titer. A similar experiment will be conducted for biological characterization of the isolate BR-MG-N09. This study expands the understanding of the diversity of chloroviruses in Brazil. Furthermore, the viruses will also be sequenced for comparative analysis of the genomic description, to enable a clearer understanding of these new isolates.

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PT.631

IN SITU GENOMIC EVOLUTION OF TWO PANDORAVIRUSES ISOLATED FROM THE SAME ENVIROMENTAL SITE ACROSS TIME

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Pandoraviruses were first described in 2013. They comprise giant viruses that infect amoebae and are notable for their large genomes, which can reach up to 2.77 Mb, with up to 90% of their genes lacking known homologs. This study investigates the genomic features of two pandoraviruses isolated from *Acanthamoeba castellanii* from the same collection point in the São Francisco River, in Almenara, Minas Gerais, Brazil, at different time points (October 2021 and January 2022). To our knowledge, this is the first report of the same giant virus being isolated from the same location at different time points, providing an opportunity to study Pandoravirus evolution *in situ*. The viruses were morphologically characterized by transmission electron microscopy, and their genomes were sequenced, annotated, and analyzed for mutations. Both exhibited oval-shaped particles approximately 1 µm in length, with an apical ostiole. The October 2021 isolate has a genome of 1,828,525 bp, while the January 2022 isolate has 1,827,464 bp, sharing 99.49% whole-genome nucleotide identity. Functional analysis revealed that most genes are uncharacterized, alongside genes involved in diverse functions such as cell signaling, DNA replication, and RNA processing. A total of 19,432 mutations were identified, 67.83% in coding regions. The most mutated genes include serine/threonine kinases and hypothetical proteins. Although the number of replication cycles cannot be estimated, a rate of one mutation per 94.04 nucleotides was observed over three months, which is unexpected for a dsDNA virus. We hypothesize that paralogs and pseudogenization enable high mutation tolerance, promoting diversity and environmental adaptation.

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PT.632

PHYLOGEOGRAPHIC STUDY ON THE SPREAD OF VACCINIA VIRUS STRAIN CANTAGALO THROUGHOUT BRAZIL FROM 1999 TO 2024

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Cantagalo (CTGV) is a vaccinia virus (VACV, Poxviridae) field strain, first identified in 1999 in Cantagalo-RJ, causing vesicular lesions in dairy cattle and milkers. CTGV outbreaks have since been reported in states of Southeastern, Midwestern, Northern and Northeastern Brazil. The spread of CTGV in Brazil represents a unique opportunity to study the natural evolution of VACV, otherwise restricted to vaccine strains. This work aims to characterize the genomes of 40 CTGV clinical isolates obtained in different Brazilian states throughout 25 years of this strain's known circulation. Viruses were isolated from lesion scabs in BSC-40 cells. Genome sequencing used either virosomal or purified virus DNA on Illumina MiSeq and NextSeq platforms. Contigs were assembled using SPAdes/Geneious, and genomes were annotated using GATU/CLC. The 40 genomes obtained ranged from 180,989bp to 187,690bp with >96.23% identity across the whole genome. Phylogenetic analysis clustered all CTGV isolates as a sister group to the Brazilian vaccine strain VACV-IOC and horsepox viruses. Four phylogenetic subgroups were detected, each comprised of isolates obtained geographically and/or temporally close to each other. Close relationship between isolates from Mato Grosso (2008) and Rondônia (2009) suggests a possible spread route. The isolates obtained in Southeast Brazil between 1999-2003 exhibited a 3.7-kbp deletion in the 5' end and appear to form a separate group from the others found throughout Brazil to this day. When comparing the first and most recent CTGV genomes, 409 SNPs accumulated across the whole genome, which suggests a lower mutation rate than VACV vaccine strains. Funding: CNPq/CAPES/FAPERJ.

