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PT.001

Effect of RECK protein on DNA repair in HPV-transformed cells

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Human papillomavirus (HPV)-associated malignancies, mainly cervical cancer, are a global public health problem, especially in developing regions such as Latin America. HPVs causes approximately 5% of all human cancers including almost 100% of all cervical, 88% of anal, 78% of vaginal cancer, 25% of vulvar cancer, 51% of penile carcinomas and a proportion of head and neck cancer that depends on the specific anatomical area and geographic region. Currently, a prophylactic vaccine is available for the management of HPV associated malignancies, although its implementation on a large scale has been a challenge. Likewise, the existing vaccine has no effect on existing or advanced infections. Therefore, it is essential to search for new and efficient therapeutic alternatives, including for already established infections. Previous studies from our laboratory found that some proteins involved in DNA repair, namely ATM, ATR and CHK2, are critical for the survival and oncogenic potential of HPV-transformed cells. Besides, we reported that the tumor suppressor protein RECK is consistently down-regulated in HPV-associated tumors and that its' over-expression reduces the tumorigenic potential of HPV-transformed cell lines. Therefore, the aim of our project is to investigate the effect of RECK protein overexpression on some important repair pathways in the HPV life cycle. For this purpose, cell lines that super-express RECK was treated with inhibitors of ATM, ATR and CHK2 proteins and their viability and clonogenic potential was evaluated. The results obtained may contribute to establishing a new therapeutic role for RECK in HPV-associated tumors and will be presented during the congress.

PT.002

ASSESSMENT OF MURINE NEUTROPHIL RESPONSE DURING OROV INFECTION

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Oropouche (OROV) is an emerging virus associated with outbreaks of rash febrile illness in the Brazilian Amazon region and in other countries in South America. More than 30 OROV fever outbreaks have been documented in Brazil with more than 500,000 suspected cases. OROV is neurotropic in animal models and can cause encephalitis and meningitis in humans, and despite the great impact, little is known about the pathogenic aspects that guide the development of the disease and neuroinvasion. Circulatory neutrophils seem to be the main cell type that drives the development of encephalitis by other arboviruses, raising the possibility that these cells serve as a Trojan horse in a process of neuroinvasion. We decided to characterize the effects of OROV replication on neutrophil activation and function, as well as the role of these cells in the protection and/or pathogenesis of this infection. For this, we analyzed cell viability, viral replication, and release of extracellular neutrophil traps (NETs) ex vivo and cell migration in C57BL/6 mice after OROV infection in a subcutaneous chamber. So far, results indicated that neutrophils migrate and are infected by OROV in the site of infection. *In vitro* infection showed that neutrophils are susceptible to infection. The virus induced the death of neutrophils, and does not lead to the activation of some classic defense mechanisms of these cells, such as the release of NETs and granular proteins (myeloperoxidase), and production of reactive oxygen species (ROS).

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PT.003

In vitro evaluation of the anti-herpetic activity and modulation of inflammatory mediators of *Salvia uliginosa*

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The Herpes Simplex Viruses (HSV) cause recurrent symptoms throughout life, from oral and genital infections to severe complications like encephalitis. It is estimated that billions worldwide are infected with HSV-1 or HSV-2. HSV-2 infection can lead to genital ulcers, with the possibility of reactivation under stress or immunosuppression. The immune response is crucial in defense, coordinating cytokine production that limits viral replication. Despite antiviral treatments like acyclovir, resistant strains highlight the need for alternatives. Plants of the Salvia genus, rich in bioactive compounds like terpenes, have been studied for their medicinal properties. This project aimed to assess the *in vitro* antiviral activity of *Salvia uliginosa* against HSV-2 and analyze its impact on inflammatory mediators, exploring new treatment perspectives. The study involved plaque assays to verify the virucidal effect, pre- and post-treatment, and modulation of pro- or anti-inflammatory cytokine production in macrophages. Results showed that *S. uliginosa* exudate has significant antiviral activity, inhibiting HSV-2 replication in a dose-dependent manner in direct virucidal, pre-treatment, and post-infection assays. However, Icetexone, the major compound in the exudate, did not exhibit any effect in either assay. This suggests other compounds in the exudate contribute to the antiviral effect, possibly interfering with viral adsorption. Additionally, the exudate did not cause a significant increase in IL-1β and IL-10 cytokine production, indicating an absence of direct immunomodulatory stimulation. Further assays are needed to fully understand the exudate's immunomodulatory potential. These results underscore the potential of *S. uliginosa* extracts as effective therapeutic agents.

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PT.004

GENETIC DIVERSITY AND IN VITRO ADAPTABILITY OF YELLOW FEVER VIRUS (YFV) VACCINE STRAIN 17DD AT INTRA-HOST AND INTER-HOST LEVELS.

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The yellow fever virus (YFV) presents high genetic variability, infecting both vertebrate and invertebrate hosts. Additionally, the 17DD vaccine strain corresponds to a heterogeneous viral pool, presenting phenotypically distinct plaques. This study aims to analyze the genetic diversity and adaptive dynamics of the YFV vaccine strain by mimicking, *in vitro*, inter and intrahost infection cycles. Initially, a plaque purification assay was performed, isolating small (<2mm) and large (>2mm) plaque variants. Biological characterization of the isolates showed morphological changes in the smaller plaque variant, which was identical in size to the larger plaque, as well as a similar viral titer (smaller plaque 10.10^4 PFU/ml vs large plaque $11,7.10^5$ PFU/ml). Subsequently, consecutive infections were initiated with each isolate in *Aedes aegypti* embryonic lineage (Aag2) and human hepatocarcinoma lineage (HepG2), representing intrahost cycles, totaling 5 cycles. After 120 hours, the supernatant was collected, titrated and stored, with one cycle performed on each cell so far. For both isolates, the first passage in HepG2 didn't show plaque lysis during titration, suggesting a loss of infectivity influenced by cell selective pressure and possible transcomplementation between the isolates. To confirm this, HepG2 cells were infected with the viral pool prior isolation and coinfected with the two isolates to investigate recovery of infectivity. In parallel, the interhost infection cycle was initiated for each isolate, alternating between human and mosquito cells. In the future, each YFV isolate collected before, during and after each cycle will be submitted to NGS, evaluating emerging mutations and their impact on viral fitness.

Financial support: CAPES/CNPq

PT.005

Development of a cell line from the bat *Desmodus rotundus* and its susceptibility to infection by *Lyssavirus rabies* (RABV), *Betacoronavirus pandemicum* (SARS-CoV-2), and some arboviruses.

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New World bats have been shown to carry several viruses of health importance. This study aimed to develop a cell line from the hematophagous bat *Desmodus rotundus* and evaluate its susceptibility to *Lyssavirus rabies* (RABV), *Betacoronavirus pandemicum* (SARS-CoV-2), and some arboviruses. Primary cultures of *D. rotundu*s fetal kidney cells were transformed with the plasmid pSV1 containing the *Large T* and *Small T* antigen gene sequences of *Simian Virus 40* (SV40) for immortalization. The clones obtained were selected by limiting dilution and transformation was confirmed by PCR for SV40, resulting in a cell line named FKDR (*Fetal Kidney Desmodus Rotundus).* The karyotype of the primary and immortalized cell cultures was determined. FKDR cells were infected with *Lyssavirus rabies* (RABV), *Alphavirus mayaro* (MAYV), *Alphavirus chikungunya* (CHIKV), *Orthoflavivirus dengue* (DENV-2), *Orthoflavivirus zikaense* (ZIKV), and *Betacoronavirus pandemicum* (SARS-CoV-2). Susceptibility was measured by observation of cytopathic effect and staining by immunoperoxidase or direct immunofluorescence using virus-specific antibodies. The karyotype of the primary cell culture showed diploid cells with 28 chromosomes. Once immortalized, the cell adopts a fusiform appearance. The karyotype of FKDR cells showed 54–56 chromosomes, these anomalies are typical of SV40 immortalization. Infection of FKDR with *Lyssavirus rabies* did not show a cytopathic effect but showed positive staining by immunofluorescence. All arboviruses and coronaviruses tested in this study induced cell rounding, lysis, and positive immunoperoxidase staining. The FKDR cell is a valuable tool for studying the pathology and biology of these viruses in *D. rotundus* bat.

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PT.006

GOLD(I) COORDINATED COMPOUNDS AS A POTENTIAL CLASS OF ANTIVIRAL DRUGS AGAINST MAYARO VIRUS

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Introduction: Mayaro virus (MAYV) is an arbovirus responsible for Mayaro fever (MAYF), a neglected emerging disease that causes dengue-like symptoms. Aggravatingly, the infection can develop into chronic arthralgia and polyarthralgia conditions. Currently, there are no vaccine or specific against MAYF. Here, we assessed the antiviral potential of new gold(I) [Au(I)] coordinated compounds against MAYV. **Methods**: Vero-E6 cells were treated with the highest non-cytotoxic concentration of Au(I) compounds in the presence or absence of MAYV-expressing *nanoluciferase* (MAYV-*nanoluc*). Dose-response assay was performed with concentrations of compounds ranging from 0,39 to 50 µM. After 24h, viral replication was measured using *renilla*- luciferase assay and cell viability was evaluated by MTT assay. Time of drug-addition assays were also performed to investigate the effects of the compound on different stages of MAYV replicative cycle. **Results:** Au(I)-1 at 10 µM and Au(I)-2, Au(I)-3, Au(I)-4, Au(I)-5 at 2 µM inhibited MAYV replication in 76%, 84%, 81%, 90%, and 84%, respectively. In the dose-response assay, compounds Au(I)-4 and Au(I)-5 presented highest selectivity index (SI) of 9.0 and 8.9, while Au(I)-1, Au(I)-2 and Au(I)-3 obtained SI of 2.4, 3.1 and 1.8, respectively. The strongest activity of Au(I) compounds was on post-entry stage, in which all compounds were able to inhibit viral activity in up to 98% (Au(I)-1: 80%; Au(I)-2: 48%; Au(I)-3: 98%; Au(I)-4: 86%; Au(I)-5: 97%). **Conclusion:** Data presented here demonstrated the potential of Au(I) coordinated compounds against MAYV, making it an interesting source of molecules for the development of future antiviral drugs to manage Mayaro fever.

Financial Support: FAPEMIG, CAPES, CNPq, FAPESP

PT.007

ANTIVIRAL ACTIVITY OF PALLADIUM(II) COORDINATED COMPOUNDS AGAINST MAYARO VIRUS

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Introduction: Mayaro Fever is an emerging negligent disease that causes hyperthermia, muscle pain, and skin rashes. Additionally, arthralgia and polyarthralgia symptoms can persist for months or years after the infection. The etiologic agent of this disease is Mayaro virus (MAYV), which can be transmitted by the bite of mosquitoes of the genus *Haemogogus sp* and *Aedes sp*. The wide distribution of these vectors in Latin America favors the endemic nature of the infection in this region. Aggravatingly, there are no vaccine or antiviral drugs against MAYV infection. Here we investigated the antiviral activity of palladium(II) coordinated compounds Pd(II)-1, Pd(II)-2, and Pd(II)-3 against MAYV infection. **Materials and methods**: Dose-response assays were performed with Pd(II)-1, Pd(II)-2, and Pd(II)-3 in presence or absence of MAYV expressing the nanoluciferase gene (MAYV-*nanoluc*) at a Multiplicity of infection of 0.1 for 24 h. 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. **Results:** Pd(II)-1, Pd(II)-2, and Pd(II)-3 showed selectivity indexes of 6.4, 10.3 and 3.7, respectively. In time of drug-addition assay Pd(II)-1 and Pd(II)-2 inhibited viral activity >80% and >90% in virucidal, entry and post-entry stages of infection, respectively. Alternatively, Pd(II)-3 impaired MAYV activity >73% in virucidal and post-entry steps. **Conclusion:** Pd(II) coordinated compounds demonstrated an interesting anti-MAYV activity *in vitro*, presenting potential as source of molecules for the development of novel antivirals against MAYV.

Financial Support: FAPEMIG, CAPES, CNPq, FAPESP

PT.008

HETEROLOGOUS DENGUE INFECTION OF IMMUNOCOMPETENT MICE LEADS TO MULTIORGAN DAMAGE

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In dengue, severe outcomes seemed to be related to heterologous infection with the dengue virus (DENV) and particularly to types -2 and -3. To study those aspects, most of the proposed murine models resort to neonatal, humanized or immunodeficient mice or significantly diverge from infections as they occur naturally, such as use of neuroadapted viruses, lethal doses and invasive routes of inoculation (e.g. intracerebral). Considering this, our aim was to develop a model of immunocompetent mice intravenously inoculated with clinical non-neuroadapted strains of DENV. Two-month-old BALB/cJ mice were infected with 10^4 PFU of DENV-3 and, two months later, infected with 10^4 PFU of DENV-2. The negative control group was MOCK-inoculated. After 3, 7, 10, 14 or 21 days after infection, mice were euthanized (N=5/time). The organs (liver, kidneys, spleen, lungs, heart, brain and cerebellum) were collected, fixated in formalin, dehydrated in alcohol, clarified in xylene and embedded in paraffin wax. The histological slides were stained with hematoxylin and eosin for histopathological and morphometric analyses. All organs showed some level of vascular impairment, such as vascular congestion and thrombi formation, except the heart that show no critical damage. Necrosis was relevant in hepatocytes and renal tubular cells. Inflammatory infiltrate in lungs lead to massive alveolar septa thickening. In spleen, the most prominent alteration was the red pulp hyperplasia. Despite the formation of clots in the braincase of some animals, histologically the changes in brain and cerebellum were moderate, including neuron death and disorganization of Purkinje cells.

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PT.009

ULTRASTRUCTURAL CHARACTERIZATION OF SECONDARY DENGUE INFECTION IN MICE TISSUES

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Among many gaps in knowledge about dengue, the effects of the infection on organs are undoubtedly a highlight. However, studies focusing on the ultrastructural aspects of these effects are lacking. Our aim was to characterize some alterations in an immunocompetent murine model for secondary dengue virus (DENV) infection with aid of transmission electron microscopy (TEM). For this purpose, two different groups were evaluated: primary infection by DENV-1 and secondary infection by DENV-2 and primary infection by DENV-3 and secondary infection by DENV-2. Two- month-old BALB/c mice were intravenously infected with clinical dengue virus strain and, two months later, infected again with the heterologous serotype. After 3 days after infection, mice were euthanized (N=5/time). For TEM analysis, the tissue samples (liver, lung and heart) were fixed in glutaraldehyde 2,5%, post-fixed in osmium tetroxide 2%, dehydrated in acetone and embedded in epoxy resin. The ultrathin sections (50-70 nm) were obtained and picked up using uncoated copper grids to be observed. In both lung and heart, there was intense trafficking of transport vesicles in endothelial cells, suggesting its activation. In the liver, hepatocytes with expanded lysosomes, autophagosomes and thickening of rough endoplasmic accompanied by numerous thicker electron-dense ribosomes were observed, as well as discoid platelet circulating in capillary. In the heart, rarefied mitochondria and disorganization of intercalated discs, as well as disorganization of cardiac fibers and DENV-like particles were observed. These findings underscore the importance of studying the ultrastructural aspects in DENV infection, complementarily to other morphological evaluation techniques.

Financial Support: CAPES, FAPERJ.

PT.010

ELUCIDATING MONKEYPOX VIRUS MORPHOGENESIS THROUGH DIFFERENT APPROACHES OF ELECTRON MICROSCOPY

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Monkeypox, caused by monkeypox virus (MPXV), led the World Health Organization to declare a global public health emergency on July 23, 2022, due to an unprecedented outbreak. Several aspects of MPXV replicative cycle remain unknown, representing an obstacle for the area of drug repositioning. With this thought in mind, we carried out ultrastructural studies using electron microscopies to describe the step-by-step morphogenesis of MPXV. In this study, Vero cells were infected with a MPXV strain obtained from an oropharyngeal swab of a patient suspected of monkeypox. This sample was collected during an observational cohort study conducted in Rio de Janeiro, Brazil, from June to August 2022. At 6 days post-infection (dpi), for Transmission Electron Microscopy (TEM) and Scanning-TEM, the cells were fixed in glutaraldehyde, post-fixed in osmium tetroxide, dehydrated in acetone and embedded in epoxy resin. With aid of Focused Ion Beam (FIB) Scanning Electron Microscopy, after 1200 sections were taken under the FIB, the cellular structures were manually segmented, and the image series were aligned for 3D reconstruction. For Helium Ion Microscopy, the cells were fixed at 3 dpi, dehydrated in ethanol, submitted to critical-point-drying and carbon-coating. Infected cells exhibited cell lysis plaque formation, altered chromatin profiles, rough endoplasmic reticulum (RER) thickening, myelin figures, and mitochondrial and RER disorganization around viral factories. Virus entry occurred through endocytosis, with MPXV particles adhering to cytoskeletal filaments, and progeny were extruded via exocytosis. These techniques collectively shed light on virus-cell interaction and alterations throughout the MPXV morphogenesis.

Financial Support: CNPq, FAPERJ.

PT.011

SARS-CoV-2 SPIKE-INDUCED CELL CHANGES

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COVID-19 pandemic was the most relevant global public health emergency of the last century. COVID-19 is caused SARS-CoV-2, a positive-sense single-stranded RNA virus, and its entry into permissive cells occurs through the binding of the receptor-binding domain (RBD) of the Spike protein (SP) to the angiotensin-converting enzyme 2 (ACE2). Therefore, this study aims to analyze the effects of SARS-CoV-2 recombinant SP in cells, following binding and internalization. Therefore, VERO E6 (African green monkey kidney epithelial cells) and HUVEC cells (human umbilical cord endothelial cells) were cultured and subjected to cell viability assays using the MTT colorimetric method, after treatment with the SP at seven different concentrations (100 to 1.5 µg/mL, 1:2 ratio) over 30 min, 1, 2, 3, 4, and 20 h. Subsequently, binding and internalization were assessed by flow cytometry (S protein at 50 µg/mL for 30 minutes). MTT results revealed that the cell viability was not affected in both cell lines, preserving the levels of living cells above 90% across all tested conditions. Moreover, the results indicate that both cell types are susceptible to the Spike protein. In HUVEC cells, uniform levels of binding and internalization were observed, while VERO cells exhibited higher binding than internalization. In summary, the findings suggest that the Spike protein can bind and internalize in these cell lines without affecting their viability. Further research is ongoing, focusing on measuring metabolic alterations in these cells by analyzing LDH, glucose, and lactate levels.

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PT.012

LIPID METABOLISM MODULATION AGAINST CHIKUNGUNYA VIRUS INFECTION

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In 2024, Chikungunya affected over 180,000 brazilians, a 67% increase compared to the same period in 2023. Chikungunya is caused by the Chikungunya virus (CHIKV), and its main symptoms include fever, arthralgia, and myalgia, which can persist for months or even years post-infection. Our group recently demonstrated that adipose tissue is an extrapulmonary target for SARS-CoV-2 infection; however, the CHIKV infection of adipocytes has not been reported so far. In the present study, we found that human adipocytes from the A41 lineage are susceptible and permissive to CHIKV infection. Human adipocytes show an approximately $6x10^3$-fold increase in viral production after 24 hours of infection, reaching a peak of 1.05×10^8 PFU/ml that sustains up to 48h, as shown by plaque assay. We also characterized the dynamics of lipid droplets in CHIKV-infected human adipocytes by immunofluorescence. Additionally, CHIKV infection induced lipolysis in mature adipocytes as shown by a 3.4-fold increase in HSL protein phosphorylation 24h post-infection detected by western blot. Now, we intend to investigate whether the modulation of lipid metabolism observed in CHIKV-infected human adipocytes also occurs in skeletal muscle cells from C2C12 cell line. Skeletal muscle tissue plays a fundamental role in the development and progression of disease and is also central to one of the main symptoms and sequelae of CHIKV infection: myalgia. Thus, we aim to contribute to a better understanding of the metabolic impacts of CHIKV infection in muscle cells and investigate the role of lipid droplets in this context.

Financial support: CAPES, FAEPA, FAPESP

Key words: Chikungunya, lipid droplets, skeletal muscle

PT.013

COORDINATION WITH PALLADIUM, PLATINUM OR ZINC INCREASES ANTIVIRAL ACTIVITY OF PYRIDINEMETHANAMINE AGAINST MAYARO VIRUS

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Introduction: Mayaro virus (MAYV) is the etiological agent of Mayaro fever, characterized by dengue-like symptoms. Additionally, the infection can progress to a chronic condition in over 50% of patients, characterized by severe and prolonged arthralgia. Nonetheless, there are no specific antiviral drugs or vaccines available against MAYV, 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 palladium (Pd), platinum (Pt), and zinc (Zn) coordinated to pyridinemethanamine (BBAMP) against MAYV. **Methods:** BBAMPHCl, PdBBAMP, PtBBAMP, and ZnBBAMP were screened for their cytotoxicity in Vero-E6 cells. Then, the highest non-cytotoxic concentration of each complex was selected to be evaluated on MAYV replication using the MAYV-*nanoluciferase*. Dose-response assay was performed with active compounds at concentration ranging from 2.34 to 300 µM, in the presence or absence of MAYV-*nanoluc*. Cell viability and viral infectivity were evaluated through MTT and luminescence assays, respectively. **Results:** BBAMPHCl, PdBBAMP, PtBBAMP, and ZnBBAMP at 10 µM reduced viral replication by 31%, 50%, 34%, and 44%, respectively. In dose-response assays, the complexes had EC_{50} of 20.1 µM, 20.7 µM, 11.4 µM and 10.5 µM, and selectivity indexes of 11.6, 9.1, 16.7, and 68.9, respectively. **Conclusion:** The data presented here indicates the anti-MAYV potential of metal complexes, considering that coordinated compounds displayed strong activity against MAYV replication, representing promising candidates for the development of future antiviral drugs.

Financial support: FAPEMIG, CNPq, CAPES, FAPESP.

PT.014

MAYARO VIRUS IS STRONGLY INHIBITED BY COPPER(II) COMPLEXES

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Introduction: Mayaro virus (MAYV), an arbovirus from the *Togaviridae* family, is the causative agent of Mayaro fever, a disease characterized by dengue-like symptoms that can progress to a chronic condition. Further, there is no approved antiviral drug against MAYV, being the current treatment based on controlling the symptoms. Metal complexes have been described by their therapeutic potential against different viruses, representing promising sources for the development of antivirals. The present work aimed to evaluate the anti-MAYV activity of copper (Cu) complexes with 2′-hydroxy-4-benzyloxychalcone analogues (BC and BCF). **Methods:** The cytotoxicity of BC, BCF, CuBC, CuBCF, and CuBCPhen complexes was evaluated using Vero-E6 cells, and the highest non-cytotoxic concentration of each complex was selected for antiviral assays using MAYV-*nanoluciferase*. Compounds were subjected to dose-response assay at concentration ranging from 2.34 to 300 µM (BC, BCF and CuBC), 1.17 to 150 µM (CuBCF), or 0.39 to 50µM (CuBCPhen), in the presence or absence of MAYV-*nanoluc*. **Results:** BC (10 µM), BCF (50 µM), and CuBC (50 µM) reduced viral replication in 67%, 58% and 89%, respectively, while CuBCF (2 µM) and CuBCPhen (0.4 µM) decreased viral replication by 40% and 51%, in this order. BC, BCF, CuBC, CuBCF, and CuBCPhen had an EC_{50} of 14.8 µM, 269.6 µM, 26.8 µM, 18.9 µM, and 6 µM, resulting in selectivity indexes of >20.3, >1.1, >11.2, >7.9, and 8.2, respectively. **Conclusion:** The data presented here highlight copper complexes as promising candidates for the development of future antiviral drugs against MAYV.

Financial support: FAPEMIG, CNPq, CAPES, FAPESP.

PT.015

Role of IRF5 in dendritic cells for Mayaro virus infection in a murine model

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Climate and environmental changes caused by anthropological actions have intensified contact of humans with new viruses in recent decades. In Brazil, Mayaro virus (MAYV) is spreading from the Amazon region and it is probable that it can emerge in other regions of America. MAYV infection induces an arthritogenic febrile illness similar to Chikungunya. The control of infection by these viruses is usually related to innate immunity with a large participation of APCs (antigen-presenting cells) and production of type I interferon by activation of interferon regulatory factors, such as IRF5. The aim of this work is to investigate the role of IRF5 for MAYV restriction, analyzing mortality, morbidity and viral tropism in $CD11c^{cre+}Irf5^{f/f}$ MAYV-infected mice in comparison with control animals. Interestingly, about 30% of the Irf5 KO mice were vulnerable to MAYV infection. It succumbed to 20 dpi and showed a high viral load in the brain and a significantly increased paw swelling. In addition, part of survivors revealed a high viral load in several sites, such as paw, inguinal lymph nodes and kidney, even after 40 days after infection. Thus, the results indicate that IRF5 in DCs is essential for controlling the viral replication and inflammation during the initial and chronic phases of MAYV infection in murine model.

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PT.016

EFFECT OF *PHTHORIMAEA OPERCULELLA GRANULOVIRUS* INHIBITOR OF APOPTOSIS PROTEINS ON INSECT CELL SURVIVAL AND INFECTION

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Keywords: *Phthorimaea operculella granulovirus*, PhopGV, baculovirus, IAP, apoptosis, cell viability

The potato, *Solanum tuberosum*, is a globally important crop. The main pest is the caterpillar *Phthorimaea operculella*, from the Gelechiidae family and Lepidoptera order. Baculoviruses exploit cellular machinery for their benefit, one of these mechanisms is the interference with the apoptosis pathway. In this study, we aimed to characterize *in vitro* the activity of apoptosis inhibitor proteins (IAPs) from the baculovirus *Phthorimaea operculella granulovirus* (PhopGV). These proteins, in other baculoviruses, inhibit apoptosis, promoting viral replication and boosting viral particle production. To characterize the activity of these genes, we constructed recombinant baculoviruses with the apoptosis inhibitor gene p35 deleted to express the IAP1 and IAP5 proteins from PhopGV. We used these viruses to analyze the effects of expressing these proteins in different insect cell lines: Sf21, Tn5B, and UFLAg, over a 96-hour post-infection period. Initially, we examined morphological changes and cell death using light microscopy. Based on the kinetics of IAP expression, the UFLAg cell line exhibited a cell viability rate of only 13%; in Sf21 cells, viability was 62%, while in Tn5B cells, it was 91%. Subsequently, these viruses will be used to evaluate the effects of these proteins in mitochondrial cell viability assays, caspase 3/7 activities, and cellular DNA fragmentation. Analyzing the differences in the progression of infection by the recombinant viruses, we found that the IAPs alone are not sufficient to completely inhibit apoptosis in insect cells, and their activity is cell line-dependent, suggesting that the inhibitory activity is dependent on the virus-host interaction.

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PT.017

GAMMA DELTA T CELLS ARE CRUCIAL PREVENTING SEVERE PATHOGENESIS BY ALPHAVIRUS MAYARO.

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Historically, among the three arboviruses proposed as candidates for the next arboviral epidemic in the Americas, one of them is Alphavirus mayaro (MAYV). This RNA virus causes an exanthematous disease that can lead to chronic symptoms such as arthritis and myositis. As a challenge, searching for pathogenetic aspects of arboviruses is necessary to discover factors related to the severity of symptoms. To determine whether the protective response against the Mayaro virus is related to T cells or B cells, we sought to study the pathogenesis of the Mayaro virus in the TCR gamma delta knockout murine model. We measured the production of pro- and anti-inflammatory cytokines by protein quantification using Luminex technology. We measured the activation of the cellular antiviral response's components pathway by mRNA quantification using RT-qPCR with Sybr Green probes. We evaluated viral tropism using RT-PCR with TaqMan probes and also titration. We established signs of morbidity and mortality. Our results demonstrate factors associated with morbidity and death in the model are the absence of gamma delta T cells plus an imbalance of inflammatory mediators without weight loss in the individuals. 38.65% of the individuals presented sudden death and viable viruses in the brain tissue. More notably, our results obtained markers linked to arthritis in TCRdelta ko mice survivors after evaluation forty days post-infection. Overall, these preliminary insights show that gamma delta T cells are one of the targets for studying the severity of this disease and possible future therapeutic targets.

Financial support: FAPESP

PT.018

Antiviral Potential of Chondroitin Sulfate-Coated Iron Oxide Nanoparticles Against Dengue Virus

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Arboviruses, particularly the Dengue virus, are responsible for significant global morbidity and mortality, with severe impact in tropical regions. Despite advancements in pharmaceuticals, there are still no effective antiviral treatments for dengue, with current therapies focusing solely on symptom relief. This underscores the urgent need for the development of specific antiviral therapies. Iron oxide nanoparticles (IONPs) have gained attention due to their unique properties and antiviral potential. Studies indicate that IONPs can inhibit viral entry, interfere with viral replication and assembly, and reduce viral infectivity. Coating IONPs with biomolecules such as chondroitin sulfate, known for its antiviral properties, may enhance these effects. This study explores the antiviral potential of chondroitin sulfate-coated IONPs for dengue treatment. The IONPs were synthesized by co-precipitation and characterized, showing an average size of 20.7 nm, zeta potential of -37.8 mV, and a polydispersity index of 27.81. To assess their antiviral effect, the cytotoxic concentration (CC50) was first determined in Vero CCL-81 cells using the MTT assay. Cell viability was measured after 5 days of exposure to IONPs at concentrations ranging from 0 to 1600 µg Fe/ml. The IONPs exhibited low toxicity, maintaining over 90% cell viability even at high concentrations up to 800 µg/ml. The maximum reduction in viability was 87% at 1600 µg/ml. Assays are being conducted to determine the effective concentration (EC50) and selectivity index (SI). Initial results suggest that chondroitin sulfate-coated IONPs have low cytotoxicity and hold promise as effective antiviral agents.

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PT.019

EVALUATING THE ANTIVIRAL POTENTIAL OF PLANT EXTRACTS ON VACCINIA VIRUS

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Vaccinia virus (VACV), a member of the *Poxviridae* family, is a key subject of study due to its historical role in eradicating smallpox and its use as a vector in recombinant vaccine development. Although smallpox has been eradicated, other poxviruses, such as monkeypox (MPXV). This underscores the importance of studying VACV as a prototype for understanding poxvirus biology and developing antiviral therapies. In this context, plant-based compounds are increasingly explored for their therapeutic potential. This study investigates the in vitro antiviral activity of 160 plant crude extracts against VACV. Vero cell monolayers were treated with crude extracts (20 µg/mL) and infected with VACV. After 48 hours, cell viability and viral replication inhibition were evaluated by optic microscopy followed by densitometry using the ImageJ software to assess the cytopathic effect (CPE). Of the 160 extracts tested, three demonstrated significant antiviral activity with no cytotoxicity, achieving over 80% inhibition of viral replication. Extract EX6558 was the most effective, showing 100% inhibition of viral replication, followed by EX6557 (96.5%) and EX6556 (80.7%) all of them obtained from different parts of a plant of the Asteraceae family. Bioassay-guided fractionation of the extract EX6558 was performed using ultra-high-performance liquid chromatography (UHPLC) coupled with high-resolution mass spectrometry (HRMS), resulting in the annotation of nine compounds in the active fractions. Computational and in vitro anti-VACV analyses of these compounds are ongoing. This study underscores the potential of plant extracts as antiviral agents against VACV, laying a strong foundation for the development of new antiviral therapies derived from natural sources.

Financial Support: CNPq 406441/2022-7 (INCTPox)

PT.020

VACCINIA VIRUS INHIBITION BY ACRIDONE

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Vaccinia virus (VACV), a member of the *Poxviridae* family is the etiologic agent of bovine vaccinia. VACV has been extensively studied due to its historical significance in the eradication of smallpox, and its use as a vector in various recombinant vaccine platforms. Despite the successful eradication of smallpox, concerns remain regarding the safety of related viruses, particularly in the context of potential use as bioterrorism agents. This underscores the need to develop effective antiviral therapies. In this study, we evaluate the inhibitory effect of a acridone, named FAC22, on VACV replication in vitro using Vero cells. Cell viability was assessed via a densitometric assay, while a plaque-forming unit (PFU) assay quantified the viral load. To elucidate potential mechanisms of action, we conducted virucidal, pre-treatment, and post-entry assays. FAC22 exhibited a potent anti-VACV activity and high selectivity index (IS = 15.8), reducing the viral load by 4 logs after 24 hours of post-entry assays, eliminating plaque formation and by 3 logs after 48 hours of post-entry assays (150 PFU/mL) at 30 µM, compared to the viral control (1.2 x 10^4 PFU/mL and 8.0 x 10^5 PFU/mL at 24 and 48 hours, respectively), achieving a 99.9% reduction in viral replication. The compound exhibited limited virucidal activity, reducing the viral load by nearly one log. These findings suggest that FAC 22 effectively disrupts theVACV replication cycle, highlighting its potential as a candidate for the development of novel antiviral drugs against Vaccinia virus infections and hopefully against other poxviruses.

Financial Support: CNPq 406441/2022-7 (INCTPox), Fapemig, Fiocruz, CAPES

PT.022

MODULATION OF CELLULAR PARAMETERS OF MICROGLIA BY THE NUCLEOCAPSID (N) PROTEIN OF SARS-COV-2

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SARS- CoV-2 infection can lead to severe respiratory symptoms and has been shown to affect the central nervous system by crossing the blood-brain barrier, causing pre-activation of microglial cells upon contact. This study aimed to evaluate whether the nucleocapsid (N) protein of SARS-CoV-2 influences specific cellular parameters of human microglia C20 lineage. Our results demonstrated that varying concentration of the N protein did not alter cell viability, as measured by the MTT assay. Additionally, cell death profile modulation, assessed using Annexin-V and PI staining, showed no alteration in the cell death profile due to the N protein. Lipid droplets, indicators of cellular activation associated with energy metabolism and inflammation, increased in biogenesis in cells stimulated with the N protein compared to non-stimulated cells. Reactive oxygen species (ROS) generation, often linked with increased lipid body biogenesis, was analyzed using DCFH-DA staining and flow cytometry. The N protein did not induce ROS generation in microglia. Thus, the N protein of SARS-CoV-2 promotes microglial activation and lipid metabolism in microglia without causing oxidative stress or cell death, suggesting its potential as a target for specific therapies. Further investigations are required to understand how the N protein affects microglial function and the implications for disease progression or recovery.

Financial Support: FAPDF

PT.023

IN SILICO ANALYSIS OF STRUCTURAL PROPERTIES OF 6K AND TF PROTEINS ACROSS THE SEVEN ALPHAVIRUS SEROCOMPLEXES

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Introduction: Alphaviruses are enveloped arboviruses that cause various human and animal diseases, including encephalitis and arthritis. The 6K and TF proteins, minor structural components of these viruses, play key roles in virus budding and act as virulence factors. This study aimed to explore the structural differences of these proteins across the seven alphavirus serocomplexes. Materials and Methods: Amino acid sequences of 6K and TF proteins from prototypical species representing each serocomplex – VEEV, EEEV, WEEV, NDUV, BFV, MIDV, and SFV – were obtained from GenBank in FASTA format. These sequences were analyzed using CLUSTAL OMEGA for sequence alignment, PEPSTATS for side-chain polarity, PREDATOR for secondary structure prediction, and PROSCAN for post-translational modification (PTM). Results: Sequence alignment revealed the highest identities among 6K and TF proteins between MIDV/SFV (58.33%) and VEEV/EEEV (52.11%), with the lowest between NDUV/SFV (19.05%) and NDUV/MIDV (11.11%). Both proteins exhibited predominantly apolar profiles, with mean apolarity of 72.75% for 6K and 60.43% for TF. The 6K protein had a higher proportion of alpha-helices (46.69%, on average), while TF was mainly composed of random coils (59.73%, on average), with WEEV, BFV, and MIDV lacking beta-sheets. PTM analysis indicated 6K's propensity for CK2 phosphorylation, N-glycosylation, and PKC phosphorylation, except in EEEV and NDUV. Similar PTMs were found in TF, with additional amidation in VEEV, EEEV, and WEEV. Conclusion: While these proteins share structural similarities across alphaviruses, certain functional characteristics appear to be regionally specific, reflecting potential adaptations to primary endemic areas.

Financial Support: National Council for Scientific and Technological Development (CNPq).

PT.024

INVESTIGATION OF HUMAN CORONAVIRUSES IN NON-HUMAN PRIMATES, MINAS GERAIS, BRAZIL, 2023

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In 2019, SARS-CoV-2 emerged, causing a pandemic that affected millions of patients worldwide. Since then, SARS-CoV-2 has continued to circulate globally. In addition to infecting humans, SARS-CoV-2 has been found to infect domestic, pet, or wild animals. One example is the natural infection of coatis in urban parks in Belo Horizonte, Minas Gerais (MG), illustrating the occurrence of viral spillback in urban settings. Some non-human primates (NHPs) have a synanthropic and opportunistic nature, which facilitates their interaction with humans, especially in urban parks, increasing the risk of interspecies viral transmission. Given that NHPs are susceptible to coronavirus infections, this study aimed to perform the molecular screening of human coronaviruses in samples collected from NHPs in MG from June to November/2023. For this purpose, oral swabs were collected from 50 animals *(Callithrix penicillata n=47, Callicebus nigrifrons n=2, Sapajus nigritus n=1).* Total RNA was extracted and used as a template for two different one-step RT-qPCR reactions, targeting the RNAse P gene (endogenous control), and the N1 and N2 viral genes (2019/nCoV RUO Kit, IDT), respectively. The samples tested positive for the endogenous control, but none of them amplified the viral genes. The samples will be further tested for other human coronaviruses associated with the common cold. The results could indicate that none of the animals were actively infected by SARS-CoV-2. However, given the close interaction of NHPs with humans in urban areas and their susceptibility to coronavirus infections, surveillance must be continued.

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PT.025

IMPLEMENTATION OF VIRAL DETECTION METHOD AT THE CENTRAL PUBLIC HEALTH LABORATORYOF MINAS GERAIS (LACEN/MG) FOR HEALTH SURVEILLANCE PURPOSES

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Health Surveillance aims to continuously analyze the population's health situation, in order to control determinants, risks and damages, ensuring comprehensive health care. Public Health strategies should focus on the concept of “ONE HEALTH”, which deals with the integration between human, animal and environmental health. Foodborne and waterborne illnesses represent one of the most serious concerns in public health and one of their main etiological agents is viruses. Thus, viral detection, epidemiology and control have been targets of interest for food hygienists and policy-makers. The confirmation of an outbreak is based on clinical, epidemiological and laboratory evidences from clinical, food and water samples. Furthermore, wastewater-based epidemiology (WBE) is a highly efficient tool for detecting and characterizing public health targets. In Brazil, research for enteric viruses in samples involved in outbreaks is not routine in public health laboratories, due to the complexity, unavailability and high cost of isolation methodologies. Therefore, it is urgent to include this parameter in the analytical scope of LACENs to strengthen health surveillance actions. In pursuit of more comprehensive and effective laboratory strategies, we implemented a viral detection method in water and wastewater samples, that consists at viral concentration through membrane adsorption-elution followed by nucleic acid extraction using a commercial kit and detection by Real Time PCR. For food samples, we are still making adjustments to the methodology in accordance with ISO 15216-1. We detected viruses in all three matrices, that support the urgency to expand the analytical scope of the public health laboratory network in Brazil.

PT.026

SEARCH FOR PHAGE DISPLAY-DERIVED INHIBITORS FOR NSP2 FROM CHIKUNGUNYA AND MAYARO VIRUSES

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Chikungunya (CHIVK) and Mayaro (MAYV) viruses are important emerging mosquito-borne alphaviruses capable of causing recurrent clinical and epidemiological manifestations in Brazil. Despite advances in the structural and molecular understanding of their viral particles, inhibition of their infectious capacity remains linked to prophylactic methods targeting the transmitting vectors. Furthermore, treatment after infection relies mainly on drugs to reduce symptoms. Consequently, the search for highly specific antiviral agents has become essential. The cysteine protease belonging to the C-terminal region of the nonstructural protein 2 (nsP2p) of both viruses plays a key role in viral RNA replication and virus-host interactions. Stereochemical mapping using the phage display technique will be used to search for potential antivirals against the activity of these enzymes. Phage display is a technique based on the expression of peptides in phage capsids, with the purpose of selecting specific protein binders from genomic libraries. This technology allows the screening of functional peptides for the development of drugs with inhibitory potential. To date, the nsP2p proteins of CHIKV and MAYV were expressed in 1 liter of Terrific Broth medium at 18ºC and induced with 0.4 mM IPTG. Purification of nsP2p-CHIVK under soluble conditions guaranteed a yield of 8 mg/ml per liter of culture medium. The phage display for CHIKV was performed using the Ph.D. TM -12 Phage Display Peptide Library kit, based on the M13KE phage vector. The purified phages will be sent for next-generation sequencing. After the synthesis of the peptides, they will be tested in cell culture, and thus evaluate the inhibition potential against arboviruses.

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PT.027

Evolutionary dynamics to the arising of HIV-1B GWGR

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HIV-1 subtype B has been shown to present multiple combinations of the four amino acids that form the tip of the gp120 V3 loop. The most prevalent motif around the world is the GPGR; however, in Brazil, the GWGR variant circulates with high frequency. This motif has been previously related to a higher T-CD4+ count and a lower rate of disease progression. Therefore, this study aims to understand the evolutionary dynamics of GWGR, seeking to uncover the role of Brazil in the emergence and dissemination of this variant. To perform that, 21,325 gp120 sequences and its corresponding metadata were downloaded from the Los Alamos National Laboratory database. The dataset was aligned using muscle v3.8.425 and edited in Aliview v1.28. After removing short sized and non-subtype B sequences, the final dataset included 5,454 sequences of maximum 1,515 base pairs in length. The IQTree v2.0.7 was then used for phylogenetic inference and ancestral state reconstructions were performed using TreeTime v0.11.3. Preliminary results indicate that the GWGR motif appears to form a paraphyletic clade, arising multiple times among Brazilian HIV-1B GPGR sequences. Previous studies assumed that the GWGR lineage is monophyletic; however, our findings show that this motif emerged independently multiple times from a monophyletic clade of Brazilian GPGR sequences. Furthermore, additional results reveal that the GLGR motif originated from viruses harboring the GWGR motif.

PT.028

ORIGIN AND DISPERSAL OF HIV-1 SUBTYPE F1 IN BAHIA

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From 2007 to 2021, Brazil reported 381,793 cases of HIV-1 infection, with the Northeast region ranking as the second most affected area. Within the Northeast, Bahia, as the most populous state, holds the highest absolute number of cases. Although subtype B prevails in the Northeast, non-B variants, like F1, represent a significant proportion, needing comprehensive phylogenetic analyses. This study employed genetic and epidemiological data to explore the origin and spread of HIV-1 subtype F1 in Bahia. We analyzed 24 new pol sequences from people living with HIV from the state of Bahia, Brazil. To understand viral phylodynamics, we collected HIV-1 subtype F1pol sequences from the Los Alamos database, resulting in a dataset of 5,195 sequences. After filtering out recombinant and low-quality sequences, the sequence alignment was performed with MAFFT. The initial phylogeny was reconstrcuted by the Maximum Likelihood (ML) method in IQ-TREE and temporal signal was evaluated using TempEst v1.5.3. Bayesian reconstruction was performed for two subclades with high temporal signal quality selected directly from the larger ML tree. The primary finding of the Maximum Clade Credibility (MCC) trees revealed multiple introductions in Bahia between the 1980s and early 2000s, with the Southeast-BR region being the main source of introductions to the Northeast-BR and Bahia. Despite the initial dissemination of F1 from the Southeast to Bahia, our analysis indicate a more recent connection with other states in the North and Northeast regions of Brazil.

Financial Support: CAPES/CNPq

PT.029

Newcastle Disease Virus detection in wild birds from the southern Brazilian coast during the circulation of Avian Influenza Virus.

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The Newcastle Disease Virus (NDV) is an *Avulavirus* belonging to the *Paramyxoviridae* family and causes one of the most clinically important diseases in birds, affecting wild and domestic species. Consequently, causes significant global damage to the poultry sector, the diagnosis must be reported to the World Organization for Animal Health (WOAH). The aim of this study is to detect the presence of NDV in migratory birds along the Brazilian southern coast, focusing on evaluating its relevance and impact as a clinically significant and economically important pathogen. A total of 624 fecal samples were analyzed, collected from migratory flocks of shorebirds and seabirds which passed through the beaches of the southern coast of Brazil, specifically in Parque Nacional da Lagoa do Peixe (PNLP), located on coast of Rio Grande do Sul (RS). The analysis was performed after centrifugation of raw sample, extraction of genetic material using magnetic beads, and purification through ethanol precipitation, followed by RT-qPCR analysis. Four samples tested positive for NDV detection, all originating from birds of the genus *Calidris* sp. These samples are being isolated in embryonated chicken eggs for later be sequenced using the Sanger method to obtain more information, enabling genotyping of its spread among migratory birds. Although the complete viral detection process is not yet concluded, it's already possible to maintain an alert regarding research in the locality, not only in the search for signs of infection by the NDV but also in infection by other zoonotic viruses important, such as Avian Influenza Virus (AIV).

Financial Support: CNPq, PREVIR.