PT.633

First report of Euphorbia ringspot virus infecting *Euphorbia milli* in Brazil

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The international trade of ornamental plants represents an important pathway for the dissemination of plant viruses worldwide. In 2025, crown-of-thorns (*Euphorbia milii*) plants exhibiting mosaic, chlorotic ringspots, and flower color breaking symptoms were observed in public green areas of the city of São Paulo, SP, Brazil. To investigate whether these symptoms were associated with a viral infection, symptomatic tissues were analyzed by transmission electron microscopy (TEM), revealing cylindrical inclusions and flexuous filamentous particles characteristic of members of the family *Potyviridae*. Virus identification was performed by RT-PCR using degenerate primers targeting the CI region of potyviruses, yielding an amplicon of approximately 700 bp. The PCR product was sequenced by the Sanger method, and BLAST analysis showed ~95% nucleotide identity with a Euphorbia ringspot virus (EuRSV, *Potyvirus euphorbiae*) isolate from Florida (GenBank accession PQ661197). Phylogenetic analysis based on the amplified CI region, including representatives of different genera within the family *Potyviridae*, placed the Brazilian isolate in a clade distinct from previously characterized EuRSV isolates. These findings provide molecular evidence of EuRSV infecting *E. milii* in Brazil and suggest the occurrence of a genetically divergent isolate. This study expands the current knowledge of EuRSV distribution and genetic diversity and highlights the importance of virus surveillance in ornamental plants to prevent the introduction and spread of potentially emerging viral pathogens.

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PT.634

Seasonal Detection and Quantification of Cyanophages and Toxigenic Cyanobacteria in the Santiago River Using Molecular Tools

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The seasonal dynamics of toxigenic cyanobacteria and cyanophages were investigated at two sites of the Río Santiago (open river and canal) in Ensenada, Buenos Aires Province, Argentina. Sixteen samples were collected in March, June, October, December, and January. In situ physicochemical parameters, chlorophyll-a, and microcystins were measured. For molecular analyses, 100 mL of water per sample were filtered, and DNA extracted from retained material was examined by conventional PCR targeting the cpcBA, mcyJ, and g91 genes, and by qPCR for quantification of mcyJ and g91.The cpcBA gene was consistently detected, confirming cyanobacterial presence. The toxigenic marker mcyJ was amplified in 11 of 16 samples (68.8%), distributed across both sites (5 in the open river, 6 in the canal), with qPCR quantification ranging from 1.78×10^{1} to 1.66×10^{4} total copies. Microcystins were detected in 10 of 11 mcyJ-positive samples, evidencing concordance between gene presence and toxin occurrence.Detection of the cyanophage marker g91 was less frequent (31.3%), restricted to March and June (2 samples from the open river, 3 from the canal). Quantification was achieved in 3 of 5 positive samples, ranging from 2.63×10^{0} to 3.20×10^{1} total copies.These results provide one of the first records of seasonal detection and quantification of cyanophages in the Río Santiago and establish a foundation for understanding virus–cyanobacteria interactions in fluvial ecosystems of Buenos Aires Province.This study was conducted with the support of CONICET grant PIP 11220200102990CO.

PT.635

ISOLATION AND GENOMIC CHARACTERIZATION OF PANTANAL VIROPHAGE, A NEW SPUTNIKVIRUS SPECIES FROM THE BRAZILIAN PANTANAL BIOME

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Amoeba-infecting giant viruses, such as mimiviruses, challenged traditional virology paradigms due to their complex genomes and particle structures. Interestingly, these viruses can form atypical relationships with other organisms, including parasitism by virophages, and may carry mobile genetic elements called transpovirons that code for their own proteins. These elements may use the giant virus's replication machinery and possibly exploit virophages for dissemination, forming intricate tripartite systems. Here, we describe isolation of such a system involving *Moumouvirus pantanense*, its associated transpoviron, and a new virophage named Pantanal virophage. A sewage sample collected in Porto Murtinho, at the southern end of Brazil's Pantanal biome, was screened using *Acanthamoeba castellanii* as the isolation platform. Transmission electron microscopy revealed the moumouvirus with a scarce amount of surface fibrils on its multi-layered capsid, a hallmark of the genus, and smaller virophage particles inside vesicles. Genomic analysis confirmed >90% nucleotide identity with *Moumouvirus maliensis*, leading to its identification as *Moumouvirus pantanense*. The Pantanal virophage has a 17,964 bp genome with 21 coding sequences, including two ORFans that code for proteins with completely unknown functions. Phylogeny placed Pantanal virophage as a divergent branch within the *Sputnikvirus* genus. Average nucleotide and amino acid identity analyses comparing different virophage sequences indicated that Pantanal virophage represents a new *Sputnikvirus* species. These findings shed light on giant virus-virophage-transpoviron triplet diversity, evolution, and ecology, contributing to a future update in virophages taxonomy. The identification of Pantanal virophage as a new *Sputnikvirus* species expands known phylogenetic diversity, underscores the importance of exploring underrepresented ecosystems, and raises questions about virophage-host coevolution and ecological impact.

PT.636

Sequencing and phylogeny of the insect-specific virus *Phasivirus phasiense* collected in Southern Brazil

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Phasivirus phasiense is an insect-specific virus (ISV) commonly found in mosquitoes, where it has been shown to modulate arbovirus replication. As it is transmitted only horizontally, it may serve as a marker of host dispersion patterns. Therefore, research on ISVs may contribute to epidemics control, particularly in regions with high arbovirus prevalence and limited studies on ISVs, such as Southern Brazil. Adult *Aedes* mosquitoes were collected by active capture in March 2026 in Estância Velha, Rio Grande do Sul, Brazil. Samples were macerated with liquid nitrogen and mantained in MEM medium. Mosquito pools underwent 12000xG centrifugation, and RNA was extracted from the supernatant using the TRIzol™ Reagent (Invitrogen™) Kit. Sequencing ibraries were prepared, and shotgun metagenomic sequencing was performed on an Illumina NextSeq platform. The genome was assembled using the virus typing tool from Genome Detective. The L segment, which encodes the virus' RdRP protein, was completely sequenced and submitted to BLASTn, resulting in 205 hits in the NBCI database. A phylogenetic tree was constructed using IQ-TREE. The resulting phylogenetic tree revealed complex divergence patterns, with samples being divided geographically, but in divergence with previous phylogenetic studies. This may be attributed to the limited number of samples from this region; to the best of our knowledge, this is the first *Phasivirus* genome reported from Southern Brazil. This study provides novel data on the species and contributes to a broader understanding of ISVs. This study was funded by Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS).

PT.637

GENOMIC DIVERSITY OF TUBULAVIRUSES ASSOCIATED WITH *Ralstonia* IN PRODUCTIVE INFECTIONS AND PROPHAGE STATES

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Tubulaviruses are filamentous bacteriophages that can establish chronic infections or enter the prophage state. The bacterial genus *Ralstonia* occupies diverse ecological niches as plant pathogens, human pathogens, and environmental species. Approximately 100 tubulaviruses infecting *Ralstonia* have been described in productive infections. In contrast, *Ralstonia* genomes harbor hundreds of tubulavirus-like prophages, although a comprehensive genomic analysis of these integrated elements is still lacking. It remains unclear whether *Ralstonia*-associated tubulaviruses constitute a biologically, genomically, and phylogenetically cohesive group. To address this, we used comparative genomic approaches to analyze these viruses in both productive infections and integrated prophages. We obtained viral and bacterial genomes from GenBank in May 2026. We identified and classified prophages using the geNomad tool. We compiled 141 tubulaviruses genomes from productive infections from the literature and screened 1,623 Ralstonia genomes, identifying 1,015 putative prophage sequences. Tubulaviruses in productive infections exhibited a median genome size of approximately 11 kb, whereas prophage-derived sequences displayed a median size of 15 kb and ranged from 0.4 to 40 kb. GC content also differed between productive and integrated viral forms, with some prophages showing GC values closer to those of the bacterial host genome. These findings suggest that integrated tubulavirus prophages may undergo progressive domestication within bacterial chromosomes. Overall, *Ralstonia*-associated tubulaviruses show distinct genomic characteristics depending on whether they occur in productive infections or as prophages, suggesting different evolutionary trajectories within this viral group. Additional comparative, ecological, and evolutionary analyses are underway to investigate their diversity, host adaptation, and evolutionary dynamics.

PT.638

GENOMIC AND BIOLOGICAL DIVERSITY OF BACTERIOPHAGES INFECTING BACTERIA OF THE *RALSTONIA SOLANACEARUM* SPECIES COMPLEX IN AGRICULTURAL SYSTEM