PT.030

VESTITOL AS A POTENTIAL ANTIVIRAL AGENT AGAINST CHIKUNGUNYA VIRUS: IN VITRO ASSESSMENT

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Vestitol is an isoflavonoid found in various natural sources with known anti-inflammatory and antimicrobial properties. However, few studies have investigated its antiviral activity. Chikungunya virus (CHIKV) is associated with persistent inflammatory polyarthralgia and currently lacks approved antiviral treatments. This study aimed to investigate the antiviral activity of vestitol against CHIKV in vitro. Initially, cell viability, the maximum non-toxic concentration (MNTC), and the 50% cytotoxic concentration (CC50) were determined for the compound. Vero E6 cells were seeded in 96-well plates and treated with vestitol at concentrations ranging from 3.125 to 400 µM. After 48h of incubation, cell viability was assessed using an MTT colorimetric assay, which identified MNTC = 100 µM and CC50 = 201.6 µM. For the antiviral assay, cells were infected with CHIKV and treated with vestitol concentrations ranging from 3.12 to 100 µM for 48 hours. The MTT assay was subsequently used to determine the degree of viral inhibition (%), the EC50 and the selectivity index (SI=CC50/EC50). Vestitol treatment reduced the viral cytopathic effect, with an EC50 of 37.99 µM and an SI of 5.37. Additionally, the percentage of CHIKV-positive cells was evaluated via intracellular flow cytometry 36h after treatment with 100 µM vestitol, and a significant reduction in the percentage of CHIKV-infected cells was observed. In summary, these findings suggest that vestitol is a promising antiviral candidate against CHIKV. Further research is needed to elucidate the mechanisms underlying its antiviral activity.

Financial Support: CAPES, CNPq and FAPEAL.

PT.031

Activity Against Vaccinia Virus of crude extracts of soil fungi.

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Outbreaks and epidemics caused by emerging and re-emerging viruses, such as monkeypox (MPXV) and Vaccinia virus (VACV), are significantly impacting global health. Given the limited availability of approved antiviral drugs, this study aimed to screen crude extracts obtained from cultures of fungi isolated from soil for their potential activity against Vaccinia virus (VACV-WR). To evaluate the cytotoxicity and antiviral activity of these extracts, Vero CCL-81 cell monolayers were treated with crude extracts (20 µg/mL) and subsequently infected with VACV-WR. Cell viability was measured after 48 hours using densitometry using ImageJ software to quantify the cytopathic effect (CPE). VACV replication inhibition was also assessed using plaque reduction assays. Of the 40 extracts tested, five demonstrated significant antiviral activity with VACV replication inhibition exceeding 40%. Extracts EX19426 and EX19442 were the most effective, showing 80.7% and 100% inhibition of viral replication, respectively. Extracts EX19446 (87.1%), EX19428 (76.8%), and EX19450 (52.4%) also exhibited notable inhibition levels. These findings indicate that EX19442 and EX19426 are particularly promising, meriting further fractionation and analysis. This study highlights fungal extracts with potential antiviral activity against VACV-WR, providing a solid foundation for the development of new antiviral agents from natural sources.

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PT.032

Densitometric Analysis as an alternative method for high-throughput antiviral screening against poxviruses

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The search for effective antiviral agents requires methods that are efficient, cost-effective, and suitable for high-throughput screening. Traditional techniques, such as the plaque reduction assay, are widely used but can be time-consuming. Recent advancements in technology offer methods that streamline this process, enhancing accuracy and consistency. One such method is densitometric analysis using ImageJ software, which evaluates the cytopathic effect (CPE) of antiviral compounds. ImageJ is a free, versatile image processing tool that provides precise measurements of cell density changes in monolayer cultures, offering reliable viral inhibition assessment. This study evaluates the effectiveness of using Vero cell monolayer staining with crystal violet combined with densitometric analysis via ImageJ as a practical, economical, reproducible, and sensitive approach for assessing antiviral activity against Vaccinia virus (VACV). Two established assays were used: the CPE assay and the plaque reduction assay, considered the gold standard for evaluating viral replication. Vero cells were cultured, infected with VACV, and treated with 40 extracts. For the CPE assay, infected monolayers were stained with crystal violet and analyzed with ImageJ to measure cytopathic effects, while the plaque reduction assay involved counting viral plaques to determine antiviral efficacy. The results showed that ImageJ densitometric analysis provided a sensitive and consistent measure of antiviral activity, comparable to the gold standard plaque reduction assay, but was faster and required fewer reagents and less manual labor. Thus, combining monolayer staining with densitometric analysis using ImageJ offers a valuable, efficient alternative for high-throughput antiviral screening against poxviruses.

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PT.033

Effective Protocol for Mycoplasma Decontamination of Vaccinia Virus (VACV) Stocks.

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Mycoplasmas are the smallest self-replicating organisms known to science and cannot be observed using standard microscopy. They have significant limitations in synthesizing components for their metabolism and require host cell components for replication and survival, leading to alterations in cellular functions and eventually cell death. Mycoplasma contamination of viral stocks has negatively impacted a wide range of virology studies.Here, we describe a protocol for decontaminating Vaccinia virus (VACV) viral stock contaminated with mycoplasma. Initially, Vero cells were cultured in 6-well plates in DMEM medium supplemented with 1%FBS containing ciprofloxacin (10µg/mL) and tylosin (8µg/mL). An aliquot of VACV-WR contaminated with mycoplasma was clarified by centrifugation and serially diluted. The cell monolayers were infected with these dilutions and incubated at 37°C, for 48 hours. Afterward, the cells were scraped, subjected to three freeze-thaw cycles, clarified by centrifugation, and the supernatants were stored at -70°C. DNA extraction from the infected cells was performed using the PureLink® Genomic DNA Mini Kit, followed by qPCR testing with specific primers for mycoplasma detection. Following the decontamination treatment, a 10^{-6} dilution of the viral stock showed no cytopathic effect without amplification of mycoplasma DNA, confirming the absence of mycoplasma contamination. This sample was used to produce a new VACV seed pool in Vero cells treated with both antibiotics, which was retested by qPCR to confirm the absence of mycoplasma. This protocol, involving the simultaneous use of two antibiotics and rigorous molecular testing, proved to be effective for decontaminating VACV viral stocks.

Financial Support:CAPES INOVAFioCruz, FAPEMIG, CNPQ, INCTPox.

PT.034

TITLE: EVALUATION OF THE IMPACT OF THE COVID-19 PANDEMIC ON THE CIRCULATION OF RESPIRATORY VIRUS IN CHILDREN.

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INTRODUCTION. Children infected with SARS-CoV-2 rarely progress to respiratory failure, which prompted a rapid and immediate scientific effort to encompass knowledge about the virus in pediatric patients and the possible interference of other respiratory viruses. **OBJECTIVE** evaluating the incidence, clinical and epidemiological characteristics, in addition to determining the seasonality of infection by SARS-CoV-2 and other respiratory viruses in children treated in 6 public hospitals in São Paulo during February 2020 to February 2023. 16,604 distinct events from 12,936 patients According to gender, 7,154 were male and of these, 5,433 were positive for at least 1 virus. From females, 5,663 samples were collected, of which 4,084 were positive. The patients were classified into 4 groups, according to the WHO, namely: Neonates with 572 positive, infants with 5,921 positive, children with 2,464 positive and adolescents with 632 were positive and 71 were positive but without age information. RT-qPCR was detected 57% positive samples for at least 1 tested virus, with RSV being the most prevalent with 39.6% of cases, followed by AdV and BoV with 13% each, PIV with 11% and Sars-CoV-2 with 6.3% positivity and the others with less than 6% each. Concluded that RSV continues to be the most important viral pathogen in childhood infections. In 2020, there was only the circulation of Sars-Cov-2 and Adenovirus, but the other viruses returned in the following years, however the seasonality of the diseases studied changed when compared to studies prior to the pandemic.

KEYWORD: SARS-COV-2, VIRUS, RT-PCR, PEDIATRIC, RSV, HOSPITALS, RESPIRATORY, VIRUSES.
Financial Support: Ministry of science, technology and innovation (MCTI).

PT.035

LEGACY OF CORONAVIRUS INFECTION: INTERFERENCE IN BONE METABOLISM AND ACCENTUATED OSTEOPOROSIS

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Long COVID, a delayed consequence of COVID-19, is associated with several extrapulmonary effects that are still unknown. Some of them are suggested to be the impact on bone metabolism, which can be even more critical in the elderly, the population with high risk for COVID-19 and to osteoporosis. This study aimed to investigate the impact of coronavirus infection on alterations in the musculoskeletal system of healthy and osteoporotic mice. Osteoporosis was induced in 5 weeks old female C57BL/6J mice through ovariectomy, and infection was conducted after 15 days with $3x10^4$PFU/30µL of the murine coronavirus (MHV-A59), intranasally. Mice were monitored daily until euthanasia at 2, 5, 30 and 60 days post-infection, and the collected organs were processed for histology, ELISA, viral titration, PCR, and MICRO-CT analysis. MHV-A59 infection resulted in body mass loss, leucopenia, and moderate to mild lung injury, characterized by hemorrhage, leukocyte infiltration, and alveolar wall hyperplasia, which was associated to similar viral loads in ovariectomized and non-ovariectomized mice (SHAM). Infection induced bone resorption in the femurs of healthy mice and worsened ovariectomy-induced osteoporosis, reducing bone volume and trabecular thickness. There was an alteration of expression of different molecules associated with bone metabolism, which was distinct in osteoporotic and SHAM mice, including increased concentration of RANKL, IL-1β, CCL2, CCL3 and reduces IL-6 in the femur. Thus, MHV-A59 infection induced acute lung injury associated with bone resorption in healthy mice and exacerbation of pre-existing osteoporosis, which is related to the change expression of inflammatory mediators.

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PT.036

EXPERIMENTAL VIRAL EVOLUTION TO ENHANCE THE ONCOLYTIC CAPACITY OF ZIKA VIRUS IN GLIOBLASTOMA STEM CELLS IN VITRO

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Glioblastoma (GBM) is the most common, aggressive and lethal primary brain cancer in existence. The poor prognosis is attributed to extensive heterogeneity, as GBM is composed of different subpopulations of tumor cells, including stem cells (GSCs) and differentiated cells (DGCs), which promote tumor growth and invasion. Although ZIKV has oncolytic activity in GBM, the virus preferentially infects GSCs, while the majority of tumor cells remain uninfected. Thus, the objective of this work is, through experimental evolution, to adapt ZIKV to the GBM stem cell, enhancing its oncolytic activity, amplifying ZIKV tropism for these tumor cells. In our work, we will carry out serial passages of ZIKV in GSCs to generate adapted strains of ZIKV. In this initial step, we performed the characterization of the infection of human glioblastoma stem cells (TG1) with the ancestral ZIKV). TG1 cells were infected with ZIKV (MOI 1 and 0.1) and submitted to quantification of viral RNA by RT-qPCR and the formation of PFUs by plaque assay. RNA quantification and the formation of PFUs at 48h, 72h, and 96h dpi suggest that these cells are susceptible to the virus and produce infectious viral particles, mainly at MOI 1. However, we did not observe a reduction in cell viability up to 4 dpi in all the MOI tested (1, 5 and 10). Our preliminary results suggest that ancestral ZIKV is capable of infecting TG1 cells and generating viral progeny, but does not yet exhibit cytotoxic activity. We will evaluate whether ZIKV adaptation increases virulence in GSCs.

PT.037

VIRUCIDAL ACTIVITY OF NEW COMPOUNDS AGAINST MURINE CORONAVIRUS

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To date, there is an urgent need to develop new therapeutic approaches for the treatment of COVID-19 and new compounds are being studied for their potential virucidal action. The murine Coronavirus MHV-A59 has significant genetic and structural similarity with SARS-CoV-2, making it a great alternative model to SARS-CoV-2 and can be worked on in conventional safety level laboratories. Therefore, the objective of this work is to evaluate the virucidal effectiveness of 8 new compounds against coronaviruses. The experiment was carried out by exposing a suspension of activated compound in the presence of MHV-A59 and carrying out serial dilution of each suspension/treatment in a monolayer of L929 cells. Dilutions were made to concentration 10^{-4} and inoculated in duplicate in 24-well plates. After 48 hours of incubation, the cells were fixed and, after fixation, the cell monolayer was stained with Crystal Violet. After counting the number of lysis plaques in each treatment, a potent virucidal activity of 4 of the 8 compounds tested was observed, with a significant reduction in the viral titer. New tests will be carried out to evaluate the activity of these compounds against SARS-CoV-2.

Financial Support: FAPEMIG; CAPES; CNPQ; EMBRAPII; CIMOL; Mucpharm; Novandina;

PT.038

Using MALDI-MS as a new methodology for giant virus identification

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Since the discovery of the first giant virus in 2003, hundreds of new species have been described as a result of prospecting experiments in the most diverse environments on Earth. After isolation and morphological characterization, the identification and taxonomic classification stage is performed using molecular biology data via genome sequencing, which comprise cumbersome and expensive techniques. Based on this, we propose the use of matrix-assisted laser disorption mass spectrometry (MALDI-MS) as a fast and efficient approach for the identification, classification and triage of giant viruses. Virus samples were used as analytes, comprising 8 species belonging to 3 families. In order to explore the potential for differentiation of closely related species, 5 species from the same family, from the 4 main subgroups, were used. A database was constructed and standardized from technical and biological replicates. The experiments were performed as double-blind assays. Spectra obtained by MALDI-TOF were analyzed based on the presence/absence of peaks and intensity. Our data show that viruses from the same family have a high degree of similarity among themselves, and therefore it is possible to distinguish the three families used based on a direct analysis of the spectra. This observation is confirmed by the phylogeny constructed with the spectra, where each family forms independent branches containing its member species. Finally, it was also shown that it is possible to differentiate viruses at the genus level, indicating a high potential of this technique for descriptive approaches of new species.

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PT.040

PROSPECTION OF NEW COMPOUNDS WITH ANTIVIRAL ACTIVITY AGAINST POXVIRUSES

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Poxviruses are double-stranded DNA viruses, among which are *Orthopoxvirus vaccinia* (VACV), *Orthopoxvirus monkeypox* (MPV), and *Orthopoxvirus variola* (VARV). In recent years, the number of human infection cases associated with poxviruses, especially MPV, has been increasing. Considering the impact caused by these viruses, the current scenario emphasizes the critical need for the development of control methods and specific treatments to tackle the mpox public health challenge. This work focuses on the antiviral screening of four new synthetic compounds against VACV. First, the cytotoxicity of the compounds was evaluated, and the concentration that caused 50% cytotoxicity (CC50) was determined. Subsequently, the antiviral activity of the compounds was tested, and the 50% inhibitory concentration (IC50) of infective VACV viral particles was determined. Ultimately, the selectivity index (CC50/IC50) was calculated. The tested compounds showed no significant toxicity in BSC-40 cells, with CC50 values exceeding 750 µM. The results of antiviral activity showed that the four evaluated compounds have anti-VACV activity, protecting BSC-40 cells from infection, with IC50 values of 1.76 µM, 2.22 µM, 10.27 µM, and 1.93 µM for LV6, LV7, LV8, and LV9, respectively. Therefore, the compounds showed a wide therapeutic window, with a selectivity index (SI) of 568 (LV5), 540 (LV6), 74.9 (LV7), and 518 (LV8). The compounds have proven to be highly selective and will be studied in MPV, as they could be a promising strategy to treat poxvirus infections.

Financial support: INCT Pox, CNPq, CAPES, FAPEMIG, EMBRAPII, MucPharm, Novandina.

PT.041

Encapsulation of bacteriophage with bovine serum albumin nanoparticles for control of *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is an antibiotic-resistant gram-negative bacterium widely isolated in hospital environments. This bacteria is able to cause nosocomial infections in immunocompromised patients. A new therapeutic alternative to antibiotics would be the application of viruses that can infect resistant strains of *P. aeruginosa.* Bacteriophages or phages have the ability to infect bacterial strains and promote their lysis without causing severe immunological reactions in human hosts. One method to improve the delivery of *P.aeruginosa* infecting bacteriophages to specific sites and also improve the stability of phage cocktails would be the encapsulation of phages in nanoparticles. In this work, we used bovine serum albumin nanoparticles (BSA-NPs) as an encapsulation structure for bacteriophage able to infect *P. aeruginoisa* PA14 strain. NPs with phages (NPF) and empty (NPV) were produced by acetone-induced coacervation. The encapsulation efficiency (EE%) was calculated by the difference in viral title in the solution before and after encapsulation. Results demonstrated that the EE% of NPF was 99%. A stability assay also showed that NPF are very stable, since no phage are release from the matrix when these nanoparticles were incubated at 37ºC for until 72h. BSA-NPs proved to be excellent bacteriophage encapsulation matrices and can be used as viral delivery systems in tissues infected with *P. aeruginosa.* Future studies will evaluate the efficiency of these nanoparticles as antimicrobial agents on clinical isolates and biofilms of *P. aeruginosa.*

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PT.042

ANTIVIRAL POTENTIAL OF NATURAL COMPOUNDS FROM AN AMAZON PLANT AGAINST CHIKUNGUNYA VIRUS

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Introduction: Chikungunya virus (CHIKV) is the etiological agent of chikungunya fever, a disease characterized by painful arthralgias and dengue-like symptoms. CHIKV infection can progress to a chronic condition that may last for years, and because it has spread to over 123 countries worldwide, it is considered a public health problem. However, there are no available antiviral drugs against chikungunya fever. In this scenario, natural compounds isolated from the amazon plant *Eugenia punicifolia* have previously shown important activities such as antioxidant and hypoglycemic. In the present work, we evaluated the antiviral potential of 15 compounds isolated from *Eugenia punicifolia* against CHIKV. **Materials and Methods:** Cell viability of the 15 amazon compounds was evaluated at 50, 10 and 2 μM for 24 hours through MTT assay. The viral infectivity analysis was performed employing CHIKV-*nanoluciferase*, a viral construct inserted of a *nanoluciferase* reporter gene, which was used to infect BHK-21 cells in a multiplicity of infection of 0.1 in the presence or absence of compounds at non-cytotoxic concentrations for 16 hours. The replication rates were assessed by measuring luminescence levels using renilla luciferase assay. **Results:** All tested amazon compounds inhibited CHIKV replication, resulting in replication rates from 38% up to 86%. The highest inhibitory rates were observed for the compounds Eugenia-B, Eugenia-F and Eugenia-J, which impaired CHIKV replication in 85%, 80% and 86%, respectively. **Conclusions:** Our data demonstrate that compounds isolated from *Eugenia punicifolia* possess antiviral activity against CHIKV, representing candidates for the development of future antiviral drugs against chikungunya fever.

Financial support: FAPEMIG; CNPq; CAPES; FAPEAM.

PT.043

DESIGN AND VALIDATION OF A MOLECULAR TOOL FOR HPIV2 GENOME SEQUENCING IN THE CONTEXT OF LIMITED GENOMIC SURVEILLANCE

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Human parainfluenza virus type 2 (HPIV2) infects humans causing respiratory infections. Despite its frequent circulation and high mutagenic potential, there is no significant genomic surveillance for HPIV2, with only 151 complete genomes deposited on GenBank. In our study, we validated a protocol for complete genome sequencing of the HPIV2 by designing a molecular protocol to amplify the whole HPIV2 genome. Fifteen primers were designed based on the dataset with all available complete genome sequences, using Primer Scheme and Primer 3, with further manual checking. To validate the protocol, 7 positive samples for HPIV2 collected between 2014 to 2022 in England were used (CT ranges from 20 to 30). Using the developed protocol, after the amplification by PCR, these samples were subjected to the MinIon sequencing process. As result, 71% of processed samples generated complete genome sequences, and partial genome sequences were obtained from 29% of samples. Phylogenetic reconstruction with the 7 sequences generated here and the 151 sequences available on GenBank demonstrated that 5 of the 7 sequences from this study grouped with sequences from the USA from 2014-2017 (95% bootstrap), and 1 sequence grouped into the branch with sequences from different countries around the world (97% bootstrap), suggesting genetic modification. In conclusion, these findings demonstrate that our protocol fulfills its purpose, resulting in the sequencing of HPIV2 complete genome. Given the lack of genomic data on this virus and its wide spread worldwide, we reinforce the importance of developing molecular tools to carry out proper genomic surveillance.

PT.044

Dynamics of SARS-CoV-2 Variants of Concern: Tales of a cohort from a Brazilian city

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SARS- CoV-2 Variants of Concern (VOCs), have exhibited increased viral virulence, and Brazil has reported five VOCs circulating. In this study, RT-qPCR analyzes were conducted for VOCs identification on 633 clinical samples collected from a cohort of patients with respiratory symptoms, between December 2020 and February 2022, in Uberlandia, a city in Minas Gerais state. Additionally, one hundred positive samples for SARS-CoV-2 were submitted to sequencing of the spike genomic region of SARS-CoV-2. Initially, VOC Alpha was predominant, but with the emergence of the Gamma variant in January, 90% of samples in March 2021 have already been identified as Gamma. From August to December 2021, all sequences were identified as Delta. By the end of December 2021, the Omicron was detected and, subsequently, all samples were identified as this variant. Analyzes of the spike genomic region of virus isolated confirmed VOCs identified by RT-qPCR and revealed amino acid substitutions. Our findings suggest that over time, with flexibility of social isolations measures and due to advantageous mutations, the viral spread of VOCs became predominant in the region.

Financial support: CAPES, CNPq, FAPEMIG, FAPESP

PT.045

ANTIVIRAL ACTIVITY OF NEW BENZOTHIAZOLE ANALOGS AGAINST CHIKUNGUNYA VIRUS: *IN VITRO* EVALUATION AND MECHANISTIC INSIGHTS

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Chikungunya virus (CHIKV) can cause persistent inflammatory polyarthralgia and antiviral treatment are currently lacking. This study aimed to evaluate the antiviral efficacy of novel benzothiazole analogs against CHIKV *in vitro*. Cytotoxicity assays were performed using Vero E6 cells treated with different concentrations of 19 benzothiazoles, and cell viability was assessed by MTT assay after 48 hours to determine the CC50. No cytotoxic effect at 50 µM were observed for eleven compounds and were thus tested to antiviral activity at concentrations ranging from 6.25 to 50 µM against CHIKV (MOI 0.01). The compounds EdCHIK10, EdCHIK39, and EdCHIK82 demonstrated over 83% viral inhibition in the MTT assay following 48 hours of treatment. EC50 values were determined and were 9.73 ± 2.92 µM, 5.88 ± 0.93 µM, and 9.58 ± 1.16 µM, with selectivity indexes (SIs) > 20.55, > 30.58, and > 20.87, respectively. Time-of-drug-addition assay revealed moderate significant antiviral activity during pretreatment for EdCHIK10 and EdCHIK39, no significant activity during adsorption, and pronounced activity during posttreatment for all three compounds. Notably, EdCHIK82 exhibited significant antiviral effects even when it was added up to 6 hours after adsorption. Intracellular CHIKV staining and flow cytometry analysis revealed a reduction in infection rates from 40.96% ± 9.84% (untreated) to 8.40% ± 1.81% (EdCHIK10), 11.13% ± 0.35% (EdCHIK39), and 21.86% ± 12.04% (EdCHIK82). In summary, these data suggest that novel benzothiazoles are promising bioactive agents against CHIKV and further investigation are needed to understand its molecular mechanisms of action.

Financial support: FAPEAL, CNPq, CAPES.

PT.046

Structural and functional studies of the bacteria-phage interface by cryoelectron tomography

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Bacteriophages (phages) are viruses that thrive by infecting bacteria. Recently, the roles and applications of phages in health, agriculture and biotechnology has gained recognition, ushering in “The Third Era of Phages”. The phage infection is highly dynamic, in particular the early steps involving adsorption and genome delivery. However, inevitably, most biostructures visualization techniques tend to distance the target from its natural context and introduce artifacts into samples. In this sense, cryoelectron tomography (cryoET) emerges as an alternative, since vitrification methods preserve fine ultrastructures in a native state. Deciphering molecular mechanisms underlying viral replication is basis for devising ways to control the infection or to predict its effects. Therefore, this project aims to apply cryoET to study processes involved in early infection steps by ΦXacM4-11 and ZC03, both modern isolates of lytic phages against *Xanthomonas citri* and *Pseudomonas aeruginosa*, respectively. Previous studies of our group solved these phages’ structures by Single-Particle Analysis, identifying the main protein structures. We now propose the collection of new data in order to clarify their major changes during infection. Phages will be propagated by double-layer plaque assays and purified by CsCl gradient ultracentrifugation. The best conditions for the infection will be defined based on one-step growth trials. Samples with different post-infection times will be frozen, imaged using a high-end electron microscope and processed by tomographic approaches. Up to now, we successfully propagated both phages and imaged them using negative staining electron microscopy. Plus, some cryogenic grids have been screened, although just preliminarily to assess initial conditions.

Keywords: bacteriophage, cryoelectron tomography, structural biology
Financial support: FAPESP, CAPES

PT.047

Bacteriophage-loaded bovine serum albumin nanoparticles increase viral infection rate against *Pseudomonas aeruginosa*

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Nanoparticles and nanocarriers are used as delivery systems for antimicrobial drugs to multi-resistant bacteria. In this study, we developed bovine serum albumin nanoparticles loaded with *Pseudomonas aeruginosa* PA14- infecting bacteriophages (NPF) and evaluated their effects on the planktonic cultures of this bacteria. Empty nanoparticles (NPV) were produced as a control. The morphological characteristics of both nanoparticles were analyzed by dynamic light scattering and scanning electron microscopy. NPF or NPV were incubated with *P. aeruginosa* cultures for 48 hours and absorbance was analyzed at 0, 24, and 48 hours. Viral titers were determined by double agar methods at the same intervals. The cytotoxicity of the nanoparticles was evaluated by the MTT method. Viral nanoparticles showed irregular contours and homogeneous sizes. Empty nanoparticles resemble smooth spheres of varying sizes. NPF had smaller sizes than NPV, 220.3± 3.6nmand 403.2 nm± 23.2nm, respectively. The Zeta potential both NPs was -10± -0.9 mV. The polydispersity index for NPF was 0.090 Pdl (± 0.02Pdl), while for NPV it was 0.536±0.01Pdl). The bacterial population density was significantly reduced in cultures treated with NPF and more markedly compared to non-encapsulated phage. Viral titers were 5 to 2 logs increased, at intervals of 24 and 48 hours, respectively if compared to non-encapuslated phage. NPF or NPV did not show cytotoxic effects in treated cell cultures. Bovine serum albumin nanoparticles loaded with phages increase the rate of viral infectivity and can control the population density of *P. aeruginosa* more efficiently than free phages.

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PT.048

Prophage impact on *Ralstonia solanacearum* Species Complex ecology and evolution

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The mobilome embraces all mobile genetic elements (MGEs) in the microbiome, such as plasmids, transposable elements, and viruses. It influences the composition of microbial communities and facilitates the transmission of antimicrobial resistance genes and virulence factors through horizontal gene transfer. Prophages are bacteriophages that are integrated into the host genome. They play an essential role in the ecology, physiology, and evolution of their bacterial hosts by lysogenic conversion. Beyond this process, they serve as carriers for disseminating genetic information within a bacterial community and contribute to the competitive interactions among their hosts within the microbial community. Nevertheless, few studies have focused on the role of prophages in the distribution of genes across bacterial species. *Ralstonia solanacearum* is a Gram-negative beta proteobacterium belonging to the family Burkholderiales and, alongside two more species, *R. pseudosolanacearum* and *R. syzygii*, forms the *Ralstonia solanacearum* Species Complex (RSSC) a worldwide important group of phytopathogens. The genus *Ralstonia* is an excellent candidate for studying mobilome systems due to its wide range of species, including non-phytopathogens, and its multiple strategies to evade viral infections. To examine the role of the mobilome in this species complex, we compared 619 genomes belonging to the genus *Ralstonia* that were available from public databases and annotated the defense mechanisms, effector genes, and prophages encoded in the genomes. Essentially, our study wants to amplify the knowledge of how these prophages and other viral species interfere with their host's ecology and how they are dispersed among the species, including the non-pathogenic species.

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PT.049

Expression and characterization of a crystallized SARS-CoV-2 membrane (M) protein fused with the C-terminal portion of the Bacillus thuringiensis Cry1C for enhanced stability and cellular response analyisis

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The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) triggered a global pandemic starting in 2019, resulting in millions of deaths worldwide. Consequently, understanding both structural and non-structural proteins of the virus has become crucial for developing diagnostic kits and vaccines. This study focused on expressing the structural M protein fused to the C-terminal of the Cry1C protein, naturally produced by Bacillus thuringiensis, resulting in a crystallized protein potentially offering greater stability. Using specific oligonucleotides, the M protein gene was amplified via polymerase chain reaction (PCR) and cloned into the transfer vector pFast1CT transfer vector, which contains the 3'end sequence of the cry1C gene. Employing the Bac-to-Bac® Baculovirus Expression System (ThermoFisher Scientific), we achieved the expression of the fused M protein in insect cells, as confirmed by Western blot analysis. Additionally, the protein's crystals' microstructure was examined using scanning electron microscopy (MEV). To assess the cellular effects of protein M protein, the MTT colorimetric assay indicated that M protein did not induce cell death. Further evaluation of cellular parameters, including lipid body biogenesis and reactive oxygen species generation, was conducted upon protein upon solubilization. The findings revealed that the M protein was non-cytotoxic and did not cause oxidative stress. However, it did activate lipid body production, indicating cellular activation. Thus the crystallization of the M protein not only suggests enhanced stability but also provide a foundation analysis that could be instrumental in future diagnostic and vaccine development efforts, as highlighted by previous research.

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PT.050

ORTHOPOXVIRUS IN NON-HUMAN PRIMATES: INVESTIGATION OF VIRAL CIRCULATION FROM DIFFERENT SITES, BAHIA, BRAZIL

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The *Orthopoxvirus* genus comprises some of the most important viruses in human history. Some species in this genus, such as vaccinia virus and monkeypox virus, can be related to a large animal host range, and it is essential to understand how these viruses reach these animals to maintain itself in nature. In light of this, between 2021 and 2023, serum samples from 105 monkeys (*Callithrix sp., Allouata caraya* and *Leontopithecus chrysomelas*) were collected from 14 different municipalities in Bahia, Brazil, in order to investigate the orthopoxvirus evidence of circulation. The serum aliquots were diluted at 1:10 and tested by real-time PCR, as described in a published protocol. Parallel to this, other serum samples were submitted to PRNT tests in order to investigate the presence of orthopoxvirus neutralizing antibodies. None of the samples showed any viral DNA detection. In contrast, one sample tested positive in PRNT tests, demonstrating the presence of neutralizing antibodies, indicating a previous contact to an orthopoxvirus species. The seropositive animal, a Wied's Black-tufted-ear Marmoset (*Callithrix kuhlii*), was captured in February of 2023 in Ilhéus, Bahia. To our knowledge, this is the first time it is detected anti-orthopoxvirus antibodies from *Callithrix kuhlli*, a marmoset species that is frequently found in urban areas. Although it is not possible to define which orthopoxvirus species the animal had the previous contact, and considering the recent monkeypox virus circulation in Brazil, this detection of anti-orthopoxvirus antibodies highlights the needs for further surveillance in these animals, in order to prevent future zoonotic challenges.

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

PT.051

EFFECT AND MECHANISM OF ACTION OF SEMISYNTHETIC CARDENOLIDE DERIVATIVES AGAINST HERPES SIMPLEX VIRUS

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Herpes Simplex Virus (HSV) is a prevalent and highly contagious pathogen that can cause a range of clinical manifestations. Herpes Simplex Virus type 1 (HSV-1) infects about 70% of the world's population, posing a globally relevant health problem. In recent years, this virus has been associated with acquired resistance to available treatments, presenting global health challenges that necessitate the exploration of new therapeutic alternatives. Cardenolides, a natural product known traditionally for treating cardiac diseases, have recently been studied and acquired attention for their remarkable antiviral properties of certain molecules belonging to this class. In this perspective, the anti-herpetic activities of semisynthetic analogs of cardenolides were evaluated. The plaque reduction assay (IC_{50}) evaluated antiviral activity against HSV. Two compounds, 6b and 6d, stood out in this research context and were selected for mechanistic studies. This involves assessing their effects on HSV-1 viral adsorption and penetration, along with analysis of cardenolides' interference in the protein expression of herpesviruses, using HSV-1 29-R strain, acyclovir-resistant. Results from the study indicate that the semisynthetic cardenolides 6b and 6d do not affect viral adsorption or penetration, and do not exhibit virucidal activity. Instead, these compounds directly inhibit specific viral proteins, preventing the formation of new virions. Therefore, these semisynthetic cardenolides deserve further investigation as bioactive compounds with potential applications in drug research and development, contributing to advancing knowledge in the field and emerging as promising therapeutic alternatives.

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PT.052

IN VITRO STUDY OF THE ACTION OF BERBAMINE ON CHIKUNGUNYA VIRUS (CHIKV)

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Arboviruses are viruses transmitted through the bite of hematophagous arthropods, their main vector being *Aedes aegypt*. Among them, Chikungunya virus (CHIKV) is responsible for the disease Chikungunya fever (CHIKF) and, until now, there is no antiviral against it. Therefore, the objective of this study was to analyze the antiviral potential of berbamine against the CHIKV replication cycle. The cytotoxicity assay was performed in BHK-21 and Huh 7.0 cells incubated with berbamine from 200 to 1.5625 µM during 24 hours. The highest non-cytotoxic concentration, above 80% viability, was 6.25 µM for BHK-21 and 12.5 µM for Huh 7.0, used in the antiviral assays. The initial screening with berbamine with CHIKV-CMV- Nluc at MOI 0.1 and 2 for BHK-21 and Huh 7.0, correspondingly, presented an inhibition in the CHIKV replication cycle of of 77.3% and 94.6%, respectively, in BHK-21 and Huh 7.0 cells. The dose dependence assay were tested at different concentrations of berbamine and CHIKV-CMV-NLuc at MOI 0.1 for BHK-21 and MOI 2.0 for Huh 7.0, presenting a the selectivity index of 3.19 for BHK-21 and 4.19 for Huh 7.0. The time-of addition assay with berbamine and CHIKV-CMV-Nluc performed in BHK-21 presented higher CHIKV inhibition results at entry and post-entry, with 88.8% and 83.0% inhibition of the viral replication cycle, respectively. Berbamine showed protection in BHK-21 cells against CHIKV and an inhibitory effect at entry and post-entry. It is possible to note that significantly inhibits the action of CHIKV, being a safe compound for antiviral research against CHIKV.

Financial Support: CAPES/CNPq

PT.053

USE OF STORYTELLING TECHNIQUES AND ARTIFICIAL INTELLIGENCE FOR THE DEVELOPMENT OF AN E-BOOK TO EXPAND KNOWLEDGE ABOUT VACCINES

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Scientific dissemination about vaccines plays a important role in society, especially in the current context which the growing spread of false information and misinformation related to vaccines has generated distrust and doubts among the population, compromising adherence to immunization campaigns and the effectiveness of disease prevention measures. Translating scientific data for the general public is essential for disseminating accurate information about vaccination. Based on this, we decided to create an e-book for children that tells the history of vaccines in a playful and engaging way, showing the importance of vaccines and their evolution over time. For this, the e-book was developed using storytelling techniques, with meetings held to construct the script and define the characteristics of the characters, which were designed using the artificial intelligence tool Midjourney. The e-book has 45 pages and narrates the history of vaccines and vaccination with the help of Sofia, a curious little girl, and Clara, a researcher. Together, they embark on a time-traveling adventure through significant events in the history of vaccines, accompanied by Mensageirinha, an RNA messenger strand that carries the message about the importance of vaccines. This e-book, in addition to providing accurate information about vaccines, will foster scientific interest and understanding from an early age, helping to build a more informed generation. Furthermore, the book can be a support for parents, as it will provide scientific information aimed at promoting an informed, conscious society capable of discerning misinformation caused by fake news from scientifically-backed information.

Financial Support: FAPEMIG, CNPq and UNIFAL-MG

PT.054

DEVELOPMENT OF QUALITATIVE EVALUATION TEST OF ANTIBODIES AGAINST RABIES VIRUS IN ELUTED SERUM SAMPLES 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 neutralizing antibodies against the RABV. This study aimed to develop an alternative serological method for detection of antibodies against RABV using serum samples from filter paper. For this, twelve serum samples from cattle previously tested using the RFFIT technique were used: 4 positive, 4 negative, and 4 weak positive (low titer). These samples were transferred onto filter paper discs (2 cm diameter), and different variables were applied and tested in microtubes: a) type of eluent to be used (PBS or dilution fluid); b) incubation time of the disc in the eluent (1 hour or 24 hours); c) amount of eluent (100 µl or 200 µl); d) number of discs (1 disc or 3 discs). After applying the variables qualitatively, discs were removed from the microtubes and the eluent from each sample was processed using RFFIT technique. As results, the 4 positive samples remained positive, while the 4 negative samples remained negative. The 4 weak positive samples presented negative results. The variables that maintained positivity across the 4 selected positive samples used 3 discs and 24 hours of incubation in 200 µL of PBS. This study demonstrated the feasibility of sending samples on filter paper for easier transportation and storage without refrigeration. Further studies are needed, and prospects include testing whole blood samples on filter paper paired with serum samples in microtubes from the same animal.

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PT.055

IMPACT OF ANTIBIOTIC-INDUCED MICROBIOTA DEPLETION ON INFECTION CAUSED BY SLEV IN MICE

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The St. Louis encephalitis virus (SLEV) is a Flavivirus transmitted by Culex mosquitoes that causes severe neurological diseases and high mortality rates. Recent studies have highlighted the role of gut microbiota in viral infections, but the specific impact on SLEV susceptibility remains poorly understood. This study investigated how antibiotic-induced microbiota depletion might increase the susceptibility of mice to SLEV. We used C57BL/6 mice, previously treated with antibiotics, and an untreated control group. Both groups were inoculated with SLEV. Microbiota depletion was confirmed by reduced bacterial density. A significant alteration in the taxonomic profile of the microbiota was also observed in antibiotic-treated mice, with a decrease in the Firmicutes and Bacteroidota phyla, known for their fermentative and immunomodulatory functions, and an increase in Proteobacteria, a phylum of pathogenic Gram-negative bacteria. Although microbiota depletion did not significantly alter viral load, it did modify antiviral cytokine response, with reduced INF-γ levels in depleted mice. These mice showed greater disease susceptibility, with higher mortality rates (83%) and more significant weight loss compared to mice with intact microbiota (50%). The results highlight the importance of gut microbiota in SLEV susceptibility, underscoring the need to further investigate the interaction between microbiota and viral infections, especially those affecting the central nervous system.

PT.056

SEXUAL BEHAVIOR AND MONKEYPOX KNOWLEDGE AMONG USERS OF A HEALTH CARE UNIT IN DIVINÓPOLIS-MG

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Monkeypox (Mpox) is an infectious disease of viral etiology, caused by *Monkeypox virus.* Despite the reduction of cases in the last year, Mpox is still prevalent and the lack of knowledge about the disease indicates the need for continued education of the population. This study aimed to investigate the level of knowledge about Mpox among users of the Health Care Unit (SAE/CTA), in Divinópolis-MG. A cross-sectional study was conducted, in which the participants answered a questionnaire, with questions about sociodemographic characteristics, sexual behaviors, health, and knowledge about Mpox. Descriptive analyses were used. 167 people responded the questionnaire. Preliminary results showed that the mean age of the participants was 41 years (SD=12.4). The majority was man (59.3%), self-declared no-white (64.7%), living without a partner (65.9%), with completed high school (42.5%). In relation to sexual behaviour, the majority was self-declared heterosexual (55.9%) and had their first sexual relationship in adolescence (70.7%). Frequent condom use, in the last year, was reported by 34.5% of the participants, with the majority reporting not having used it (37.9%). Approximately 30% of the sample reported a sexually transmitted infection diagnosis last year and 82.3% reported good health. Regarding Mpox knowledge, the majority was familiar with the disease (64.7%). However, only 5.4%, 44.2%, and 20.4% reported know about transmission, symptoms and vaccine, respectively. The results point to the need to disseminate informations about Mpox to notify, treat and alert the community in order to mitigate its spread.

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PT.057

A Peptide Formulation Based on Annexin A1 as a Host-directed Therapy Against Dengue Disease

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Severe dengue is characterized by intense inflammation, cytokine storm, vascular leakage, hemorrhage, and shock. Innate immune cells and products are key in these processes. There are no licensed antiviral drugs or host-targeting therapies for dengue, highlighting the urgent need for new treatments. Our research identifies Annexin A1 (AnxA1) as crucial in resolving dengue. We found reduced AnxA1 levels in dengue patients, especially severe cases, and in DENV-infected mice. Treatment with the AnxA1 mimetic peptide (AC2-26) improved disease outcomes in mice without impairing infection response. However, systemic peptide administration faces issues of instability and rapid degradation. To overcome this, we formulated AC2-26 with cyclodextrins (CDX) to enhance stability and bioavailability. Using Hydroxypropyl-β-cyclodextrin, we improved the stability and effects of AC2-26 in vivo. Experiments on A129 mice infected with DENV-2 involved intraperitoneal or oral administration of pure or formulated peptide (CDX-AC2-26) starting 36 hours post-infection. Euthanasia occurred at day 3, during peak viral replication. Results showed both treatments reversed clinical scores and thrombocytopenia. CDX-AC2-26 had enhanced anti-inflammatory effects, reducing mast cell degranulation, plasma MCPT-1, and cytokine levels in the spleen. Importantly, CDX-AC2-26 did not affect DENV titers, indicating selective anti-inflammatory and pro-resolving properties. Combined with an antiviral drug (a nucleotide analogue), CDX-AC2-26 fully prevented disease and lethality in mice. These findings emphasize the importance of combined therapies for synergistic effects, leading to more effective strategies with fewer side effects.

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PT.058

A NOVEL DNA-LAUNCHED REVERSE GENETICS SYSTEM FOR GENERATION OF RECOMBINANT FLAVIVIRUSES AND ALPHAVIRUSES

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Viral reverse genetics systems are a powerful tool for investigating several aspects of viral biology, as well as for developing recombinant live virus vaccines, diagnostic assays, and high-throughput platforms for screening antiviral candidates. Regarding positive RNA viruses, such as flaviviruses and alphaviruses, the generation of recombinant viruses may be hampered by the need for high-quality RNA (obtained via *in vitro* transcription) and/or by the instability of some viral genomes when maintained in conventional *Escherichia coli* cloning systems. Herein, we describe a novel, stable and simple DNA-launched reverse genetics system for manipulating flavivirus and alphavirus genomes. Initially, we constructed a hybrid vector for cloning in both eukaryotic (*Saccharomyces cerevisiae*) and prokaryotic (*E. coli*), containing the citomegalovirus (CMV) promoter and the hepatitis delta virus (HDV) ribozyme at the 5' and 3' end of the cloning site, respectively, termed pRS-CMV-HDR-pA. The full genomes of chikungunya virus (CHIKV) (alphavirus) and flaviviruses [yellow fever virus (YFV) and Zika virus (ZIKV)] were then amplified and individually cloned into the pRS-CMV-HDR-pA vector in a single-step homologous recombination in *S. cerevisiae* (strain YPD-252). Clones of each virus (DNA plasmid) were transfected (FuGENE® HD reagent) into BHK-21 cells and the generation of replicative viral particles was monitored by cytopathic effect and confirmed by plaque assay. Overall, our system represents a stable, simple and low-cost platform for manipulating alphavirus and flavivirus genomes, contributing to the available strategies for generating recombinant viruses.

Financial Support: CNPq, CAPES and FACEPE

PT.059

Molecular epidemiology of Mayaro virus among febrile patients in Roraima State, Brazil, 2018-2021.

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Mayaro virus (MAYV) is a mosquito-borne alphavirus that has been endemic to Latin America and the Caribbean since the 1950s. MAYV infection can cause fever, rash, and arthralgia, which may develop into chronic conditions in some patients. MAYV infections share similar clinical manifestations with other arboviruses like dengue (DENV), chikungunya (CHIKV), and Zika viruses (ZIKV), making Mayaro fever difficult to distinguish clinically or epidemiologically. Furthermore, Mayaro fever remains a neglected disease with a limited understanding of its impact, even in endemic regions. In this study, we conducted a molecular epidemiology study to investigate the active circulation of MAYV and other endemic arboviruses in 822 patients with acute febrile illness between 2018 and 2021 in the Amazon region of Roraima State, Brazil, using quantitative reverse transcription polymerase chain reaction (RT-qPCR) and high-throughput sequencing. We detected the Mayaro virus (MAYV) in 3.4% (28/822) of febrile patients tested. We also isolated and sequenced MAYV strains and confirmed that these cases were caused by genotype D. Additionally, we detected CHIKV RNA in 2% (16/822) and DENV in 17.8% (146/822) of patients, including 7.6% (63/822) of DENV-1 and 10.8% (89/822) of DENV-2. All samples tested negative for ZIKV, Oropouche virus, and DENV serotypes 3 and 4. In conclusion, our study identified the active co-circulation of MAYV, DENV, and CHIKV in patients with febrile illness in Roraima State, Brazil. Our data underscore the need to improve MAYV surveillance to better determine its impact in the Amazon region.