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The *Ralstonia solanacearum* species complex (RSSC) is a group of Gram-negative phytopathogenic bacteria that cause bacterial wilt in crops such as tomato, potato, and eucalyptus. These bacteria colonize xylem vessels, leading to vascular blockage, irreversible wilting, and plant death. Current disease management strategies remain limited, and bacteriophages have emerged as alternatives for the biological control of bacterial wilt. In this study, soil samples collected from sugarcane, tomato, and eucalyptus fields were used to isolate bacteriophages infecting members of the RSSC. Phage genomic DNA was extracted and sequenced using the Illumina MiSeq platform. A total of 38 bacteriophages were isolated. Taxonomic analyses identified 37 phages belonging to the class *Caudoviricetes*, characterized by tailed virions with icosahedral capsids and dsDNA genomes, and one filamentous phage belonging to the order *Tubulavirales*, characterized by non-enveloped virions with helical symmetry and circular positive-sense ssDNA genomes. The isolated bacteriophages exhibited diverse infection strategies, including lytic, lysogenic, and chronic infection cycles. In addition, host susceptibility without productive infection was observed in some virus–host interactions, suggesting the presence of bacterial defense barriers that affect phage replication. Together, these isolates represent a diverse collection of bacteriophages infecting the RSSC. The diversity of infection cycles and genomic architectures highlights their potential for biotechnological applications, including the development of phage cocktails to overcome bacterial resistance mechanisms. These findings expand current knowledge of phage diversity and viral ecology in agroecosystems and provide important resources for developing sustainable strategies to manage bacterial wilt.

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PT.639

DISTINCT PLAQUE MORPHOLOGIES IN A LYTIC PHAGE POPULATION INFECTING β-LACTAM-RESISTANT *ESCHERICHIA COLI*

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The increasing prevalence of antimicrobial-resistant bacteria is a major global public health concern, reducing antibiotic effectiveness and reinforcing the need for alternative control strategies. Bacteriophages have gained renewed attention because of their ability to specifically infect and kill bacteria, including multidrug-resistant strains. We aimed to isolate and characterize lytic bacteriophages infecting a β-lactam-resistant environmental *Escherichia coli* isolate recovered from Lake Paranoá, Brasília, Brazil. Wastewater samples from Asa Norte were sequentially filtered through 0.45- and 0.22-µm membranes and used for phage enrichment. Lytic activity was detected by spot assay, followed by plaque isolation using the double-layer agar method. Two distinct plaque morphologies were consistently observed: large, translucent plaques and small, opaque plaques. Despite more than ten purification rounds, both morphotypes persisted and could not be separated. Transmission electron microscopy revealed only one morphologically distinguishable type of tailed viral particle with an icosahedral head. Phage DNA was extracted and will be sequenced to determine whether the two plaque morphologies result from distinct bacteriophages or phenotypic variation of a single phage. The phage population remained stable across pH 3-11 and after exposure to organic solvents. Additionally, 13 *E. coli* isolates recovered from the same site are being evaluated for host range, including four biofilm-producing strains. Ongoing experiments are assessing infectivity against these isolates, biofilm reduction, and bacterial load reduction in hospital wastewater. These findings reveal an unresolved pattern of plaque morphology within a morphologically homogeneous phage population and support further genomic investigation and evaluation of its biocontrol potential.

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PT.640

Biodiversity and structure of livestock-associated bacteriophage communities in Brazil and Spain

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Intensive livestock production generates swine manure and broiler litter, which serve as reservoirs for diverse microbial communities. Within these environments, bacteriophages regulate bacterial populations and mediate gene exchange. This study compared bacteriophage communities in 23 Brazilian metagenomes, including broiler litter collected in western Santa Catarina in 2019 (n = 8), 14 biodigester influent/effluent swine-manure samples collected during 2013–2019, and one organic-farm raw-manure sample during 2019 (BioProject PRJEB63971), with 29 publicly available Spanish swine-manure metagenomes from NCBI SRA (PRJNA628671). Sequencing used Illumina NovaSeq 6000 (2 x 151 bp paired-end) and NextSeq 500 platforms for the Brazilian and Spanish datasets, respectively. Reads underwent quality control, host-sequence removal, viral mapping, vOTU clustering, and taxonomic classification. Phage communities were evaluated using alpha and beta diversity metrics, PERMANOVA, PERMDISP, and differential abundance tests. Bacteriophages represented over 98% of detected vOTUs in both datasets and were dominated by Caudoviricetes. Brazilian broiler litter and swine manure differed in phage community composition (PERMANOVA: Bray-Curtis $q = 0.006$; Hellinger $q < 0.001$), whereas biodigester influent and effluent showed no significant differences (all PERMANOVA $q \geq 0.317$). Spanish regions showed no significant differences in alpha diversity but differed in abundance-based beta diversity (Bray-Curtis and Hellinger $q = 0.003$; Aitchison $q = 0.031$). These findings reveal matrix-associated differences in Brazil and regional differences in Spain, despite broad taxonomic similarity at the class level.
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Genome Characterization of Temperate Bacteriophages and Associated Genetic Features in Avian Pathogenic *Escherichia coli* from Brazilian Poultry