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PT.060

IN VITRO STUDY OF GENETIC DIVERSITY AND ADAPTATION DYNAMICS OF SAINT LOUIS ENCEPHALITIS VIRUS (SLEV) DURING TRANSMISSION CYCLE.

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The Saint Louis encephalitis virus (SLEV) is capable to circulate between vertebrate and invertebrate hosts. This study evaluated the genetic diversity and adaptive dynamics of SLEV, in cell culture, during transmission cycles in vertebrate and invertebrate hosts. The infection cycles were mimicked using mosquito (Aag2) and primate (LLC-MK2) cell lines, with cycles of infection alternating lineages (interhost) and cycles without lineage alternation (intrahost), where the genetic diversity of SLEV before, during, and after each cycle was evaluated by NGS. In the interhost cycle, synonymous mutations T3727C in NS2A and C4576A in NS2B were fixed with modulation between 30% and 70%, followed by an interruption of the cycle after the second passage due to loss of infectivity. In the intrahost cycle in LLC-MK2, a frequency of 99% for the non-synonymous R100K substitution in NS1 and synonymous T7075C in NS4B was observed, while in the Aag2 cycle, the H285R mutation in the envelope protein emerged and reached a frequency of 61%. In the interhost and LLC-MK2 intrahost cycles, the emergence of two plaque phenotypes, with distinct morphology (small and large), was observed, with an increase in viral titer. These data suggest that intrahost cycles impose distinct selective pressures that drive the rapid evolution of SLEV, impacting the viral plaque phenotype and replicative fitness, while host alternation imposes a greater barrier to viral evolution. New assays are underway to characterize the fixed mutations separately, evaluating their impact on replicative fitness and the structure of viral proteins.

Financial support: CAPES

PT.061

EVALUATION OF ANTIVIRAL ACTIVITY OF CARDIAC GLYCOSIDES AGAINST SARS-CoV-2

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Since its discovery in December 2019, Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have been countless cases of mortality and morbidity. Several compounds such as chloroquine, hydroxychloroquine, lopinavir-ritonavir, and remdesivir have been tested as potential therapies; however, no effective treatment is currently recommended by regulatory agencies. Some studies on respiratory viruses have shown the antiviral activity of cardiac glycosides, correlating their effect with Na^+,K^+ ATPase (NKA) modulation. Cardiac glycosides are secondary metabolites used to treat patients with cardiac insufficiency because they are the most potent inotropic agentes and its effects dependt on cell type, exposure time, and drug concentration. Herein, we showed the antiviral activity of ouabain, digoxin and convalatoxin on VERO E-6 cells infected with SARS-CoV-2 (MOI 0,1). All compounds showed activity antiviral 24 hours post infection. The cell viabily was evaluated by resazurin colorimetric method at 48 and 72 hours. Digoxin and Convalatoxin were not cytotoxic in the concentrations tested at 48 and 72 hours. However, ouabain was cytotoxic from 0.25µM in 48 hours and 0.5µM in 72 hours. All drugs demonstrated high efficacy upon prophylactic administration at least in on stage. Therefore, the cardiac glycosides evaluated impairs the SARS-CoV-2 infection in the therapeutic-treated cells.

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PT.062

BACTERIOPHAGES AGAINST *Pseudomonas aeruginosa* ARE VERY STABLE TO TEMPERATURE AND PH VARIATIONS

Sanchietta L[1], Cunha GA[1], Durante MFR[1], Marangoni GS[1], Terceti MS[1], Gabriel MLC[2], Carvalho BA[1], Malaquias LCC[1], Coelho LFL[1] - [1]Federal University of Alfenas, Brazil - Department of Microbiology and Immunology, [2]Federal University of Alfenas, Brazil. - Department of Microbiology and Immunology

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 alarming projection is further exacerbated by opportunistic multi-resistant bacteria, such as *Pseudomonas aeruginosa*. Taking in consideration this fact, the use of bacteriophages or phages became a promising method due to its capacity in to use the natural replication mechanism for provoke the bacterial cell lysis and thus to control the replication of these pathogenic organism. From that, this work aims to analyze the stability of 6 phages isolated form water environmental samples in to different pH and temperature conditions. The phages were amplified and titrated using the double-layer agar technique. To check the stability of the phages to different temperatures and pHs, $1X10^9$ pfu of each phage were added to 1ml of sterile buffered saline (PBS) and exposed to temperatures of 30ºC -70ºC or at pHs 3-9 for 1 hour. After this time, the phages were titrated as described above. The results showed that the phages area very resistant to pH and temperature changes. Only pH below 3.0 and a temperature above of 70ºC were able to completely inactivate the phages. In this way, it can be concluded that all bacteriophages analyzed retain their lytic activity in various pH and temperature conditions and are highly resistant to variations in these conditions.

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PT.063

ASSESSMENT OF SUSCEPTIBILITY TO DENGUE WITH WARNING SIGNS IN OFFSPRING BORN TO ZIKA VIRUS INFECTED MICE

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The Zika virus (ZIKV) caused an epidemic in Brazil in 2015, in which cases of microcephaly and other malformations were observed in newborns. Recently, a significant increase in Dengue virus (DENV) cases has been observed, ranging from self-limited symptoms to clinical presentations of Dengue with warning signs (DWWS), which include changes in hemodynamic parameters. Therefore, cases of DWWS could be related to previous infections. The aim of the study is to investigate the association between embryonic ZIKV infection and susceptibility to DWWS in adulthood. For this purpose, pregnant C57BL/6 mice (n=5) were infected with ZIKV($1x10^6$PFU/i.p) or injected with PBS on embryonic day 5.5. After 5 weeks of birth, the pups were injected with DENV ($1x10^6$ PFU/i.v) or PBS and euthanized after 48 hours. The groups obtained were: PBS|PBS;ZIKV|PBS;PBS|DENV;ZIKV|DENV. Thrombocytopenia was observed in ZIKV|DENV (246.1±147.9) compared to PBS|DENV (554.6±248.7) (p=0.0428). Furthermore, there are signs of anemia and/or hemorrhage in ZIKV|DENV due to decreased hematocrit (24.780±3.064), red blood cell (5.04±0.62) and hemoglobin (7.7130±0.9538) values compared to PBS|DENV (35.830±7.223, p=0.0345; 7.272±1.488, p=0.047; 11.41±2.442, p=0.0304, respectively). In addition, a greater presence of inflammatory infiltrate was noted in the liver of DENV-infected animals. Thus, these results suggest that congenital ZIKV infection could be an aggravating factor during DENV infection in adulthood.

Key words: Congenital Zika Syndrome; Zika virus; Dengue virus; Dengue with warning signs.
Financial Support: CNPq, CAPES, Fapemig, INCT em dengue e interação microrganismo hospedeiro.

PT.064

ASSESSMENT OF 3-ACYLHYDRAZONE-4-QUINOLONE DERIVATIVES AS ANTI-HIV-1 AGENTS USING *IN VITRO* AND *IN SILICO* APPROACHES

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Currently, HIV continues causing a significant number of deaths worldwide. Despite the availability of antiretroviral therapies, the emergence of drug-resistant strains and the side effects of the licensed drugs necessitate the search for new treatment options. Three 3-acylhydrazone-4-quinolones (2, 3 and 4) with anti-HIV-1 activity in peripheral blood mononuclear cells were identified during an early drug discovery campaign. In this study, the antiviral potential of these derivatives against HIV-1 replication in MT-2 cells, their mechanism of action and their pharmacokinetic and toxicological (ADMET) properties were investigated using both *in vitro* and *in silico* methods. All compounds exhibited low cytotoxicity, with CC_{50} values above 700 µM, while zidovudine, an antiretroviral drug, had a CC_{50} of 128 µM. Compound **2** demonstrated the strongest anti-HIV-1 activity (EC_{50} = 3.37 µM), followed by compounds **3** (EC_{50} = 3.79 µM) and **4** (EC_{50} = 4.03 µM). Mechanistic studies indicated that these compounds act on steps after entry into viral replication, such as reverse transcription. Further enzymatic assays confirmed that compounds **2** and **4**, but not **3**, inhibit HIV-1 reverse transcriptase (RT) with IC_{50} values of 29.52 µM and 22.77 µM, respectively. Molecular docking with HIV-1 RT showed that compounds **2** and **4**, in contrast to compound **3**, share a similar binding mode with the non-nucleoside RT inhibitor delavirdine, which explains their different activity profiles. In addition, ADMET studies underpinned their potential as drug candidates. Therefore, our results emphasize the antiviral potential of these three 3-acylhydrazone-4-quinolone derivatives and support the development of new anti-HIV-1 drug candidates.

PT.066

FOLIC ACID RESTRICTED DIET DURING PREGNANCY OF Zika virus INFECTED FEMALE MICE LEADS TO FETAL COMPROMISE AND OFFSPRING DEVELOPMENTAL IMPAIRMENT IN ADULTHOOD

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Zika virus (ZIKV) is an arbovirus associated with neurological complications, microcephaly and fetal anomalies. This set of fetal malformations associated with ZIKV is called Congenital Zika Syndrome. In 2015, Brazil experienced an outbreak of ZIKV where it was observed that clinical signs were aggravated in regions of social vulnerability and malnutrition, which possibly related to the low intake of important nutrients for fetal formation, like folic-acid. Thus, the objective was evaluating the effect of the absence of folic-acid in the pre and postnatal period of the offspring of mice infected with ZIKV. Pregnant female C57BL/6 mice previously subjected to control and folic-acid-free-diet received PBS or ZIKV on embryonic day (ED) 5.5, obtaining the groups: control-diet PBS and ZIKV (C/PBS;C/ZIKV); folic-acid-free-diet PBS and ZIKV(AF^-/PBS;AF^-/ ZIKV). Fetal tissues were collected on DE15.5 or the offspring were waited for birth and accompanied for 12 weeks. In the prenatal phase, a reduction in fetal weight, increase in the concentration of inflammatory mediators, viral load and a decrease in the recruitment of macrophages and neutrophils in the placenta were observed in AF^-/ZIKV. In the brain, increase in the concentration of inflammatory mediators and macrophage recruitment were observed, additionally to inadequate morphological development in AF^-/ZIKV. In the postnatal phase, the offspring presented brain, ocular, cardiac and testicular malformations, father to behavioral changes involving memory loss, anxiety and hyperactivity in AF^-/ZIKV. These results suggest an intensified maternal immune activation in ZIKV infection associated with maternal folic-acid deficiency during gestation, which is reflected in the development of the offspring.

Financial Support: CNPq, Capes, Fapemig.

PT.067

EVALUATION OF THE ANTIVIRAL POTENTIAL OF 4-QUINOLONE DERIVATIVES AGAINST HERPES SIMPLEX VIRUS TYPE 1

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Herpes Simplex Virus Type 1 (HSV-1) currently affects more than 3 billion people worldwide. Its characteristic clinical manifestations include orolabial and/or genital lesions as well as skin ulcers. However, more serious manifestations can occur, such as encephalitis, meningitis, and neurodegenerative diseases such as Alzheimer's. Current drugs do not eliminate the virus from the body and are ineffective against new resistant strains. This study aims to evaluate the antiviral activity of 4-quinolone derivatives (M01-M10) against HSV-1. Initially, the cytotoxic profile of these molecules was evaluated in Vero cells using the MTT method and it was observed that the derivatives showed a low cytotoxic effect ranging from CC_{50} = 434.82 µM to >1000 µM, except for derivative M10 (CC_{50} = 51.78 µM). Subsequently, for antiviral activity evaluation, Vero cells were infected with HSV-1 (KOS strain; MOI 1) and then treated with molecules (50 µM) for 24 h. The lysates were collected, and the viral titers were determined by plaque reduction assay. Derivatives M01, M07, M08, and M10 showed the best percentages of inhibition (72,09%; 92,63%; 74,34%;73,68%) respectively. The most active molecules had their EC_{50} and selectivity index determined. The M08 derivative (EC_{50} = 30.83 µM / SI = 32.44) was the most potent, followed by M07 (EC_{50} = 31.02 µM / SI = 14.02) and M01 (EC_{50} = 35.33 µM / SI = >28,31), respectively. Finally, according to in silico studies, the derivatives showed good bioavailability and a low toxicological profile. Therefore, the 4-quinolone derivatives presented possible molecules as antiviral candidates against HSV-1.

Financial support: CNPq, CAPES, FAPERJ, UFF (PROPPI)

PT.068

EVALUATION OF AMINO SUGAR-BASED ISOQUINOLINE-5,8-DIONES AND NAPHTHOQUINONES AS POTENTIAL ANTIVIRALS AGAINST CHIKUNGUNYA VIRUS

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Chikungunya virus (CHIKV) is endemic in tropical regions, including Brazil, with over 243,000 suspected cases reported in 2024. This virus is primarily transmitted by *Aedes* mosquitoes and CHIKV infection can lead to symptoms such as fever, joint pain, and myalgia, with some patients experiencing persistent joint-related symptoms. Although a vaccine has been approved by the FDA, there are no specific antivirals available yet, which stimulates the development of new drugs. In this context, isoquinolines and naphthoquinones are known to possess potential antiviral activity against RNA viruses. This study aims to evaluate the cytotoxicity and antiviral activity of synthetic amino sugar-based isoquinoline-5,8-diones (**W1**-**W5**) and naphthoquinones (**W6**-**W9**) against CHIKV. First, Vero cells were treated with compounds (50-1,000 µM) for 72h, and cytotoxicity was measured using the MTT assay. Then, the antiviral activity of the compounds was evaluated. Vero cells were infected with the CHIKV BRA/RJ/18 strain using a MOI of 1.0 for 1h. After that, cells were treated with compounds (50 µM) for 24h and supernatants were collected for virus titration using plaque reduction assay. Among the tested compounds, only compounds **W1**, **W2**, **W6**, **W8,** and **W9** showed low cytotoxicity with 50% cytotoxic concentration (CC_{50}) values higher than 240 µM. Besides, compound **W2** showed significant antiviral activity and inhibited 78.93% of virus replication, while the other compounds showed low activity (<40%). In conclusion, compound **W2** exhibited low cytotoxicity and strong inhibitory activity, indicating its potential as an antiviral agent against CHIKV for future investigation.

Financial Support: CNPq, CAPES, FAPERJ

PT.069

SEASONALITY OF AVIAN INFLUENZA VIRUS IN BRAZIL / SOUTH AMERICA

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There is an abundance of data on influenza incidence in human beings. This tends to be from temperate regions and show a strong seasonality characterized by a single peak in the winter months lasting between 8 and 12 weeks. Although seasonal human influenza patterns in tropical and subtropical regions are not as pronounced as temperate regions, there are numerous studies that demonstrate a certain viral seasonality. Although there is some evidence that survival of the avian influenza virus (AIV) is temperature dependent, there has been limited investigation into the seasonal patterns of incidence in birds. In this work, individual avian influenza isolates were aggregated by month and place. Data collection were provided by Influenza Virus Resource from the National Center for Biotechnology Information. The circulation of avian flu strains does not seem to be restricted to a single short season, but rather, the risks appear to be greater during two months of the year, April and November in Brazil, and April and December in South America. In Brazil peaks coincide with the arrival and departure of migratory birds. The study of the seasonality of AIV is quite complex as it can be influenced by a series of factors, including climatic and geographic conditions, bird migration patterns and interactions between different bird species. The purpose of this work is to show that there is a seasonal pattern (incidence peaks) in avian influenza detection in Brazil. This may be important when considering the time and target populations for future scientific expeditions.

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PT.070

SARS-COV-2: ULTRASTRUCTURAL CHARACTERIZATION OF MORPHOGENESIS IN AN IN VITRO SYSTEM

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The pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has impacted public health and the world economy and fueled a worldwide race to approve therapeutic and prophylactic agents, but so far there are no specific antiviral drugs. Understanding the biology of the virus is the first step in structuring strategies to combat it, and in this context several studies have been conducted with the aim of understanding the replication mechanism of SARS-CoV-2 in vitro systems. In this work, studies using transmission and scanning electron microscopy and 3D electron microscopy modeling were performed with the goal of characterizing the morphogenesis of SARS-CoV-2 in Vero-E6 cells. Several ultrastructural changes were observed—such as syncytia formation, cytoplasmic membrane projections, lipid droplets accumulation, proliferation of doublemembrane vesicles derived from the rough endoplasmic reticulum, and alteration of mitochondria. The entry of the virus into cells occurred through endocytosis. Viral particles were observed attached to the cell membrane and in various cellular compartments, and extrusion of viral progeny took place by exocytosis. These findings allow us to infer that Vero-E6 cells are highly susceptible to SARS-CoV-2 infection as described in the literature and their replication cycle is similar to that described with SARS-CoV and MERS-CoV in vitro models.

Financial Support: CNPq, Fiocruz-IOC

PT.071

TESTS OF ANTIVIRAL ACTION MOLECULES IN VERO E6 CELLS INFECTED WITH SARS COV-2: AN ULTRASTRUCTURAL VIEW

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Coronaviruses (CoV) drew worldwide attention for causing outbreaks of severe acute respiratory syndrome (SARS-CoV) in Asia 2002-03 and respiratory diseases in the Middle East (MERS-CoV), 2012. In December 2019, once again a novel coronavirus (SARS-CoV-2) was first identified in Wuhan, China, and was associated with a severe respiratory infection, now known as COVID-19. Vero E6 cells are shown as a well-characterized standardized in vitro system for viral mass production, for testing drug candidates and vaccines against the novel coronavirus. Given the impact on global public health, there is a rush to protocol an effective therapy, this project proposes: to analyze the impact on the biosynthesis of SARS-CoV-2 in the Vero-E6 lineage when treated with molecules with antiviral action. For this, we evaluated by transmission electron microscopy monolayers infected with viral isolates and exposed to drugs that have already been identified as interesting from the point of view of inhibiting SARS-CoV-2 replication, such as mefloquine and daclatasvir at 10 µM. Viral titer in supernatants was assessed by detection of viral RNA using the quantitative technique of RT-PCR. All procedures involving experimental infection were performed in a safety level 3 laboratory, in accordance with the standards of the WHO. Preliminary studies revealed that 10 µM of mefloquine promoted inhibition of viral replication while daclatasvir inhibited replication by around 80%. These medications could be good candidates for clinical research in animals and humans. The ultrastructure promotes the generation of refined data and high value for biomedical research.

Financial support: CNPq; Fiocruz-IOC

PT.072

DETECTION OF DENGUE IN SALIVA OF MICE: FIRST DETECTION IN AN IMMUNOCOMPETENT MURINE MODEL

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The lack of an experimental animal model for the study of dengue pathogenesis is a limiting factor for the development of vaccines and drugs. In previous studies, our group demonstrated the susceptibility of the BALB/c mice to infection by dengue virus (DENV) 1 and 2, and the virus was successfully isolated in several organs. In this study, BALB/c mice were experimentally infected via intravenous route with DENV-4, and samples of their saliva were collected. The viral RNA was extract from the saliva samples, which was in turn subjected to qRT-PCR, with detection limit of 0.002PFU/mL. The presence of DENV-4 viral RNA was detected in the saliva of two mice, presenting viral titers of 10^9 RNA/ml. While the detection of DENV RNA via saliva sampling is not common practice as a diagnosis tool, due to the lower detection rates in human patients, the results observed in this study seem to indicate that, as in humans, saliva sampling in mice also produces lower detection rates, correlating the infection in both cases. This study reports the first DENV detection in saliva of BALB/c immunocompetent mice experimentally infected with non-neuroadapted DENV-4.

Financial support: CNPq; Fiocruz-IOC

PT.073

EVALUATION OF THE HUMORAL IMMUNE RESPONSE AGAINST YELLOW FEVER VIRUSES (VACCINE AND SYLVATIC) IN VACCINATED INDIVIDUALS

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High- impact outbreaks of Yellow Fever (YF) occurred in Brazil between 2016 and 2018, with more than 2,000 cases and over 700 deaths recorded. The primary form of protection against yellow fever is the 17DD vaccine. In this context, recent data indicate that there is a difference in the humoral immune response generated against the vaccine virus compared to the sylvatic virus. This study aimed to evaluate the humoral immune response produced against the wild and vaccine YF viruses in individuals vaccinated with the 17DD vaccine. To this end, the plaque reduction neutralization test, which is considered by the World Health Organization to be the gold standard for assessing the humoral immune response in vaccinated or YF patients, was carried out. A total of 23 serum samples from healthy individuals vaccinated with a full dose of the 17DD vaccine, who were part of a study on vaccine response conducted in São Paulo, Brazil, in 2018, were tested. The samples were collected pre-vaccination and 30-45 days after immunization. The results revealed the absence of neutralizing antibodies against the vaccine and wild yellow fever viruses in samples collected before vaccination. Additionally, vaccinated individuals showed higher levels of neutralizing antibodies against the 17DD virus compared to the wild virus, revealing different homotypic and heterotypic humoral immune response profiles. Thus, the findings described here are relevant when considering guidelines for population immunization programs, especially in areas endemic for the YF disease.

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

PT.074

HOST RANGE ANALYSIS OF LYTIC BACTERIOPHAGES OF *PSEUDOMONAS AERUGINOSA ISOLATED FROM SOUTHEAST BRAZIL*

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Pseudomonas aeruginosa is a Gram-negative bacterium capable of biofilm formation and some isolates exhibit high resistance to antibacterials. Bacteriophages represent an alternative to conventional drug therapy, offering potential solutions to the issues of antibiotic resistance and biofilm barrier. Bacteriophages were isolated using wastewater samples from several cities from southeast Brazil and the host bacterial strain PA14, resulting in the purification of 28 bacteriophages in the last three years. Host range analyses were performed using five multi-drug resistance (MDR) strains of *P. aeruginosa*. The MDR phenotype was confirmed using disk-diffusion method, to assess the susceptibility of these strains to antibacterials (Meropenem, Ceftazidime, Piperacillin + Tazobactam, Imipenem, Amikacin, Ciprofloxacin, Cefepime, Aztreonam, and Polymyxin B). The presented resistance varied between complete resistance, where bacterial growth occurred directly over all antibiotics tested, and intermediate resistance. Two MDR strains (PA SPM 37166 and PA 37117) exhibited resistance to all bacteriophages, while the remaining three strains displayed susceptibility, with sensitivities to 14 (PA KPC 37174), 12 (PA 37090), and 1 bacteriophage (PA KPC 37121) respectively. Therefore, phage therapy emerges as a promising strategy in light of the challenges posed by biofilm formation and antibacterial resistance in Pseudomonas aeruginosa.

Financial Support: FAPEMIG, CNPq and UNIFAL-MG

PT.075

CHALLENGES IN THE CHIKUNGUNYA VIRUS EMERGENCE DURING A DENGUE OUTBREAK IN BRAZIL

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Chikungunya virus (CHIKV) and the Dengue virus (DENV) exhibit an overlapping of signs and symptoms and frequently co-circulate in hyperendemic tropical areas for dengue, which challenge the clinical diagnosis and highlight the need of more accurate diagnostic tests. Between November 2023 and April 2024, during a dengue outbreak accompanied by the emergence of CHIKV, 2,342 serum samples were analyzed by RT-PCR, of which 126 were positive for DENV and 133 for CHIKV. Additionally, comorbidities, signs, symptoms, and hospitalization frequency from the patients were assessed. The analysis revealed a higher frequency of leukopenia. (34.92% vs. 20.30%, $p = 0.008$), thrombocytopenia (13.49% vs. 5.26%, $p = 0.025$) and ocular pain (20.63% vs. 8.27%, $p = 0.004$) in dengue-patients, while rash (14.29% vs. 4.76%, $p = 0.009$), arthralgia (44.36% vs. 12.7% $p < 0.001$), and occurrence of hospitalizations (49.62% vs. 31.75%, $p = 0.003$) were more frequent in Chikungunya-patients. In addition, eight Chikungunya-patients presented neurological manifestations, in contrast of no cases in dengue ($p = 0.015$), being them encephalitis, meningoencephalitis, encephalopathy, and stroke. Once the clinical or laboratory manifestations are not exclusive of each disease, these findings underscore the need for etiological diagnosis in hyperendemic areas for dengue to support the clinical approach for managing these viral infections.

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PT.076

INACTIVATION OF SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2 (SARS-COV-2) BY PAINT FORMULATIONS WITH BIOCIDES ADDITIVES

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Public awareness of emerging pathogens and infection transmission by contaminated surfaces has increased the interest in materials with biocide activity. Materials with additives that can inactivate viral particles are a sound alternative for hard to decontaminate places such as those with high circulation of people, like mass transit stations, or with naturally higher chance of contamination, like hospitals. Reactive oxygen species can nonspecifically oxidize lipids and proteins, rendering viral particles inactive. Addition of pro-oxidant agents to paints could decrease virus dissemination by fomites. We investigated the ability of different acrylic or epoxy paint formulations to inactivate enveloped viruses. Viral inactivation assay was performed by incubating 1000 DI_{50} of Severe Acute Respiratory Syndrome Coronavirus 2 (*Betacoronavirus pandemicum,* SARS-CoV-2 P.1/Gamma variant) for five minutes on a coated surface (1.9 cm^2) with a paint with (tannin, zinc pyrithione (PtZn), copper(I) oxide or copper(II) oxide) or without (control) additives. After, viruses were recovered from the surfaces and pipetted into a well of a 96-well plate seeded 24h prior with 2.10^4 VERO cells per well. At three days post-infection, RNA was extracted, and RT-qPCR was performed. Three independent samples of each material were tested, and each qPCR reaction was performed in duplicates. Viral genome load recovered from all epoxy paints with additives were significantly lower than genome load from control ($p \leq 0.008$), in which tannin presented the best result with a greater than 1000-fold reduction. No acrylic paint formulation presented viral inactivation properties. Further studies include testing against non-enveloped viruses and inactivation mechanism confirmation.

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PT.077

Experimental Infection with Respiratory Syncytial Virus during Pregnancy

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Respiratory syncytial virus (RSV) is considered the most important viral agent of lower respiratory tract diseases in pediatric populations worldwide, and RSV infections are particularly severe in infants, especially preterm neonates. Experimental studies about RSV transmission to fetuses through the placenta and to neonates by lactation have been limited. We developed an experimental model of vertical transmission of RSV in BALB/c mice and detected the presence of viral RSV genome in lungs from the dams, as well as in placentas, brains and fetal lungs from fetuses in mid and late gestation. The offspring gestated and nursed by sham-inoculated dams was susceptible to RSV challenge on the 5th day of life, as evidenced by increased lung cellularity, thickening of alveolar septa and significantly higher viral loads. Conversely, neonates born from and nursed by RSV-infected dams were resistant to challenge. Remarkably, RSV genome was detected in mammary gland tissue by RT-qPCR and primary placental cells obtained from mice were permissive to *in vitro* RSV infection. These findings suggest that RSV can be vertically transmitted from mother to offspring, providing in utero and postnatal exposure that potentially influences neonatal susceptibility to subsequent RSV infections. The detection of the viral genome in both fetal tissues and mammary glands highlights the complex dynamics of RSV transmission and suggests potential for further research into maternal-fetal interactions and the development of protective strategies for at-risk neonatal populations.

Financial Support: CAPES, CNPq

PT.078

GENOMIC ANALYSES OF VACCINIA VIRUS STRAIN CANTAGALO FROM DIFFERENT REGIONS OF BRAZIL, 1999 - 2022

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Cantagalo (CTGV) is a field strain of vaccinia virus (VACV, Poxviridae), first identified in 1999 in Cantagalo-RJ, causing vesicular lesions in dairy cattle and milkers. Since then, outbreaks of CTGV infection have been reported in states of Southeastern, Midwestern, and Northern Brazil. The spread of CTGV in Brazil represents a unique opportunity to study the natural evolution of VACV, otherwise mainly restricted to smallpox vaccine strains. This work aimed to characterize the genomes of 14 clinical isolates of CTGV obtained in different states throughout 23 years of its circulation in Brazil. Lesion scabs were used to isolate virus in BSC-40 cells. Genome sequencing used either virosomal or purified virus DNA on Illumina MiSeq and NextSeq platforms. Reads were assembled using Spades and Geneious, and genomes were annotated using GATU/CLC. The genomes ranged from 180,989 bp to 187,259 bp with 96.6% to 99.99% identity. Phylogenetic analyses clustered all CTGV isolates as a sister group to the Brazilian vaccine strain IOC and horsepox viruses. Three SNP-related subgroups were detected, that is, ‘Southeastern (1999-2004)’, ‘Rondônia (2009-2012) and Espírito Santo (2007)’ and ‘Goiás (2022) and Tocantins (2008)’, also supported by Median Joining network analysis. Bootscan analysis of virus isolates from Goiás revealed a >70% supported genetic contribution of viral genomes from TO. All Southeastern subgroup shared a 3.7-kb deletion in the 5´end. Overall, CTGV genomes exhibited fewer SNPs and INDELs when compared to VACV strains used as smallpox vaccine. An in-depth analysis of genomic and populational structure is in course.

Funds: CNPq, CAPES, FAPERJ

PT.079

Ultrastructural alterations in mammalian cells infected with Ilheus virus

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The orthoflavivirus ilheusense (ILHV) is a neglected arbovirus belonging to the genus Orthoflavivirus and the family Flaviviridae. Cases in humans occur periodically, mainly in Latin America, indicating the epidemic potential of this pathogen. Brazil is the country with the highest number of cases. Orthoflaviviruses, during infection in cells, induce various morphological and cellular changes. These alterations are related to both the replication process and the structures formed from cellular damage. However, although there is clearer data in the literature for other orthoflaviviruses, the relationship between ILHV and the formation of these structures, its replication cycle, and cellular alterations remains unknown. One of the main objectives of this study is to characterize the primary morphological and ultrastructural changes in infected green monkey kidney epithelial cells (Vero), as well as to map their replication cycle, virion structure, and genome. To achieve these objectives, monolayers of Vero cell lines were infected with an MOI of 0.01 and collected at different times post-infection. The cytopathic effects (CPE) on the monolayers were evaluated using bright-field microscopy. For ultrastructural analysis, the Hitachi HT 7800 transmission electron microscope was used. Ultrastructural analyses confirmed that ILHV can induce the formation of double-membrane vesicles, convoluted membranes, and vesicular packets. These structures, similar to those observed in Zika (ZIKV) and Dengue (DENV) viruses, constitute replication complexes that assist in the ILHV replication process in cells. Our preliminary results reveal that ILHV infection induces a cytopathogenesis like that observed in in vitro studies for other orthoflaviviruses.

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PT.080

Thermodynamic Analysis of the Structural Stability of Chikungunya Viral Particles Physically Inactivated by High Hydrostatic Pressure and their Immunogenicity.

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Chikungunya fever is caused by an arbovirus transmitted by mosquitoes of the genus Aedes. Belonging to the genus Alphavirus and the Togaviridae family, Chikungunya (CHIKV) can cause musculoskeletal and joint pain and death. The objective of the work is to evaluate the structural stability and immunogenicity of CHIKV particles inactivated under high hydrostatic pressure (HHP). Viral particles were incubated under HHP (1 to 16h) at 2.8kbar and evaluated for infectivity by plaque-forming unit assay. Purified viral samples were used for transmission electron microscopy (TEM) and spectroscopic assays. Intrinsic fluorescence spectroscopy and light scattering data were collected with virus samples at increasing pressures (0.7 to 2.8 kBar) and increasing urea concentrations (0 to 8 M). For inactivation assays, viral particles were incubated at 2.1 kBar for 30 hours or 2.8 kBar for 16 hours. Humoral immunogenicity assays were performed in BALB/c mice that received 3 doses of HHP-inactivated viral antigen at 14-day intervals. As a result, virus inactivation was observed when pressures of 2.1 and 2.8 kBar were used. Light scattering and intrinsic fluorescence spectroscopy data suggest that pressures up to 2.8 kBar do not lead to particle aggregation and quaternary and tertiary structural proteins remain relatively stable. However, TEM images indicate loss of the mature structure and loss of specific humoral immunogenicity, as shown in ELISA and microPRNT assays. We conclude that HHP induces CHIKV inactivation and, under our experimental conditions, leads to the loss of its specific humoral immunogenicit.

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PT.081

Investigating the antiviral potential of tamsulosin hydrochloride against dengue virus

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The replication of the dengue virus (DENV) involves the activation of lipophagy pathways, leading to the degradation of triglycerides and the release of fatty acids from lipid droplets, which is crucial for ATP generation and viral replication. Targeting these pathways presents a promising antiviral strategy. Transcriptomic analysis of DENV-2-infected AML12 cells showed differential expression of genes involved in lipid metabolism, including the alpha-1 adrenergic receptor (ADRA1A), which mediates responses related to lipophagy regulation and fatty acid β-oxidation. This study investigates the role of ADRA1A in DENV replication, using ADRA1A antagonists as potential therapeutic targets. Among commercially available ADRA1A antagonists in Brazil, we selected Tamsulosin Hydrochloride (TH) based on its low cost, minimal hepatotoxicity, and non-psychotropic effects. Initial assays were conducted to determine the CC50 and EC50 of TH with ≥98% purity (HPLC). Both non-infected and DENV-2-infected AML12 cells (m.o.i. 1) were exposed to eight different concentrations of TH. After three days, the supernatant was frozen and cell viability was analyzed. Non-infected cells exposed to all concentrations exhibited 100% viability. To determine the EC50, the supernatant was titrated. Infected AML12 cells exposed to 100 µM of TH showed a 90% reduction in PFU compared to the vehicle control. The EC50 was determined to be 35 µM. Currently, mechanism-of-action assays are being conducted. These results suggest that TH reduces DENV-2 particles in AML12 cells. By targeting ADRA1A, this study offers insights into DENV replication mechanisms and potential therapeutic implications.

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PT.082

A LECTIN FROM *VATAIREA MACROCARPA* AS A POTENT INHIBITOR OF ZIKA VIRUS

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Introduction: Zika virus (ZIKV), a virus from the *Flaviviridae* family has raised global concern due to its neurological and congenital complications. It is the causative agent of Zika fever and was associated with Guillain-Barré syndrome and microcephaly in newborns during the outbreak in 2015 in Brazil. This underscores the urgent need to develop new therapeutic strategies. Lectins are proteins widely distributed across the Plantae kingdom. *Vatairea macrocarpa*, a legume genus within the *Fabaceae* family, produces lectins that reported therapeutic properties, including antioxidant, anti-edematogenic, anti-inflammatory, and antibacterial. Given these biological activities, this study evaluated the antiviral potential of a lectin isolated from *Vatairea macrocarpa* (VML) against ZIKV *in vitro.* **Materials and methods:** Vero-E6 cells were infected with ZIKV-*nanoluc* at a multiplicity of infection of 0.01 for 72 hours in the presence or absence of VLM at non-cytotoxic concentrations, determined by WST-I assay. Virus replication levels were quantified by measuring *nanoluciferase* activity using the *Renilla* luciferase Assay System. **Results:** VML was able to reduce ZIKV replication in 61%. In addition, the compound demonstrated a dose-dependent activity, resulting in an effective concentration of 50% (EC_{50}) of 3 µg/mL, a cytotoxic concentration of 50% (CC_{50}) of 76.6 µg/mL, and a selective index of 25.5. **Conclusion:** In summary, these findings highlight the potential of *Vatairea macrocarpa*-derived lectins for developing antiviral treatments against ZIKV *in vitro*.

Financial support: FAPEMIG, CNPq, CAPES, FUNCAP.

PT.083

EXPLORNG THE ANTIVIRAL EFFECTS OF LECTINS FROM *CANAVALIA* AGAINST ALPHAVIRUSES *IN VITRO*

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Introduction: Alphaviruses such as Mayaro (MAYV) and Chikungunya (CHIKV) are mosquito-borne viruses posing significant public health threats, especially in Brazil. These infections are often characterized by fever and severe arthralgias. Current treatment options are limited, emphasizing the need to identify effective antivirals. In this context, lectins are non-immune proteins biding carbohydrates, widely found in Plantae. Canavalia, a legume genus in the Fabaceae family, produces lectins with reported antimicrobial effects against candida and antiviral activity against SARS-CoV-2. This study evaluated the antiviral properties of two lectins from Canavalia (ConA and ConM) against MAYV and CHIKV. **Materials and methods:** Vero-E6 or BHK-21 cells were infected with MAYV-*nanoluc* at a multiplicity of infection of 0.1 for 24 and 16 hours, respectively in the presence or absence of lectins at non-cytotoxic concentrations, determined by MTT assay. Virus replication levels were quantified by measuring nanoluciferase activity using the Renilla luciferase Assay System. **Results:** ConA and ConM inhibited CHIKV replication in 96% and 93%, respectively. ConA resulted in an effective concentration of 50% (EC_{50}) of 9.44 µg/mL, CC_{50} of 139.9 µg/mL, and a selective index of 14.7. Additionally, ConA showed inhibition of MAYV replication in 39% and ConM in 97%. **Conclusions:** In summary, ConA and ConM strongly inhibits MAYV and CHIKV *in vitro*, representing candidates for the development of future antiviral drugs against alphaviruses.

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PT.084

EXPRESSION AND OCCLUSION OF DENGUE VIRUS NS1 PEPTIDES INSIDE POLYHEDRA OF A CYPOVIRUS FOR SEROLOGICAL DIAGNOSIS .

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The dengue virus (DENV) is an arbovirus of global health concern. The challenges of existing serological diagnostic kits in the market include delayed detection of IgG/IgM antibodies and susceptibility to cross-reactivity with other serotypes and different arboviruses. Cypoviruses, members of the Spinareoviridae family, stand out for their ability to form highly stable and protease-resistant crystallized protein structures known as polyhedra. These structures hold promise for the development of serological diagnostic tests by incorporating recombinant proteins within them. This study aims to express both multi-epitope and individual epitopes from the NS1 region of the four dengue virus serotypes enclosed within polyhedra. To achieve this, a chimeric gene encompassing multiple epitopes was designed, incorporating segments of the NS1 protein from all four DENV serotypes and subsequently synthesized. Individual epitopes of each serotype were amplified using PCR. These constructs were individually cloned into a plasmid containing the cypovirus polyhedrin, controlled by a baculovirus promoter and the sequence of the alpha-helix 1 (H1) of polyhedrin. The H1 has been demonstrated to associate with the polyhedrin protein to form polyhedra. Following cloning, the genes were fused to the H1. Recombinant baculoviruses containing these various constructs were generated and used to infect insect cells. Infected insect cells exhibited the presence of polyhedral crystals. The expected molecular mass of the recombinant proteins were analyzed and confirmed by SDS-PAGE and Western-blot techniques. These recombinant crystals will be used as diagnostics inputs employing serum from patients previously diagnosed with dengue by RT-PCR and/or viral isolation.

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PT.085

EFFICACY OF CANDIDATE COMPOUNDS FOR A NEW DISINFECTANT AGAINST SARS-CoV-2, MPXV AND BACTERIA

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The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in 2019 and Monkeypox virus (MPXV) outbreaks in 2022 necessitated the adoption of strategies to contain their spread in the environment, such as personal hygiene and disinfection procedures. These measures increased the demand for antiseptic products, leading to the depletion of raw materials used in their production. In this study, we evaluated the virucidal efficacy of natural and chemical compounds from abundant sources in Brazil against SARS-CoV-2 and MPXV for use as raw materials in disinfectants. We also performed bactericidal assays with the final solution that showed the best results against viruses. We selected target compounds (in silico) for in vitro tests to determine solubility, cytotoxicity, and virucidal activity against SARS-CoV-2 and MPXV. Oxone was able to completely inactivate SARS-CoV-2 (B.1 and Omicron strains) and MPXV after 2 minutes of incubation. Consequently, a 2% Oxone solution was used to produce a new disinfectant that was also tested against *Staphylococcus aureus* and *Klebsiella pneumoniae* under various conditions. Our results demonstrate the necessity for individualized testing, as different bacteria and viruses can exhibit different behaviors when exposed to a compound and may require different contact times. Furthermore, we show the potential application of a 2% Oxone formulation with broad-spectrum virucidal and bactericidal activity.

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PT.086

The pharmacological inhibition of histidine triad binding protein (HINT1) promotes infectivity enhancement of Dengue, Chikungunya and Zika viruses

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Zika (ZIKV) and Dengue (DENV) viruses are members of the Orthoflavivirus genus and Chikungunya virus (CHIKV) of the Alphavirus genus. They promote apoptosis by modulating apoptotic factors. HINT1, a ubiquitous protein with phosphoroamidase activity, acts as a tumor suppressor by regulating p53, Bax, and Bcl-2. During antiviral research, we found that inhibiting HINT1's enzymatic activity increased DENV, ZIKV, and CHIKV virus production/infectivity in different cell lines. This study aims to characterize HINT1's role during these viral infections. Basal levels of HINT1 in cell cultures didn't alter over 96 hours and slightly increased at 72 hours in SH-SY5Y when analyzed by western blot. Compounds inhibiting HINT1 increased infectious particle production in HUH7 and SH-SY5Y cells: ZIKV (50- and 16-fold, respectively), (DENV 8-fold at 24 HPI), and (CHIKV 5-fold in SH-SY5Y at 6 HPI). A time-of-addiction experiment was performed for two compounds in SH-SY5Y cells infected with ZIKV. We observed an increase in virus production regardless of the treatment time for one of the compounds. Further, SH-SY5Y cells pretreated with IFN-β, infected with ZIKV, and treated with the compounds, showed no difference in HINT1 levels and virus production. Therefore, we conclude that HINT1 plays an important role in the regulation of these arboviruses' replication independent of the type 1 IFN cellular response. Further studies to understand the mechanism underpinning this regulation are being done.

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PT.087

GIANT VIRUSES INHIBIT SUPERINFECTION BY DOWNREGULATING PHAGOCYTOSIS IN ACANTHAMOEBA

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In the context of the virosphere, viral particles can compete for host cells. In this scenario, some viruses block the entry of exogenous virions upon infecting a cell, a phenomenon known as superinfection inhibition. The molecular mechanisms associated with superinfection inhibition vary depending on the viral species and the host, but generally, blocking superinfection ensures the genetic supremacy of the virus's progeny that first infects the cell. Giant amoeba-infecting viruses have attracted the scientific community's attention due to the complexity of their particles and genomes. However, there are no studies on the occurrence of superinfection and its inhibition induced by giant viruses. By employing techniques such as electron microscopy, one-step growth curve and immunofluorescence microscopy, this study shows that mimivirus, moumouvirus, and megavirus, exhibit different strategies related to the infection of *Acanthamoeba*. For the first time, we reported that mimivirus and moumouvirus induce superinfection inhibition in amoebas. Interestingly, megaviruses do not exhibit this ability, allowing continuous entry of exogenous virions into infected amoebas. Our investigation into the mechanisms behind superinfection blockage reveals that mimivirus and moumouvirus inhibit amoebic phagocytosis, leading to significant changes in the morphology and activity of the host cells. In contrast, megavirus-infected amoebas continue incorporating newly formed virions, negatively affecting the available viral progeny. This effect, however, is reversible with chemical inhibition of phagocytosis. This work contributes to the understanding of superinfection and its inhibition in mimivirus, moumouvirus, and megavirus, demonstrating that despite their evolutionary relatedness, these viruses exhibit profound differences in their interactions with their hosts.

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PT.088

HIV-1 NEF PROTEIN INHIBITS INTERFERON TYPE I SIGNALING PATHWAY BY DOWNREGULATION OF STAT1.

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Type I interferons are an important antiviral immune cytokine. IFN-I binding to their cell surface receptors (IFNAR1/IFNAR2) leads to receptor kinases (JAK1/TYK2) and signal transducers (STAT1 and STAT2) phosphorylation to induce the synthesis of several antiviral ISGs. To viruses can interrupt the activation of this pathway to evade the immune response and establish a productive infection. Previous work indicates that Nef, an accessory protein of HIV-1, decreased the total levels of phosphorylated STAT1, however, the mechanisms underlying this effect are not explored. This work investigated the mechanism by which Nef controls the JAK/STAT pathway. To this end, A3.01 CD4+ T lymphocyte cells expressing GFP or GFP/Nef were generated, and the total levels of this this pathway's components were evaluated under unstimulated and INF-α2b stimulated conditions. In this context, we observed that Nef reduces the total levels of STAT1 in basal and stimulated conditions leading to undetectable pSTAT1 levels. Consistently with this, Nef led to a blockage of antiviral ISGs expression. In contrast, Nef did not change the total levels of STAT2. Finally, Nef mutated in myristylation domain (G2A) does not downregulate STAT1, suggesting that the Nef's capacity to associate with cell membranes is important for this phenotype. Interestingly, treatment of Nef expressing cells with the proteasomal inhibitor MG132 partially recovered STAT1 expression. In conclusion, these results suggest that Nef expression in A3.01 T lymphocytes downregulate the JAK/STAT pathway stimulated by INF-α2b, possibly through the proteasomal degradation of STAT1.