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The poultry industry is an important animal protein sector worldwide, with Brazil among the leading producers. *Escherichia coli* is a normal inhabitant of avian intestinal microbiota, and some strains can cause respiratory and systemic infections. Extensive use of antimicrobial agents has created selective pressure, favoring emergence and dissemination of antimicrobial-resistant *E. coli* strains, including extended-spectrum β-lactamase (ESBL) and plasmid-mediated AmpC β-lactamase-producing isolates. While plasmids are recognized as vehicles for the dissemination of antimicrobial resistance genes, temperate bacteriophages have emerged as additional contributors to horizontal gene transfer. Through mechanisms such as transduction and lysogenic conversion, these viruses influence bacterial adaptation and genome evolution. Their role in the spread of resistance genes in poultry-associated bacteria remains incompletely understood. This study analyzed temperate bacteriophages in *E. coli* isolated from femurs of Brazilian broiler chickens, most meeting molecular criteria for avian pathogenic *E. coli* (APEC), using whole-genome sequencing. Because femoral bone marrow is normally sterile, recovery of *E. coli* indicates systemic dissemination and invasive potential. Temperate phages were widespread across APEC genomes and showed ecological structuring, but were not enriched for antimicrobial resistance genes, virulence factors, or plasmid-associated markers. Instead, phage regions were significantly enriched in insertion sequences, suggesting that these elements, rather than resistance determinants, are the main genomic features associated with temperate phages in these isolates. The findings indicate that temperate bacteriophages contribute to genome plasticity and mobilome organization in pathogenic avian *E. coli*, but are not reservoirs of resistance or virulence genes in this setting.

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Discovery of *Orthoflavivirus nelsonfei*, a Novel Species of the Genus *Orthoflavivirus*, Isolated from *Haemagogus janthinomys* Mosquitoes Collected in the Brazilian Amazon

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The genus *Orthoflavivirus* (family *Flaviviridae*) comprises genetically, ecologically and evolutionarily diverse viruses, including those transmitted between arthropods and vertebrates, host-range restricted viruses in insects or vertebrates, and viruses with no known vectors. In this study, we report the discovery of Nelsonfe virus (NFV), representing the novel species *Orthoflavivirus nelsonfei*, isolated from *Haemagogus janthinomys* mosquitoes collected in the Brazilian Amazon. The virus was named in honor of entomologist Nelson Ferreira Fé in recognition of his contributions to Amazonian entomology. Two mosquito pools that tested positive by a pan-orthoflavivirus SYBR Green RT-qPCR assay were subsequently inoculated into C6/36 and Vero cell cultures for virus isolation. Cytopathic effects were observed exclusively in C6/36 cells. Viral RNA from both isolates was subjected to metagenomic Illumina sequencing, and de novo assembly generated two NFV genomes of 10,869 and 10,856 base pairs, each containing a single open reading frame (ORF) encoding a 3,441-amino-acid polyprotein. Pairwise nucleotide identities ranged from 42.5% to 72.6% relative to other orthoflavivirus species, with the highest similarity observed with Guapiaçu virus. Phylogenetic analyses placed NFV within the insect-specific orthoflavivirus (ISOF) clade, specifically within the dual-host-associated insect-specific orthoflavivirus (dISOF) lineage, where it was identified as a new member of this group. Therefore, based on genomic divergence and phylogenetic placement, NFV represents a novel species within the genus *Orthoflavivirus*. The identification of NFV expands the known diversity of orthoflaviviruses and provides insights into their evolution, highlighting the importance of integrated virological and genomic approaches for exploring viral diversity.