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PT.089

Antiviral action of different molecules obtained from invertebrates against avian coronavirus

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Coronaviruses cause epidemics not only in humans, but also in animals. Therefore, the search for new antivirals has become an urgent need. Arthropods is soured for bioprospecting for news antivirals. In this study, the objective was to identify compounds with antiviral effect against avian Coronavirus in propolis from *Scaptotrigona aff postica,* hemolymph from *Lonomia obliqua* and mygalin. The propolis was obtained *Scaptotrigona aff postica*. *Lonomia obliqua* hemolymph was obtained from wild caterpillars. Mygalin was obtained from the tarantula *A. gomesiana.* The isolation and purification of antiviral substances performed by a ReversePhase HPLC DAD-ESI-MS/MS. The antiviral assay was performed by reducing infectious foci in VERO cells. Cells were treated 1 hour before infection or 1 hour after infection, with 2, 5 and 10% of propolis or hemolymph and its purified or synthetic components. For purified mygalin or it synthetic analogue was used in test 26, 52, 104 and 160 µM. Treated and untreated cells were infected with different amounts of coronavirus (100 to 1000 DCTI50). Result. Propolis and crude hemolymph reduced avian coronavirus by an average of 256x when used at a concentration of 5% v/v and an average reduction of 8x when 160µM of mygalin was used. Synthetic mygalin (26 uM), reduced viral replication by 16 times and the purified propolis reduced the virus replication an average of 32 times. The antiviral responses of the 3 substances were dose dependent, being 2 times more intense when added 1 hour before cell infection with the virus.

PT.090

Antiviral action of aqueous extracts of propolis from Scaptotrigona af. postica (Hymenoptera; Apidae) against zicavirus, chikungunya, and mayaro virus

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The limited availability of antivirals for new highly pathogenic strains of virus has become a serious public health. Insect exudates or their products are some sources for news antibiotics and antivirals. Since the ancient civilizations, the products obtained from the bee have been as antimicrobial, anti-infammatory and antitumor. Investigations of biological activity of propolis have been carried out mainly in the species Apis mellifera. However, for the Meliponini species there are few studies. The importance of studying these bees is because they come from regions with native forests and therefore with many species of plants. Previous studies by our group demonstrated an intense antiviral activity against Herpes, infuenza, and rubella viruses. Therefore, this study aimed to identify, isolate and characterize compounds with antiviral efects from aqueous propolis extracts from *Scaptotrigona af postica*, in viruses such as zicavirus, chikungunya, and mayaro virus. The evaluation of the antiviral activity of the crude and purifed material was performed by reducing infectious foci in VERO cell cultures. The results obtained with crude propolis, indicate a high reduction of zicavirus (64×) mayaro (128×) chikungunya virus(256x). The chemical characterization was performed by HPLC and mass spectrometry. The purifed fraction of propolis reduced zicavirus 16x, mayaro virus 32x and chikungunya virus 512x. Likewise, it was observed that the antiviral response was concentration dependent, being more intense when propolis was added 2 h before the viral infection. Now we are carrying out the chemical characterization of the purifed compounds that showed antiviral action.

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PT.091

Intranasal Liposomal Remdesivir causes SARS-CoV-2 Clearance in the Lung and Brain of K18-hACE2 Mice, Ensuring Complete Survival

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A huge challenge after the emergence of COVID-19 has been the discovery of effective antiviral drugs. Although remdesivir (RDV) emerged as one of the most promising drugs, its pharmaceutical formulation Veklury® is limited by moderate efficacy, high toxicity and need for parenteral administration. The aim of the present work was to develop a liposomal formulation of RDV for pulmonary administration and evaluate its efficacy in models of COVID-19. Liposomal RDV nanoformulation (LRDV) was selected based on high drug encapsulation efficiency, sustained drug release property and high in vitro selectivity index. LRDV was then evaluated for its efficacy in transgenic K18-hACE2 mice infected with SARS-CoV-2 after repeated intranasal administration at 10 mg/kg/bid for 5 days. Veklury® given intraperitoneally at 20 mg/kg was used for comparison. Mice receiving LRDV remained alive up to 15 days post-infection (dpi), in contrast to control groups given PBS and empty liposomes that showed 100% death at 6 dpi and the Veklury® group with 62.5% death at 8 dpi. A remarkable effect of intranasal LRDV is the complete elimination of SARS-CoV-2 in both the lungs and the brain of mice. This is in contrast with Veklury® that did not significantly reduce virus titer in the brain. This effect was correlated with significant inhibition of neutrophils and monocytes recruitment in the lungs. Histopathological evaluation of the liver showed marked hydropic degeneration in the Veklury® group, but not in the LRDV group. Intranasal LRDV emerges as a promising therapeutic strategy for COVID-19, including “Long COVID”.

Keywords: COVID-19, intranasal, liposomes, K18-hACE2 mice, remdesivir, SARS-CoV-2

Financial support: CAPES, CNPq and FAPEMIG

PT.092

GENOTYPIC AND BIOLOGICAL CHARACTERIZATION OF VACCINIA VIRUS STRAIN CANTAGALO ISOLATED IN RONDÔNIA, 2018 – 2021

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Vaccinia virus strain Cantagalo (CTGV, Orthopoxvirus, Poxviridae) causes a pustular skin disease in dairy cows and milkers, and has been detected in several states of Brazil since 1999. Rondônia (RO) has the highest number of cases in the Amazon region. Previous genomic studies suggest that CTGV isolates circulating in Brazil are genetically diverse, which needs further investigation. Our goal is to characterize the genotypic and biological diversity of CTGV isolates from RO collected from 2018 to 2021, a period not covered by previous studies. Fifteen clinical samples obtained from infected cows from different municipalities were inoculated into BSC-40 cells. Forty viral plaques of each isolate were analyzed, showing a similar size, nearly 1 mm^2. However, the AFO-06 isolate revealed a mixed pattern of viral plaques, some measuring 2.5±0.5205 mm^2, but most were 1±0.4140 mm^2. We then isolated representative viral clones of each plaque size, AFO-06L and AFO-06S, respectively, after three rounds of plaque purification in BSC-40 cells. The A56R, K2L, and C7L genes were PCR-amplified, followed by Sanger-sequencing. Phylogenetic inference confirmed both clones as CTGV. For genomic analysis, we selected 11 isolates, including AFO-06L and AFO-06S, to isolate virosomal DNA from infected cells. Six genomes have already been sequenced in Illumina MiSeq. Reads were assembled in Spades and Geneious, generating genomes of 185,955 to 186,857 bp that were 96.81% to 97.36% identical to the CTGV reference isolate CM-01-1999. The genomic structure, the diversity of SNPs and INDELs, and other genomic analyses are being investigated.

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PT.093

P OTENTIAL OF CAPRYLIC ACID TO REPLACE B-PROPIOLACTONE IN THE INACTIVATION PROCESS OF THE RABIES VIRUS FOR EQUINE IMMUNIZATION

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The prophylactic approach to rabies is carried out through vaccination and application of anti-rabies serum. Anti-rabies serum is produced by hyperimmunizing horses with the rabies virus inactivated with β-propiolactone (β-PL) which brings biosafety concern and is difficult to acquire in Brazil. Then, the objective of this project is to investigate alternative products to replace β-PL in the rabies virus inactivation process for equine immunization. Two batches of viruses were divided into 4 aliquots and inactivated with β -propiolactone and 1%, 0.75% and 0.5% caprylic acid, respectively. Residual activity assay was performed *in vitro* and *in vivo* to confirm viral inactivation. Next, 3 groups of rabbits were immunized with the rabies virus inactivated in different conditions, mimicking the immunization cycles used for horses. The development of the immune response was monitored by ELISA and the level of neutralizing antibodies by the RIFFT assay. The results showed that only 0.5% caprylic acid was not able to completely inactivate the rabies virus. Rabbits immunized with the different inactivated virus preparations showed a high antibody titer regardless of the groups and all antigen preparations were capable of inducing the production of neutralizing antibodies. However, the group immunized with rabies virus inactivated with β -propiolactone gave a much higher titer compared to those with caprylic acid. Therefore, caprylic acid is able to inactivate the rabies virus which maintains immunogenic properties to induce neutralizing antibodies. However, acid concentration conditions and immunization schedule need to be improved to achieve higher neutralizing antibody levels.

Financial support: FAPEMIG

PT.094

ANTIVIRAL ACTIVITY OF BRAZILIAN RED PROPOLIS COMPOUNDS AGAINST CHIKUNGUNYA VIRUS: *IN VITRO* EVALUATION AND *IN SILICO* ANALYSIS

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Chikungunya virus (CHIKV) is an arbovirus transmitted by *Aedes* mosquitoes that induces persistent polyarthralgia. Currently, there are no licensed antivirals for this virus. Therefore, this study evaluated the effects of 12 Brazilian red propolis (BRP) constituents against CHIKV in Vero E6 cells *in vitro*. Cytotoxicity and antiviral screening were performed via posttreatment assays at concentrations of 25 µM and 50 µM via an MTT assay. Promising compounds identified in the antiviral screening were further tested with serial dilutions to determine the CMNT, CC_{50}, EC_{50}, and selectivity index (SI) values. For compounds with the highest SIs, the percentage of CHIKV-positive cells was detected by intracellular flow cytometry. Additionally, a time-of-addition assay and in silico analysis of potential interactions between these compounds and CHIKV targets using molecular docking were performed. Of the 12 compounds evaluated, 6 exhibited promising activity in the screening assays. Among these, isoliquiritigenin (SI=4.87) and catechin hydrate (SI=18.31) presented high SIs and significantly reduced the percentage of infected cells. These compounds did not show activity at -2h or during viral adsorption (0h). However, in the posttreatment assay, both compounds exhibited promising activity up to 8 hours postviral adsorption. *In silico* analyses revealed strong interactions between these two compounds and the CHIKV nsP3. Taken together, these results suggest a possible inhibition mechanism of these compounds during the viral replication cycle. In brief, the BRP compounds demonstrate promising antiviral potential against CHIKV, thus contributing to the discovery of new bioactive agents.

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PT.095

SCREENING OF SEMISYNTHETICS FLAVONES WITH POTENTIAL ANTIVIRAL ACTIVITY AGAINST CHIKV IN VITRO

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Chikungunya Fever (CHIKF) is caused by the Chikungunya virus (CHIKV), an arbovirus from the *Togaviridae* family transmitted by mosquitoes of the *Aedes* genus. The disease affects all age groups, but factors such as age and comorbidities can worsen symptoms, which include self-limiting fever and severe arthralgia. Despite the FDA's approval of the Ixchiq® vaccine, the search for effective treatments remains crucial. Flavones, bioactive plant compounds with antiviral properties, and their semisynthetic analogs have emerged as promising alternatives. To evaluate the antiviral activity of nineteen semisynthetic flavones with 5-aryl, 8-aryl, and 5,8-bisaryl modifications in the native structure of a flavone found in the rhizomes of *Kaempferia parviflora* and targeting CHIKV non-structural protein 2, MTT reduction assays were conducted in BHK-21 cells to assess the compounds cytotoxicity. Of these nineteen flavones, eleven were found to be non-cytotoxic at concentrations of 18.75, 37.5, 75, and 150 µM and were selected for further antiviral activity evaluation using plaque assays with CHIKV. One 8-aryl flavone reducing the viral titer from $4.3x10^6$ to $4.7x10^2$ PFU/mL at concentration of 150 µM and showing a dose-dependent reduction in viral titer. Additionally, assays with a CHIKV replicon-transfected cell model revealed that 8-aryl flavones reduced fluorescence by more than 50%, suggesting effective interaction with the virus's non-structural proteins. Among the flavones tested, those with 8-aryl modifications, especially 8-phenyl-dimethoxychrysin, demonstrated significant potential as antiviral agents against CHIKV.

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PT.096

ZOOMING IN ON VIRAL MUTATION RATES: WHAT CAN WE SEE?

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We propose defining mutational rates as a probability distribution and investigating their properties. We based our work on *in vitro* data from cellular infections with HIV-1 in a single replication round, which had been previously published. Mutation probabilities were estimated using a Bayesian approach with Dirichlet distributions. We analyzed the data from the *in vitro* experiment by constructing a probability distribution of mutational rates rather than considering a single mean value. This allowed us to account for the variability of the data and have some insights into the virus population dynamics. The results show that the mean mutation rates range from 6.34×10^{-5} to 5.96×10^{-3} substitutions per site per replication cycle. This is consistent with the HIV-1 mutational rates previously reported in the literature, which vary from 10^{-5} to 10^{-3} substitutions per site per replication cycle. We also showed the robustness of the distribution of mutation rates concerning restriction to genes or genomic fragments. The minimum mean values observed across distinct genes ranged from 6.37×10^{-5} (*env*) to 9.13×10^{-5} (*tat*) and maximum mean values from 4.81×10^{-3} (*vpu*) to 5.90×10^{-3} (*pol*). The orders of magnitude of mutation rates were consistent and also preserved across distinct arbitrary fragments of sizes up to 200 nucleotides. Therefore, our method can be used to estimate mutation rates, whether for RNA viruses or other viruses by high-throughput sequencing.

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PT.097

HCV GENOTYPE PREDICTION USING A DNA-BASED DEEP LEARNING FOUNDATION MODEL

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The determination of viral genotypes is critical for antiviral therapy effectiveness. The Hepatitis C Virus (HCV) has 7 known genotypes and numerous subtypes, which exhibit different responses to treatment. Therefore, identifying the specific genotypes and subtypes of those pathogens is essential for the success of possible treatments. Over the past decade, high-throughput sequencing methods have produced abundant genomic data, which can be used to evaluate new technologies for classification and prediction tasks. Deep learning models such as GPT (Generative Pre-training Transformer) and BERT (Bidirectional Encoder Representations from Transformers) are currently the most adopted for tasks that involve large strings of text because they tokenize and learn the context of their elements, which is useful when dealing with large sequences of characters, such as genomic data. We used a pre-trained foundation model based on the transformer architecture of BERT, called dnaBERT, which is the current state-of-art for natural language processing (NLP) tasks. Our version of dnaBERT was fine-tuned to predict 6 out of 7 HCV genotypes, using HCV whole genome sequences fragmented to 1/10 of their original length for optimal performance. That process resulted in an accuracy of more than 97%, with minimal loss and only genotype 5 presented the least precision due to data scarcity. Due to its characteristic way of processing DNA sequences the same way it would do to words in a phrase, the model was proven efficient for the HCV genotype classification task.

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PT.098

IN VITRO EVALUATION OF GENETIC DIVERSITY AND ADAPTATION DYNAMICS OF MAYARO VIRUS (MAYV) IN DIFFERENT HOSTS

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The Mayaro virus (MAYV) is a rising concern with potential to become a significant health issue, especially due to a possible adaptation to new hosts and vectors. This study investigates the genetic variability and adaptive dynamics of MAYV across transmission cycles involving mosquitoes and humans using in vitro models. Initially, HFF1 cells were infected with MAYV at a multiplicity of infection (MOI) of 1. After 72 hours, the virus was collected and used to infect AAG2 cells. This process was repeated 5 times, mimicking the transmission cycle between mosquito and human cells. Additionally, sequential infections were conducted in AAG2 and HFF1 independently, without alternating hosts, for a total of 4 cycles in each cell line. The collected virus from each cycle was submitted to Next-generation sequencing (NGS), evaluating inter and intrahost changes across each infection cycle, associating mutation emergence and fluctuation with viral fitness. Key mutations emerged in high frequency in MAYV proteins, such as: nsP1 W456G (80%) in interhost cycle; nsP3 D31N (85%) and E2 E162K in intrahost HFF1 cycle; and E2 G95S (50%), nsp1 W456G (42%) and nsp1 W456R (56%) in intrahost AAG2 cycle. Our findings reveal that selective pressures from different host cell lines and infection cycles rapidly shape viral populations, impacting viral titer changes. Mutated variants were isolated and further analyzes are in progress to evaluate, by viral growth kinetics and *in silico* assays, the impact on viral *fitness* and protein structure respectively.

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PT.099

DETECTION OF NEUTRALIZING ANTIBODIES AGAINST ARBOVIRUSES FROM LIVER HOMOGENATES

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Yellow fever virus (YFV) circulates in a sylvatic cycle between non-human primates (NHPs) and mosquitoes. NHPs are sentinels for outbreaks in Brazil, and yellow fever surveillance is based on the investigation of NHP's epizootics. NHP carcasses are a source for epizootic investigations and are suitable for molecular tests, but not serological. Based on PRNT using sera, we tested the utility of liver homogenates from experimentally infected (YFV; Mayaro virus [MAYV]; chikungunya virus [CHIKV]; mock) mice. Homogenates from mock-infected mice showed a low level of nonspecific virus neutralization against YFV, MAYV, or CHIKV, homogenates from YFV, MAYV-, and CHIKV- infected mice demonstrated significantly higher levels of median virus neutralization compared to controls. Receiver operating characteristic (ROC) curves analyses were performed showing scores ≥0.97 (95% CI ≥ 0.89-1.0) for the area under the curve (dilutions 1:20-1:80), showing differentiation in infected and mock groups. Homogenates from 25 NHP carcasses (from 2017 YFV outbreak in Brazil) were tested using the adapted PRNT and rapid anti-YFV IgM immunochromatographic tests. Neutralization activity was detected in 6 samples that were also positive by PCR and anti-YFV IgM tests. Our results demonstrate the feasibility of using liver homogenates as an alternative approach for serological investigation in viral surveillance. The use of liver homogenates in serological tests expands the possibilities for outbreak investigation and epizootics. Here, we detected antibodies against YFV in liver homogenates of NHPs using rapid tests and PRNT, and we propose this approach as a complementary strategy for YF surveillance.

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PT.100

ANTIVIRAL ACTIVITY OF THE CALCIFIED RED MACROALGAE *DICHOTOMARIA MARGINATA* AGAINST HERPES SIMPLEX VIRUS TYPE 1

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Herpes simplex virus type 1 (HSV-1) is the etiologic agent of herpes, an infection characterized by vesicular ulcerative cutaneous lesions, affecting over 3.7 billion people. Severe manifestations, such as encephalitis, and the increasing risks of neurodegenerative diseases raise concern. Although there are drugs available to treat these infections, the emergence of resistant strains boosts the search for novel therapies. This work aims to evaluate the antiviral potential of the calcified red macroalgae *Dichotomaria marginata* against HSV-1. The cytotoxicity of the extract was evaluated in Vero cells after 72-h treatment using MTT assay. The extract showed low cytotoxicity with a concentration required to decrease cell viability by half (CC_{50}) of 209.01 µg/mL. We assessed its antiviral activity against HSV-1 (KOS strain) replication in Vero cells (MOI 1) after treatment with different concentrations (1.25-50 µg/mL) for 24 h. After that, total lysates were harvested, and virus titers were determined by plaque reduction assay. The extract presented a significant antiviral activity, with a concentration required to decrease the production of viral particles by 50% (EC_{50}) of 21.09 µg/mL. Further, we investigated whether the extract has protective effects after a 1-h treatment (50 µg/mL) of Vero cells before infection (MOI 1) and virucidal activity after treating HSV-1 suspensions ($2x10^5$ PFU) for 1 h. The extract showed a strong virucidal activity (100 %), but no protective effects on cells. In conclusion, the extract exhibited a promising antiviral potential as a drug candidate to combat HSV-1 infection and other mechanisms of action are still under investigation.

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PT.101

Prospecting and characterization of swinepox virus in the Brazilian Amazon

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Poxviruses stand out among emerging viruses and the genus *Suipoxvirus* is important for veterinary health. The species *Swinepox virus* (SWPV) is the only representative of the genus and is responsible for causing swine pox, a pustular vesicular disease that affects pig herds around the world. The disease caused by SWPV is associated with precarious sanitary conditions, in which transmission can occur through direct contact with lesions or through mechanical transmission. This work aims to prospect, characterize and isolate SWPV in biological samples from pigs from the Brazilian Amazon. Samples of lesion (n=1), serum (n=51) and blood (n=4) from pigs in the state of Pará (PA) collected in 2021 and 2022 were subjected to molecular investigation by qPCR using the C20P-C1L gene region as target. For sequencing, the Ion Torrent PGM platform (Thermo Fisher Scientific) was used, evolutionary analysis was performed using viral DNA polymerase as a reference and the phylogenetic tree was constructed using the maximum likelihood method with the Tamura-Nei model. Viral isolation was performed using the PK-15 cell line. Of the samples tested, one pig lesion from the municipality of Eldorado do Carajás (PA) collected in 2021 was positive for SWPV. The virus from this sample was isolated in PK-15 cells and genome sequencing showed the grouping with other SWPV sequenced in Brazil and around the world. Our data demonstrate the circulation of SWPV in pig herds in the Brazilian Amazon, showing the importance of increasing efforts to better understand the epidemiology of the disease in the country.

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PT.102

The establishment and structuring of an institutionalized Virus Collection

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Establishing a biobank is crucial for storing biological material and associated data to support research and understanding disease mechanisms to improve public health by understanding disease etiology. In response to the COVID-19 pandemic, many biobanks were set up worldwide to help curb the spread of the virus through accurate diagnosis. The Biobanks must adhere to good laboratory practices, quality control systems, and legal and ethical guidelines, developing standard sample collection, processing, and storage procedures. Due to long-term storage, dedicated software is essential for sample registration, cataloging, tracking, and quality assurance. The Biobank houses the first institutionalized Virus Collection, that is being established with the highest standards of quality and precision. The collection started producing a cell culture bank and four virus specimens getting to improve virus titles after deposit, increasing from 10^2 to 10^6 PFU/mL. Additionally, Virus Collection performs taxonomic characterizations taking into account mainly molecular and phylogenetic characteristics of the viruses. This service can be offered independently of the deposit request. Regarding the deposit of viruses in the biobank, three modalities are established: open, which allows the supply without depositer consent; restricted deposit, in which supplies are done under depositer consent; and closed, in which no supply is permitted under a defined period. The establishment of high-quality virus collections, such as the BBS-Fiocruz Virus Collection with support from UFRJ and Fiocruz-Manaus, demonstrates our commitment to public health and to improving scientific reproducibility, ensuring that research findings can be independently verified and validated, thereby strengthening the foundation of scientific knowledge.

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PT.103

CLINICAL-EPIDEMIOLOGICAL ANALYSIS OF THE 2019 MEASLES OUTBREAK IN SÃO PAULO, IN HOSPITAL PATIENTS

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Measles is a global concern and in 2019, Brazil faced a significant measles outbreak, with almost 12,000 cases reported. We evaluate the incidence of measles in the city of São Paulo, during the 2019 outbreak and determine the clinical and epidemiological characteristics considering populations vaccinated and not vaccinated with MMR, medical records and immunization data from 360 patients. More than 90% of patients had a rash and/or fever at the same time, followed by 74% of cases with cough and runny nose, and to a lesser extent conjunctivitis and Koplick. Of the total patients, 35% (126/360) were positive for measles, with more than 65% (83/126) of them not immunized and 65% (234/360) were negative for measles with 38.1% (89/234) without immunization. Of the negatives, more than 95% of cases were positive for Cytomegalovirus. Of those positive for Measles, 21.4% (27/126) were admitted to the ICU/wards, with 70.3% (19/27) of those admitted not being immunized. The 31/38 (81.5%) positive patients, aged between 18 and 29 years and 57.6% (34/59) of positive patients aged less than 1 year, have no history of immunization. These data show that the high rate of hospitalization in ICUs and wards reflects the severity of the disease, especially among those who are not immunized. These data reinforce the need for awareness campaigns and easy access to vaccines, to increase vaccination coverage and protect the population against outbreaks of preventable diseases.

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PT.104

ANTIVIRAL ACTIVITY OF NOVEL IMINE COMPOUNDS AGAINST THE VACCINIA VIRUS

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The world has recently experienced an outbreak of Orthopoxvirus monkeypox (MPV). Cases of MPV were reported in countries where the disease is not endemic, bringing an alert to the swiftly increase in viral spread. Orthopoxvirus vaccinia (VACV) is the prototype of the Poxviruses family, which also include the Variola virus, one of the most health-threatening viruses that existed. Thus, VACV becomes an ideal viral model for antiviral screening trials enhancing drug discovery efforts. In this work, the cytotoxicity and antiviral activity of 5 novel compounds were evaluated. They exhibited low cytotoxicity to BSC-40 cell line up to a concentration of 1000 µM. Furthermore, the examination of viral replication of VACV-WR at different multiplicities of infection (moi) for up to 60 hours post infection (hpi) allowed the establishment of antiviral assay set up conditions such as time of infection (48 hpi) and moi (0.01). In those conditions, the compounds demonstrated in vitro antiviral activity against VACV infection. The reference compound showed an IC50 of 1.51 µM, while its synthetic derivatives showed a significant reduction on IC50 values, which fell down to 0.22 and 0.29 µM for two top compounds. The selectivity index (SI) values for these two top antiviral candidates were greater than 1,000, indicating their efficiency and/or selectivity. Together, our results show that the derivatives studied here have putative antiviral activity against VACV and potentially against the life-threatening MPV. New studies are being developed in order to identify antiviral mechanisms of these top two candidates.

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PT.105

IN SILICO DRUG REPURPOSING UNCOVERED THE ANTIVIRAL POTENTIAL OF OXIBENDAZOLE AGAINST CHIKUNGUNYA VIRUS

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Chikungunya virus (CHIKV) infections can present a debilitating and persistent nature that can drastically compromise the quality of life of patients. Yet, specific drugs are lacking, making the search for new antivirals essential. In this context, nsP2 protease emerged as a promising therapeutic target because it is key for CHIKV replication and pathogenesis. This work aimed to identify potential CHIKV nsP2 protease inhibitors with antiviral potential within DrugBank using in silico and in vitro methods. A virtual screening combining ligand- and target-based strategies was conducted within DrugBank libraries comprising 5,944 compounds. Six hits were obtained and two, oxibendazole (OBZ) and capecitabine (CPB), were purchased for experimental validation. The antiviral activity of the compounds was assessed by plaque reduction assays. OBZ inhibited the replication of CHIKV 181-25 strain in Vero cells at 50 µM (66.4%), but not CPB. Additionally, OBZ inhibited the replication of the CHIKV BRA/RJ/18 strain with similar activity (61.8%) and a 50% effective concentration of 11.4 µM. The cytotoxicity of OBZ was evaluated in Vero cells using the MTT assay. This drug showed low cytotoxicity with a 50% cytotoxic concentration higher than 500 µM and a selectivity index higher than 44. Further, its mechanism of action was investigated through a set of experiments, like time-of-drug addition, virucidal, attachment, entry, and release assays. OBZ showed the highest activity when added until 4 hpi and moderately inhibited virus entry (33.8%), but not other steps. Collectively, our data support further investigations on the antiviral potential of OBZ to fight CHIKV infections.

Financial Support: CAPES, CNPq, FAPERJ, NIH

PT.106

ANGIOTENSIN-(1-7) MITIGATES THROMBOCYTOPENIA AND VASCULAR LEAKAGE IN SEVERE DENGUE INFECTION

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The host immune response plays a critical role in dengue pathogenesis and severity, with severe cases linked to excessive inflammation and impaired resolution mechanisms. Therapeutic approaches using pro-resolving molecules show promise in treating acute inflammatory infectious diseases. Angiotensin-(1-7) [Ang-(1-7)], a peptide from the renin-angiotensin system, facilitates inflammation resolution via its receptor Mas (MasR). This study explores Ang-(1-7)'s therapeutic potential in dengue infection. A129 mice infected with DENV-2 were treated with Ang-(1-7) (100 µg/kg, subcutaneously, twice daily) starting 36 hours post-infection. Euthanasia was performed 3 and 5 days after infection. The infection with DENV-2 in A129 mice led to increased clinical scores, such as weight loss, thrombocytopenia, and leukocytosis, along with elevated levels of inflammatory mediators in the plasma and spleen (MCPT-1, CCL2, CXCL1, CCL5 and INF- γ), increased permeability, and viral titers in the liver, spleen, and lungs. However, Ang-(1-7) treatment mitigated these effects, preventing clinical score increases, thrombocytopenia, and leukocytosis. It also reduced circulating leukocytes, particularly granulocytes, and decreased plasma levels of CCL2 and MCPT-1, associated with plasma leakage. The treatment also reduced mast cell degranulation and vascular permeability in the spleen, preventing 25% of animal lethality. Additionally, Ang-(1-7) lowered viremia, although it did not significantly impact viral titers in target organs. In summary, Ang-(1-7) enhances inflammation resolution by increasing lymphocyte numbers and activation, preventing thrombocytopenia, and boosting early pro-inflammatory responses, leading to reduced viral titers and improved clinical outcomes.

Keywords: Angiotensin-(1-7), dengue, pro-resolving, thrombocytopenia

Financial Support: CNPq, CAPES, FAPEMIG, INCT

PT.107

MOLECULAR ANALYSIS OF rs9277535 (HLA-DPB1) IN THE PROGRESSION OF LIVER DISEASE IN HEPATITIS B AND DELTA CARRIERS IN AMAZONIC REGION

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Considered the most severe form of chronic viral hepatitis, Hepatitis Delta is classified by its accelerated pathological progression to cirrhosis and hepatocellular carcinoma. This progression can be influenced by various factors, including human genetics. Therefore, this study aims to molecularly analyze SNP rs9277535 and the expression of the HLA-DPB1 gene. Fifty chronically diagnosed Hepatitis B and/or Delta carriers were recruited and clinically monitored. Patients were assessed using the METAVIR and FIB-4 scores for hepatic fibrosis. Experiments were conducted using customized kits for human genotyping (rs9277535) and gene expression (HLA-DPB1) by real-time PCR, with results calculated as fold change ($2^{\wedge(-\Delta\Delta CT)}$). The study population included 74% with HBV and 26% with HDV, with 64% being men and an average age of 43 years. In genotyping, F0/F1 had 50% A/A, 40% A/G, and 10% G/G; F2/F3 had 50% A/A, 30.77% A/G, and 19.23% G/G; F4 showed 64.29% A/A, 21.43% A/G, and 14.28% G/G. HLA-DPB1 gene expression was detected in 38% of cases, with 42% low, 42% normal, 10% undetectable, and 6% overexpression. The study identified a high prevalence of the A allele in the population and gene overexpression in an individual with cirrhosis and chronic liver disease. Research like this is crucial for understanding the genetics and factors that affect the progression of hepatitis B and Delta in the Amazon, aiming to improve diagnostics and prognostics.

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PT.108

EVALUATION OF THE PROGRESSION OF LIVER FIBROSIS STAGES IN HEPATITIS B CARRIERS IN THE AMAZON REGION

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Hepatitis B represents a significant public health challenge in Brazil and worldwide. Between 2000 and 2022, 750,651 cases were reported, with 36.9% attributed to Hepatitis B and 14.5% of cases occurring in the North region. This study aims to analyze the clinical progression of Hepatitis B carriers in the Amazon region. A total of 107 medical records of chronic Hepatitis B patients (1994-2022) were analyzed, evaluating liver fibrosis using METAVIR and FIB-4, along with laboratory, serological, biochemical, and treatment assessments. Of the total, the most identified fibrosis stages were 44.85% F0/F1, 34.58% F2/F3, and 20.57% F4. Initial average laboratory values for F0/F1 were: creatinine (CR) 0.6 mg/dL, alpha-fetoprotein (AFP) 0.72 IU/mL, AST 23 U/L, ALT 24 U/L, and albumin (ALB) 3.36 g/dL. Initially, 81.3% had positive HBsAg. Current average values were: CR 0.6 mg/dL, AFP 3.3 IU/mL, AST 22 U/L, ALT 21 U/L, and ALB 4.14 g/dL, with detectable HBsAg in 54.16% of cases. Advanced stages (F2/F3 and F4) showed increased liver markers initially, but these normalized with treatment. In patients without fibrosis, the laboratory profile remained stable over time, with 27% achieving spontaneous HBV clearance. In patients with moderate/advanced liver fibrosis, treatments proved effective in controlling liver damage. This study highlights that research on Hepatitis B and its clinical aspects is extremely important for understanding the disease's behavior in the Amazon region.

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PT.109

A NEW IN SILICO METHODOLOGY TO AID IN THE DEVELOPMENT AND INITIAL TESTING OF AN HIV VACCINE

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The HIV represents one of the biggest global health challenges. A comprehensive solution remains elusive despite progress in understanding the virus and developing antiretroviral therapies. Therefore, developing an anti-HIV vaccine continues to be relevant and an alternative to mitigate the difficulties in developing this vaccine is the use of a multi-epitope protein design. Thus, we created a chimeric multi-epitope vaccine using the he Modified Vaccinia Ankara (MVA) viral vector as a genetic delivery agent. The epitope selection process for constructing this vaccine was carried out using databases such as IEBD with an integrated technique based on convolutional neural networks considering the location of epitopes in conserved regions. We created training from IEBD also using genetic conservation information and trained a neural network model to create the vaccine chimera. The construction of models capable of identifying the three-dimensional structure of the protein created through AlphaFold, modeling the problem as a graph, using the properties of this graph to help identify conserved epitopes non-linear in B and T cells. We used meta-heuristics and Bayesian optimization models to narrow the space of chimeras, focusing only on those that were stable and had valid physicochemical properties. The construction of the plasmid encoding the chimeric designed was subsequently transfected into cells containing the designed plasmid and infected with MVA, followed by selection of recombinant MVA vectors expressing the intended HIV-based chimera.

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PT.110

Investigating Respiratory and Gastrointestinal Symptoms in Children: Co-Occurrence of Viral Pathogens in São Paulo, Brazil, 2021

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Pneumonia and diarrhea are leading causes of death in children under 5 worldwide. This study investigates viral agents causing respiratory illness in children ≤3 years-old with diarrhea in São Paulo, Brazil, during spring 2021. Twenty paired samples (oropharyngeal swab and feces) were screened for bocavirus (HBoV), adenovirus (HAdV), rotavirus (RVA), enterovirus (EV), parechovirus (PeV-A), norovirus (NoV), Influenza A/B (Flu A/B), respiratory syncytial virus (RSV) and astrovirus (HAstV) using commercial and in-house (RT)-qPCRs, and conventional nested (RT)-PCR. Positive samples were sequenced and analyzed phylogenetically. HBoV and NoV were detected in 75% (15/20) of cases, with co-infections in 65% (13/20), suggesting their role in respiratory and gastrointestinal symptoms. HAdV, EV, PeV-A and RSV were each found in 10% (2/20) and Flu A in 5% (1/20). Dual-infections included HBoV+NoV (40%;8/20) and NoV+PeV-A (5%;1/20), while triple infections stricken HBoV+NoV+RSV (10%;2/20), HBoV+NoV+HAdV (10%;2/20), HBoV+NoV+EV (5%;1/20) and NoV+EV+PeV-A (5%;1/20). Samples tested negative for RVA, HAstV and SARS-CoV-2. NoV strain GII.4_Sydney[P16] predominated (69.2%;9/13), followed by GII.2[P16] (27.1%;3/13) and GII.4_Sydney[P31] (7.7%;1/13). HBoV was identified as HBoV-1, EV as Coxsackievirus A6 (CVA6), HAdV as type C6 (HAdV-C6), and PeV-A as PeV-A1. Phylogenetic analysis revealed no community outbreak, with distinct clusters for HBoV-1 and NoV genotypes, indicating independent origins. HAdV-C6, CVA6 and PeV-A1 molecular analysis also suggested distinct genetic sources. Our findings highlight the co-occurrence of respiratory and enteric diseases, revealing significant local viral diversity. Despite the small sample size, this underscores the complexity of differential diagnoses when symptoms overlap and emphasizes the crucial role of syndromic surveillance.

PT.111

GENETIC VARIABILITY OF THE LONG CONTROL REGION OF HUMAN PAPILLOMAVIRUS TYPE 16 IN PARAGUAYAN WOMEN PARTICIPATING IN THE ESTAMPA STUDY ACCORDING TO SEVERITY OF CERVICAL LESION.

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Introduction: Human papillomavirus type 16 (HPV16) is responsible for 60% of cervical cancer cases worldwide. HPV16 variants are classified into 4 lineages (A-D) and 16 sub-lineages (A1-3 European, A4 Asian, B1-4 and C1-4 African, D1-4 Asian-American). Studies have shown association between HPV16 variants and lesion severity, which may present different distribution by geographic region. **Objective:** To determine the genetic variability of the LCR region of HPV16 in cervical samples from Paraguayan women and the possible association with cervical intraepithelial neoplasia grade 2 or higher (CIN2+). **Methodology:** Observational cross-sectional study. Forty-four HPV16-positive cervical samples from the ESTAMPA Paraguay study were included, 19 without CIN2 and 25 with CIN2+ (1CIN2, 16CIN3 and 8Cancer). Circulating variants were identified by analysis of the LCR region of the genome. Fisher's exact test was used to determine the association between mutations, variants and lesion grade. **Results:** A1 (81.2%), A4 (2.3%), D2 (2.3%), D3 (11.4%) and D4 (2.3%) sublineages were detected. Forty-nine point mutations were identified at 48 sites. A statistically significant association with the presence of CIN2+ was observed in 11/49 mutations ($p<0.05$). 10/11 mutations associated with the presence of CIN2+, were preferentially observed in non-European variants. In addition, statistically significant association was observed between non-European variants and presence of CIN2+, $p=0.0065$. **Conclusion:** These results contribute with variant distribution data, detecting for the first time an Asian HPV16 variant in cervical samples from Paraguayan women, as well as demonstrating an association between non-European variants and lesion severity.

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PT.112

HLA-A2-RESTRICTED PEPTIDES SIGNATURE DERIVED FROM HUMAN CORONAVIRUSES REVEAL LOSS OF EPITOPES IN SARS-CoV-2 VARIANTS

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Four years after the onset of SARS-CoV-2 pandemic, COVID-19 remains one of the biggest challenges of modern medicine, causing over 3000 deaths in April/2024 and leaving over 65 million individuals experiencing long-term effects, referred to as "long-COVID". Since its emergence, SARS-CoV-2 has given rise to several strains and substrains, characterized by mutations in the Spike (S) protein. Such mutations can impact the efficiency of current SARS-CoV-2 vaccinal platforms, emphasizing the importance of tracking peptide signature and immunogenicity of new variants. In that sense, this work sought to evaluate the presence of MHC-I-restricted immunodominant epitopes from human coronavirus (hCoV) subfamilies and five of the main SARS-CoV-2 strains (alpha, beta, gamma, delta, and omicron) using in silico and in vitro mapping tools. Our findings indicate HLA-A*02 haplotype as the preferred MHC-I for binding hCoV peptides. However, these peptides presented the lowest combined prediction scores compared to peptides restricted to other MHC-I haplotypes. Furthermore, SARS-CoV-2 variants exhibited lower prediction scores than the Wuhan virus, indicating a potential evasion of the $CD8^+$ T-cell response. In vitro assessment of peptide-HLA-A*02 binding revealed major epitope loss for Spike and ORF1ab proteins of SARS-CoV-2 variants compared to the Wuhan strain. Conversely, peptides from the Nucleocapsid (N) were conserved among all five variants and remained as important targets for HLA-A*02 binding. Overall, the data presented in this study offers new insights regarding the antiviral $CD8^+$ T-cell response against SARS-CoV-2 variants and subvariants, potentially contributing to the development and validation of new immunizers to induce broad cellular antiviral response.

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PT.113

Epidemiological Surveillance of Rotavirus A and Norovirus in Children with Gastroenteritis between 2013 to 2020 in Sergipe, Northeast Brazil

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Acute gastroenteritis (AGE) remains one of the leading causes of death in children under 5 in developing countries, resulting in the deaths of more than 400,000 children annually. Several pathogens, including bacteria, viruses, and intestinal parasites, can cause AGE in children. Among the viruses, group A rotavirus (RVA) and norovirus (NoV) stand out, which are responsible for millions of cases of diarrhea worldwide every year. In this context, this study aimed to verify the prevalence of RVA and NoV in cases of childhood diarrhea in Sergipe, Brazil, between 2013 and 2020. Viral diagnosis was performed by RT-qPCR on diarrheal stool samples from children under 10 years of age, collected in two pediatric emergency rooms in the state. From January 2013 to March 2020, 912 samples were collected, of which 43.3% (n = 395) were positive for at least one of the viruses tested. For RVA, 190 (20.8%) were positive, and for NoV, 205 (22.5%) were positive. Besides diarrhea, fever (74.5%) and vomiting (70.7%) were the most frequent symptoms. A higher prevalence of NoV infection can be observed when compared to RVA (20.8% vs 22.5%), which may reflect vaccination coverage for RVA. Considering that RVA continues to contribute to more than 20% of cases of childhood diarrhea, and that norovirus also remains an important etiological agent, constant epidemiological surveillance of NoV and RVA in the region is necessary.

Financial Support: CNPQ

PT.114

LABORATORY PARAMETERS AND MACHINE LEARNING IN PREDICTING THE CLINICAL OUTCOME OF COVID-19 PATIENTS

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The pandemic caused by the SARS-CoV-2 virus is a crisis affecting the public health system. Although some hematological and biochemical parameters are altered during moderate and severe COVID-19, there is still a lack of tools to combine these parameters to predict the clinical outcome of a patient. Hematological and biochemical parameters of patients diagnosed with COVID-19 and machine learning algorithms were used to predict clinical outcome. The 81 patients included in this study were admitted to the Risoleta Neves Hospital in Belo Horizonte, of which 28 were discharged and 53 died. Laboratory parameters were taken at three points in time: T1 - 0 to 6 days of hospitalization, T2 - 7 to 14 days and T3 – after 14 days. Five machine learning models (obtained in a cross-validation process) were used to test the probability of patients progressing to death. Among the parameters evaluated, the ones that stand out in T1 time are urea, lymphocytes and basophils count, glucose and age, which could be possible biomarkers for the severity of COVID-19 patients. This study shows that urea is the parameter that best classifies the severity of the patient, making it a crucial analyte to be used in machine learning algorithms to predict the patient's clinical outcome.The machine learning algorithms were utilized for each time point. However, the order of importance of other parameters changes for each time point, demonstrating the importance of a dynamic approach for the effective prediction of patient outcome.

Financial support: FAPEMIG, CAPES,CNPq, EMBRAPII, MucPharm, Novandina

PT.115

HANTAVIRUS IN BRAZIL AND ALAGOAS: AN EPIDEMIOLOGICAL STUDY (2022-2023)

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Hantavirus is an RNA virus transmitted by rodents, responsible for causing Hantavirus Pulmonary Syndrome (HPS). Due to its nonspecific symptoms, early identification is challenging, leading to underdiagnosis because of its similarity with other diseases. This study aims to evaluate the incidence of hantavirus infection and the epidemiological profile of patients between 2022-2023, using a quantitative, descriptive, and retrospective analysis, conducted in July 2024. Data was provided by the National Disease Notification System (SINAN). The results revealed 51 laboratorial confirmed cases in 2022 and 65 in 2023. There is a predominance of males, with 66.66% in 2022 and 83.07% in 2023. Young adults are predominant, with 50.98% between 20-39 years in 2022 and 44.61% between 40-59 years in 2023. The patients are primarily found in the Southeast, Central-West, and South regions, with cases reported by eight states. In the North and Northeast regions, only two states reported hantavirus cases during this period. Recently, Alagoas has shown an increase in severe acute respiratory syndrome cases with no defined etiological agent; however, there have been no hantavirus reports in this state for the past 23 years, which differs from studies conducted between 2010-2012 that identified the presence of IgG and IgM antibodies for hantavirus locally, indicating possible underreporting. This study is relevant for identifying areas and epidemiological profiles vulnerable to infections, reinforcing the development of effective public policies and highlighting the importance of broad hantavirus diagnosis in Brazil, especially in the Northeast region.

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PT.116

AN IN SILICO CHARACTERIZATION OF TREM-1 AND TLT-1 AS NOVEL PLAYERS IN DENGUE VIRUS INFECTION

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The dengue virus (DENV) envelope (E) and non-structural 1 (NS1) proteins, are related to its adsorption and clinical outcome, respectively. High severity cases are associated with excessive immune responses, cytokine storms, and augmented vascular permeability. Thus, based on the role of the the Triggering Receptor Expressed on Myeloid Cells (TREM-1/TLT-1) family members in inflammation besides their expression in different cells, including platelets, we sought to identify, through in silico tools, a putative interaction among these receptors and the DENV E and NS1 proteins. Our results indicate that TREM-1 may play an important role both in viral adsorption and amplification of inflammation, as it virtually interacted with the E and NS1 proteins. These interactions occurred mainly through the complementarity-determining regions (CDRs), especially CDR3. Additionally, TLT-1 interacted with NS1, mainly involving the CDR3 region, indicating a possible role of these receptors in disease severity and viral adsorption. Among the E protein residues interacting with TREM-1, L487, was detected within the four serotypes. Epitopes described as antigenic in NS1 (W28, Q31, Y32, F160, and T165) that are common to all DENV serotypes were identified as mediators of antigen recognition by TREM-1 and TLT-1. Subsequently, random mutations were performed in key receptor residues for NS1 recognition, which drastically compromised antigen recognition. We suggest TREM-1/TLT-1 as novel receptors in dengue pathophysiology, that may be involved in viral adsorption and immune response amplification. Together, these results offer new perspectives for understanding dengue pathogenesis and fostering the development of therapeutic strategies.

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PT.117

MODELING INFLUENZA A VIRUS INFECTION IN NEURAL CELLS DERIVED FROM INDUCED PLURIPOTENT STEM CELLS (IPSC)

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The Influenza A virus, the etiological agent of "flu," belongs to the Orthomyxoviridae family and is classified into four main subtypes: Influenza A, B, C, and D. In addition to affecting the respiratory tract, Influenza may also impact the nervous system, particularly influencing the cerebral development of fetuses, though these mechanisms are not yet fully elucidated. To investigate the pathophysiology of Influenza within the central nervous system (CNS), this study employed neuroprogenitor cells (NPCs) derived from induced pluripotent stem cells (iPSCs) to examine the effects of infection on the brain. This endeavor necessitated the standardization of the viral infection protocol, given that the viral strain utilizes a specific protease to complete its infectious cycle. Accordingly, we developed a protocol to determine the optimal trypsin concentration for facilitating viral transduction. Our findings demonstrated that the Influenza A virus (H1N1 subtype, pdm09 strain) is capable of infecting NPCs. Infection was confirmed through molecular qPCR assays on the cell supernatant and cellular assays utilizing immunofluorescence with antibodies. Furthermore, the cells showed an inflammatory tendency. Comparison of infected and uninfected NPCs (control), both subjected to the established trypsin concentration to enhance viral transduction, revealed that control cells succumbed to death before the virus-exposed cells, suggesting that Influenza A may induce the expression of proteins that activate cellular survival pathways in neural cells. Notably, the potential of the Influenza virus to extend cellular survival may be associated with the synergistic action between viral and cellular proteins, which could be explored for future therapeutic interventions.

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PT.118

ORTHOPOXVIRUS MONKEYPOX INTRAFAMILIAL CLUSTER TRANSMISSION IN MINAS GERAIS, BRAZIL

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Orthopoxviruses are pathogens of global public health concern. Since 2022, Orthopoxvirus monkeypox (MPV) has caused a worldwide public health emergency, with human cases observed in several countries where Mpox was not considered endemic and with no reported human-to-human transmission. This work aims to isolate and characterize MPV from clinical specimens from an HIV-positive patient and his family contacts. Clinical samples collected in 2023 from five individuals were submitted for viral isolation, including crusts and swabs. The processed samples were inoculated into Vero cell monolayers to visualize cytopathic effects after 48 or 72 hours. The supernatant was collected, and DNA was extracted using High Pure Viral Nucleic Acid Kit, Roche. Three samples from two patients were positive, considering the combination of viral isolation and qPCR analysis targeting the E9L gene. MPV was isolated from nasal and abdominal crusts from an HIV-positive adult male and from a crust from a female child living in the same house. DNA underwent multiplex PCR amplification for sequencing, followed by library preparation with an Illumina CovidSeq kit. Subsequently, sequencing was performed on the MiSeq platform with the v3 reagent kit and 600 cycles. Genomes were assembled using SPAdes, minimap2, and Geneious, resulting in 3 consensus sequences with >96% coverage and >500X depth. Evolutionary analysis classified them as clade IIb, lineage B.1.9. In light of these findings, we provide evidence for intrafamilial transmission of MPV. Our results highlight the need to improve case control measures to prevent transmission at very early disease stages and close contacts.

Financial Support: INCT-POX; CNPq; FAPEMIG; Capes.

PT.119

UNDERSTANDING THE MOLECULAR EPIDEMIOLOGY OF THE MEASLES VIRUS 2019 OUTBREAK IN SÃO PAULO – BRAZIL BY WHOLE GENOME SEQUENCING

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Measles is a highly contagious exanthematous disease that has a R0 of 18, th0lus requiring a high vaccination coverage of 95% to prevent the active circulation. The etiological agent of measles is *Morbillivirus hominis* (formerly *Measles morbillivirus* - MeV), a virus from the *Paramyxoviridae* family. The objective of this work was the molecular study of the measles virus responsible for the 2019 outbreak in Brazil, through sequencing of the complete genome of the virus present in samples from infected patients using Next Generation Sequencing (NGS). Therefore, 117 samples from 216 measles-positive patients with an original cycle threshold (ct) of up to 31 were selected. Sequencing was carried out by the Ion Torrent platform, using an Ion AmpliSeq NGS Panel™ (ThermoFisher Scientific), which performs targeted sequencing for the MeV genome based on a reference genome of the D8 genotype (MK161348.1). The number of reads obtained for each sample varied considerably, but even with this variation it was possible to obtain a good coverage of 98% of the genome considering that of the 117 samples sequenced, 54.7% presented up to 2% N, 27.3% presented 2 to 4% and a smaller part of 17.9% had a high percentage of N (5% or more). Nearly all sequenced samples were identified as belonging to the D8 genotype, except for one that was identified as belonging to the vaccine genotype (genotype A). This study generated new MeV complete genome sequences contributing to the understanding of the molecular epidemiology of the virus.

PT.120

DESIGN OF MULTI-EPITOPE PROTEINS FOR SEROLOGICAL DIAGNOSIS OF *Orthoflavivirus zikaense*

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The *Orthoflavivírus zikaense* (ZIKV) is an arbovirus that causes Zika fever, a disease with few symptoms, although it has recently been associated with Guillain-Barré Syndrome and congenital syndromes. Our group used immunoinformatics to identify potential immunogenic epitopes with low similarity to other arboviruses, high conservation within ZIKV, and high population coverage, making them good candidates for the development of diagnostic tests (doi.org/10.1016/j.nmni.2019.01.002). Five previously identified structural peptides were tested in an ELISA assay with serum from patients previously infected with ZIKV, showing high reactivity and proving to be promising. The objective of this study was to design chimeric proteins using the five epitopes: one HLA-I epitope (Pep01) and four HLA-II epitopes (Pep02; Pep03; Pep04 e Pep05). Deep learning-based molecular modeling was used to resolve the protein structures, and parameters such as epitope accessibility, instability index (reference value < 40), membrane binding propensity, solubility (reference value > 0.45), and DNA synthesis complexity were evaluated. Two stable proteins were designed with epitopes connected by flexible linkers of glycine and serine with two copies of the epitopes (GSGSGS) and rigid linkers of alanine, glutamine, lysine with one copy of the epitopes (AEAAAKEAAAKA). The flexible and rigid chimeras demonstrated an instability index of 21.84 and 16.16 respectively and were highly soluble with solubility values of 0.513 and 0.597 respectively, which will be applied in new assays for the development of differential serological diagnostic tests for the *Orthoflavivírus zikaense*.

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PT.121

MPOX: EVALUATING TRANSMISSION AMONG CONTACTS

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Mpox results from infection with the Orthopoxvirus monkeypox (MPXV). It initially presents with fever, myalgia, and lymphadenopathy, followed by a skin rash that progresses into papules, vesicles, and pustules, which eventually form crusts and heal. In recent years, recurrent outbreaks of the MPXV have been reported in various countries, with Brazil being one of the most affected. Therefore, monitoring suspected cases is necessary for rapid identification and control of the disease. A total of 13 volunteer individuals, some of whom had signs and symptoms suggestive of mpox, were contacts of a patient with confirmed MPXV infection. Clinical and sociodemographic data were obtained, and serum samples were collected from each individual. Sera from contact animals were also collected. The serum samples were used to perform the plaque reduction neutralization assay (PRNT), which was conducted using Orthopoxvirus vaccinia and BSC-40 cells in a 24-well plate system. The average age of the volunteers was 32.8 years, with the majority being female (61.5%). Through PRNT we observed the presence of anti-orthopoxvirus neutralizing antibodies only in one sample of the contacts in a titer of ≥ 1:40. This individual was vaccinated for smallpox, as can be deferred from their age. This finding underscores the importance of vaccination history and immune response in the context of mpox outbreaks. Continued monitoring and surveillance of suspected cases are imperative for prompt disease identification and control, particularly in regions experiencing recurrent MPXV outbreaks like Brazil.

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PT.122

VIRAL HEPATITIS IN THE WESTERN AMAZON: CONNECTIONS BETWEEN IMMUNOGENETICS AND VIROLOGY

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Background: Hepatites B (HBV) and Delta (HDV) are among those chronic infections with genetic susceptibility, being most notable in the western Brazilian Amazon region, exibiting fibrotic pathophysiological changes. Methodology: A total of 100 individuals were included (50 HBV-50 HDV), subdivided into: 22 monoinfected without chronic liver disease (CLD); 28 monoinfected with CLD; 21 co-infected without CLD; and 29 co-infected with CLD. We applied qPCR and customized targets (rs1800795/IL-6; rs8099917/IFNL3/4), as well gene expression using *fold change*, correlating with serum cytokine. Results: HDV carriers with detectable viral load had high levels of ALT and AST, as observed in HBV-immune carriers. The cytokines IL17A and IL4 were detected, with IL17A being observed at high levels in co-infected with detectable HDV-RNA, as well as for monoinfected immunoactive. To IL4, interleukin was dosed in co-infected with undetectable HDV-RNA and identified in HBV without liver disease. The CG/GG genotypes (rs1800795) were associated with CLD ($p<0.05$). However, there was no detection of IL-6 by gene expression, only IFNL3/4, HLA-DPB1 and TGF-β, latter with normalized *fold change* levels among carriers. Overexpression of the IFNL3/4 was seen monoinfected group without CLD and HLA-DPB1 in monoinfected with CLD. Conclusions: Detectable viral load for HDV and polymorphisms in the IL-6 gene were associated with greater severity of advanced hepatitis. This study contributes to identification of virological and immunogenetic factors associated with a worse CLD prognosis in HBV and HDV co-infection.

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PT.123

EVALUATION OF SUPERINFECTION IN THE CLINICAL PROGRESSION OF HEPATITIS DELTA VIRUS CO-INFECTORS IN THE WESTERN AMAZON

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Background: There are approximately 15 million individuals co-infected with HBV and HDV spread across several countries in the world, however this number is underestimated. HDV superinfection is more frequently associated with liver failure, cirrhosis and even the development of hepatocellular carcinoma (HCC) when compared to HBV monoinfection. In Brazil, HDV infections have an endemicity profile in the Western Amazon region with characteristic circulation of HDV genotype 3 strongly associated with clinical progression to disease severity, representing a national public health problem. Objective: This research presents cases of young people with possible superinfection who showed a rapid development of liver disease in the severe form of HDV infection. Methods: Samples were collected July 2023. The clinical and laboratory data of the participants was collected through electronic medical records. To characterize the HDV genotype, a Nested-PCR was used to obtain a 406 bp fragment of the HDV genome and subsequent genetic sequencing was performed. Results: Individuals from a rural community in the municipality of Lábrea showed a profile of rapid progression to chronic liver disease. Phylogenetic analysis showed that the sequences of all the cases were classified as HDV genotype 3. Conclusion: This result demonstrates the impact of Delta Hepatitis as an important public health problem for the young-adult population and a major challenge in the context of the Brazilian Western Amazon region.

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PT.124

Structural Impacts of VP1 Protein Substitutions of Echovirus 6 and 30 on the Interaction with Uncoating Receptor FcRn

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Enterovirus B species (EVB) are widespread and often associated with human central nervous system infections. The viral capsid protein VP1 is central in interacting with the cell surface neonatal receptor (FcRn), which initiates capsid uncoating and cell entry. Despite cryo-EM structures of VP1-FcRn complexes already established, the interaction rules remain unclear. In this study, we used in silico analyses to assess the impact of VP1 substitutions on the interaction with FcRn. Sequences of the two most relevant EVBs circulating in Brazil, Echovirus 6 (E6) and Echovirus 30 (E30), were collected from the GenBank database. Datasets were aligned, clusterized based on identified mutations and analyzed using SIFT to predict their impact on protein function. Structural models of prototype sequences and mutants were generated at the asymmetric unit and five-fold axis levels, applying the Rosetta Pmut-Scan module and VMD. Changes in the electrostatic potential surface map were evaluated using PyMOL. Significant VP1 substitutions were found in regions interacting with FcRn, including the BC, GH, and EF loops, and at the C-terminal and hydrophobic pocket regions. Based on SIFT prediction, some substitutions could have affected protein function. Comparative analysis of electrostatic potential surface maps revealed significant changes in the surface charge profiles. Our results provided valuable information on changes in essential regions of the virus capsid that could cause modifications in receptor binding.

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PT.125

ASSESSING THE ANTIBODY RESPONSE AND SOLUBLE MEDIATOR PROFILES INDUCED BY WILD-TYPE AND VACCINE STRAINS OF YELLOW FEVER: LESSONS FROM THE 2016-2018 OUTBREAK IN BRAZIL

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Between 2016-2018, Brazil experienced major sylvatic yellow fever (YF) outbreaks. Patients from these outbreaks represented a unique opportunity to assess the immune response triggered by wild-type (WT) strains of yellow fever virus (YFV). Our study evaluated the systemic immune response of patients from these outbreaks, compared to healthy vaccinees and seronegative individuals. Using a 48-plex Luminex assay, we quantified circulating levels of pro-inflammatory (IFN-α2, IFN-γ, TNF-α, TNF-β, IL-1α, IL-1β, IL-6, IL-12, IL-15, IL-16, IL-18, TRAIL, MIF, LIF) and regulatory cytokines (IL-1ra, IL-2ra, IL-3, IL-4, IL-5, IL-9, IL-10, IL-13, IL-17A), chemokines (CXCL1, CXCL8, CXCL9, CXCL10, CXCL12, CCL2, CCL3, CCL4, CCL5, CCL7, CCL11, CCL27), and growth factors (basic-FGF, PDGF-β, VEGF, G-CSF, GM-CSF, M-CSF, β-NGF, HGF, SCGF-β, SCF, IL-7, IL-2), in serum samples from YF patients and vaccinees, collected 30 to 60 days post-infection/vaccination, and from seronegative individuals. Neutralizing antibodies (nAb) levels were also measured for all participants, through plaque reduction neutralization tests. Preliminary findings revealed the occurrence of a storm of mediators with a mixed immune response profile in YF patients, with significant elevation of 36 mediators compared to vaccinees and seronegatives, and of 43 mediators when compared only to vaccinees, suggesting that vaccine and WT strains of YFV can induce distinct immune profiles. nAb levels were also elevated in YF patients, compared to vaccination. Further analysis is being performed. Thus far, our study has provided a better understanding of important differences between the responses to natural infection and vaccination, and helped fill the gap of knowledge concerning the immune response against WT YFV.

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PT.126

EVALUATION OF HEPATITIS B AND DELTA VIRUS GENOTYPES AND SUBGENOTYPES IN PATIENTS WITH LIVER DISEASE

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Background: hepatitis B and Delta are one of the most important public health problems worldwide. The state of Rondônia, located in the northern region of Brazil, is highly endemic for viral hepatitis. Despite this, studies on the genetic diversity of the Delta hepatitis virus (HDV) and the hepatitis B virus (HBV), especially in patients with Delta hepatitis, are still scarce in the Amazon region. Based on this context, this project aims to evaluate the genomic variability of HBV and HDV simultaneously in patients diagnosed with hepatitis Delta superinfection in the Amazon region. Methodology: a total of 22 carriers of hepatitis Delta superinfection were included. Conventional PCR followed by Sanger sequencing was used for HBV and HDV genotyping. Results: the study population was 77.3% male (17/22) and had an age mean of 38 years. A total of 100% (22/22) were classified as HDV genotype 3 (HDV-3) and showed a closer phylogenetic relationship with Brazilian sequences. As a partial result, 27.3% (6/22) were positive in the conventional PCR for HBV and these samples will subsequently be sequenced. Conclusion: it is hoped that the results of this study will contribute to understanding the profile of genetic variability and the clinical profile of patients with HDV. In addition, the analysis of the two different viruses simultaneously in the same patient can provide information on viral behavior in the host.

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PT.127

Justicidin B inhibits the multiplication of Zika virus in post-infection stages

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Zika virus (ZIKV) is an emerging arbovirus that in recent years has been associated with cases of severe neurological disorders, such as microcephaly in newborns and Guillain-Barré syndrome in adults. As there is no vaccine or treatment, the search for new therapeutic targets is of great relevance. In this sense, plants are extremely rich sources for the discovery of new bioactive compounds and the species *Phyllanthus brasiliensis* (native to the Amazon region) remains unexplored. n a previous study, the antiviral prospection of bioactive compounds from this plant, pointed out Justicidin B as the most promising, which was highly selective for ZIKV and reduced the viral load by 100%. Therefore, this study aimed to investigate the mechanisms of antiviral action of this lignan. For this, treatment with Justicidin B was carried out at different moments of the ZIKV multiplication cycle (adsorption, internalization and post-infection), in addition to evaluating the virucidal activity. The results revealed that Justicidin B does not have virucidal activity, nor does it affect the adsorption stage, exercising its antiviral effect during post-infection stages, resulting in a remarkable 99.9% reduction in viral load when treatment was initiated until 24 hours after infection, with significant difference to the positive control (ribavirin). Our findings suggest that Justicidin B inhibits endosomal internalization and acidification, effectively interrupting the viral multiplication cycle. Therefore, the findings shed light on the promising potential this compound isolated from *P. brasiliensis*, which could contribute to the drug development and treatments for Zika virus infections.

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PT.128

THE POTENTIAL OF EXTRACT FROM LEAVES OF *Tontelea micrantha* AND POSSIBLE MECHANISMS OF ACTION AGAINST CHIKUNGUNYA VIRUS

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A recent study explored the activity and mechanisms of action of methanolic leaf extract from *Tontelea micrantha* against the Chikungunya virus (CHIKV), known for its painful, prolonged arthritic symptoms. Despite ongoing vaccine clinical trials, no specific treatment or approved vaccine is currently available. Initially, the cytotoxic concentration for 50% (CC_{50}) of the methanolic leaf extract (EMF) was determined. Subsequently, the effective protective concentration for 50% of infected cells (EC_{50}), selectivity index (SI) at different multiplicities of infection (MOI), and potential mechanisms of action were investigated. Antiviral assays were conducted on Vero cells infected with CHIKV and treated with the extract for 48 hours. The methyl-thiazol-tetrazolium method was used to evaluate both cytotoxicity and antiviral activity. Results indicated EMF extract exhibited no cytotoxicity (CC_{50} > 300 µg/mL). Regarding antiviral activity, the extract demonstrated potency with an EC_{50} of 43 µg/mL and SI of 8.16 for MOI 0.1. For other MOIs (1 and 5), EC_{50} values were similar (41 and 44 µg/mL), reducing viral load by up to 3 logs and maintaining SI above 4, indicative of selective antiviral activity. Evaluating mechanisms of action, the extract exhibited a virucidal effect, also acting during viral adsorption and after internalization, suggesting direct action on the viral particle and/or host cell. These promising findings suggest compounds present in *T. micrantha* are potential candidates for drug development against the Chikungunya virus.

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PT.129

ANTIVIRAL ACTION USING DIFFERENT FLAVONOID STRUCTURES AGAINST MAYARO VIRUS

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Alphavirus Mayaro (MAYV) is endemic especially in South America, but has already been reported in more than 10 Brazilian states. The symptoms of Mayaro Fever are often fever, rash, myalgia and arthralgia. The virus spreads primarily through the bite of infected mosquitoes of the genus *Hemagogus.* To date, there is no available vaccine or antiviral against MAYV. Flavonoids exhibit some recognized biological properties such as immunomodulatory and anti-inflammatory. Considering such activities, this study aims to explore the activity of monomeric, dimeric and oligomeric flavonoids against MAYV. Firstly, the flavonoids were tested for their cytotoxicity on Vero cells, which were maintained in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 5% Fetal Bovine Serum (FBS) and a cocktail of antibiotics. In the cytotoxicity assay, the colorimetric methyl thiazole tetrazolium (MTT) method was used. From this experiment, the value of the cytotoxic concentration of the flavonoids for 50% of the treated cells (CC50) was obtained, which proved to be 337,01µM; 521,15µM; 500,61µM and 500,77µM for PCA2, PCA1, HBC and GNT respectively. Next, the antiviral assay was performed, for which the cells were treated with the flavonoids and infected with MAYV. This assay led to obtaining a Selectivity Index (SI) of 35,63; 41,97; 1,00 and 1,01 for PCA2; PCA1; HBC and GNT respectively. Although the results are not yet conclusive, they indicate that molecules with a trimeric structure have better action and a better SI for MAYV.

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PT.130

EVALUATION OF THE ANTIVIRAL EFFECT OF MONOMERIC, DIMERIC AND OLIGOMERIC FORMS OF FLAVONOIDS AGAINST *THE ALPHAVIRUS CHIKUNGUNYA*

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Arboviruses are a major cause of morbidity worldwide. Therefore, studies on molecules with pharmacological activities to combat them are of paramount importance. The *Alphavirus chikungunya* (CHIKV) belongs to the *Alphavirus* genus and the *Togaviridae* family, causing fever, prolonged polyarthralgia, rashes, and headache. In addition to vaccines, the search for antiviral molecules is extremely important, with flavonoids being a promising group of molecules. In this study, the antiviral activity of ten molecules with different chemical structures, i.e., monomeric, dimeric, and oligomeric, against CHIKV was investigated. Vero cells were used in 96-well microplates with 2.0 x 10^4/well infected with CHIKV at a multiplicity of infection (MOI) of 0.1 and treated with different concentrations of these molecules (500-15.625 µM) for 48 hours. The study initially addressed cytotoxicity, and in the antiviral assay, satisfactory activity against CHIKV was demonstrated for 4 of these molecules (PCA2; PCA1; HBC and GNT), which showed a half-maximal cytotoxic concentration (CC50) of 337.01µM; 521.15µM; 500.61µM and 500.77µM respectively. For these molecules, antiviral assays showed action against CHIKV, with a selectivity index (SI) of 6.32 for PCA2, 19.12 for PCA1, 1.87 for HBC and 11.57 for GNT. Although a direct correlation between the structures of the molecule set and the antiviral action was not demonstrated, relevant effects against CHIKV were verified, which encouraged the search for more details of these and other molecules against this important virus. It also opens up perspectives for studies on other related viruses.

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PT.131

Brain and Lung Immunopathogenesis of SARS-CoV-2 Delta Variant in K18-hACE2 Transgenic Mice

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The COVID-19 pandemic has highlighted the diverse clinical profiles of SARS-CoV-2 variants, reflecting the virus's adaptation to evade the host's immune system. This study aims to elucidate the immunopathogenesis of COVID-19 in different tissues using the K18-hACE2 transgenic mouse model, which expresses the human ACE2 receptor utilized by the virus for entry. We focused on brain and lung tissues. We established an infection model for the Delta variant by assessing acute illness at varying virus concentrations (10^3, 10^4, and 10^5 PFU). We performed gene expression and viral load quantification via qPCR. In the brain, viremia is higher according to the inoculum. In the lung, the group with the highest inoculum showed the highest viremia, but no statistical difference in viral load was observed between the groups, indicating controlled infection. Our study also found that SARS-CoV-2 infection induced leukopenia in the animals, and there was an increase of human ACE2 expression in the lung but not in the brain post-infection. Those with the highest inoculum exhibited more severe clinical signs. In contrast, the group with the lowest inoculum showed higher expression of cytokines (IL-6) and interferon-stimulated genes (IFIT1, IFITM3, and ISG15), suggesting an effective antiviral innate immune response. These findings demonstrate the utility of the K18-hACE2 model in reproducing COVID-19 and highlight the tissue-specific responses to SARS-CoV-2 infection.

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PT.132

THE INFLUENCE OF B-ADRENERGIC RECEPTOR (ß-AR) AGONIST AND ANTAGONIST DRUGS IN THE PERMISSIVENESS AND RESPONSE PROFILE OF HUMAN MICROGLIA TO SARS-COV-2 INFECTION

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COVID-19, an infection caused by SARS-CoV-2, can trigger manifestations in the central nervous system, such as headaches, dizziness, strokes, and post-infection neurocognitive sequelae. Due to the increased production of pro-inflammatory cytokines, leading to a cytokine storm, there may be disruption of the blood-brain barrier and activation of T cells and macrophages. Microglia, a specialized macrophage in the central nervous system, can exhibit a pro-inflammatory profile (M1) that contributes to pathogen elimination but also can cause neuronal damage; and when presenting an anti-inflammatory profile (M2), it promotes protective effects. β-adrenergic receptors (ß-AR) can suppress the pro-inflammatory response by a variety of mechanisms, including the stimulation or inhibition of important cytokines. This study aimed to evaluate the influence of ß-AR agonist and antagonist drugs on the response of human microglia to SARS-CoV-2 infection. The C20 human microglia lineage was characterized as CD11b+, CD45low, and Iba-1low, expressing receptors used by SARS-CoV-2 (ACE2, TMPRSS2, NRP1) and β-ARs. Microglial activation was analyzed by interferon expression after stimulation with Poly I:C or SARS-CoV-2 infection; as well as the effects of salbutamol (β2-AR agonist) and propranolol (β-AR antagonist) on interferon expression, viral load, and modulation of receptors used by the virus. Both treatments reduced IL-6 and IL-8 secretion in the first hours after activation with Poly I:C but did not influence after SARS-CoV-2 infection. Treatment with salbutamol did not affect viral load and reduced the expression of ACE2 and NRP1.

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MEASLES IN SÃO PAULO: FROM THE INTRODUCTION OF THE VACCINE IN 1964 TO SEROEPIDEMIOLOGY IN THE 2019 EPIDEMIC.

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Measles is a disease caused by the virus *Morbillivirus hominis*, highly contagious among non-immunized individuals of (R0) 9 and 18, it is necessary that at least 95% of the population be immunized to reach herd immunity. In Brazil, the vaccine for measles was introduced in 1964, but only with the creation of the "Programa Nacional de Imunização" (1973) a more organized distribution was achieved. However, the immunization rate against the MeV took long to reach the recommended levels; in the year 1979 the state of São Paulo only reached 80% vaccination against measles. A great post-vaccination outbreak happened in the same region during 1997 with 42.058 confirmed cases and 42 deaths. The endemic circulation of the virus ended in 2000 in the state of São Paulo, whereas in Brazil the autochthonous transmission was recognized as interrupted in 2002 and declared as eliminated in 2016. In 2019, an epidemic struck São Paulo with 15.598 confirmed cases, 2.039 hospitalized and 18 deaths. Serology for IgM and IgG was performed by LVCM and confirmed by IAL in 332 serum samples patients. From July to September of 2019, 332 serum samples were collected, about 92/332 (28%) were not vaccinated and 52% (173/332) were immunized with at least one dose of MMR vaccine. IgM was reactive for measles in 33,5% samples (111/332) and IgG in 63,5% (211/332) of cases. In conclusion the low vaccination rate against measles was one of the reasons for the 2019 outbreak.

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PT.134

OBTATION OF THE REPLICATION COMPLEX (DNA POLYMERASE) OF MONKEYPOX VIRUS FOR THE IDENTIFICATION OF ANTIVIRAL DRUG CANDIDATES

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In May 2022, the WHO was notified of a case of monkeypox in the United Kingdom caused by the Monkeypox virus (MPXV). This virus, belonging to the genus Orthopoxvirus, contains double-stranded DNA and two essential polymerases: DNA polymerase (DNApol) and DNA-dependent RNA polymerase (DdRp). The DNApol, composed of three subunits (F8-polymerase, A22, and E4-glicosilase), is critical for viral replication. This research aims to obtain the MPXV DNApol complex with its three subunits and characterize them functionally through kinetic and structural assays. The nspE4 gene was synthesized in the pET28a vector for expression in *Escherichia coli*. Expression in the BL21 (DE3) strain showed better yields in TB and ZYM media than LB medium, regardless of IPTG concentration tested (0.25, 0.5 or 1 mM). The protein was purified by affinity chromatography using Ni^{2+} resin and gel filtration with Superdex 75 10/300 column. The pure protein was subjected to crystallization assays using commercial kits, resulting in small crystals observed from 21 days onwards. The subunits F8 and A22 were subcloned into pFastBac vectors for expression in insect cells, *Spodoptera frugiperda* (Sf9), using bacmids system to cells transfection. The nspF8 was purified like described above. The nspE4 and F8 subunits underwent thermal denaturation assays, resulting in melting temperature (T_m) of 39 and 46,6°C, respectively. Kinetics assays will be now conducted for compounds screening to identify potential inhibitor molecules. These results provide a solid foundation for future studies of the DdRp complex, contributing to a better understanding of MPXV and the search for antiviral drugs.

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PT.135

COMPARISON OF VIRAL LOAD AND CLINICAL CHARACTERISTICS IN HOSPITALIZED CHILDREN WITH SARS-COV-2: PRE- AND POST-OMICRON VARIANT EMERGENCE

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Children experience COVID-19 differently from adults, presenting milder symptoms but occasionally severe outcomes. Higher viral loads lead to worse outcomes. The Omicron variant has increased pediatric hospitalizations, highlighting the need for research. This study compares clinical and epidemiological characteristics, including viral load, among 54 hospitalized children with SARS-CoV-2 in Brazil before and after the Omicron variant. It included 21 children (G1, from August 2020 to June 2021 - pre-Omicron emergence), and 33 children (G2, from January 2022 to May 2022, post-Omicron emergence). Clinical samples (nasopharyngeal/oropharyngeal swabs or saliva) were tested for viral load using RT-qPCR. Demographic, clinical, and symptomatic data were obtained from questionnaires or medical records with informed consent. Most children were male (68.5%) with a median age of 19.5 months (IQR:6.0–97.75). The most frequent symptoms were fever (76.0%), cough (76.0%), and rhinorrhea (63.0%), with no differences between the groups. Comorbidities were more frequent in G2 (70.0%), though without statistical significance (p=0,346). The majority of children required oxygen support correlated with longer hospital stays (p=0.002). Age influenced significantly the requirement for oxygen support (p=0.018). Viral load was significantly higher (p=0.006) in G1 compared to G2 ($3.3x10^7$ copies/mL, IQR=$1.3x10^5$–$2.1x10^8$ versus $2.9x10^4$ copies/mL, IQR=$3.0x10^3$–$5.3x10^6$). Sex, presence of fever and cough, and use of oxygen therapy were associated with viral load (p=0,035). Comorbidities significantly increased the probability of hospitalizations longer than 10 days (OR=6.289; p=0.011). This study highlights the clinical challenges and complexities in managing hospitalized children with COVID-19, showing clinical and viral load differences between two pandemic periods.

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PT.136

Clinical Aspects and SARS-CoV-2 Viral Load in COVID-19 Patients Admitted to an Intensive Care Unit in Brazil

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COVID-19 can range from mild symptoms to severe organ failure, yet understanding how socioeconomic, clinical, laboratory factors, and viral load influence disease progression in ICU patients remains limited. This study analyzed clinical outcomes and clinical characteristics, including viral load (Ct values), in 231 ICU patients infected with SARS-CoV-2. Viral loads from clinical samples (nasopharyngeal/oropharyngeal swabs) were measured using RT-PCR, and clinical data were obtained from medical records with informed consent. Overall survival was assessed using Kaplan-Meier survival analysis and the log-rank test for curve comparison. Men comprised 50.4% of the deaths, and the median age of deceased patients was 68.2 years. Hypertension (65.6%, $p=0.017$) and diabetes (37.4%, $p>0.05$) were the most common comorbidities among those who died, while dyspnea (84.7%) and desaturation (80.9%) were the most frequent symptoms. Severe complications, such as acute kidney injury and septic shock, were more common among deceased patients. Non-invasive ventilation was used equally between the groups, but 42.7% of deceased patients required tracheal intubation. Older patients had significantly lower survival rates, highlighting age as a key risk factor. The median Ct value in samples from deceased patients was slightly lower (23.5) compared to those who were discharged (24.2), but this difference was not statistically significant ($p>0.05$). Of all patients, 55 (24.2%) had high, 133 (58.6%) had moderate, and 39 (17.2%) had low viral loads. Identifying these factors is crucial for developing more effective therapeutic interventions and improving clinical outcomes in hospitalized populations.

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PT.137

EFFECTS OF DRUGS USED TO TREAT ASHTMA AND HYPERTENSION ON SARS-COV-2 INFECTION AND REPLICATION IN CARDIOMYOCITES

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SARS- COV-2, the etiological agent responsible for the disease known as COVID-19. Among the symptoms caused by COVID-19, respiratory and cardiovascular complications are deeply associated with severe cases of the disease which can lead to death. Many patients afflicted with SARS-CoV-2 infection had prior health issues, such as ashtma and hypertension, and had treatment with drugs such as salbutamol and propranolol. During the pandemic, little was known about SARS-CoV-2-host interaction and how treatment with drugs from previous conditions could affect the pathogenesis. Thus, this study aimed to evaluate the replication and infectious capability of SARS-CoV-2 *in vitro* in *hiPSC* cardiomyocytes in the presence of either propranolol and salbutamol. Cells were treated with either salbutamol or propanolol at a 1μM concentration and infected 24h later with SARS-CoV-2 of the Wuhan lineage. Supernatant and/or cellular pellet were then collected for analysis of gene expression. Treatment with either drug reduced the expression of TMPRSS2 and AT1, while treatment with salbutamol increased expression of CD147, all of which are proteins associated with viral infection. Propanolol-treated cells displayed a significant reduction in viral load in the supernatant compared to positive controls. Conversely, the same group displayed a higher viral load than positive controls in the pellet analysis. During a 24h-period, treatment with salbutamol increased expression of IL-1β, IL-6 and IFNγ at different time stamps. Similarly, propanolol increased expression of IL-6 and TNF-α. Altogether, the study highlights an important reduction in TMPRSS2 in cells treated with propranolol, which could lead to a reduced infection of SARS-CoV-2.

PT.138

PERFORMANCE OF RAPID ANTI-IGG, IGM, AND NS1 TESTS FOR DENGUE DIAGNOSIS IN A HYPERENDEMIC AREA

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Primary dengue virus infection provides immunity against that serotype, but secondary infection with a different serotype increases the risk of severe dengue. Early and accurate diagnosis is crucial due to the lack of effective antiviral therapy and reliance on supportive care like fluid replacement. This study assesses the performance of rapid anti-IgG, IgM, and NS1 tests in hospitalized patients from a hyperendemic region. We retrospectively analyzed 1023 clinical specimens collected during a dengue outbreak from August 2019 to December 2021 at the Hospital de Base de São José do Rio Preto, SP, Brazil. These specimens underwent RNA extraction and PCR testing for DENV (1-4) and Panbio Rapid Test, resulting in 270 PCR-positive and 752 negative samples. The rapid tests' results (specificity, sensitivity, PPV, and NPV) for NS1, IgM, IgG, and their combination were evaluated in Global (DV+ and DV-), DENV-negative (DV-), and DENV-positive (DV+) groups. The NS1 test demonstrated high specificity across all groups, with sensitivity highest in the DV- group (71.64%) and lowest in the DV+ group (50.00%). The IgM test showed low sensitivity in the DV- group (23.88%) and moderate in the DV+ group (59.56%). The IgG test performed better in the DV+ group (78.68%) and worse in the DV- group (15.67%). Combined, the NS1, IgM, and IgG tests showed high sensitivity across all groups, indicating effectiveness in identifying infected individuals and minimizing false negatives. Such an approach is especially crucial in hyperendemic regions for DENV, where accurate and rapid detection is essential for proper disease management.

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PT.139

NOVEL AMAZONIAN EXTRACTS AS ANTIVIRAL CANDIDATES AGAINST SARS-CoV-2 INFECTION *IN VITRO*

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The SARS-CoV-2 pandemic boosted the search for antivirals aimed at treating COVID-19. The antivirals for COVID-19, today, are very expensive, impeding access to these treatments. Therefore, it is urgent to develop new drugs to treat this disease. This study aimed at evaluating the cellular effects and the antiviral anti-SARS-CoV-2 activity of two amazonian extracts as candidates for the COVID-19 treatment. B1 and B2 extracts showed previous promising results against other viruses and were selected for the study. They were tested using six dilutions for cytotoxicity and plaque-reduction assays to assess antiviral potency in BSL-3. Both extracts showed low cytotoxicity, with above 80% of viability in Vero CCL-81 cells. Also, both extracts had an antiviral activity against SARS-CoV-2 (Wuhan and Omicron). In tests based on the Wuhan, B1 decreased 3-log at a dilution up to 1:40, presenting a dose-dependent effect. In tests based on the Omicron, B1 reduced 6-log up to 1:40 dilution and almost 2-log in the 1:80 dilution. This effect was more significant in Omicron variant as compared to the Wuhan strain. In assays using extract B2, a reduction of ~6-log was shown for both strains in the first dilution (1:2), however, a smaller effect on the next dilutions was observed compared to B1. This data indicate a dose-dependency of those extracts and a promising and significant interference in the viral replication cycle, specially for B1. Therefore, further tests are underway in order to shed light regarding the mechanisms of viral inhibition and its potential as anti-SARS-CoV-2 antiviral candidates.

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PT.140

PREVALENCE OF HUMAN T CELL LYMPHOTROPIC VIRUS (HTLV)-1 AND 2 IN AMAPA, BRAZIL, AND THE NEED FOR SCREENING IN DIFFERENT POPULATION SEGMENTS

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Human T-cell lymphotropic virus (HTLV) is often neglected despite causing severe manifestations in 3-5% of carriers, including HTLV-associated myelopathy (HAM). Its silent nature and varied transmission routes necessitate widespread screening. Few studies on HTLV seroprevalence in Brazil exist, primarily targeting specific groups like blood donors and pregnant women.
This study estimates HTLV-1/2 seroprevalence in the general population of Amapá, Brazil, and gathers data from other states to provide a comprehensive view of the current HTLV situation. Samples from Amapá were tested for IgG anti-HTLV1/2 through ELISA, and seroprevalence studies were reviewed using PubMed and Google Scholar.
Among the 900 serum samples collected from adults in 2022-2023, 400 were already tested revealing a 0.75% positivity rate, three times higher than previous estimates based on blood donors. Investigating the publicly available Brazilian data, we found that most studies are focused on blood donors and most states have outdated information, about seven years old. Recent findings described a prevalence of 0.1% in Minas Gerais, Goiás, and Ceará, and 0.2% in Pernambuco and Pará.
Despite their heterogeneity, blood donors represent a specific population and may not accurately reflect HTLV prevalence in the general population. While HTLV-1/2 testing is mandatory for blood donors and has recently been included in prenatal programs, it is not yet part of routine sexually transmitted infection (STI) screening at health clinics. Therefore, understanding the actual prevalence of HTLV-1/2 in the general population is crucial for effective public health planning and intervention.

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PT.141

DEVELOPMENT OF A NEW PROTOCOL FOR HTLV-1 WHOLE GENOME SEQUENCING USING NANOPORE TECHNOLOGY

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Human T-lymphotropic virus type 1 (HTLV-1) is often neglected despite causing severe conditions in 3-5% of carriers. There is a scarcity of complete HTLV-1 genome sequences in databases, and current sequencing protocols for HTLV-1, are expensive and time-consuming. Obtaining more complete genomes of a virus is crucial for elucidating its basic biology, conducting epidemiological studies, and exploring how viral genetics influence its various manifestations. Thus, we aimed to develop a method for amplifying and sequencing the complete HTLV-1 genome using Nanopore technology with the MinION device. A custom set of 60 primers to cover nearly the full length of the HTLV-1 genome was designed using the Primal Scheme tool. Various amplification conditions were tested, including primer concentrations and annealing temperatures. Then, six samples from infected patients were amplified and sequenced. The fastq reads were assembled in CLC Workbench using the NC_001436.1 as the reference sequence. Comparison between input primer concentrations of 10 µM and 20 µM showed that 20 µM resulted in faint bands and numerous primer dimers on the agarose gel. A 10 µM primer concentration and lower annealing temperatures (63-61ºC) offered better results, although all samples had good sequencing parameters and vertical sequencing coverage ranging from 2,000 to 3,000 nucleotides. Our results demonstrate an effective method for HTLV-1 whole-genome sequencing using Nanopore technology, offering a cost-effective and practical alternative to traditional methods. Nanopore can be easily implemented in any laboratory, potentially expanding viral studies with sequencing methods.

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PT.142

QUANTIFICATION OF SOLUBLE TRIGGERING RECEPTOR EXPRESSED ON MYELOID CELLS-1 (sTREM-1) IN CHIKUNGUNYA PATIENTS

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Paraguay experienced one of the largest chikungunya epidemics in the Americas at the end of 2022 and beginning of 2023. This arbovirosis, caused by the chikungunya virus (CHIKV), is characterized by fever and joint pain and is usually self-limiting, but can cause serious manifestations. It has been reported that the triggering receptor expressed in myeloid cells-1 (TREM-1), such as neutrophils and monocytes, increases its expression as an amplifier of the immune response. Soluble TREM-1 (sTREM-1), the released form of TREM-1, has been investigated as a biomarker for infectious diseases, including prognosis prediction. Our study aimed to evaluate the levels of sTREM-1 in serum of chikungunya patients. We have included 64 hospitalized chikungunya patients confirmed by rRT-PCR and classified into two groups according to clinical manifestations: classical (32/64), characterized mainly by musculoskeletal manifestations, and systemic (32/64) if the cases presented some organic alteration or systemic deterioration. The median level of sTREM-1 was 671 pg/mL (IQR 1060). The median sTREM-1 level was higher in male patients and those over 60 years of age ($p<0,0211$ and $p<0,0005$ respectively). Patients with systemic chikungunya (SC) had a significantly higher median level of sTREM1 compared to patients with classical chikungunya ($p<0.0001$). Moreover, 16/32 patients classified as SC died. It was observed that for every 100-unit increase in sTREM-1, the odds of systemic CHIKV increase by 19.6%. These findings provide the first data on sTREM-1 in chikungunya patients and highlight the need for studies to evaluate its use as a biomarker of severity.

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PT.143

ANALYSIS OF THE SPATIO-TEMPORAL TREND OF HEPATITIS A IN THE STATE OF PARÁ, 2009 TO 2019

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Hepatitis A is a serious global public health problem , caused by the Hepatitis A Virus, considered to be one of the most common and resistant human enteric viruses, mainly affecting children, an important cause of waterborne diseases. Infection occurs via the fecal-oral route through contaminated food and water and by sexual transmission via the anal-oral route. The vaccine is the most effective form of prevention. It aimed to analyze the spatio-temporal profile of hepatitis A in the state of Pará between 2009-2019 by means of an ecological design using data extracted from the DataSUS Information System; with tabular organization in the Excel program and descriptive statistical analysis and Prais-Winsten regression were carried out to calculate the annual percentage variations in incidence rates. Finally, the coroplastic thematic maps of the average and incidence rate of hepatitis A in Pará were made by importing the data and georeferenced meshes, using the QGIS 3.12 software. In 117 of the 143 municipalities in the state of Pará, 4,065 cases were reported, with an average of 369.54 cases/year, 54% were men; the highest incidence was among children aged 5-9 (30%); among brown people (77%), and elementary school children (41%). The average incidence rate between 2009-2019 ranged from 0 to 85.42 cases/100,000 inhabitants, with a drop in notifications between 2009-2019, with the exception of the Marajó I region, whose analysis by the surveillance sectors will be necessary due to a possible underreporting due to the COVID-19 pandemic.

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PT.145

EVALUATION OF THE ANTIVIRAL ACTIVITY OF OLIGONUCLEOTIDES AGAINST THE ZIKA VIRUS

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Zika virus (ZIKV) is an emerging arbovirus with increasing importance in global public health. This study aims to use RNA interference technology for silencing regions in ZIKV genome and thus inhibiting viral replication in Vero cells. Double-stranded RNA (dsRNA) molecules were constructed by bioinformatics programs, 5'UTR, protein C, prM, NS1 and NS2A target sequences were identified in ZIKV genome. ZIKV MR766 strain was amplified and titered in Vero cells. Six primers were designed and used in the construction of the dsRNA molecules. Six single-stranded oligonucleotide sequences (ssDNA) were designed and synthesed. After transfection tests, the antiviral activity of dsRNA and ssDNA molecules was evaluated by Reed Muench. Cell viability was not affected by the twelve synthesized molecules and Lipofectamine. NSF/ NSR dsRNA showed significant results in reducing viral titer in protocols one and six. dsRNA F2/R2 showed higher values in the rate of reduction of viral titer in the third and sixth protocols. ssDNAs showed significant results in Protocol N°2, however, values presented by all ssDNAs were even better in the third protocol. Combinations VI and X were the ones with the highest values of reduction in viral titer, 91.68% and 97.6%, respectively. ssDNA X3 showed significant results in the second and third protocols and also in Combinations II, IV and X. Analysis strategies by bioinformatics tools and transfection of dsRNAs and ssDNAs revealed by the profile of oligonucleotide sequences with possible ability to inhibit ZIKV MR766 in mammalian cells.

Financial Support: CAPES, CNPq, FAPERJ.