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VIRAL SURVEILLANCE IN *APIS MELLIFERA* COLONIES: INVESTIGATION OF THE OCCURRENCE OF MULTIPLE VIRAL PATHOGENS ASSOCIATED WITH COLONY HEALTH

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Growing concern over the “pollinator crisis” has highlighted the decline of managed honey bee and native bee populations in recent years, which has been associated, among other factors, with the increasing occurrence of viruses that compromise colony health, productivity, and survival. Herein, our goal was to monitor viral pathogens in *Apis mellifera* across different seasons and landscapes. Initially, was investigated the occurrence of viruses in *Apis mellifera* colonies during the rainy season. A total of 35 colonies were analyzed, including two colonies from Mirassolândia, São Paulo (SP) (comprising four pools of 20 individuals per colony) and 33 colonies from Engenheiro Coelho-SP, distributed across orange orchards, eucalyptus plantations, and native forest areas, with pools consisting of 25 individuals. Samples were homogenized in chilled PBS, filtered, and subjected to ultracentrifugation for viral particle clarification and concentration. RNA was extracted using the QIAamp kit. RT-PCR was performed using specific primers targeting pathogenic bee viruses reported in the literature, and amplicon were confirmed by Sanger sequencing. Among the colonies evaluated, *Acute bee paralysis virus* was detected in five colonies (14.3%), including one colony from Mirassolândia and four from Engenheiro Coelho. *Sacbrood virus* was identified in two colonies located in orange-growing areas, whereas *Black queen cell virus* was detected in two colonies, one from an orange orchard and the other from a eucalyptus plantation. These preliminary findings suggest greater viral circulation in anthropogenically modified landscapes and highlight the need for continuous monitoring to better understand the ecology and dynamics of viral pathogens in bee populations.

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PRELIMINARY INSIGHTS INTO THE EUROPEAN HONEY BEE VIROME USING VIRAL METAGENOMICS IN SÃO PAULO, BRAZIL

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Pollinators play a crucial role in ecosystem stability and global food security, and the European honey bee (*Apis mellifera*) is among the most important pollinator species. However, colony health is increasingly threatened by pathogens, particularly viruses. Despite their importance, the diversity, distribution, and impact of viral pathogens associated with pollinators remain poorly characterized, especially in Brazilian populations. Viral metagenomics based on next-generation sequencing (NGS) provides a powerful approach for comprehensive discovery and characterization, enabling the investigation of complex viral communities. This ongoing study aims to characterize the viral diversity associated with the European honey bee collected from urban, semi-urban, and native forest environments in São José do Rio Preto, Mirassolândia, and Engenheiro Coelho, Brazil. Samples will be analyzed through bioinformatics and phylogenetic approaches and subsequently validated by PCR and Sanger sequencing. Additionally, selected viruses of epidemiological interest will be investigated for subsequent in vitro isolation using AmE-711 cells. As an initial validation step of the proposed workflow, a PCR-positive *Apis mellifera* sample for Acute Bee Paralysis Virus (ABPV) was analyzed by viral metagenomics. The complete ABPV genome (9,665 bp) was recovered with 76,563 mapped reads and a mean sequencing depth of 1,516.83×. The analysis also revealed the partial genomes of Lake Sinai Virus and Apis rhabdovirus 1. These findings demonstrate the efficiency of the workflow and highlight viral metagenomics as a valuable tool for uncovering honey bee-associated viromes and supporting viral surveillance programs in Brazil.

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THE PROBLEM OF VIRUS DEFINITION IN HIGHER EDUCATION TEXTBOOKS

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The definition of a virus remains a significant gap in biology, creating challenges for teaching and learning in the field of virology. Therefore, we analyzed how viruses are defined in virology textbooks used in higher education in Brazil. To do this, we examined and classified virus definitions found in virology and microbiology textbooks available at the Federal University of Western Bahia library, prioritizing the most recent editions. We also compared these definitions with others, such as the one used by the International Committee on Taxonomy of Viruses. Among the ten books evaluated, three presented definitions based exclusively on physical properties, and only two offered negative definitions, while five provided hybrid definitions. Most authors relied on cell theory to define viruses as non-living, acellular organisms; however, only three books classified viruses based on these characteristics, and only one identified the virus as a living entity. Thus, we believe there is a need to revise the concept of the virus in higher education textbooks. A clear definition of a virus is essential to avoid semantic issues during the teaching and learning process, particularly in the field of virology.