PT.146

MOLECULAR DIVERSITY OF DENGUE VIRUS SAMPLED IN PARANÁ, BRAZIL, BETWEEN 2016 AND 2024

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Dengue is a viral disease of extreme importance to public health. In Brazil, rising temperatures have contributed to the disease expansion southwards, with Paraná state presenting alarming increasing incidence over the years. Using high-throughput genome sequencing and bioinformatics tools, we sequenced and genotyped 646 DENV genomes, obtained from serum specimens collected from laboratory-confirmed dengue cases, sampled in Paraná between 2016 and 2024. Of the total, 499 (77,2%) were classified as serotype I, all belonging to genotype V (DENV1-GV), that has prevailed in the American continent since dengue's reintroduction in the 1980s. Other 135 (20,9%) were serotype II, with 80 (59,3%) classified as genotype III (DENV2-GIII, Southern Asian-American), and the remaining 55 (40,7%) classified as genotype II (DENV2-GII, Cosmopolitan). The other 12 genomes (1,9% of the total) were all classified as serotype 3, genotype III (DENV3-GIII). DENV2-GIII was the only genotype of serotype II ever detected in South America until 2021, when the DENV2-GII introduction in the continent was detected. Our sampling results show that, although DENV1 continues to be the prevalent serotype in Paraná through the screened period, DENV2-GII appears to be replacing GIII as the current prevalent DENV2 genotype. Furthermore, the detection of DENV3 in Paraná, a serotype that did not circulate in Brazil for 15 years until its recent reemergence in North Brazil in 2023, indicates that DENV3 is dispersing throughout the country. Taken together, these results contribute to the knowledge of DENV circulating in areas of recent expansion and low populational immunity.

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PT.147

GENOMIC SURVEILLANCE OF RESPIRATORY VIRUS SARS-COV-2 IN ARARAQUARA, SAO PAULO, BRAZIL

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The COVID-19 pandemic has reinforced the importance of effective genomic epidemiological surveillance in a local approach. Araraquara, one of São Paulo's most industrialized cities with 242,228 inhabitants, is 270 km from the state capital and is well-suited for monitoring emerging variants due to its demographic and epidemiological profile. The monitoring involved 584 SARS-CoV2 samples sequenced by the laboratory where this study was conducted by NGS using the Illumina COVIDSeq test for library preparation and the Illumina MiSeq system for data generation. Analysis was performed using the DRAGEN COVID Lineage pipeline, and sequences were submitted to the GISAID database. Phylogenetic analysis was then conducted with the Nextclade platform, incorporating all 657 (584/657 in-house) sequences from 2020 to 2024 available on GISAID from the same location. The phylogeny of Araraquara's samples showed very similar patterns to those seen in Brazil and globally. All of the mutations found were previously described. The main lineages identified were P.1 for the Gamma variant and BQ.1.1 for Omicron, and the predominant clades were 20J (115/155), 21J (120/121) and 22E (119/119). Surveillance is crucial for timely public health decisions and measures, hence the extreme importance of tracking SARS-CoV-2 variants and subtypes to enhance global genomic monitoring.

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PT.148

FULLEROL REDUCES THE OXIDATIVE DAMAGE CAUSED BY ZIKA VIRUS INFECTION IN J774.A1

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The *Zika virus*, responsible for Zika fever, is an arbovirus of significant public health concern in Brazil due to challenges in vector control, diagnostic delays, and its association with severe diseases. Currently, there are no approved treatments for the disease and its complications. The role of reactive oxygen species (ROS) in Zika infections is known, affecting the imbalance in redox homeostasis, thus prompting the search for compounds capable of combating ROS and restoring redox homeostasis. Fullerol (C60OH [22-24]) is a nanomaterial shown to scavenge ROS, with previous studies demonstrating its antioxidant and antiviral action in Zika infections. This study aimed to explore fullerol's ability to mitigate oxidative damage caused by ZIKV in J774.A1 cells. The cytotoxicity of the nanomaterial was evaluated, and antioxidant activity was measured through intracellular ROS testing post-infection. Superoxide dismutase activity was also assessed, along with the effects of fullerol treatment on lipid peroxidation and protein carbonylation, important markers of oxidative stress. Results indicated no cytotoxic effects of fullerol at the tested concentration and a reduction in ROS levels during Zika infection at the optimal concentration of [3.125nM]. Fullerol also increased SOD activity and significantly reduced lipid peroxidation and protein carbonylation, suggesting a potent antioxidant action capable of mitigating oxidative damage induced by ZIKV infection in cellular models.

PT.149

Evaluating Chikungunya Severity through Chymase, LBP, and Demographic Factors: Evidence from the 2023 Paraguay Outbreak

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In 2023, Paraguay experienced its most severe chikungunya epidemic to date, highlighting the emergence of atypical and severe forms of the disease. Proteins such as lipopolysaccharide-binding protein (LBP) and chymase have been associated with the severity of various diseases, suggesting their potential as markers for assessing severity. This study aimed to identify factors linked to chikungunya severity, focusing on serum levels of chymase and LBP, as well as demographic factors like age and sex. The study included 64 hospitalized patients from Asunción, Paraguay, recruited between January and June 2023. These patients were between one and nine days from the onset of symptoms (average 4±1.8 days) and tested positive for chikungunya RNA via rRT-PCR. Of the participants, 52% were male and the mean age was 57±27 years. Patient severity was classified according to Brito et al.'s criteria, dividing them into classic chikungunya (CC, n=32) and systemic chikungunya (SC, n=32). Chymase median concentration was significantly higher in SC compared to CC ($p=0.0040$; $\alpha=5\%$). Logistic regression analysis revealed that age and sex are significant predictors of severity, while chymase and LBP levels showed non-significant but potentially relevant trends. The model demonstrated a good fit, with an AIC of 59.405 and a residual deviance of 47.405 with 46 degrees of freedom, indicating a well-balanced model. These findings highlight the importance of considering demographic factors and biomarkers in the clinical assessment of chikungunya patients.

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PT.150

Epidemiological Surveillance of SARS-CoV-2 in Minas Gerais between 2023 and 2024

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SARS- CoV-2, the virus responsible for COVID-19, emerged in Wuhan, China, in 2019, rapidly spreading worldwide. Since 2021, partnerships between UFMG, private sectors, and state and municipal health agencies have led to the OViGen project, one of Brazil's largest genomic surveillance initiatives. With the resurgence of COVID-19 cases in late 2023, the project was resumed to monitor the virus's dynamics and assess the impact of mutations in variants between epidemiological weeks 20 of 2023 and 22 of 2024 in Minas Gerais. Ten Regional Health Management units were selected to send 20 positive samples per epidemiological week for PCR testing and full genome sequencing, using the Illumina MiSeq and NextSeq platforms. The obtained sequences were classified and submitted to GISAID, while phylogenetic and phylogeographic analyses are ongoing to map the virus's spread within the state. During the analyzed period, the Omicron GK subvariants, dominant in 2023, were replaced by the JN and JD subvariants in 2024, alongside the detection of new recombinants such as XDD and XDR.

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PT.151

IDENTIFICATION AND DESIGN OF PEPTIDES TARGETING MAYARO (MAYV) AND OROPOUCHE (OROV) VIRUSES FOR DIAGNOSTIC PURPOSES

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Recent outbreaks of neglected arboviruses, Oropouche (OROV) and Mayaro (MAYV), have caused significant morbidity in South America, with the Oropouche fever recently causing the world's first deaths in Brazil. Moreover, current diagnostic tests for these viruses are either scarce or have been very little explored. To address these issues, this work proposed designing small proteins using the state-of-the-art bioinformatics techniques to serve as raw material for the diagnosis of OROV and MAYV. Viral genomic sequences from human hosts were obtained from the Virus Pathogen Resource (ViPR) public database, quality-checked, and aligned using MAFFT. Evolutionary analyses were performed with BEAST 1.10, generating 10,000 maximum clade credibility trees of posterior probability for the encoding glycoproteins gene of MAYV's (E2) and OROV (Gc). Conserved regions over time were identified and subsequently analyzed for divergence at the species and genus levels, along with structural properties such as solvent-accessible surface area. Using these regions, 20,000 protein backbones were generated with RoseTTAFold Diffusion, with the most compact ones selected for sequence design via ProteinMPNN, both deep-learning programs. For each best-scored sequence, structure prediction was performed using AlphaFold3 and ranked based on folding propensity and RMSD relative to their backbones and native regions. Finally, five proteins containing OROV and MAYV glycoprotein-derived peptides were identified as promising candidates. Future efforts will involve synthesizing these proteins and validating them with target-specific aptamers generated either computationally or experimentally, which are hoped to be specific and may be explored in potential diagnostic tools to detect these neglected arboviruses.

Financial Support: FAPESP

PT.152

EXPLORING THE SPREAD OF HIV-1 SUBTYPE C IN BRAZIL THROUGH TRANSMISSION CLUSTER ANALYSIS

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HIV has a remarkable capacity to generate genetic diversity, resulting in a wide spectrum of types, subtypes, and recombinant forms. Brazil is characterized by an epidemic dominated by HIV-1 subtype B, except for the southern region, where subtype C (HIV-1C) prevails. In the present study, we seek to expand the knowledge about the genetic diversity of the HIV-1C circulating in Brazil. We reconstructed the phylogeny of HIV-1C circulating in Brazil from a dataset consisting of 3,693 sequences (partial *pol*), retrieved from the LANL and GenBank databases, in addition to 7 new sequences identified in Salvador, Bahia. The dataset was aligned using MAFFT, followed by a maximum likelihood (ML) phylogenetic reconstruction using IQTree. Transmission clusters were inferred from the ML tree using the Cluster Picker 1.2 software. Our results indicate a strong transmission flow of HIV-1C from Santa Catarina and Paraná to São Paulo state. Correlation analysis suggests that the number of HIV-1C transmission clusters has increased recently in São Paulo, pointing to a growing epidemic within the state. In addition, São Paulo might have acted as a transmission hub in Brazil, linking the epidemic in southern states to other regions of the country. We hypothesize a possible role of Goiás in the dissemination of HIV-1C to the Northeastern region of Brazil. On the other hand, Rio Grande do Sul, despite being one of the states with the higher prevalence of HIV-1C, shows a very localized epidemic, with no significant influence on transmission to other states.

PT.153

EVALUATION OF THE HUMORAL IMMUNE RESPONSE TO THE 4TH OF COVID-19 VACCINE BOOST IN ELDERLY.

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Elderly is a group that has a high incidence, hospitalization and worsening of COVID-19, in addition to a lower proportion of humoral response than younger people. Our research aimed to evaluate the hypothesis of decreases in Immunoglobulin G (IgG) levels produced by immunization against SARS-CoV-2 in the elderly, also evaluating variables such as physical exercise, comorbidities, age, race and gender. A cohort study of 35 physically active participants (mean age 73 years) and 82 sedentary participants (mean age 75 years) was performed. Serum samples were collected from participants at predefined times, in which the 4th dose of the COVID-19 vaccine was used as reference. The indirect ELISA technique was used to obtain the measures, with the RBD of the Spike protein (S) and the recombinant Nucleoprotein (N) (FPZ0513) as the primary antigens, both from the ancestral strain (Wuhan). Moreover, the Cytopathic Effect Neutralization Technique (CPE-VNT) was used as the gold standard in search of correlation. The results showed that the averages of the IgG measurements for N at the 3 times were statistically different ($p= 0.0012$) and no statistically difference when analyzing the data between the groups studied ($p = 0.24$). Regarding RBD, the 3 times were statistically different ($p= 0.000003$) and when associated between groups, a statistically significant difference was found in the first collection ($p = 0.008$). Our data show a better projection of RBD levels in the physically active group compared to the period before vaccination, without a significant difference after fourth dose.

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PT.154

Osteopontin and Chikungunya infection: key molecule for inflammation and disease progression?

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The SPP1 gene encodes osteopontin, a protein implicated in chronic inflammatory and autoimmune diseases. This protein functions as a Th1 cytokine, modulating T cell immune responses by inhibiting the anti-inflammatory cytokine IL-10 and enhancing Th1-mediated pro-inflammatory responses. This study compared SPP1 gene expression in monocytes infected in vitro with Chikungunya virus (CHIKV) to those infected with another co-circulating alphavirus in Brazil, Mayaro virus (MAYV). Despite the clinical similarities between the diseases caused by these viruses, Mayaro disease generally presents milder symptoms and less frequent joint inflammation recurrence than Chikungunya. Transcript analysis revealed that SPP1 gene expression was approximately 12-fold higher at early time points following CHIKV infection compared to both MAYV infection and uninfected cells. Although SPP1 expression decreased progressively at later infection stages, it remained elevated. In contrast, MAYV infection did not significantly alter SPP1 expression relative to uninfected cells. This increase in gene expression was positively correlated with levels of inflammatory cytokines and chemokines, including IL-6, IL-15, and eotaxin. Our findings suggest that targeting osteopontin production and secretion could mitigate the inflammatory response induced by CHIKV, providing a potential molecule for therapeutic intervention.

PT.155

Evolutionary-Adaptative Process and Phenotypic Characterization of Chikungunya and Mayaro Viruses

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Chikungunya (CHIKV) and Mayaro (MAYV) viruses, two important *Alphaviruses, are* the primary focus of our study. These viruses cause health problems in humans with similar clinical manifestations. The adaptive success of CHIKV is sustained by transmission cycles that explore the evolutionary landscape of the urban environment, potentially undergoing adaptive mutations. This has been observed in three of four main CHIKV lineages and none of the MAYV viruses, indicating that the causes of failure or adaptive success of these viruses still need to be explored. To shed light on these evolutionary processes and characteristics, we curated and analyzed datasets of genomic sequences from these two viruses using phylogenetic analysis tools and molecular evolution analyses. Our study involved curating and analyzing over 637 high-coverage sequences, with recombination analysis and assessing selection pressures. We also evaluated the viral fitness of lineages for each virus available in the laboratory, analyzing the infection kinetics of mammalian and mosquito cells and the presence of specific mutations in the stocks and supernatants with RT-qPCR to correlate the genotypic and evolutionary findings with phenotypic observations. Together, our findings outline the prevalent drives of purifying selection on the evolution of these viruses while underscoring the pivotal role that positive selection pressure plays in shaping their adaptive capacity. Furthermore, we identified critical genomic viral characteristics influencing these viruses' evolutionary and adaptive processes.

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PT.156

ANTIVIRAL ACTIVITY OF HONEY AND AROEIRA HONEY EXTRACT AGAINST *Mayaro virus*

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The north of the state of Minas Gerais has a diversity of plant species, including the *Astronium urundeuva* (Aroeira) species. In this region, in dry season, the species is the only one that flourishes, being the only source of nectar for bees, providing the production of honey with unique properties, called monofloral aroeira honey. The presence of phenolic compounds gives this honey antioxidant and antimicrobial action. To date, no study has investigated its action against arboviruses, such as Mayaro virus (MAYV), which is considered an emerging and endemic virus in Latin America. Thus, considering that there is no available treatment or vaccine and that there are no studies that investigated the antiviral action of Aroeira honey, the objective of this present work was to evaluate the *in vitro* activity of honey and Aroeira honey extract on MAYV. For this, cytotoxicity (CC_{50}) and antiviral action were evaluated in Vero cells by the colorimetric method. Regarding the cytotoxic effect both honey and Aroeira extract were not toxic to cells, presenting CC_{50} of 1000 and 2000 µg/mL, respectively. However, even at the highest concentrations tested for antiviral assays, cell viability was similar to that of the viral control. Therefore, honey and Aroeira extract did not showed anti-MAYV activity. To better understand the antiviral activity of aroeira honey, additional studies must be carried out to concentrate possible secondary metabolites with medicinal properties present in aroeira honey.

PT.157

PREVALENCE OF MAYARO INFECTIONS IN AMAZONIAN PERI-URBAN AREAS

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Arboviruses are a public health problem in Brazil, presenting similar symptoms, which makes clinical diagnosis of these infections difficult. The molecular diagnosis available in the country is still limited to Dengue, Zika and Chikungunya viruses, leaving gaps in relation to the possible circulation of other species that cannot be detected routinely. Molecular investigation of *Alphavirus*es circulating in the Western Amazon. Samples screened negative for Malaria by thick drop and arboviruses such as Zika virus, Dengue (serotypes 1, 2, 3 and 4) and Chikungunya by RT-qPCR were subjected to conventional PCR tests for the detection of targets belonging to the genus *Alphavirus*, Sanger sequencing was used to confirm and characterize the positive results obtained. A cohort of 967 individuals previously negative for DENV (serotypes 1, 2, 3 and 4), ZIKV, CHIKV and Plasmodium spp. showed positivity of 0.20% (2/967) for the genus tested, *Alphavirus*. Phylogenetic analysis of the sequences obtained indicated that the samples belonged to genotype D of the Mayaro virus. This finding highlights the importance of differential diagnosis associated with genomic surveillance in laboratories, identifying new viruses with the potential to spread at an early stage.

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PT.158

DETECTION OF HIGH-RISK HUMAN PAPILLOMA VIRUS IN BREAST CANCER BIOPSIES USING REAL-TIME PCR, PERU

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Breast cancer is a major public health problem worldwide, with the Human Papillomavirus (HPV) increasingly being linked in the last three decades as a possible causal agent. Several studies have found HPV genetic material in women with breast cancer, suggesting a possible association. However, it remains to be confirmed whether HPV is a direct causal factor or whether it acts simply as a passenger or intracellular parasite.
Recent research has identified the presence of high-risk HPV types, specifically HPV-16 and HPV-18, in breast carcinoma biopsies. However, further studies are needed to understand the exact role of HPV in the development of breast cancer.
The aim of this study was to determine the presence of Human Papillomavirus (HPV) types 16 and 18 in paraffin-embedded breast tissue biopsies from patients with a clinical diagnosis of breast cancer. Fifty paraffin-embedded breast cancer biopsies were analyzed for HPV DNA by real-time molecular PCR, primers targeting the HPV E6 gene. 78% (39) were HPV positive, of which 56% (28) were HPV-16 positive and 22% (11) were HPV-18 positive. This study provides evidence for the presence of HR-HPV (16 and 18) in breast cancer biopsies, highlighting the need for further research to understand the role of HPV in breast carcinogenesis. These findings may have important implications for the prevention and treatment of breast cancer.
Keywords: Human papillomavirus type 16 and 18, breast cancer, biopsy, qPCR.

Financial Support: VRIP – UNMSM.

PT.159

EVALUATION OF THE *IN VITRO* ANTIVIRAL ACTIVITY OF *PIPER ADUNCUM* ESSENTIAL OILS AGAINST THE DENV-2 VIRUS

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Currently, there are no therapies to prevent severe dengue disease. Essential oils (EOs) can serve as foundational sources for research and the discovery of phytomedicines for alternative therapies. EO samples from *Piper aduncum*, a plant used in traditional medicine in Peru, were obtained through hydrodistillation. A cytotoxicity test was performed on cell cultures using the Vero-76 cell line, resulting in CC50 values indicating low toxicity. Subsequently, the reduction of the cytopathic effect (CPE) generated by dengue serotype 2 (DENV-2) was evaluated using a plaque reduction assay, revealing the antiviral activity (AA) of these oils. *Piper aduncum* EOs exhibited weak AA, with a 35% reduction in DENV-2 CPE at a concentration of 100 µg/mL. Phytochemical analyses suggest that the antiviral activity of the EOs could be attributed to the presence of sesquiterpenes and monoterpene alcohols. This study reports the antiviral activity of EOs against DENV-2, related to their composition of monoterpenes and sesquiterpenes. The results provide a foundation for future research into the development of antiviral phytomedicines.

Financial Support: VRIP – UNMSM.

PT.160

ANTIVIRAL RESISTANCE CONFERRED BY MUTATIONS IN HUMAN INFLUENZA A VIRUS IN SÃO PAULO STATE FROM 2022 TO 2024.

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The influenza virus is characterized by its frequent genetic alterations, leading to diverse subtypes and clades. In this study, we evaluated mutations associated with antiviral resistance in human influenza viruses of the H1N1 and H3N2 subtypes. Positive samples detected by RT-qPCR for Influenza A with a Cq value below 29 were sequenced using Next-Generation Sequencing, analyzed with bioinformatics tools, and assessed in silico on GISAID to identify resistance-related mutations. Among the eight H1N1 viruses sequenced, one exhibited the V27A mutation in the M2 protein, which alters the ion channel structure and reduces the efficacy of adamantanes. Of the 24 H3N2 viruses sequenced, six had the S329N mutation in the Neuraminidase (NA) segment, which is associated with reduced efficacy of Oseltamivir and Zanamivir. Additionally, one NA of H3N2 virus harbored the D251N mutation, potentially leading to reduced or significantly reduced inhibition by Zanamivir. These findings underscore the importance of genomic surveillance for the early detection of viral changes, which is crucial for ensuring effective public health measures.

Keywords: Antiviral Resistance; Surveillance; H3N2; H1N1.

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PT.161

ANALYSIS OF THE SARS-COV-2 MUTATIONAL PROFILE IN IMMUNOCOMPROMISED INDIVIDUALS WITH PROLONGED VIRAL SHEDDING

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COVID-19 infectiousness typically wanes after ten days. Prolonged SARS-CoV-2 infections with continued viral shedding can occur. Whole-genome sequencing (WGS) is crucial for identifying and managing these prolonged infections. This study explored the SARS-CoV-2 mutational profile by iSNVs in immunocompromised individuals with prolonged viral shedding. WGS in positive SARS-CoV-2 samples by RT-PCR with a ct<30. Consensus were assembled with ViralFlow, and lineages assigned by PangoLineage. Viruses were isolated from Vero E6 cells in BSL-3 facility. Thirteen immunocompromised individuals (C1 to C13) positive for SARS-CoV-2 were monitored, including Delta-AY.99.2 (n=1), Gamma-P.1 (n=3), Omicron-BA.1/BA.1.* (n=6), BA.5 (n=2), and one recombinant XBB.1.5.86. A total of 66 nasopharyngeal samples yielded 55 complete genomes. Median age was 37,4 years-old (range: 22 to 83 years-old), with eight HIV positive (C6-C13), and three were receiving corticoids (C2/C3/C11). Clinical outcomes revealed three cases (C2/C5/C11) died, and others discharged. We highlight the C7 with 15 positive samples collected over 816 days since symptom onset. Active viruses were isolated from C7 (386 days post-infection). Mutational analysis over time revealed different punctual mutations predominant ADAR-like signatures, especially in Spike, followed by APOBEC-like and ROS mutations. ADAR-like mutations in the spike region may alter ACE2 receptor binding, potentially producing SARS-CoV-2 variants with modified infectivity and transmissibility. This study underscores the complexity and variability of prolonged SARS-CoV-2 infections in immunocompromised individuals, showing persistence viral shedding and emergence of iSNVs. The viral isolation highlights the need for rigorous surveillance and control measures to prevent to prevent reintroduction of these lineages into the population.

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PT.162

Replication Kinetics of EVA71 in Neural Cells

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Enterovirus A71 (EVA71) is a human pathogen with emerging neurotropism. However, the behavior of the infection in neural cells is poorly understood. Viruses selectively exploit host cell elements for replication, impacting the metabolic states of infected cells. Mitochondria plays a crucial role in cellular metabolism. We aimed to characterize the EVA71 infection in neural cells (SK-N-AS) and its impact on mitochondria. For this, SK-N-AS cells were infected with EVA71 at 3 different multiplicities of infection (MOIs): 0.01, 0.1, and 1. The infection was monitored for 24 hours by cytopathic effect (CPE) in cell monolayer, and the viral titer and viral load of the supernatant were quantified using TCID50 and RT-qPCR, respectively. CPE was observed in a MOI-dependent manner after 6 hours post-infection (hpi), and viral particles were released to the supernatant after 4 hpi, with a replication peak at 6 hpi. We also observed fluctuations in viral load throughout the time. The mitochondrial morphology was assessed using transmission electron microscopy at 3 points (4hpi, 6hpi, and 16hpi) and two different MOIs (0.1 and 0.01). The viral kinetics of EV-A71 were established in an important neural cell over time and MOI, as well as the impact on mitochondrial morphology. Additional experiments are required to confirm the impact of EV-A71 replication on mitochondria metabolism in neural cells.

Financial Support: capes, cnpq, fiocruz, faperj

PT.163

DEVELOPMENT AND CHARACTERIZATION OF AN ARTIFICIAL GENETIC CONSTRUCT CAPABLE OF GENETICALLY TURNING VIRUSES SUSCEPTIBLE TO ACYCLOVIR-LIKE DRUGS

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To this day, a great deal of antiviral therapies has been developed for plenty of viruses, yet only a select few of them are widely administered, such as the ganciclovir-driven treatment for Herpesvirus 1 (HSV-1) infected patients. Unfortunately, virologists have been having difficulty creating specific therapies and long-lasting vaccines against some widespread viruses such as SARS-CoV-2 and Influenza. Therefore, surfaces the need of crafting finer vaccines and treatments to outcome the current and upcoming viral outbreaks. This project intends to build a genetic construct to be inserted into viral genomes and genetically make them susceptible to acyclovir-like drugs. Three genes make up the designed construct, the thymidine kinase gene from HSV-1, 2A, an autocatalytic gene from foot and mouth disease virus (FMV), and the last one being the coding sequence for the enhanced green fluorescent protein (eGFP). Upon cloning in an expression vector and subsequent transfection into HEK293T cells, the construct showed decent signals of fluorescence from eGFP expression. Furthermore, when transfected into plates of HEK293T cells that were afterwards treated with ganciclovir, the construct was able to induce cell death in preliminary experiments. Hence, this genetic construct appears as a multipurpose tool to be used in an array of applications. Its ability to sensitize viral genomes to existing antiviral drugs opens doors to innovative therapeutic strategies.

Financial Support: FAPERJ.

PT.164

ANTIVIRAL EVALUATION OF BROMAC® IN CELLS AND TRACHAEL ASPIRATE SAMPLES AGAINST SARS-CoV-2 OMICRON VARIANT

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COVID-19 represents a challenge of modern civilization and SARS-CoV-2 is a very resilient virus that can mutate, and escape antibodies generated by either viral exposure or vaccination, and, therefore, SARS-CoV-2 remains an eminent public health threat. Due to the varied symptoms and the large number of infections and deaths caused by COVID-19, it is important to study alternative approaches to contain the impact of the new variants. Currently, there is no 100% effective antiviral treatment for severe COVID-19, and many patients still succumb to infection. In this regard, BromAc® is a combination of Bromelain and N-acetylcysteine, that has shown important mucolytic effects as well as anti-inflammatory properties. Therefore, in the present study, we performed *in vitro* and *ex vivo* analyses to assess the effect of BromAc® in containing viruses at different levels. Here, there is evidence that the compound showed virucide activity and with a dose-dependency manner *in vitro* in the Vero-ACE2/TMPRSS2 cell line infected by the Omicron variant. Also, BromAc® can abrogate genomic copies of the SARS-CoV-2 virus in tracheal aspirate samples (TAS) from critically ill COVID-19 patients. In addition, in those *ex vivo* samples, BromAc® promotes cleavage of the S1 Spike subunit which brings novel evidence of antiviral activity in COVID-19 patient TAS. Finally, we observed that BromAc® decreased spike expression in low-granularity cells from those TAS samples. These results bring evidence of *in vitro* and *ex vivo* antiviral activity of the BromAc®, which suggests its potential as a nebulized approach for the treatment of COVID-19.

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PT.165

TLR3 RS3775291C/T POLYMORPHISM IS ASSOCIATED WITH A GREATER NUMBER OF LONG COVID SYMPTOMS AND HIGH IL-6 LEVELS

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SARS- CoV-2 infection can result in long COVID, characterized by a variety of symptoms that persist after resolution of the acute infection. Long COVID may be associated with an alteration in the inflammatory response. The *TLR3* rs3775291C/T polymorphism generates an amino acid change in the receptor structure (Leu412Fen) and has been associated with a more intense inflammatory response. The study included 116 blood samples from individuals with long COVID and 67 without long COVID. Genotyping for *TLR3* rs3775291C/T was performed by real-time PCR and cytokine quantification by flow cytometry. For statistical analyses, the chi-square, G, Mann-Whitney and Kruskal-Wallis tests were performed. There was no difference in genotypic and allelic frequencies for TLR3 rs3775291C/T between the group with and without long COVID. The TT polymorphic genotype was more frequent among individuals who presented number of symptoms ≥7 (p=0.0318). Genotypes for the *TLR3* rs3775291C/T polymorphism were not associated with the duration of long COVID symptoms. TNF-α levels were not different between carriers of the genotypes for the *TLR3* rs3775291C/T polymorphism. Individuals carrying the CT and TT polymorphic genotypes had higher levels of IL-6 than individuals with the wild genotype (p=0.0063). Patients with long COVID who presented a greater number of symptoms (≥7) had higher levels of TNF-α and IL-6 (p= 0.0369 and p= 0.0366, respectively). The *TLR3* rs3775291C/T polymorphism appears to contribute to the development of a greater number of long COVID symptoms, possibly by increasing levels of the inflammatory cytokine IL-6.

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TLR3 RS3775291 (C/T) POLYMORPHISM IS NOT ASSOCIATED WITH COVID-19 SEVERITY

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COVID-19 can present several clinical manifestations, ranging from mild and moderate cases to the most severe, which include severe acute respiratory syndrome (SARS). Alterations in the immune response can favor the development of severe COVID-19. The innate immunity receptor, TLR3, recognizes double-stranded RNA (dsRNA) and induces the secretion of inflammatory cytokines and type I interferons. The *TLR3* rs3775291C/T polymorphism can influence the inflammatory response and consequently the severity of the viral infection. The study included 370 blood samples from people infected with COVID-19, 117 had severe COVID-19 and 253 had non-severe COVID-19. All samples were genotyped for *TLR3* rs3775291C/T by real-time PCR, and cytokine quantification was performed by flow cytometry. For statistical analyses, the chi-square, Mann-Whitney, and Kruskal-Wallis tests were performed. The frequency of the *TLR3* rs3775291C/T polymorphism was not different between the groups with severe and non-severe COVID. Individuals with severe COVID had higher levels of the cytokine TNF-α than non-severe individuals, but there was no statistically significant difference. Individuals with severe COVID had higher levels of the cytokine IL-6 when compared to the non-severe group (p= 0.0181). There was no statistically significant difference in the levels of the cytokines TNF-α and IL-6 between individuals with the different genotypes for *TLR3* rs3775291C/T. Although TLR3 is essential in antiviral defenses, the *TLR3* rs3775291C/T polymorphism does not appear to influence higher levels of cytokine production, the inflammatory response of COVID-19, and consequently the severity of the disease.

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PT.167

TLR7 RS3853839 (C/G) POLYMORPHISM IS ASSOCIATED WITH HIGH LEVELS OF TLR7 EXPRESSION IN HTLV-1 INFECTION

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Human T-lymphotropic virus 1 (HTLV-1) can cause serious diseases such as adult T-cell leukemia/lymphoma (ATLL), HTLV-1-associated myelopathy (HAM), and other inflammatory diseases. TLR7 detects ssRNA and induces the synthesis of type I interferons. The gene has some genetic variations, including *TLR7* rs3853839C/G (located in the 3' UTR), which is associated with changes in the receptor's gene expression and may influence the control of viral infection. The study included 159 blood samples from people living with HTLV-1, 66 had clinical manifestations (HAM, rheumatological manifestations and uveitis) and 93 were asymptomatic. All samples were subjected to genotyping for *TLR7* rs3853839C/G, quantification of gene expression and proviral load, performed by real-time PCR. For statistical analyses, the chi-square and Mann-Whitney tests were performed. The frequency of carriers of the polymorphic allele was not different between the asymptomatic and symptomatic groups. Symptomatic individuals had higher levels of *TLR7* gene expression than asymptomatic, but there was no statistically significant difference. Individuals with HTLV-1 carrying the G allele had higher levels of *TLR7* expression than those with the C allele ($p= 0.0215$). Among asymptomatic individuals, carriers of the polymorphic allele (G) also had higher expression levels compared to those with the A allele ($p= 0.0336$). Proviral load levels were not different between the groups evaluated. The *TLR7* rs3853839C/G polymorphism may contribute to increased *TLR7* expression and influence inflammatory manifestations associated with HTLV-1 infection but does not influence virus replication.

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PT.168

METABOLIC EFFECTS OF GERANIOL DURING HRSV INFECTION IN CELL CULTURE

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Introduction: Human respiratory syncytial virus (hRSV) is the most common pathogen responsible for lower respiratory diseases in children, causing bronchiolitis and pneumonia, and is associated with significant morbidity and mortality. There are currently no approved antiviral drugs. Natural compounds, such as chalcone, have shown potential benefits against other viruses, inhibiting the viral cycle through several pathways. **Methodology:** Three geraniol derivatives were tested on HEp-2 cells at non-cytotoxic concentrations before and after hRSV infection, and their virucidal activity was evaluated using the MTT assay. Derivatives that showed viral inhibition were investigated using plaque reduction assays to identify specific stages of the viral cycle. The evaluation of the metabolic changes of the derivative with the most promising inhibitory effect was carried out by 1H NMR. **Results and conclusion:** The results showed that geraniol derivatives (G1543, G1544, and G1541) inhibited infection with EC50 estimated at 5 μM for G1543, G1544, and 28 μM for G1541. Analysis of the induced metabolic changes revealed that the G1541 derivative acts on metabolic pathways, suggesting action on protective pathways in hRSV-infected cells. Thus, geraniol derivatives can prevent hRSV infection through different mechanisms during the replicative cycle, making them promising compounds in the treatment of hRSV infections.

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SCREENING OF NEW ANTIVIRAL CANDIDATES AGAINST SARS-CoV-2 USING MHV-A59 AS A MODEL

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COVID-19 became a global pandemic in 2020 and still represents a serious threat to public health. To this day, there is a need to develop therapeutic approaches that enable the distribution of drugs through universal health systems in a more accessible way. Therefore, it is important that new antiviral candidates are tested to expand the therapeutic possibilities against SARS-CoV-2. However, working with this virus requires special training and BSL3 laboratory conditions, creating challenges for these studies. Thus, the murine hepatitis virus (MHV-A59), which belongs to the Betacoronavirus genus, would be a good screening model for the study of coronaviruses, due to its phylogenetic proximity and the possibility of manipulation in BSL2 laboratories. Thus, this project aims to perform an initial screening of compounds synthesized against SARS-CoV-2, evaluating their antiviral and virucidal activity against MHV-A59, as a preliminary step prior to final tests on BSL3. To this purpose, 30 compounds were tested in a cell viability assay with Alamar Blue™ on Vero-CCL81 and L929 cells. Next, a preliminary antiviral test was carried out using Alamar Blue™ after 16 hpi with MHV-A59 in L929 cells treated with the antiviral candidates. After that, 14 promising compounds were selected for the dose-response and virucidal assay. 6 out of these showed promising antiviral activity with a high selectivity index and 1 candidate showed virucidal activity against MHV-A59. In conclusion, MHV-A59 viral models allowed for the selection of the most promising candidates to proceed with the final tests against SARS-CoV-2, which reduced costs and working time.

Financial Support: FAPEMIG; CNPQ; CAPES; EMBRAPII; Mucpharm; Novandina.

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GAMMA INTERFERON PLAYS AN ESSENTIAL ROLE IN YELLOW FEVER VIRUS-INDUCED DISEASE: EFFECT OF AN ADENOSINE NUCLEOSIDE ANALOG

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Yellow fever virus (YFV) infection remains a significant public health threat. Low vaccination rates underscore the urgent need for antiviral therapies. Nucleoside analogs have been used to inhibit viral replication by targeting the RNA polymerase of several viruses. This study aimed to establish a murine model of YFV infection and to evaluate the *in vivo* antiviral efficacy of an adenosine nucleoside analog. A129 and AG129 mice, deficient in type I or type I and II interferon receptors, respectively, were intravenously inoculated with 10^4 PFU of a wild-type YFV strain. Mice were monitored for survival and clinical signs for up 14 days (dpi) or euthanized at 1, 3, and 5 dpi for comprehensive pathological assessments. To evaluate the therapeutic potential of a novel adenosine nucleoside analog, AG129 mice were infected and treated (10 mg/kg, i.p, B.I.D, from 1h until 5 dpi. AG129 mice exhibited heightened susceptibility to YFV infection with progressive disease manifestation leading to 100% lethality by day 8 in comparison to A129, in which presented a self-limited disease. AG129 mice displayed leukocytosis and hepatic injury characterized by elevated viral loads, tissue damage, and impaired liver function. YFV-induced disease in AG129 mice was in part dependent on uncontrolled viral titers recovered from livers and plasma. Treatment resulted in antiviral effects, associated by reduced viral RNA levels in the liver and amelioration of YFV-induced disease. Overall, this study established a novel and suitable platform for study YFV pathogenesis and antiviral drugs testing.

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PT.171

TARGETING PI3Kγ PATHWAY FOR TREATING DENGUE VIRUS INFECTION

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Dengue disease is a major problem worldwide, impacting millions of people annually with no specific approved treatments. The pathogenesis of dengue is a complex interplay of viral and host factors, driven in particular by an excessive inflammatory response triggered by the infection. Several viruses can modulate the PI3K/Akt signaling pathway to aid replication, and the underlying mechanisms remains unclear. The study aims to explore the impact of PI3Kγ inhibition during Dengue virus (DENV) infection *in vivo*. Experiments were performed using wild-type (WT) and PI3Kγ knockout mice inoculated with DENV. Parameters, including survival rates, hematologic, virologic, histopathologic, and inflammatory analyses, were evaluated. Additionally, the therapeutic potential of a selective PI3Kγ inhibitor (AS605240) was investigated in DENV-infected A129 mice. PI3Kγ deficiency resulted in lower lethality and protected DENV-induced thrombocytopenia, decreased hemoconcentration, vascular permeability, and liver damage compared to DENV-infected WT littermates. In addition, PI3Kγ deficiency correlated with reduced viral replication in the blood, spleen and liver alongside decreased production of inflammatory mediators in plasma and spleen. Pharmacologic inhibition of PI3Kγ not only ameliorated DENV-induced thrombocytopenia and liver injury, but also reduced DENV replication in target organs. Treatment with AS605240 reduced the concentration of IL-6 in the spleen and plasma. This study sheds light on the significant pro-viral effects of the PI3Kγ signaling pathway during DENV infection and its central role in pathogenesis by curbing excessive DENV-induced inflammation. Inhibition of PI3Kγ shows promising host-directed targets for developing novel Dengue disease therapies, offering substantial benefits to hosts.

Financial Support: Capes, CNPq, Fapemig, INCT Dengue

PT.172

GENOMIC SURVEILLANCE FOR ARBOVIRUS IN EMERGENCY CARE UNITS IN SALVADOR, BAHIA, BRAZIL.

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Arboviruses such as Dengue (DENV), Zika (ZIKA), and Chikungunya (CHIKV) have been endemic in Brazil, posing significant challenges to Public Health. This project aims to implement genomic surveillance for arboviruses to understand their emergence in new regions. This cross-sectional study was conducted at two emergency care units in Salvador, Bahia. Patients with fever or rash and associated symptoms within the last 7 days were enrolled. Data collection was done through REDCap, and blood samples were tested for Zika, Dengue, and Chikungunya using Multiplex RT-qPCR and anti-IgM serology, along with Dengue NS1 serology. Sequencing was performed with MinIon Oxford Nanopore. The sequences were aligned with the MAFFT program, phylogenetic analyses were performed in IQ-TREE, and the temporal phylogenetic was performed with BEAST. Between April 2023 and March 2024, 351 participants were recruited, with 327 providing samples. The majority were women (218; 62.1%), of mixed race (156; 44.8%), with a median age of 29. Multiplex qPCR identified 25 cases of DENV1, 2 of DENV2, and 8 of CHIKV. Sequencing identified DENV-1 as genotype V and DENV-2 as genotype II. Serology results showed 64 (25.9%) positive for NS1, 124 (68.9%) for Dengue IgM, 6 (6.6%) for Chikungunya IgM, and 1 (1%) for Zika IgM. This study provides an overview of arbovirus infections in Salvador, Bahia. Phylogenetic analysis revels the ongoing presence of Dengue Virus-1V and the identification of Dengue Virus-2 Cosmopolitan Genotype in Northeast Brazil. The findings will contribute to enhanced surveillance and a deeper understanding of arbovirus dynamics.

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EPIDEMIOLOGICAL INVESTIGATION OF A FOODBORNE OUTBREAK OF GASTROENTERITIS CAUSED BY A RARE NOROVIRUS GII.10 — BRAZIL, AUGUST 2023

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This study aimed to establish the agent responsible for a gastroenteritis outbreak during a lunch event in Aracruz city, Espírito Santo state, Brazil. On August 1st, 2023 the health surveillance agency of Espirito Santo received a notification of a set cases of acute gastroenteritis (AGE) from patients that attended an event organized by municipal health department from Aracruz. A local catering company provided food for lunch on July 29 (30 lunch boxes). The menu provided was macaroni, rice, tropeiro beans and cooked potatoes. The ingredients used in the preparation of tropeiro beans were beans, banana, cabbage, sausage and cassava flour. No salad was sent on the side nor any drinks. Diarrhea, nausea, vomiting, abdominal pain, headache and weakness were the main reported symptoms peaked between July 30 and 31. Stool samples of 5 ill persons were analyzed using the quantitative polymerase chain reaction (RT-qPCR) to detect rotavirus A (RVA A) and norovirus GI/GII (NoV) using primers and probe targeting the NSP3 gene segment and ORF1/2 junction, respectively. All samples tested (30-50 years old) were positive for NoV GII with high cycle threshold values (21-30), no coinfection was observed, nor hospitalization reported. For virus characterization samples were amplified using One-Step RT-PCR kit with Mon431 and G2SKR primers (557 bp) targeting ORF1/2 junction region. Sequence analysis revealed that the genotype associated was GII.10[P16]. These findings indicated circulation of a rare NoV GII.10 in the Brazilian population linked with a foodborne transmission AGE outbreak.

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PT.175

PREVALENCE OF ANTIBODIES AGAINST RESPIRATORY SYNCYTIAL VIRUS AND INFLUENZA A AND B IN PUERPERAL WOMEN IN THE CITY OF MANAUS

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Introduction: Viral Acute Respiratory Infections (ARIs) are considered a public health problem, especially in developing countries such as Brazil. ARIs caused by viruses account for a large proportion of hospitalization and mortality rates in infants and children. Among the viruses that can cause this condition are Influenza and Respiratory Syncytial Virus (RSV). Knowing that the immune system of newborns depends on passive immunity from their mothers, it is important to assess the prevalence of antibodies in these women. **Aim:** This study aims to analyze whether puerperal women who have been vaccinated and/or infected with influenza or RSV have IgA and IgG antibodies against these blood viruses and to quantify their prevalence. **Methodology:** In order to understand the prevalence of antibodies against the Influenza A and B viruses and RSV in puerperal women, 375 blood samples (serum) from women participating in the study were selected for the ELISA enzyme-linked immunosorbent assay. **Results:** 60.48% of the samples showed IgG and 31.18% IgA antibodies to Influenza A. As for Influenza B, 97.04% tested positive for IgG, while only 6.45% were positive for IgA. As for Respiratory Syncytial Virus, 43.55% tested IgG and 31.45% IgA. **Conclusions:** A high prevalence of respiratory viruses, especially Influenza B, was observed among the study participants. Based on the data, ELISA tests will be carried out on the milk samples, as well as avidity and neutralization tests, to determine whether maternal antibodies protect babies against these respiratory viruses.

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PT.176

ENHANCING GENE THERAPY WITH ADENOVIRAL VECTORS COATED IN TUMOR CELL MEMBRANES.

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Introduction: Cancer remains a major global health challenge, necessitating innovative therapies. Gene therapy using oncolytic viruses, specifically non-replicative adenoviral vectors encoding p19Arf and interferon-β (IFNβ), shows promise in triggering immunogenic cell death and anti-tumor immunity. However, enhancing the specificity of these vectors towards tumor antigens is crucial for improving therapeutic outcomes. **Objectives:** This study aims to develop a synthetic biology approach to coat adenoviral vectors with cell membranes displaying tumor antigens. Utilizing the tANCHOR system, a novel cell surface display technology, we aim to integrate peptides into the cell membrane via a transmembrane tetraspanin loop, presenting antigens like the OVA peptide (SIINFEKL) to direct immune responses against tumors. **Methodology:** Cells were expanded and harvested for membrane extraction. Adenovirus expressing LacZ was coated onto cell membranes, with coating efficiency assessed through ONPG absorbance, electron microscopy (EM), and dot blot analysis. Oligonucleotides encoding the OVA peptide were synthesized and inserted into the pEXPO63.1 vector of the tANCHOR system. Peptide anchoring was visualized in transfected B16 cells using confocal microscopy. **Results:** ONPG assays showed that enveloped viral particles had transduction efficiency compared to uncoated viruses. EM and dot blot confirmed successful viral envelope formation. The tANCHOR plasmid construct was verified by sequencing, with successful transfection confirmed by microscopy and flow cytometry. **Conclusion:** We are optimizing adenovirus coating and expect to demonstrate the tANCHOR system's effectiveness in antigen presentation, aiming to improve cancer immunotherapy specificity and efficacy.

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PHYLOGENETIC CHARACTERIZATION OF CIRCULATING INFLUENZA B VARIANTS IN BRAZIL (2000-2024)

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Influenza B virus (IBV) is a pathogen that causes respiratory diseases in humans, being responsible for 20% of influenza infections and causing seasonal epidemics. The peak of infections occurs mainly in the coldest periods of the year. Beginning in the 1980s, two distinct lineages of IBV were recognized, B/Victoria and B/Yamagata, and have since continued to co-circulate in the global population. The aim of this study was to evaluate the genetic diversity and the consequent phylodynamic relationship between human IBV lineages in Brazil. To analyze the evolutionary relationship and diversity, a phylogenetic analysis was conducted using sequences downloaded from GISAID and GenBank. Four datasets were obtained, one for the HA gene and one for the NA gene of each lineage. The phylogenetic reconstruction revealed 136 clusters of HA B/Victoria, 123 NA B/Victoria, 41 of HA B/Yamagata and 29 of B/Yamagata. The analysis of the geographical distribution of the sequences belonging to these clades allowed us to observe an isolated circulation of the virus in the equatorial and tropical northeastern climate of Brazil and a high rate of transmission from the tropical climate to different parts of Brazil, mainly the southeast region. These findings show that the climatic diversity and seasonal variations present in Brazil can provide conditions for IBV strains to persist in the country. Thus, constant surveillance of the evolution and transmission of influenza in Brazil is necessary to improve strategies for flu prevention.

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PT.178

IDENTIFICATION OF VIRAL HEPATITIS, HIV AND SYPHILIS IN REFUGEES LIVING IN THE STATE OF RIO DE JANEIRO

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Viral hepatitis is a significant global health issue, impacting over 400 million people. To meet the World Health Organization's target of eliminating viral hepatitis transmission by 2030, it's crucial to study these viruses in vulnerable groups like refugees, since early diagnosis and treatment are key to controlling the spread. In this study, one hundred and seven volunteers (refugees or immigrants) assisted by partner support institutions received blood tests and completed socio-epidemiological surveys. Serological tests for hepatitis A(HAV), B (HBV), and C (HCV) were conducted using electrochemiluminescence (Liason XL-DiaSorin), and HIV and syphilis were identified using Abbon Rapid Tests (Abbot). Two individuals (2/107; 1.1%) were positive for HBsAg, a marker of HBV infection. A prevalence of 16% (18/107) was identified for the marker of exposure to this virus (anti-HBc), and of these, three samples had viral DNA detected by Polymerase Chain Reaction (PCR), denoting occult infection by HBV. Additionally, 60% (65/107) had immunity to HBV. For HAV, 71% (77/107) tested positive for total antibodies, and 0.9% (1/107) were positive for HCV. HIV prevalence was 4.7% (5/107) and syphilis was 7.5% (8/107), with 2.8% (3/107) co-infected with both HIV and syphilis. Regionally, 50% of HBV-exposed individuals were from Africa, specifically Angola, while 87% of HAV cases and the sole HCV case were from Latin America, particularly Venezuela. These findings reveal higher disease prevalence in the refugee group compared to the general Brazilian population, underscoring the need for targeted healthcare and ongoing monitoring for infectious diseases in this population.

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PT.179

The effect of RECK overexpression in HPV-transformed murine cells

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Human Papillomavirus (HPV) infection is related to a relevant fraction of genital, anal, head and neck carcinomas, and to almost all cervical cancer cases. The high death rate of people related to those cancers per day show the importance of studies related to this virus. Different molecular mechanisms are involved in HPV-mediated carcinogenesis, including the alteration of some extracellular matrix (ECM) components. Among these alterations we described the downregulation of RECK (Reversion-inducing Cystein-rich protein with Kazal motifs), that inhibits some matrix metalloproteinases (MMPs). Previously we observed that RECK overexpression in cervical cancer derived cell lines was associated with delayed tumor establishment and growth kinetics in nude mice. The aim of the present study was to evaluate the role of RECK superexpression in the tumorigenic potential of the murine cell line TC1, transformed with HPV16 E6, E7 and c-Ha-ras oncogenes. TC1 cells superexpressing RECK were generated by retroviral transduction and RECK levels were confirmed by Western blot. Even though RECK superexpression did not affect TC1 proliferation rate, high RECK levels were associated with reduced clonogenic potential and anchorage independent colony formation efficiency. Spheroids obtained from cells superexpressing RECK exhibited a higher number of dead cells, but also increased volume. Altogether, our observations indicate that RECK superexpression affects the tumorigenic potential of TC1 cells. We are now conducting experiments to determine the effect of RECK in vivo in an immunocompetent background using TC1 cells.

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Phenotypic and functional profile of CD1c+ DCs and their association with the lipid pathway in severe COVID-19

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The immune response in severe COVID-19 involves hyperinflammation, cytokine storms, and lymphocyte dysfunction. While the roles of neutrophils, monocytes, and lymphocytes are well-documented, the contribution of dendritic cells (DCs) is still unclear. Specifically, a deep understanding of the functionality and DC activation pathways during SARS-CoV-2 infection is lacking. In this study, we sought to evaluate the phenotypic and functional profile of $CD1c^+$ DCs and their association with the lipid pathway in severe COVID-19. Whole blood and serum samples collected from severe COVID-19 patients for flow cytometry, Luminex®, and lipidomics analyses. The COVID-19 patient ex vivo analysis showed a lower percentage of $HLA\text{-}DR^{high}CD11c^+$ mature DCs. Furthermore, it was observed a depletion of $CD141^+$DCs and higher percentages of $CD1c^+$DCs. Interestingly, double positive $CD1c^+CD141^+$DCs were found ex vivo and in association with death outcome. Moreover, an in vitro short term culture stimulation with inactivated SARS-CoV-2 and viral proteins S and N confirmed the role of $CD1c^+$ and $CD1c^+CD141^+$ within mature DCs in responding to the iSARS-CoV-2 stimuli. Lipidomics analysis identified 97 lipids in the mass spectrometry analysis, including increased lysophospholipids, triacylglycerols, sphingomyelins and phospholipids, which correlate strongly with CD1c expression specially in patients with fatal outcomes. These findings may indicate that CD1c+ DCs could contribute negatively to the inflammatory process during severe COVID-19, bringing insight for future therapeutic interventions.

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IMPLEMENTATION AND VALIDATION OF THE HEMAGGLUTINATION INHIBITION ASSAY TO EVALUATE THE IMMUNOGENICITY INDUCED BY PANDEMIC INFLUENZA VACCINE (H5) PROTOTYPE IN ANIMALS

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Influenza A virus (IAV) is recognized for their ability to cause seasonal epidemics and pandemics with high mortality and lethality rates, remaining a major challenge worldwide for public health. The World Health Organization recommends the Hemagglutination Inhibition Assay (HIA) to validate the correlates of vaccine-induced protection to Influenza A. However, the inner high variability of the HIA assay is known, showing variations of 22 to 54% in the same lab. This work aimed to establish and validate the methodology employed in pre-clinical assays of new vaccine prototypes of pandemic Influenza A/H5Nx. The tests were carried out using 1% *Cavia porcellus* red blood cells, purified non-fragmented IAH5 vaccine strain as antigens and anti-serum anti-H5N1/H5N8, H1N1, H3N2, Influenza B, and serum of immunized animals with the new pandemic vaccine prototype (IAV/H5). In addition, purified fragmented vaccine virus as antigen, enzymes and serum treatment protocols to remove non-specific inhibitors were also evaluated. After the establishment of the analytical methodology, the HIA performance for precision and selectivity and the respective acceptance criteria were established. The assays met the proposed validation criteria, presenting satisfactory results in terms of selectivity, after evaluation with heterologous and homologous antisera to the vaccine virus and precision, after evaluation of the coefficient of variation (CV%) between replicates of sera with low, medium and high antibody titers. The CV% inter and intra test was ≤20%. Protocol was established and validated regarding the parameters analyzed for the measurement of specific hemagglutination-inhibiting antibodies anti-H5 in animal sera.

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PT.183

THERAPEUTIC EFFICACY OF THE ALGA *DICTYOTA MENSTRUALIS* AGAINST ZIKA VIRUS

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The Zika virus (ZIKV), from the Flaviviridae family, is an arbovirus primarily transmitted by the bite of mosquitoes from the *Aedes* genus. Several studies indicate that transmission can occur through sexual or placental routes. Data in the literature suggest that ZIKV is responsible for causing neurological alterations. The absence of vaccines and available medications for the treatment of ZIKV infection makes the search for new substances that can act against this virus. Results from other studies with the FAc-2 fraction of the alga *Dictyota menstrualis* demonstrated low cytotoxicity and high antiviral activity when evaluated in infected Vero cells. This study aims to evaluate the antiviral activity of the fraction of the alga *D. menstrualis* against ZIKV in neurons cells and *in vivo.* For these evaluations, neurons from chick embryo retinas were cultured in 24-well plates for subsequent treatment. The neurons were infected with ZIKV (MOI 1) and treated with different concentrations of FAc-2, incubated for 72 hours, and evaluated daily to determine the inhibitory effect. The FAc-2 fraction demonstrated a potential reduction in the viral cytopathic effect, inhibiting it in a dose-dependent manner, with the greatest evidence at the concentration of 20 µM, preserving the structure of the neurons. In our *in vivo* studies, we evaluated the therapeutic efficacy of the FAc-2 in the testis of Balb/c mice. We found that the FAc-2 fraction was able to prevent testicular damage caused of ZIKV infection. Given these results, the FAc-2 fraction may be promising for the development of drugs against ZIKV.

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Antibody neutralization profile against SARS-CoV-2 omicron subvariants BA.1 and BA.5 induced by CoronaVac primary protocol in children and adolescents.

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Immunization against SARS-CoV-2 was a key point in COVID-19 control, reducing number of cases, hospitalizations and deaths. However, vaccination protocols were initially exclusive for adults. This fact delayed the immunization of infant individuals, which started after the emergence of immunoresistant variants, such as omicron. Thus, this study investigated the antibody response against omicron subvariants (BA.1 and BA.5), induced by CoronaVac primary protocol, in children and adolescents over time. A total of 526 serum samples were separated by age groups (3-11 and 12-17 years old) and subgroups according to previous infection (seronegative and seropositive). Sera was collected over time, before (V0) and after vaccination (1m, 3m, 6m and 12m) and submitted to a viral microneutralization assay (VNT) to evaluate neutralization profile against BA.1 and BA.5 subvariants. In general, vaccination induced and maintained, over time, high levels of neutralizing antibodies and seroconvertion, even for the immunoresistant subvariant BA.5. However, neutralization mean titers against BA.5 for adolescents were lower when compared to children. This was observed for both subgroups, with VNT_{50} means, at 12m, of 907.9 (adolescent) vs 1893.4 (children) for seronegative and 778.5 (adolescent) vs 2333.2 (children) for seropositive against BA.5, showing that previous infection had no influence on improving neutralization of this subvariant in this situation. Our data shows that an inactivated virus immunization platform provided durable serological protection for children and adolescent, including against subvariants capable to evade immune system. Further analyses are in progress to elucidate the lower antibody response presented by adolescents when compared to children.

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VACCINE RESPONSE TO HEPATITIS B VIRUS IN A RIVERSIDE POPULATION OF THE MARAJÓ ARCHIPELAGON, AMAZON, BRAZIL

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Hepatitis B is an infection caused by the Hepatitis B Virus. Vaccination is the most effective method of prevention and, in Brazil, its vaccine has been available on the SUS since the 1990s. This study aimed to assess the response to HBV vaccination in a riverside population in a municipality in the eastern Brazilian Amazon. This is a cross-sectional and descriptive study using the serological markers HBsAg, total anti-HBc and anti-HBs through enzyme-linked immunosorbent assay. A total of 406 individuals were examined, in an age range of 1-101 years; 57.9% were female; 52.5% had no difficulties in being vaccinated, 30% had a vaccination record (of which 81.1% had a complete schedule). Isolated anti-HBs (≥10mIU/mL) was detected in 26.3% of individuals, indicating vaccine protection, 61.3% were susceptible to infection, of which 21.6% had a vaccination card with a complete schedule. The investigation reflected the real vaccination coverage in this population, producing information that was not available in the PNI information system. It should be noted that among immunocompetent children and adolescents, vaccine efficacy was well below that recommended by the program. The transportation, storage and application of the vaccine schedule may be related to the ineffectiveness of the schedule received; genetic factors, obesity, difficulties in accessing basic health networks, lack of knowledge, acceptance or non-acceptance of vaccination may also interfere with this effectiveness. It is thought that access to serology for the anti-HBs marker could confirm the effectiveness of the vaccination schedule received.

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PT.186

PREDICTION OF IMMUNODOMINANT PEPTIDES SPECIFIC TO CLASS I MHC MOLECULES IN *BETACORONAVIRUS PANDEMICUM*

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Controlling the SARS-Cov-2 pandemic required both vaccination and collective isolation. Viral epitopes that elicit a strong immune response are required for the production of vaccines. These antigenic epitopes are presented by major histocompatibility complexes (MHC), encoded in the highly variable HLA genes. By considering the most common MHC 1 haplotypes in the Brazilian population (HLA-A1, -A2 and A3), we predicted immunodominant peptides of the reference genome of *Betacoronavirus pandemicum*, sequenced in Wuhan. This consisted of an *in silico* analysis of possible immunodominant epitopes in the reference strain, highlighting those with a robust immunological response. The sequence was obtained from the Nucleotide database and submitted to NetCTL-1.2 for epitope prediction. NetCTL-1.2 organizes results based on TAP processing, cleavage and affinity of these epitopes, returning a combined score of parameters. Any score greater than 0.75 is considered a potential epitope. HLA-A2 had 410 reactive epitopes, while A1 and A3 possessed 354 and 339. HLA-A2 remarkably had the most exclusive epitopes, with only one epitope from the nsp3 protein common to all haplotypes. Immunodominant epitopes were observed in non-structural proteins as well as the spike and nucleocapsid proteins, present in all haplotypes. This study provides the foundation for the development of future vaccine targets, as well as a greater understanding of the virus and its interaction with the host.

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PT.187

MOLECULAR INVESTIGATION OF ORTHOPOXVIRUS IN ARTISANAL CHEESE PRODUCED IN MINAS GERAIS, BRAZIL

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Viruses of the Orthopoxvirus (OPV) genus have a significant impact on the global scenario of emerging infectious diseases. In Brazil, especially in rural areas, the vaccinia virus (VACV) is responsible for outbreaks of bovine vaccinia, an exanthematous disease that is typically spread to humans through contact with infected dairy cattle during the hand-milking process. However, there is evidence of the circulation of VACV in urban environments and in individuals who report no contact with dairy cattle, leading to hypotheses about the alternative routes of exposure to VACV. Furthermore, recent studies have detected VACV DNA in artisanal cheese produced in the state of Minas Gerais. This data raises concern over the possible role of artisanal cheese and other dairy products in the circulation of VACV, since these goods are sold to the entire Brazilian territory, and even internationally. Therefore, the aim of this study is to investigate the presence of Orthopoxvirus DNA in artisanal cheese produced in different municipalities of Minas Gerais in 2023. For that, 94 samples were processed and submitted to DNA extraction. Subsequently, qPCR was performed targeting the C11R gene in order to detect viral DNA. At present, one sample was positive, suggesting a low but still persistent VACV contamination of artisanal cheese produced in Minas Gerais dairy farming.

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Rapid spread of the SARS-CoV-2 Omicron XDR lineage derived from recombination between XBB and BA.2.86 subvariants circulating in Brazil in late 2023

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Recombination plays a crucial role in the evolution of SARS-CoV-2. Since November 2023, a new recombinant lineage between Omicron subvariants XBB and BA.2.86, designated XDR, has been detected mainly in Brazil. In this study, we reconstructed the spatiotemporal dynamics and estimated the absolute and relative transmissibility of the XDR lineage. The XDR lineage displayed a recombination breakpoint in the ORF1a coding region, and the most closely related sequences to the 5' and 3' ends of the recombinant correspond to JD.1.1 and JN.1.1 lineages, respectively. The first XDR sequences were detected in November 2023 in the Northeastern Brazilian region, and their prevalence rapidly surged from <1% to 25% by February 2024. The Bayesian phylogeographic analysis supports that the XDR lineage likely emerged in the Northeastern Brazilian region around late October 2023 and rapidly disseminated within and outside Brazilian borders from mid-November onwards. The median effective reproductive number of the XDR lineage in Brazil during the initial expansion phase was estimated to be around 1.5. In contrast, the average relative instantaneous reproduction numbers of XDR and JN* lineages were estimated to be 1.37 and 1.29 higher than that of co-circulating XBB* lineages. In summary, these findings support that the recombinant lineage XDR arose in the Northeastern Brazilian region in October 2023, shortly after the first detection of JN.1 sequences in the country. In Brazil, the XDR lineage exhibited a higher transmissibility level than its parental XBB.* lineages and is spreading at a rate similar to or slightly faster than the JN.1* lineages.

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PRE-CLINICAL STUDY OF VACCINE CANDIDATES AGAINST ZIKA VIRUS, USING RECOMBINANT VIRAL ENVELOPE PROTEIN, FUNCTIONALIZED OR NOT IN GOLD NANORODS

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The association of *Orthoflavivirus zikaense* (ZIKV) with microcephaly and neurological complications turned this arbovirus into a global public health problem in the last decade. With the biotechnological advancement of new approaches in the development of efficient vaccines, nanotechnology has stood out. This work presents the evaluation of the cellular and humoral immune response developed by two experimental vaccines developed (ZIKV-E and ZIKV-E+GNR) against ZIKV in the C57BL/6 model, followed by a challenge with ZIKV in previously immunized pregnant female C57BL/6 mice, in order to observe possible alterations in the prenatal neurodevelopment of the fetuses, verifying vertical transmission as reported in the literature. After immunization of the mice, high levels of anti-ZIKV neutralizing antibodies were detected. Furthermore, experimental vaccines were able to induce the activation of immune system cells, increasing IFN-γ secretion in CD8+ CD107a+ cells and a tendency to increase IFN-γ and TNF secreted by CD8+ effector and central lymphocytes in memory phenotype cells. These results were observed mainly in the group immunized with ZIKV-E+GNR. Fetal viability has been shown to be affected in an infection scenario without the vaccination (65,44%), with a greater number of resorptions compared to the number of fetuses in the offspring in a scenario without the challenge (98,33%). Surprisingly, the animals immunized with ZIKV-E+GNR and subsequently challenged had a fetal viability value of (94,78%), similar to the animals in the non-challenged group, suggesting that the experimental vaccines were satisfactorily protective.

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PT.190

CORRELATION OF THE INDEX VALUE OF ELISA IGG WITH DENGUE HISTORY AND SEVERITY IN A HYPERENDEMIC REGION

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The detection of IgG antibodies is widely used to differentiate primary from post-primary dengue infections. However, the anti-IgG ELISA method can present cross-reactivity and non-specific reactions. This study correlated the ELISA index ratio (EIR - the ratio between sample absorbance and the average of calibrators, adjusted by a correction factor) with the dengue history, defined by FRNT, and the case severity. A total of 1,617 serum samples from patients at our health service, during a dengue epidemic from January to December/2021, were retrospectively analyzed. Of these, 245 samples positive by RT-PCR for DENV were tested by anti-dengue IgG ELISA and FRNT for DENV. Analyses demonstrated that ELISA presented high sensitivity (89.87%) and moderate specificity (71.08%) compared to FRNT. The analysis of EIR compared to FRNT using the ROC curve revealed satisfactory ELISA performance, with an area under the curve greater than 0.70 ($p<0.001$). The cutoff point of 1.548 for EIR ensured a sensitivity of 85% and specificity of 77%. Higher EIR levels were observed in post-primary infections, especially tertiary and quaternary ($p<0.001$), followed by secondary ($p<0.001$) and primary ($p<0.001$). Besides that, higher EIR values were observed in patients with warning signs ($p=0.034$). At last, samples from mild cases with tertiary or quaternary infection had higher mean of EIR compared to groups with one previous dengue with or without warning signs ($p < 0.05$). The EIR can be an effective alternative to FRNT, being less costly and time-consuming, and capable of correlating with different infection groups and disease severity.

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PT.191

EVALUATION OF CYTOKINE PROFILES IN INDIVIDUALS WITH LONG COVID-19 IN RELATION TO PRE-EXISTING VIRAL HEPATITIS.

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INTRODUCTION: Long COVID-19 impacts millions globally, with 10-30% of infected individuals experiencing persistent symptoms. Dysregulated immune responses, particularly involving pro-inflammatory cytokines, may contribute to ongoing symptoms by causing prolonged tissue damage.
OBJECTIVES: This study aims to analyze the cytokine profiles of IL-17A and IL-6 in plasma samples from individuals according the presence of Long COVID-19 and hepatitis infection to determine potential biomarkers associated to clinical profiles.
MATERIALS AND METHODS: Plasma was obtained from 58 individuals distributed in 3 groups: (I) Hepatitis and Long COVID-19 (n=20); (II) Long COVID-19 only (n=18); (III) healthy subjects without both conditions (n=20). Plasma levels of the cytokines IL-17A and IL-6 were determined using the enzyme-linked immunosorbent assay (ELISA) method.
RESULTS: The concentration (µg/mL) of the cytokine IL-17A in the plasma of individuals in group III was higher (30.66 ± 11.38) compared to groups I (18.82 ± 20.60) and II (25.09 ± 33.97) ($P<0.0001$). For IL-6, a statistically significant difference was observed ($P=0.0054$) between the groups, with group II exhibiting higher levels of this cytokine (82.90 ± 12.88) compared to groups I (70.47 ± 12.45) and III (74.77 ± 9.91).
CONCLUSIONS: These findings suggest distinct cytokine responses among the groups. In healthy individuals, elevated IL-17A levels may indicate immune surveillance, whereas increased IL-6 levels in Long COVID-19 patients suggest ongoing inflammation. Lower cytokine levels in the Hepatitis and long COVID-19 group could signify immune exhaustion or a regulatory phase. Further research involving additional cytokines is needed to fully elucidate these immune mechanisms and their implications.

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PT.192

U6 SMALL NUCLEAR AS AN INTERNAL CONTROL FOR miRNA EXPRESSION ANALYSIS IN PLASMA OF COVID-19 INFECTED PATIENTS.

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The Covid-19 pandemic has underscored the urgent need for biomarkers to predict and stratify patients risk. MicroRNAs (miRNAs) have emerged as potential biomarkers due to their association with disease severity. However, the selection of internal reference genes for miRNA expression analysis by RT-qPCR is critical, as it can significantly impact expression level quantification. In this study, we evaluated a set of miRNAs as possible endogenous controls to identify stable internal reference genes in plasma samples from Covid-19 patients and healthy controls. We assessed the expression levels of miRNA candidates in 24 Covid-19 patients and 24 SARS-CoV-2-negative controls using BestKeeper, Normfinder, geNorm, and Delta CT. The results revealed that RNU6B exhibited high variability between groups and samples. Mean expression analysis identified miR-320a (p=0.0019) as the most stable reference gene. BestKeeper indicated snU6 and miR-320a as the most stable, whereas Delta CT, geNorm, and Normfinder highlighted snU6 and miR-342-3p as the most stable. These findings suggest that data interpretation can be significantly influenced by the normalization strategy and the combination of algorithms enhances robustness in reference gene selection. snU6 emerged as a strong candidate for use as a reference gene in plasma miRNA analysis in Covid-19.
Keywords: SARS-CoV-2; pandemic, circulating miRNAs, normalization, endogenous reference genes.

Financial Support: CAPES, PPSUS, FAPESB

PT.193

Differential expression of miR-155 and miR-126 in SARS-CoV-2 infection

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Covid-19, caused by SARS-CoV-2, has emerged as a global pandemic, demanding many questions and studies in order to understand the pathogenesis and related mechanisms. MicroRNAs are small non-coding RNA molecules with a fundamental role in the post-transcriptional regulation of genes and may be involved in the immune and inflammatory response. Therefore, analysis of the miRNA expression profile can provide valuable information about the pathophysiology of Covid-19. Here, we analyzed the expression of miR-155 and miR-126 in 24 individuals positive for SARS-CoV-2 (RNA detectable) and 24 negative. Plasma samples were collected, total RNA was extracted and cDNA synthesis was performed using reverse transcriptase. Relative quantitation of miRNAs was performed by qRT-PCR using the TaqMan system. Previously, the miRNAs U6, RNU6B and 320 were tested as internal normalizers. The miRNA U6 showed the best result, and it was used in the normalization of the expression of the evaluated miRNAs. Our results showed a reduction in the expression of miR-155 and miR-126 in individuals infected with SARS-CoV-2, when compared to those not infected, suggesting a possible role associated with the immune response to the virus. In addition, these results provide new perspectives on the molecular mechanisms involved in the pathogenesis of Covid-19 and for new therapeutic or diagnostic strategies.

Key words: microRNAs, miRNA signature, molecular biomarker, miRNA therapy
Financial Support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Programa de pesquisa para o SUS (PPSUS).

PT.194

INCIDENCE AND SEASONALITY OF RESPIRATORY SYNCYTIAL VIRUS AFTER COVI-19 PANDEMIC

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Respiratory Syncytial Virus (RSV) is the main etiological agent causing hospitalization for acute respiratory infections (ARI) in infants and children under 5 years of age worldwide. However, data on its circulation and incidence during the Covid-19 pandemic and post-pandemic period are still scarce. With the objective of evaluating the incidence and seasonality of RSV in the post-pandemic period (2022-2024), we analyzed 9,265 samples collected from pediatric patients from 6 public hospitals in the state of São Paulo using the real-time PCR technique. As a result, we obtained 4,548 samples that were negative for RSV, and 2,392/$_{9,265}$ (25.8%) positive for RSV. Of these, 1,386/$_{2,392}$ (58%) were type A and 990/$_{2,392}$ (42%) were type B, RSV was co-detected with other viruses in 420 samples. In 2022, November had the highest prevalence with 218 positive samples, 154/$_{218}$ (70.6%) of genotype A and 64/$_{218}$ (29.4%) of genotype B. In 2023, May was the most prominent with 155 positive samples, 123/$_{155}$ (79.4%) RSV of genotype A and 32/$_{155}$ (20.6%) of genotype B. In 2024, from January to July, it circulated more in April, with 150 positive samples 48/$_{150}$ (32%) RSV of genotype A and 102/$_{150}$ (68%) genotype B. Given the above result, we can note that RSV had its seasonality changed after the pandemic with variations in the months. However, the alternation of genotype B that occurred every two years, pre-pandemic, continues to occur.

PT.195

BIOPHYSICAL AND BIOCHEMICAL CHARACTERIZATION OF NS2B-NS3PRO CONSTRUCTS BZIPRO, CZIPRO AND GZIPRO

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Zika virus (ZIKV), the causative agent of zika fever, still poses a significant global public health threat. The viral RNA genome encodes a polyprotein cleaved into three structural (capsid, pre-membrane, and envelope) and seven non-structural proteins (NS) by cellular and viral proteases. The N-terminal domain of NS3 (NS3pro) is a serine protease that forms a complex with its cofactor NS2B, known as the NS2B-NS3pro complex, responsible for cleaving the immature viral polyprotein into mature NS proteins. Currently, the NS2B-NS3pro constructs "bZipro" and "gZipro" are used for screening anti-ZIKV compounds. However, crystals derived from these constructs often adopt a non-catalytically active conformation. In contrast, the cZipro construct, which lacks six residues at the C-terminus of NS2B, stabilizes crystals in the catalytically active conformation. In this study, we compared these constructs using enzymatic assays to determine kinetic parameters (Km, kcat, and Vmax) with serial dilutions of the substrate. Additionally, we employed the biophysical assay Differential Scanning Fluorimetry (DSF) to measure the melting temperatures (Tm) of recombinant constructs. The constructs were expressed in *E. coli* and purified using nickel-affinity and size-exclusion chromatographies. We observed no significant differences in catalytic efficiency and stability between the cZipro and bZipro constructs, while gZipro exhibited lower catalytic efficiency. Our results suggest that cZipro could be used in crystallographic assays to generate crystals in the catalytically active conformation and is a reliable option for in vitro screenings of NS2B-NS3pro complex inhibitors.

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PT.196

Elderly Individuals Exhibit Elevated Levels of Anti-Monkeypox Virus Antibodies Compared to Adults, Youth, and Children

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The variola virus, the causative agent of smallpox, has claimed more human lives than all other infectious diseases combined. Consequently, any epidemiological event involving related viruses (orthopoxviruses) triggers significant concern, as was the case with the pandemic rise of monkeypox virus (MPXV), the causative agent of monkeypox, in 2022-2023. Smallpox vaccines, based on vaccinia virus antigens, are expected to provide cross-protection against MPXV. Studying the general seroprevalence of orthopoxviruses in populations worldwide is crucial for estimating susceptibility, risks, and the need for vaccination campaigns. In this study, we aimed to evaluate the seroprevalence of antibodies capable of recognizing MPXV in a Brazilian population and assess the potential for cross-protection from previous smallpox vaccinations. We analyzed demographic data, vaccination records, and serum levels of anti-MPXV IgG measured by enzyme-linked immunosorbent assay (ELISA) in 319 volunteers from Barreiras, Bahia, Brazil. Our main findings show that the numbers of volunteers with serum samples recognizing MPXV in ELISA, as well as their serum levels of anti-MPXV IgG, did not differ significantly according to gender or address type. However, both male and female elderly volunteers exhibited significantly higher concentrations of anti-MPXV IgG than adults, youth, and children. These results suggest that the elderly may benefit from cross-protection due to previous smallpox vaccinations, whereas adults, youth, and children appear susceptible to MPXV. Further investigations involving other populations are necessary to assess the necessity of vaccinating potentially vulnerable groups.

Keywords: mpox, seroprevalence, IgG, susceptibility, smallpox.
Financial Support: MCTI, FINEP, CNPq, FAPESB.

PT.197

DESIGN AND IMPLEMENTATION OF A ONE-STEP MULTIPLEX RT-qPCR QUANTITATIVE ASSAY FOR MONITORING CHRONIC HEPATITIS DELTA INDIVIDUAL

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Hepatitis Delta is caused by exposure to hepatitis B (HBV) and D (HDV) viruses, generally more severe than HBV monoinfection. The prevalence of HDV infection is underestimated and detection methods are scarce, especially in endemic regions, such as the Amazon. The study aimed to develop a high-sensitivity quantitative one-step RT-qPCR assay for the follow-up of patients with Hepatitis Delta. The developed assay simultaneously detects the viral target and a human endogenous control, and dilutions of a recombinant plasmid containing a partial region of HDV-RNA conserved for the 8 genotypes were used to construct the quantification curve. A total of 266 biological samples were collected in Rondônia and Acre and subjected to the test, in addition to a second quantitative assay used as a reference. The initial quantification slope was -3.321 with an efficiency of 100.04%. Three reproducibility and repeatability assays demonstrated low variation between runs, Limit of Quantification of 5 copies/reaction and a Limit of Detection (95%) of 2.83 copies/reaction. When compared to the reference test, the assay presented a sensitivity of 100%, specificity of 96.70%, and 97.37% accuracy. To date, 725 samples were tested using this method, from the states of Rondônia, Amazonas, Acre, São Paulo and Minas Gerais. Of this total, 26.6% had a quantifiable viral load. This assay proved to be highly efficient and reproducible and is being implemented by the Ministry of Health as an official method for monitoring the viral load of individuals with Delta in all Brazilian states.

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PT.198

DENV-2 OUTBREAK ASSOCIATED WITH COSMOPOLITAN GENOTYPE EMERGENCE IN WESTERN BRAZILIAN AMAZON

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Background: Dengue virus (DENV) causes an important disease and directly impacts public health, being the arbovirus that presents the highest number of infections and deaths in the Western Brazilian Amazon. This virus is divided into four serotypes that have already circulated in the region. **Methodology:** Molecular characterization of a cohort containing 841 samples collected from febrile patients between 2021 to 2023 was analyzed using a commercial kit to detect the main arboviruses circulating in Brazil: Zika, DENV-1, DENV-2, DENV-3, DENV-4 and, Chikungunya. Subsequently, Sanger sequencing was performed for positive samples. **Results:** The cohort detected 162 positive samples, 12 for DENV-1 and 150 identified as DENV-2, indicating co-circulation of serotypes. The samples were subjected to sequencing and the analysis of the sequences that obtained good quality revealed that 5 samples belonged to the V genotype of DENV-1 and 46 were characterized as DENV-2 Cosmopolitan genotype - lineage 5. **Conclusion:** The results allowed us to identify for the first time the Cosmopolitan genotype in Rondônia, Brazilian Western Amazon, and its fast spread dispersion.

keywords: Dengue; DENV-2; DENV-1; genotype Cosmopolitan.

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PT.199

Roseolovirus humanbeta6 (HHV-6) **: can be a trigger of Guillain-Barre syndrome?**

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Guillain- Barré syndrome (GBS) is a neurological disease that affects peripheral nerves, resulting in symptoms such as numbness, progressive muscle weakness, paralysis, tingling, and pain. The annual incidence ranges from 1.1 to 1.8 cases per 100,000 people. The exact cause is still uncertain, but cross-reactivity between pathogenic antigens and nervous tissue is believed to play a crucial role. Roseolovirus humanbeta6, HHV-6, is a neurotropic virus with latency capacity and is considered a significant candidate for triggering or worsening GBS. To investigate its possible role, we analyzed its frequency in 13 cerebrospinal fluid (CSF) samples from GBS patients at the Hospital Geral de Fortaleza, Ceará/Brazil, who tested negative for enteroviruses. Viral DNA was analyzed by real-time PCR (qPCR), and detailed medical records were collected for analysis. Among the 13 patients studied, 38.4% (5/13) tested positive for HHV-6, with a mean age of 57.8 years. Of these, 80% were women. Individuals positive for HHV-6 had a significantly longer mean hospital stay (25.6 days versus 11 days for negative individuals). Overall, 61.5% tested positive for herpesvirus, with 30.8% presenting co-infections and one patient presenting triple infection (HHV-6, HSV-2 and HCMV). HHV-6 was the most detected among herpesvirus-positive individuals, 62.5% (5/8). All HHV-6-positive individuals developed progressive tetraparesis. Despite limitations, such as the small number of samples, the results contribute significantly to alerting about the importance of investigating HHV-6 in patients with GBS and filling important gaps in the medical literature.

PT.200

ENCAPSULATED COUMARINS AND HESPERETIN AS ANTIVIRAL CANDIDATES AGAINST HUMAN RESPIRATORY SYNCYTIAL VIRUS (HRSV)

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Introduction: Human respiratory syncytial virus (hRSV) is a major cause of bronchiolitis and pneumonia in infants and young children, making it the most common cause of hospitalization globally. Nearly all children, by the age of 2 years have been infected by hRSV. Elderly people are also very susceptible to hRSV infection making it the second most frequent cause of death in Winter, after influenza virus. Currently, there is two licensed hRSV vaccine for adults and women, also a drug (Ribavirin) but it is not a specific drug against hRSV. In this context, coumarins and hesperetin have antioxidant, antibacterial, antiviral properties, are secondary metabolites that occur naturally in plants and fungi, which promotes significant visibility in the pharmaceutical field. **Methods:** We analyzed the interaction of coumarins (coumarin (1,2 Benzopyrone), esculetin (6,7- Dihydroxycoumarin), esculin (6,7- Dihydroxycoumarin-6- glucoside) and 4-methyl esculetin (5,7- Dihydroxycoumarin- 4- methylcoumarin) and umbelliferone (7- hydroxycoumarin)) and hesperetin with the hRSV nucleoprotein by biophysical techniques such as nuclear magnetic resonance and fluorescence, and viral inhibition (anti-hRSV) these compounds in culture cells (HEp-2 cells and HBE cultures). **Results** revealed specific interaction of 1-2 benzopyrone and hesperetin in a specific region of the nucleoprotein in which the RSV phosphoprotein binds for virus replication to occur. In vivo assays of coumarin, esculetin and umbelliferone showed inhibition when encapsulated with β-cyclodextrin. **Conclusion:** Our research has shown that coumarins and hesperetin inhibit hRSV, but further research is needed into where these compounds act in the life cycle of virus.

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PT.201

In vitro experiments of ofloxacin against rabies: a bioinformatics to in vitro pipeline

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Rabies is a viral disease that attacks the central nervous system (CNS) of mammals. It is caused by rabies virus (RABV), a negative sense single-stranded RNA virus belonging to the genus Lyssavirus in the Rhabdoviridae family. A vaccine for rabies is available and, when taken correctly, prevents the disease in virtually 100% of cases. However, there is still no treatment for the disease, and, after onset of symptoms, the virus is generally lethal. Following a bioinformatics project which looked for new antivirals for the disease, the drug ofloxacin, a fluoroquinolone antibiotic used against gram-positive and gram-negative bacterial infections, showed potential against the virus. The current project used *in vitro* techniques to check and quantify if the drug had *in vitro* capabilities against the virus. The *in vitro* experiment started with 96-well plate cell-based assays that were visualized through fluorescent microscopy. The assays were done using different concentrations of the drug and the virus and showed that ofloxacin lowered viral titer, with an inhibitory concentration (IC_{50}) of 0.92µM. Cytotoxicity assays were also done and showed a cytotoxicity concentration (CC_{50}) of 4.6µM. These values give a selectivity index (SI) of the drug of 5.01. Finally, real-time PCR was run to quantify the decrease of virus in the samples with ofloxacin, and these showed a 10x to 100x decrease of viral titer depending on the virus and drug dilution. These results show the potential of the drug against the virus, as well as a successful *in silico* to *in vitro* pipeline.

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PT.202

SPATIAL DYNAMICS OF ZETA, GAMMA, DELTA AND OMICRON BA.1 VARIANTS OF SARS-COV-2 IN THE STATE OF RIO GRANDE DO NORTE, BRAZIL

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In the state of Rio Grande do Norte, Brazil, the circulation of the Gamma, Delta, and Omicron variants coincided with peaks in the number of cases. To understand the spatial dynamics of concern variants, phylogenetic techniques with a maximum-likelihood statistical framework were applied to a dataset consisting of complete genomes between November 2020 and March 2022, representing a significant fraction (>70%) of the total genomes in Rio Grande do Norte (*N*=1706). The epidemiological profile of the epidemic in the state showed that the Gamma period coincided with peaks in the number of cases, deaths, and lethality (~4%). Phylogenetic analysis revealed a large number of introductions, with 100% from other Brazilian states during the Zeta and Delta periods, 98% from other Brazilian states and 2% from other countries during the Gamma period, while in Omicron, there were 36% from other countries and 64% from Brazil. In internal circulation between immediate regions, Natal stands out as the main source in all lineages. Therefore, with the resumption of urban mobility and early vaccination, the Zeta, Gamma, Delta, and Omicron variants in the state of Rio Grande do Norte established multiple simultaneous transmission networks, reaching all regions of the state, overwhelming the local healthcare system, and increasing infection cases. Given the continuous circulation of the SARS-CoV-2 in the country and the possibility of new viral emergencies, understanding the local dynamics of viral dissemination is an essential tool for improving epidemic prevention, response, and control strategies.

PT.203

PROFILE OF EXOSOMAL MIRNAS FROM HUMAN FIBROBLAST-LIKE SYNOVIOCYTES INFECTED WITH CHIKUNGUNYA VIRUS

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Chikungunya virus (CHIKV) is an arbovirus transmitted by *Aedes* mosquitoes, that causes severe polyarthralgia. Epigenetic modifications, including miRNA modulation, may play a role in this process. This study hypothesizes that CHIKV infection alters miRNA expression in exosomes secreted by human fibroblast-like synoviocytes (HFLS). HFLS were infected with CHIKV for 48 hours (MOI 0.5), and exosomes were isolated from the supernatant. Total RNA was extracted and miRNAs were quantified for RT-qPCR OpenArray analysis. Data were analyzed using the $2^{-\Delta\Delta CT}$ method, with hsa-miR-16-5p as the endogenous control and differentially expressed (DE) miRNAs were identified with adjusted p-values ≤ 0.05. In silico analysis identified validated target genes of DE miRNAs using miRTarBase and TarBase v9.0 databases, and possible regulated pathways were found via KEGG Pathway and ShinyGO 0.8. The amount of RNA isolated from exosomes of CHIKV-infected HFLS was greater than that from uninfected cells. RT-qPCR OpenArray identified 239 miRNAs in exosomes, with 30 DEs (16 upregulated and 14 downregulated). In silico analysis revealed 4,727 target genes in miRTarBase, including 4,604 genes modulated by upregulated miRNAs and 242 by downregulated miRNAs, with 119 genes common to both. TarBase v9.0 revealed 20 miRNAs with validated targets, totaling 295 genes. Pathway analysis indicated modulation of osteoclast differentiation, regulation of actin cytoskeleton, MAPK signaling, Wnt signaling, rheumatoid arthritis, and VEGF signaling. In conclusion, CHIKV infection modulates the miRNA profile in exosomes from HFLS, possibly affecting inflammatory pathways.

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PT.204

DENV GENOTYPES CIRCULATING IN RIO GRANDE DO SUL DURING EXTREME WEATHER EVENTS, 2024

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The world experienced a dengue emergency in the first semester of 2024, Brazil being the most impacted country worldwide. Until July 30, 2024 the country counted with four times the numbers of confirmed cases reported in the same period last year, and the circulation of all dengue virus (DENV) serotypes. In the State of Rio Grande do Sul (RS) the DENV infections are seasonal and caused by DENV-1, however, this epidemiology pattern is changing. Along with that, a major set of drastic events including extreme rainfalls, mass floodings have displaced more than 700,000 people in May. This study aimed to perform a genomic surveillance of DENV in RS over the outbreak. A total of 622 samples were provided by the Municipal Health Departments from cities of RS. DENV detection and serotyping was performed by RT-PCR and positive samples were selected for sequencing. Library was prepared using the COVIDSeq kit and primers designed for DENV-1/-2. Sequencing was implemented on Illumina MiSeq platform. The overall positivity of DENV was 57% (354/622), from the serotyping samples (305/354) 97,4% was DENV-1 (297/305) and 2.6% was DENV-2 (8/305). A total of 273 positive samples were sequenced and the DENV genomes were recovered in 97% (265/273) of the samples, confirming the serotypes. Molecular characterization is ongoing. The unprecedented surge of climate change-driven events and the dengue vaccination adoption have a direct implication in the dissemination and evolution of DENV, substantiating the continuous DENV genome surveillance.

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PT.205

EXPRESSION OF A SOLUBLE RECOMBINANT PROTEIN FROM THE NUCLEOCAPSID OF ORTHOHANTAVIRUS CIRCULATING IN THE AMAZON REGION FOR USE IN A RAPID LATERAL FLOW TEST

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Introduction: *Orthohantaviruses* are zoonotic, emerging viruses with an almost global distribution. The geographical distribution of hantavirus genotypes depends on the distribution of their respective reservoir hosts. In the Americas, these etiologic agents are related to Hantavirus Cardiopulmonary Syndrome (HCPS). This disease begins with a nonspecific clinical picture and can rapidly progress to respiratory failure, with a lethality rate of 35-40%. Early diagnosis of HCPS is crucial for proper management. There is still no rapid test available on the market to diagnose hantavirus. **Aim:** this work aims to develop a recombinant protein capable of being used as an antigen in a rapid lateral flow test for the diagnosis of hantavirus in the Amazon region. **Methodology:** a recombinant hantavirus protein was expressed in E. coli BL21(DE3), under low temperatures in order to avoid the formation of inclusion bodies. A protein comes from the consensus sequence of the nucleocapsid sequences of hantavirus species circulating in the Amazon Region, available in the GenBank and Virus Pathogen Resource. **Results:** the soluble protein of the hantavirus nucleocapsid was obtained purified using Chemical Affinity Chromatography and Molecular Exclusion Chromatography and then validated using Western Blotting anti-HisTag and anti-human IgG-anti-hantavirus. **Conclusion:** the importance of the protein developed in question is due to the need for a soluble and antigenic protein, without agents that could prevent the antigen from binding to the colloidal gold of the rapid lateral flow test.

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PT.206

PRODUCTION OF HEPATITIS E VIRUS RdRp PROTEIN: EXPLORING INHIBITORY PEPTIDES VIA PHAGE DISPLAY

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Hepatitis E viruses (HEV), *Hepeviridae* family, have four genotypes associated with human infection. Genotypes 1-2 are frequently linked to transmission through contaminated water-food, while genotypes 3-4 are primarily associated with zoonotic transmission. Interestingly, the diversity of HEV in bats is high, although there is no information regarding the transmission of these viruses to humans. In this context, considering that our preliminary metagenomic data in bats indicated the presence of HEV, the aim of this study is to purify the RNA-dependent RNA polymerase (RdRp). From the RdRp, we will seek to identify peptides that can bind to this protein and inhibit its biological activity, which is related to viral replication and dissemination. Additionally, we intend to conduct an *in silico* study to detect highly antigenic peptide targets using the DNASTAR software. For this purpose, the HEV RdRp (54 kDa) containing a histidine tag was cloned into the pET28(a) vector. This recombinant vector was used to transform *Escherichia coli* Lemo21, with the best expression achieved when cells were induced with 0.5 mM IPTG for 5h at 26°C. Protein purification using a nickel column was successful with the addition of washing-solubilization buffers. Furthermore, five epitope candidates were selected using DNASTAR ($1^{73\text{-}84}$; $2^{151\text{-}158}$; $3^{216\text{-}222}$; $4^{268\text{-}277}$; $5^{475\text{-}481}$), and it was verified that these epitopes are located in relevant catalytic domains of the enzyme and exposed on the protein surface. In summary, the produced RdRp is ready to be used for future prospecting of inhibitory peptides via Phage Display for evaluation as an antiviral candidate.