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SCIENCE COMMUNICATION: COVID-19 NETWORK INICIATIVES TO BRING SCIENCE AND SOCIETY CLOSER TOGETHER

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The “Observational Study Network for Monitoring the Effectiveness and Safety of COVID-19 Vaccination in Brazil and the Natural History of the Disease in Children and Adolescents” (COVID-19 NETWORK), structured into five axes and developed between 2021 and 2026, followed over 13,000 participants and analyzed 158 million vaccination records and 2 million records of severe COVID-19 cases. In addition to generating evidence on vaccine effectiveness and safety, the Network developed science communication initiatives to expand access to information, strengthen public trust in vaccines, and combat health disinformation. This study presents initiatives and products developed to disseminate research findings in accessible language, addressing vaccination and immunization with an emphasis on COVID-19 vaccines. To achieve this, a communication plan was structured along three main lines: digital, educational, and institutional. The visual identity of the COVID-19 NETWORK was created and applied across all products. Within the digital line, videos, social media content, and the podcast “Between Science and Time” (composed of ten episodes on the studies featuring participating researchers) were produced. The educational line, aimed at children, healthcare professionals, and educators, included educational booklets, games and also ludic activities which were carried out during eight science communication events. The institutional line compiled executive summaries and descriptive texts about the studies. To date, the materials described here have been presented at 8 regional and national events, reaching approximately 8,000 people from diverse audiences and demonstrating the broad dissemination and societal reach of these initiatives.
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SCIENCE COMMUNICATION IN VIROLOGY: ENHANCING VISIBILITY AND ENGAGEMENT THROUGH DIGITAL MEDIA

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LAFEM (Laboratory of Pharmacogenomics and Molecular Epidemiology) supports the diagnosis of viral infections in southern Bahia, Brazil, and uses social media-based science communication as a key component of its university extension activities to expand public knowledge of virology and related themes. The laboratory produces weekly Instagram content in two formats: educational posts addressing current topics and posts summarizing relevant scientific articles presented by laboratory members. Communication strategies have included a standardized visual identity, regular posting schedules and coverage of timely topics, especially related to fake news and viral disease outbreak. Reach and engagement were monitored using data from the Instagram Professional Dashboard. Over the last 90 days, the profile, with 1,305 followers, achieved 92,080 views from 20,268 accounts, of which 40.5% were followers and 59.5% were non-followers, generating 1,851 interactions. Among the best-performing posts, the publication on hantavirus reached 2,400 views, followed by posts addressing misinformation about the dengue vaccine (1,700 views) and misinformation linking vaccines to autism (1,300 views). Posts on HPV, congenital syphilis, and Ebola each received approximately 1,000 views. These findings indicate that infectious disease and vaccines topics are highly dynamic, heterogeneous and generate significant interest. Besides, the adopted communication strategies have important visibility and engagement, as shown by the data above. However, the high proportion of views from non-followers highlights the ongoing challenge of encouraging audiences to follow scientific profiles. The project received financial support from the institution’s Pro-Rectorate for Extension (PROEX).

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THREE-DIMENSIONAL MODELS AS AN EDUCATIONAL STRATEGY FOR RABIES AWARENESS

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Rabies is a zoonotic disease caused by the *Rabies lyssavirus* (formerly *Lyssavirus rabies*), with an almost 100% case-fatality rate once clinical signs appear, although it is preventable through prophylactic measures. Despite this, significant gaps remain in public knowledge regarding its risks and modes of transmission. In educational settings, three-dimensional (3D) models are widely recognized as valuable teaching tools. Previous studies have reported improvements in visual and tactile learning, knowledge retention, mental rotation skills, and satisfaction among both students and educators. This study aimed to report the experience of using 3D models of the *Rabies lyssavirus* structure to teach and raise awareness among elementary and high school students about the importance, risks, and prevention of rabies. Two 3D printers were used to produce the models. The first was a resin printer (Anycubic Photon Mono 4K), using both standard resin and water-washable resin. The second was a fused deposition modeling (FDM) printer (Creality Ender 3), using polylactic acid (PLA) filament as the printing material. The viral models were presented in seven public elementary and high schools in the state of Pernambuco, Brazil, where they served as visual aids during lectures and educational activities on rabies. At the end of each session, students were encouraged to recreate the virus using modeling clay and EVA foam, reinforcing learning through a playful, hands-on approach. The results indicate that combining 3D models with interactive educational activities enhances awareness of rabies and represents a low-cost, easily replicable strategy for use in public schools.