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PT.207

CREATE AND CONNECT: ARBOVIRUSES AT THE INTERSECTION OF RESEARCH AND OUTREACH ACTIVITIES

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Despite efforts to control arboviral diseases, recent outbreaks of yellow fever (YF), dengue, Zika, and chikungunya underscore their burden and risk in Brazil. From 2016 to 2018, Minas Gerais experienced a YF outbreak, with evidence of sylvatic YFV transmission to non-human primates (NHP) within urban areas. Since then, YFV has been detected in the state, including urban areas. Alongside the investigation of YFV in urban parks of Belo Horizonte (BH), we aimed to promote scientific literacy and understanding of the arbovirus-host-vector network among the local population. We created materials for an interactive exhibition connecting the arbovirus-vector-host network and prevention and control measures, while promoting responsible behavior towards wildlife. In the virology sector, 3D models and games for kids illustrated arboviruses characteristics and diversity. Next, mosquito life- cycle and viral transmission were presented through 3D models and games. As a field entomologist, visitors learned to catch mosquitoes using hand nets and traps and how to observe and identify them using a stereomicroscope or hand magnifying glass. Finally, visitors took on the role of primatologists, learning about NHP diversity, habits, and ecology through games and informational folders, and engaged in monkey-watching through binoculars. In collaboration with Parks administration of BH and UFMG, seven exhibitions have taken place, to date, in urban parks in BH and at UFMG, with an estimated audience of 745 persons, reaching audiences of different backgrounds and ages from children to adults. The exhibition proved to be successful with strong engagement and positive feedback from visitors.

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PT.208

Evaluation of the genetic and virological profile of pregnant women infected with hepatitis C viruses in a reference center in Rio de Janeiro

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Hepatitis C virus (HCV) infects approximately 58 million people worldwide, including 29,000 women of reproductive age. HCV can lead to chronic infections, cirrhosis, and hepatocellular carcinoma (HCC) and can be transmitted vertically, particularly by pregnant women with viral loads ≥600,000 IU/mL during the second and third trimesters. Although highly effective drugs exist, they are not safe for use during pregnancy due to teratogenic effects. This study aimed to evaluate the genetic variability of HCV in pregnant women followed at a reference center in Rio de Janeiro from 2016-2022. Samples from pregnant women with HCV viral loads above 103 log IU/mL were selected. HCV RNA was extracted and amplified in the NS5B region by RT-PCR, followed by sequencing to identify viral subtypes and clinically relevant mutations. The average age of the women was 32.9 ± 7.0 years, mostly in the second trimester (n=45; 47.9%). Amplification was successful in 61/70 (87.15%) samples, with 33/61 (54.10%) successfully sequenced. Subtype 1a was found in 36.4% (n=12/33), 1b in 51.5% (n=17/33), and 3a in 9.1% (n=3/33). Mutation analysis showed a low frequency (18.2%) of resistance mutations to direct-acting antivirals, including V321L, V321IV, and C316N. Most pregnant women were infected with genotype 1, the most prevalent in Brazil, and had a low frequency of drug resistance mutations, indicating potential benefit from treatment postpartum.

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PT.209

PRODUCTION AND EVALUATION OF THE IMMUNOGENICITY OF A VACCINE BASED ON THE MVA VIRUS AGAINST MPOX PRODUCTION AND EVALUATION OF THE IMMUNOGENICITY OF A VACCINE BASED ON THE MVA VIRUS AGAINST MPOX

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Some studies suggest that a vaccine based on Modified Vaccinia Ankara (MVA) virus is effective against Mpox. This work aims to optimize and produce a Brazilian vaccine based on the MVA virus and evaluate its in vivo immunogenicity. Balb/C mice were immunized intramuscularly with 1x108 MVA virus using priming and boosting. After 42 days of priming, blood was collected to assess immunogenicity. High titers of total IgG, IgG2a, and IgG1 were observed. Antibodies, post-prime/boost, capable of neutralizing 50% of viral particles, occurred at a dilution of 1/320. Mice immunized and challenged with the Mpox virus survived the infection. However, the unvaccinated and challenged group had 100% mortality. The viral load in challenged animals' lungs, spleen, and liver was assessed by plaque assay and qPCR; in non-immunized and challenged animals, high Mpox titers were observed in these organs. Immunized animals did not present viral load in the evaluated organs. The evaluation of the cellular response in the lungs of the animals showed significant production of CD4+ and CD8+ cells in the immunized animals. In addition, histopathological analyses showed a significant reduction in lung lesions caused by the Mpox virus. IFN, TLR, CD4+, CD8+ knockout animals, and B cells, vaccinated and subsequently challenged, show that the response depends on neutralizing antibodies, and the absence of TLR9 plays a vital role in survival. Our results show that a vaccine based on the MVA virus can provide a protective and sterilizing response when challenged with the Mpox virus.

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PT.210

SHIFTING PATTERNS OF CHIKV TRANSMISSION IN BRAZIL

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Chikungunya virus (CHIKV) is an arthritogenic arbovirus of the family Togaviridae and genus Alphavirus. Since 2014, Brazil has become the epicenter of CHIKV outbreaks in the Americas, with the CHIKV-ECSA lineage being the most prevalent. The study aims to explore the dynamic transmission and evolution of CHIKV in Brazil from 2014 to 2022. Between 2016 and 2020, our group actively recruited individuals presenting arboviral-like symptoms from Bahia state (Campo Formoso, Coração de Maria, Itabuna, Salvador, and Senhor do Bonfim) and from Ceará state (Maranguape). We also include samples from five individuals enrolled during an arbovirus surveillance study in Salvador and two samples from Teresina (Piauí) obtained through convenience sampling. We generate 43 novel partial and near full-length ECSA-CHIKV genomes. Phylogeographic analysis showed shifting patterns in CHIKV transmission across Brazil, with three distinct dissemination phases observed. The analysis underscores the influence of Bahia and Rio de Janeiro regional dynamics on these transmission patterns. The findings underscore the importance of continuous genomic surveillance strategies in mapping the spread and evolution of CHIKV, which is crucial to improve public health interventions to manage arboviral epidemic outbreaks. Furthermore, this study has increased the number of available CHIKV genomic sequences since the beginning of the outbreak expansion, enhancing our knowledge of the transmission dynamics of the ECSA lineage in Brazil.

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PT.211

MOLECULAR INTERACTIONS BETWEEN NATURAL COMPOUNDS AND THE HRSV M PROTEIN AS AN ANTIVIRAL TARGET

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Respiratory Syncytial Virus (RSV) is a significant pathogen that causes lower respiratory tract infections (LRTI) in infants, young children, immunocompromised individuals, and the elderly. The M protein play a crucial role in viral movement within the cell and the assembly of new viral particles by interacting with the actin cytoskeleton. It can also inhibit host transcription to by localizing in the nucleus during the early stages of infection. Natural products with antiviral properties have been found to target important protein binding sites such as the M protein to prevent the formation of mature RSV virions. This study aimed to investigate the interaction parameters between chrysin (C), quercetin (Q), pentacetylated quercetin (Q1), tetracetylated quercetin (Q2) and the RSV M protein. The M protein was expressed in *Escherichia coli* and purified by affinity and size-exclusion chromatography. Fluorescence spectroscopy was used to analyze the protein/ligand in interaction. The results showed that quercetin molecules had binding constants in range of 10^3 to $10^4 M^{-1}$, with Q2 exhibiting higher affinity than Q1, and Q1, showing stronger affinity than Q. Chrysin had binding constant in the order of $10^3 M^{-1}$. All ligands demonstrated a stoichiometry of one ligand/protein. Thermodynamic analysis, indicated that the interaction were spontaneous and hydrophobic, whit $\Delta G<0$, $\Delta H>0$, and $\Delta S>0$ values, which are essential for of complexes formation. These findings suggest that the interaction of these molecules with the viral protein may disrupt viral assembly or the formation of mature viruses, processes that are mediated by the M protein.

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PT.212

COMBINED IN SILICO AND IN VITRO TOOLS FOR THE ASSESSMENT OF HEPATITIS C VIRUS IMMUNODOMINANT PEPTIDE SIGNATURES RESTRICTED TO HLA-A*02:01

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Hepatitis C is a major global health problem due to its high morbidity and mortality. There are no approved vaccines against HCV and treatment is costly. During viral infection, MHC-I peptide presentation to $CD8^+$ T lymphocytes are essential to trigger an effective antiviral response. In this work, we performed a screening of HCV-derived immunodominant epitopes restricted to HLA-A*02:01 using *in silico* and *in vitro* tools. For *in silico* studies, 59 viral polyprotein sequences from 7 HCV genotypes were obtained from NCBI nucleotide and used for peptide prediction in NetCTL 2.0 software. Around 2,880 peptides were considered potential epitopes to activate $CD8^+$ T cells and further analyses were carried out. The ability of these peptides to induce cytokines from Th1 and/or Th2 axes of the cellular immune response was also assessed, indicating a clear tendency to induce Th2 axis. For *in vitro* evaluation, a peptide microarray system was carried out using peptides derived from 7 HCV genotypes and a recombinant dimeric HLA-A*02:01 molecule to identify immunodominant amino acid sequences. These results allowed for the identification of 615 peptides with high reactivity to HLA-A*02:01 with predominance of leucine and tryptophan residues, highlighting the importance of these residues for TCR-epitope binding and $CD8^+$ T activation. Finally, HCV-derived peptide patterns restricted to HLA-A2*02:01 observed in this study provide important information for the development of a multi-epitope and pan-genotypic vaccines.

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PT.213

Impact of viral variants on the epidemiological and clinical characteristics of COVID-19: analyses of two waves of cases

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The COVID-19 pandemic was the most health challenge in the last century. Multiple and successive waves of COVID-19 cases, caused, especially, by the emergence of new variants of SARS-CoV-2, had posed the world in a constant state of alert. Here, we present an observational, descriptive, cross-sectional study aimed to evaluate epidemiological, clinical-laboratory characteristics associated with the molecular detection of SARS-CoV-2, comparing two waves of COVID-19 cases (late 2021 and late 2022). A total of 368 nasopharyngeal samples were tested. Approximately 23% of the samples collected during the 2021 wave tested positive for SARS-CoV-2, while in 2022, the positivity was about 56%. The average age of individuals who tested positive in 2021 was higher, and significant differences in symptom frequencies were observed. In 2021, individuals who related anosmia or ageusia had a higher likelihood of testing positive for SARS-CoV-2 infection (OR: 5.583; 95% CI: 1.525-18.61; $p = 0.0151$). In 2022, those reporting pharyngalgia were more likely to test positive for SARS-CoV-2 (OR: 4.169; 95% CI: 2.332-7.498; $p < 0.0001$). All sequenced samples from 2021 were identified as the Delta variant, while in 2022, all sequenced samples were of the Omicron variant. The differences detected are likely related to the variants, which may differ in transmissibility and virulence and can alter the dynamics of the pandemic, reinforcing the importance of genomic surveillance to better understand the dynamics of viral infections.

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PT.214

Prevalence of CMV glycoprotein B genotypes in newborns with congenital CMV infection in southern Bahia, Brazil

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Cytomegalovirus (CMV) is a common herpesvirus causing congenital infections with significant neonatal health risks. This virus displays genomic polymorphism enabling its classification into distinct genotypes among its structural proteins, glycoprotein B (gB) is pivotal in viral entry into host cells, critical for viral dissemination and a key target for host immune responses. The gB gene sequence diversity differentiates CMV into four major genotypic variants, known as gB1 to gB4, each potentially influencing infection pathogenesis and disease outcomes. This study characterized CMV glycoprotein B (gB) genotypes among 2,595 newborns in southern Bahia, Brazil. The CMV gB genotypes were determined using PCR amplificationof the gBj gene followed by restriction enzyme digestion. CMV DNA was detected in 1.19% of cases, with gB1, gB2, and gB3 identified among the infected infants, gB2 being the most prevalent. Although follow-up evaluations showed no significant statistical correlation between genotypes and late sequelae, three infants exhibited hearing loss. These findings highlight the predominance of gB1 and gB2 in the studied region, highlighting the need for targeted prevention and management strategies. The study was approved by the Universidade Estadual de Santa Cruz Research Ethics Committee.

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PT.215

ESTABLISHMENT OF A FLUORESCENCE-BASED ASSAY FOR THE CHIKUNGUNYA VIRUS (CHIKV) NSP2 PROTEASE TO SCREEN POTENTIAL ANTIVIRALS

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Chikungunya virus (CHIKV), a member of the Togaviridae family, causes chikungunya fever, a debilitating disease that can progress to chronic arthritis. With no antiviral treatments available, there is an urgent need for specific therapeutics. The viral protease (nsP2pro) is essential for processing the nsP1234 polyprotein into four non-structural proteins (nsPs), making it an interesting target for drug development. In this study, we focused on obtaining the recombinant nsP2pro and establishing an enzymatic assay for screening potential anti-CHIKV compounds. The protease sequence (residues 212 to 535) was cloned into the pET-TRX vector, expressed in *E. coli* BL21 in TB medium and purified using nickel-affinity and size-exclusion chromatographies. A fluorescence-based assay was established using the synthetic substrate DABCYL-DRAG↓YIFY-EDENS-NH2, which includes the CHIKV nsp3/nsp4 cleavage site, a fluorescent donor (EDANS) and a quenching acceptor (DABCYL). Several buffers and concentrations of protein and substrate were tested. Protein was incubated either at 37°C or 30°C for 30 minutes, then the substrate was added to the reaction and the fluorescence was read after 30 minutes to 15 hours (excitation and emission wavelengths of 340 nm and 490 nm, respectively). The optimal condition was achieved with 600 nM nsP2 and 20 µM substrate at 37°C in 25 mM HEPES, pH 7.5. The next step involves screening the MMV Hit Generation Library 1 (HGL1), containing about 5,632 molecules, to identify potential CHIKV replication inhibitors to advance antiviral discovery.

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PT.216

SARS-CoV-2 VARIANTS AND CLINICAL SPECTRUM OF COVID-19 IN SOUTHERN BAHIA.

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The emergence of new SARS-CoV-2 variants impacted the COVID-19 over time. This observational, descriptive, cross-sectional study analyzed 184 nasopharyngeal swab positive for SARS-CoV-2, from February 2021 to February de 2022. Sequencing was performed on Illumina Sequencing Platform using the COVIDSeq kit and the reads aligned to the SARS-CoV-2 reference genome using the Illumina DRAGeN COVID Lineage v3.5.4 application. Sample selection criteria were: cycle threshold (CT) values ≤25 in the RT-qPCR and complete clinical and/or epidemiological data. Participants were aged between 18 and 85 years (with a majority between 31 and 50 years). In relation to severity of illness, they were classified as mild (52.79%), moderate (13%), and severe (7%) intensity. Over half (53%) required hospitalization, and 25% had comorbidities. Fifteen SARS-CoV-2 lineages were identified, including four Variants of Concern: Gamma (20.40%), Delta (13.77%), Zeta (3.06%), and Omicron (13.0%). The Zeta variant was detected in the first quarter of 2021, Gamma predominated in the first half of this year, Delta was predominant from September 2021 to early 2022, and Omicron detected in January 2022. Severe cases were more frequent in Gamma and Zeta (16.4% and 50%, respectively) compared to Delta (6.2%) and Omicron (0%) variants. Dyspnea was significantly more prevalent in severe cases (69%; OR 9.53, $p<0.001$). Besides, symptoms such as anosmia/ageusia, dyspnea, and myalgia were associated with Gamma and Delta variants. This study found significant differences in the clinical spectrum of SARS-CoV-2 infection, with Gamma and Zeta variants associated with more severe cases.

Keywords: SARS-CoV-2; pandemic, variants, Bahia, Sequencing
Financial Support: CAPES, PPSUS, FINEP

PT.217

DEVELOPMENT OF A RAPID, SIMPLE AND SENSITIVE DIAGNOSTIC BASED ON LAMP FOR DETECTION OF HUMAN RESPIRATORY VIRUSES

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Respiratory syncytial virus (RSV) and Influenza virus (FLU) are prevalent seasonal community viruses. Loop-mediated isothermal amplification (RT-LAMP) is a promising method for rapid and point of care (PoC) detection of respiratory viruses. RT-LAMP is simple, inexpensive and the amplification of the genetic material occurs at constant temperature and the results can be interpreted with the naked eye. In this study, we developed and optimized a viral detection based on the RT-LAMP technique for detection of RSV and FLU. Clinical samples of RSV and FLU were previously detected by q-RT-PCR were subjected to RT-LAMP reactions for standardization and validation. We selected two sets of primers from literature for the detection of RSV (A and B) and FLUV (A and B). Until now, we tested 21 of RSV-A and 2 of RSV-B samples with 100% and 50% agreement respectively, with the gold standard test. Our data showed that there was no cross-reactivity with other respiratory viruses for both selected primers for RSV. For FLU, our sensitivity was 100% for FLU-A and 88.5% for FLU-B and no cross-reactivity was observed between types. We also tested two sets of influenza A subtyping primer (H1N1), and both were specific for the samples tested. Considering the results, we can infer that RT-LAMP can be applied in the molecular diagnosis of RSV, FLU and other respiratory viruses. Therefore, we will conduct further experiments to refine this technique to validate it since the development of a PoC platform is very important for monitoring diseases caused by respiratory viruses.

Financial Support: FAPEMIG; FIOCRUZ; FINEP; CNPq;CAPES.

PT.218

GENOMIC EPIDEMIOLOGY OF A LARGE-SCALE AND PERSISTENT CHIKUNGUNYA VIRUS OUTBREAK IN SÃO PAULO, BRAZIL

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Since the chikungunya virus (CHIKV) was first detected in Brazil in 2013 (Asian lineage), recurring outbreaks illustrate the endemicity of the disease in the country. Recently, Brazil has been facing an increase in CHIKV cases, mainly linked to the East-Central-South-African (ECSA) lineage. In São José do Rio Preto, SP, Brazil, CHIKV was first detected in 2015, and until 2022, only 45 cases had been confirmed in the city, suggesting no significant outbreak. However, our arbovirus molecular surveillance of serum samples from patients with acute febrile illness revealed an increased detection of CHIKV since 2023, marking the region's most significant CHIKV outbreak. Between January/2022 and August/2024, we detected 542 cases (n=1,2022; n=21, 2023; n=520, 2024). Among these cases, 85.3% had previously had dengue fever (DENV-IgG positive). Full-genome deep sequencing and phylogenetic analyses of 300 samples classified all the sequences as ECSA lineage, closely related to sequences from Southeast and Northeast Brazil. The sequences were observed to be divided into two major clades, with one characterized by a monophyletic group with SJRP sequences from 2024. Epidemiological and clinical data were analyzed, comprising 51.1% of female patients, with most samples from patients aged 40-49 (16.2%). Myalgia (91.1%) and fever (88.6%) were the most common signs and symptoms. Our results stress the importance of active surveillance of arboviruses to detect new virus introductions and ensure control strategies to mitigate outbreaks. Additionally, we reinforce the critical role of differential diagnosis of arboviruses in areas with simultaneous circulation of different viruses with shared clinical profiles.

PT.219

IDENTIFICATION OF NOVEL ANTIVIRAL COMPOUNDS AGAINST CHIKUNGUNYA VIRUS USING *IN SILICO AND IN VITRO* APPROACHES

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The Chikungunya virus (CHIKV) is an arbovirus with increasing frequency of cases over the years and causes fever followed by inflammatory reactions with consequences that can persist after acute infection. Although a vaccine has been recently approved by the FDA, there are no specific antivirals for the treatment of CHIKV infections. The CHIKV nsP2 protease (nsP2pro) is a vital enzyme for viral replication and pathogenesis, which makes it an interesting therapeutic target. This study aimed to identify commercially available potential inhibitors of CHIKV nsP2pro with antiviral activity by combining *in silico* and *in vitro* methods. 239.486 compounds from the Sigma-Aldrich database were screened by different *in silico* strategies, such as pharmacophore screening, molecular docking, and drug-like properties filtering. We selected 47 potential nsP2pro inhibitors with promising drug-like properties and 9 compounds were purchased for *in vitro* validation. Their cytotoxicity on Vero cells was assessed after a 72-hour treatment using the MTT assay. Most of the compounds showed low cytotoxicity with values of concentration required to reduce cell viability by 50% (CC_{50}) ranging from 32.4 µM for compound **8** to >1,000 µM for compound **3**. Then, cells were infected with CHIKV BRA/RJ/18 strain (MOI 1) and treated with compounds (100 µM) for 24 h. Supernatants were harvested, and virus titers were quantified by plaque assays. Compound **4** showed the highest inhibitory activity (71,88%), followed by compound **3** (55,84%). Therefore, we discovered compounds with interesting antiviral potential that deserve further investigation to aid the development of new anti-CHIKV drugs.

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PT.221

SARS-CoV-2 Omicron XBB infections boost cross-variant neutralizing antibodies, potentially explaining the observed delay of the JN.1 wave in some Brazilian regions

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The unceasing circulation and ongoing evolution of SARS-CoV-2 leads to the constant emergence of novel viral lineages. That was the case for JN.1, which emerged in late 2023 and quickly replaced XBB, becoming the predominant Omicron variant worldwide in 2024. Although Brazil followed the same global trend, the epidemiological impact measured through the rise of severe acute respiratory infection (SARI) cases following dissemination of the JN.1 lineage greatly varied across country regions. To understand this difference, we analyzed the evolution of SARS-CoV-2 lineages and SARI cases in Brazil between July 2023 and March 2024 and we further assessed the neutralizing antibodies (NAb) levels against XBB and JN.1 in sera from a cohort of patients recently infected with XBB. The JN.1 variant replaced the XBB variant with similar temporal dynamics across all country regions. The Southeastern, Southern and Central-western regions experienced a brief XBB variant wave around October 2023, shortly before the introduction of JN.1, and no immediate upsurge of SARI cases during viral lineage replacement. By contrast, the Northeastern and Northern regions experienced no XBB variant wave during the second half of 2023 and a rapid upsurge of SARI cases driven by the rise of the JN.1 variant. We found that recent XBB infections significantly increase the levels of cross-reactive NAb against JN.1 in the Brazilian population immune-background. These results suggest that the XBB variant wave observed in the second half of 2023 in some Brazilian states functioned as a booster for population immunity, providing short-term protection against JN.1 infections and delaying the rise of SARI cases in these regions

PT.223

Construction of a recombinant clone of St. Louis encephalitis virus Infection knockout for the Envelope protein N154 glycosylation

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The St. Louis encephalitis virus (SLEV) is a Flavivirus that can be found in many countries of the Americas causing outbreaks, including Brazil. SLEV infection can result in the development of the encephalitis of St. Louis, a disease that can lead to neurological symptoms and death. The Envelope protein of flaviviruses can present up to two conserved glycosylation sites, studies show that they can impact the efficacy of flaviviruses in the process of cell entry, replication and neuroinvasiveness. Many SLEV strains present a conserved glycosylation site on the asparagine 154 of the envelope protein (N154), but the role it plays in infection is still unknown. Infectious clones are valuable tools for virus biology research, as they can be used in functional studies of viral proteins. We developed a protocol for construction of SLEV infectious clones that allowed us to construct both a wildtype and a non-glycosylated clone. The recombinant genomes were assembled using Circular Polymerase Extension Reaction and transfected in HEK 293T cells. The clones were rescued 10 days later when cytopathic effect was observed. The identity of their sequences was confirmed by sanger sequencing. We demonstrate by titration that the clones showed stability for at least three passages in Vero cell culture. The biological tool we developed here can be useful for the scientific community to explore the molecular biology of SLEV. We expect these recombinant clones will allow us to compare the dynamic of infection in vitro and in vivo of the non-glycosylated strain against the wildtype.

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PT.224

ASSOCIATION BETWEEN SNPS IN INNATE IMMUNE RESPONSE GENES AND COVID-19 SEVERITY IN CHILDREN

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The global number of COVID-19 deaths reached 7 million, 4% of these deaths occurring in children and adolescents. In Brazil, around 1,500 children up to 11 years old died from the disease. The most common symptoms in children are respiratory, potentially progressing to severe cases such as severe acute respiratory syndrome (SARS) and Multisystem inflammatory syndrome in children (MIS-C). Studies indicate that comorbidities and genetic factors, such as polymorphisms in immune response genes, can influence the severity of COVID-19. This study investigates the occurrence of single nucleotide polymorphisms (SNPs) in innate immune response genes in children with COVID-19. Seventy-three samples were analyzed from children under 13 years old hospitalized at João Paulo II Children's Hospital due to COVID-19. The evaluated SNPs were: TLR8(1) (rs3764879), TLR8(2) (rs2407992), TLR7 (rs179008), TLR3 (rs3775291), TIRAP (rs8177374), and MCP-1 (rs1024611), considering four categories of severity: mild, moderate, severe, and critical COVID-19. To identify the SNPs, PCR and sequencing were performed. The frequencies of the SNPs obtained were not significantly different when compared to the frequencies described in the Global ALFA, Global 1000 Genomes, Global gnomAD, American 1000 Genomes, and American gnomAD databases, except for the SNP in TLR7. Comparing severe and critical cases to mild and moderate cases, we found a higher relative risk associated with mutations in TLR8 (1), TLR7, TLR3, and TIRAP ($p < 0.05$). No association was found for SNPs in TLR8 (2) and MCP-1. Our analyses suggest an association between SNP in TLR7 gene and severity of COVID-19 symptoms in children.

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PT.225

EXPLORING GENETIC FACTORS OF MULTISYSTEM INFLAMMATORY SYNDROME (MIS-C) IN CHILDREN WITH COVID-19

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In Brazil, approximately 1,500 deaths due to COVID-19 occurred in children up to 11 years old, along with more than 2,400 cases of Multisystem inflammatory syndrome in children (MIS-C) associated with COVID-19. By 2022, SARS-CoV-2 infection was among the country's top 10 leading causes of child mortality (BUTANTAN, 2022). Severe cases of MIS-C have been linked to comorbidities, but the syndrome has also occurred in children without pre-existing medical conditions (CDC,2020). It is known that the pathogen-host relationship and the innate immune response are key factors in disease prognosis. In this context, this study aims to investigate whether single nucleotide polymorphism (SNP) mutations in innate immune response genes could be related to the development of MIS-C. For this purpose, the SNP profiles of 9 children diagnosed with MIS-C from Hospital João Paulo II were analyzed. The SNP profiles were analyzed for the following genes: TLR8 (1) (rs3764879), TLR7 (rs179008), and TLR3 (rs3775291) through genetic sequencing. Among the nine children, 5 were boys and 4 were girls, of which three boys died. One of the children developed hepatitis. Two of the nine samples (16.7%) showed the presence of the SNP TLR7 (rs179008), an A to T variation, which, in another study with 73 Brazilian children, has shown a frequency of 7.5% in mild and moderate cases. The frequency found for TLR8 (1) was 25%, and for TLR3 it was 8.3%. These data suggest a possible association between the SNPs rs179008 and rs3775291 in MIS-C cases.

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ASSESSMENT OF THE ANTIVIRAL ACTIVITY OF *APULEIA LEIOCARPA* EXTRACTS AGAINST OROPOUCHE VIRUS

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Oropouche virus (OROV) is an arbovirus responsible for causing nonspecific febrile illness, with symptoms indistinguishable from other arboviruses. More than half a million people have been infected in countries across America. In Brazil, Oropouche Fever is the second most frequent febrile illness in the Amazon region. Only in 2024, 1674 cases have been registered in the state of Amazonas, showing OROV potential emerging arbovirus. Despite its significance, there are no currently antiviral medications or vaccines available against the infection. Therefore, studies that aimed to investigate antiviral compounds with potential action against OROV are needed. In this scenario, the species *Apuleia leiocarpa* stands out for containing promising organic compounds with antiviral action, such as tannins and flavonoids, which has been proven effective against Zika and Dengue virus. Thus, this study aimed to evaluate the antiviral action of leaf and stem extracts of *Apuleia leiocarpa* against OROV in Vero cells. Initially, through cytotoxicity assays, the cytotoxic concentration for 50% of cells (CC50) was determined for each of the six extracts, followed by the global antiviral assay, which determined the effective concentration for 50% of cells (CE50). All six extracts, whether hydroalcoholic, ethanolic, or aqueous, from both stem and leaf, showed anti-OROV activity, with selectivity indices (SI) ranging from 6.08 to 51.50. Among them, the most promising was the ethanolic stem extract, with in SI of 51,50. These preliminary results are promising and serve as a basis for future studies in the search for potential antiviral compounds in the treatment of Oropouche Fever.

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COMPARATIVE ANALYSIS OF CD4 AND CD8 TRM SUBPOPULATIONS IN THE AIRWAYS OF PEDIATRIC PATIENTS WITH ACTIVE VIRAL INFECTION

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Introduction: Tissue-resident T cells (TRM) play a crucial role in lung immune responses during viral infections. Analyzing these cells in humans is challenging due to the invasive nature of conventional methods. This study aims to characterize CD4 and CD8 TRM subpopulations in the lower respiratory tract of pediatric patients with active viral respiratory infections using a less invasive approach. **Methods:** Tracheal aspirates were collected from intubated pediatric patients with viral infections in the Intensive Care Unit. Cells were stained with antibodies against CD3, CD45RA, CCR7, CD4, CD8, CD103, and CD69. Flow cytometry was employed for cell analysis, and non-parametric statistical tests assessed differences among TRM subpopulations. **Results:** The CD8+ T cell population was found to be 1.668 times more prevalent than the CD4+ T cell population in tracheal samples. Within the CD4+ population, median fluorescence intensity for CD69 was lower compared to CD103 (median difference: -1233), a pattern also observed in CD8+ cells (median difference: -882.0). Significant differences were detected among CD4 TRM subpopulations ($P = 0.0185$), particularly between CD69- CD103+ and CD69+CD103- subsets ($P = 0.0400$). Similar variations were noted among CD8 TRM subpopulations ($P = 0.0417$), with notable differences between CD69- CD103+ and CD69+CD103+ subsets ($P = 0.0400$). **Conclusion:** This preliminary results demonstrates significant heterogeneity within CD4 and CD8 TRM subpopulations based on CD69 and CD103 expression in tracheal aspirates from pediatric patients with viral lung infections. These findings highlight the distinct roles of these markers in TRM in pediatric respiratory viral infections.

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Encapsulation of five immunogenic peptides from the *Zika virus* in bovine serum albumin nanoparticles

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The *Zika virus*, *Orthoflavivirus zikaense* (ZIKV) is one of most important member of the *Flaviviridae* family and human infections are associated to Zika congenital syndrome in newborns resulting from maternal infection during pregnancy, and Guillain-Barré. Our group identified and validated 5 immunogenic epitopes from structural proteins of ZIKV. Bovine serum albumin nanoparticles (BSA-NPs) have been studied as vaccine platforms due to their capacity for peptide encapsulation, release, and immunostimulating adjuvants. In this study, synthetic peptides: Pep1, Pep2, Pep3, Pep4, and Pep5 were encapsulated separately in BSA-NPs. Empty nanoparticles (NPV) were produced as control. The nanoparticles were characterized for their size, Polydispersity Index (PDI), Zeta Potential, and encapsulation rate. Additionally, the MTT assay was used to evaluate the cytotoxicity of all nanoparticles on BHK-21 cells. The results showed that the average size of all 5 NPs containing peptides (NPPeps) was 196.47nm (±8.77) while NPV was 159.1nm (±0.7), the average PDI of NPPeps was 0.1727 (±0.09) and NPV was 0.0953 (±0.012), the average Zeta of NPPeps was -14.2mV (±0.68) and NPV was -15.4mV (±1.457), with an avarage encapsulation rate of 56.14%. Furthermore, cell viability (%) was as follows: NPPep1=96.61%, NPPep2=93.29%, NPPep3=92.54%, NPPep4=90.78%, NPPep5=92.80%, and NPV=94.53%. Therefore, the results showed that these Nanoparticles are non-toxic and encapsulated the immunogenic peptides. Future studies should be carried out in order to evaluate potential use of nanoparticles as immunogens for the ZIKV.

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PT.229

The role of lipid droplets and their interaction with viral proteins in SARS-CoV-2 infection

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Lipid droplets are dynamic organelles mainly responsible for storing neutral lipids and as a source of lipids for membrane synthesis. In recent years new functions have been attributed to these organelles, such as participation in the infectious process of certain pathogens, and lipid droplets play an important role during infection by Dengue, Zika and Hepatitis C viruses, among others. SARS-CoV-2 is the pathogenic agent that causes COVID-19, a disease that to date has been responsible for more than 6 million deaths worldwide, with around 700 million reported cases. In this work we aim to analyze how lipid droplets influence SARS-CoV-2 infection. In Vero E6 cells, we observed that the induction of lipid droplets by treatment with oleic acid inhibited viral production when cells were treated 24h before infection, while there was an increase in viral production when the treatment was 1h after infection. This phenomenon was not seen for hepatocarcinoma cell line HepG2, as the virus production was the same in all conditions. Another aspect we investigated was the presence of viral proteins in lipid droplets. We detected that the non-structural proteins nsp7 and ORF6 are present around lipid droplets. Also, we observed that nsp7 colocalizes with Rab18, an important cellular protein associated with lipid droplets. The results obtained so far indicate that lipid droplets play a significant role in the virus-cell interaction during SARS-CoV-2 infection. Furthermore, the non-structural proteins nsp7 and ORF6 may be involved in the interaction between lipid droplets and the virus.

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PT.230

DISCOVERY OF FLAVONOID DERIVATIVES AS SARS-CoV-2 RNA DEPENDENT RNA POLYMERASE (RDRP) INHIBITORS

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Emerging viruses with pandemic potential pose a significant threat to global public health. The COVID-19 pandemic, caused by SARS-CoV-2, has resulted in over 695 million cases and more than 6.9 million deaths worldwide as of July 2024. SARS-CoV-2, a positive-sense single-stranded RNA virus, the viral transcription and replication processes are mediated by a replicase complex of non-structural proteins (nsp7-nsp12-nsp8), with nsp12 serving as a key RNA-dependent RNA polymerase (RdRp). Due to its exclusively viral nature and high structural conservation among coronaviruses, RdRp is a promising target for antiviral drug discovery. To evaluate potential antiviral agents, we expressed the RdRp protein (nsp12) in insect cells using the Baculovirus system and purified it through three chromatography steps. Cofactor proteins were expressed in a bacterial system and purified using two-step chromatography. The polymerase activity of the RdRp complex was assessed using SYBR Green I fluorescence. Approximately 160 compounds were screened for inhibitory potential against the replicase complex, sourced from commercial collections and our proprietary library. One of the most potent compounds identified was a flavonoid derivative with an IC_{50} value of 0.9 μM. These findings motivated the synthesis and testing of new analogues to investigate the structure-activity relationships (SAR) of the series. The new derivatives are currently being evaluated, and some have demonstrated RdRp inhibitory activity, though the exact rates are still under analysis. These results suggest the potential of flavonoid derivatives as promising lead candidates for developing new RdRp inhibitors in COVID-19 drug discovery.

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ANTIVIRAL ACTIVITY OF APULEIA LEIOCARPA EXTRACTS AGAINST MAYARO VIRUS

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Mayaro virus (MAYV) is an arbovirus belonging to the Togaviridae family, genus Alphavirus. It was first isolated in Trinidad and Tobago in 1954, with the initial cases in Brazil reported in Belém (PA). Its primary vector is Haemagogus janthinomys, and urban transmission has seen a substantial rise. The disease caused by MAYV, known as Mayaro Fever, presents symptoms such as headache, myalgia, chills, and a triad of sudden fever, arthralgia, and skin rash. MAYV remains neglected, lacking both treatment and vaccine, thus necessitating increased research into its pathogenesis and potential therapeutic targets. In this context, plants continue to be significant reservoirs for discovering new bioactive compounds, given their generally low cytotoxicity and association with traditional medicinal knowledge. Accordingly, this study sought to evaluate the efficacy and antiviral activity of stem and leaf extracts from Apuleia Leiocarpa (commonly known as "Grápia") against MAYV. Experimental assays were conducted on VERO cells in 96-well plates, with cytotoxicity and global antiviral assessments performed using the methyl-thiazole-tetrazolium (MTT) colorimetric method. Results indicated that the examined extracts displayed low cytotoxicity (>250 µg/mL) and predominantly exhibited significant effects in protecting cells against MAYV (ranging from 36.52 to 97.66 µg/mL). Consequently, they demonstrated selectivity indices ranging from 5.12 to 20.93, with the compound extracted from stems in ethanolic solvent showing the highest selectivity. These findings suggest that extracts of Apuleia Leiocarpa hold potential as important targets for antiviral research, underscoring the need for further investigation to elucidate their mechanism of action.

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PT.232

Heterologous Expression and Purification of Arbovirus Proteases Using Baculovirus System

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Arboviruses, including Yellow Fever Virus (Flaviviridae) and Chikungunya and Mayaro viruses (Togaviridae), are pathogenic agents causing diseases and fatalities. These RNA viruses encode structural and non-structural proteins essential for replication and assembly, processed by viral proteases such as NS2B-NS3 in Flaviviridae and nsP2 in Togaviridae. This study aimed to use the baculovirus system for the heterologous expression and purification of these viral proteases. The viral protease genes were amplified via PCR, cloned into the pFastBac1 vector, and used to generate recombinant baculoviruses through the Bac-to-Bac® system. Recombinant baculoviruses were used to infect insect cells. At 72 hours post-infection (p.i.), cells were harvested, and protein expression was analyzed by SDS-PAGE and Western blot. Proteins were purified using affinity chromatography. Successful cloning and expression of NS2B-NS3 from Yellow Fever Virus and nsP2 from Chikungunya Virus were confirmed by PCR, SDS-PAGE, and western blot, showing the expected molecular weights. Affinity chromatography identified elution peaks containing the proteases, confirmed by SDS-PAGE and Western blot. This study demonstrates the viability of using the baculovirus system for expressing viral proteases, providing a tool for evaluating potential antiviral compounds.

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THE PRODUCTION OF S100A8/A9 CHANGED DURING THE PERSISTENT PAIN INDUCED BY CHIKUNGUNYA VIRUS

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Chikungunya fever, caused by the Chikungunya arbovirus, manifests with a febrile state, intense polyarthritis/polyarthralgia, often followed by myalgia and rash. In 40% to 80% of cases, the disease may persist for years, leading to a condition characterized by arthritis with persistent pain. Although it is an emerging disease, there is no specific treatment or vaccine yet, and the mechanism related to the persistence of symptoms remains to be elucidated. Alarmins, inflammatory mediators associated with host defense, can contribute to a pathological inflammatory response that, if left unchecked, can trigger various diseases, including arthritis. In this context, this study aims to investigate the link between the alarmin S100A8/A9 and chikungunya arthritis, and how treatment with analgesic drugs may interfere with the action of S100A8/A9 during infection. For this purpose, C57BL/6 mice infected with CHIKV at an inoculum of 1x106 PFU/animal were treated with the corticoid dexamethasone at a dose of 4 mg/Kg, between 7 and 11 days after infection and in another experiment, infected mice were treated with pregabalin, a drug with analgesic and anticonvulsant action, at a dose of 100 mg/Kg at two times, 0 to 2 and 7 to 11 days after infection. Blood and paw samples were then taken for analysis. Our results show that the level of S100A8/A9 is altered during infection, even in the period after the disappearance of hypernociception. Dexamethasone and pregabalin were effective only during treatment and pain returned after treatment was stopped. These results suggest S100A8/A9 as an important biomarker for Chikungunya persistent.

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PT.234

SEROPREVALENCE OF CHIKUNGUNYA IN AN INDIGENOUS POPULATION IN THE STATE OF BAHIA

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INTRODUCTION: Chikungunya is a global public health concern characterized by bilateral polyarthritis/arthralgia, which can persist for years after the febrile phase and is associated with significant morbidity. According to the latest census, Brazil has approximately 1.7 million indigenous people. The Ministry of Health recorded 596 probable disease cases in indigenous populations in the first months of 2024. However, there is a scarcity of studies on the prevalence of Chikungunya virus infections in this population, highlighting the importance of determining seroprevalence to understand its true impact on communities. OBJECTIVE: To assess chikungunya seroprevalence in indigenous populations in Bahia. MATERIALS AND METHODS: This cross-sectional study enrolled individuals of all sexes and both ages living in Indigenous territories in Bahia. Surveys were carried out using the REDCap 9.3.1 - © 2023 database, and serological diagnoses were made using the Enzyme-Linked Immunosorbent Assay to detect specific anti-CHIKV IgM and IgG antibodies. RESULTS: 2058 Indigenous individuals were enrolled. 60.2% were female, with a median age of 33 years, 48.5% were single, 46.1% had incomplete primary education, and 67.8% had a family income below one minimum wage. The most represented ethnic groups were the Tubinambás (33.1%), Pataxós (25.8%), Kaimbés (18.1%), and Kiriris (20.3%). The overall seroprevalence of Chikungunya infection was 14.2% and 7% for IgG and IgM antibodies, respectively. CONCLUSIONS: This population has a significant socioeconomic vulnerability, low schooling, and insufficient family income. Previous exposure to chikungunya infection (IgG) and recent infections (IgM) highlight the silent outbreaks in indigenous territories.

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INFLUENZA A(H3N2) VIRUS DOMINATES THE 2021-2022 SUMMER SEASON DURING THE SARS-COV-2 PANDEMIC IN SÃO PAULO, BRAZIL

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After the emergence of SARS-CoV-2 in Brazil, the efforts of research entities and the government have been focused mainly on monitoring this virus, and little is known about other respiratory viruses. Between November/2021 to January/2022, atypical increases in the number of individuals manifesting flu-like symptoms with negative results for SARS-CoV-2 were identified. Nasopharyngeal-oropharyngeal swabs were collected from 211 patients. Molecular analysis investigated the positivity to SARS-CoV-2, Influenza A/B, and RSV. Influenza A genomic sequencing, virus isolation, growth kinetics, and hemagglutination inhibition assays were performed, and statistical analyses were done using R and Python. Molecular diagnosis indicated that the patients were mainly positive for the Influenza A virus (IAV). Twenty-one IAV-positive samples were sequenced by NGS and revealed the circulation of H3N2. Sequenced genomes were compared to all other previously deposited genomes in GenBank since the emergence of the A(H3N2) subtype in 1968, through patterns recovered using Relative Synonymous Codon Usage and the results suggest that no antigenic shift occurred with the sequenced samples collected in São Paulo in 2021. Phylogenetic analysis based on segment 4 (hemagglutinin protein) brings together the genomes within the clade 3c.2a1b.2a.2, with other recently sequenced samples from different countries, including Brazil. The rapid increase in the cases suggests that the measures to contain the entry and spread of new respiratory pathogens and other lineages of common respiratory agents within the country would not work in the face of new emergencies, as occurred soon after the SARS-CoV-2 omicron variant.

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IN VITRO ANTIVIRAL EFFICACY OF A PHTHALOCYANINE DERIVATIVE AGAINST HERPES SIMPLEX VIRUS 1 (HSV-1)

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Herpes Simplex Virus 1 (HSV-1) is a neurotropic virus primarily spread through intimate contact, affecting mucocutaneous tissues and causing blisters and ulcerations. It establishes lifelong latency in neuronal cells and can lead to complications like herpetic encephalitis and ocular keratitis. Acyclovir (ACV) is the standard treatment, but viral resistance remains a concern. Natural and synthetic molecules resembling natural compounds are being explored as antiviral agents. Phthalocyanines, synthetic analogs of porphyrins, have shown antiviral activity under light exposure. This study investigates the antiviral activity of a tetracarboxy phthalocyanine derivative in the dark. VERO cells (2 × 10^4 cells/well) were treated with various concentrations of the compound for 72 hours at 37°C and 5% CO2. Cytotoxicity was assessed using MTT solution (0.5 mg/mL) after 2 hours. For antiviral assessment, cells were exposed to the compound and HSV-1 (MOI = 0.2) for 72 hours, with monolayers photographed every 24 hours. Total RNA was extracted and analyzed by RT-qPCR for the HSV-1 US3 gene using the 2−ΔΔCt method, with RNAse P as a normalization control. The data were analyzed using analysis of variance (ANOVA), followed by an appropriate post-hoc test. The derivative had a 50% inhibitory concentration (IC50) of 4.71 mg/mL and inhibited cytopathic effects up to 7.8 µg/mL. No US3 gene expression was detected at 7.8 µg/mL or higher. No statistically significant difference was noted at 0.9 µg/mL or lower concentrations compared to the viral control. The compound demonstrated a broad in vitro therapeutic window, indicating potential as an anti-HSV-1 agent.

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PT.237

SURVEILLANCE AND EPIDEMIOLOGIC FEATURES OF HUMAN ADENOVIRUS IN ACUTE GASTROENTERITIS CASES IN BRAZIL, 2021-2023

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Acute gastroenteritis (AGE) is a major cause of morbidity and mortality in low- and middle-income countries, particularly among children under 5 years of age. Viruses are known to play a key role, and adenoviruses, especially the enteric types F40 and F41 are important cause of AGE. Our study describes HAdV surveillance in Brazil during a three-year period, from January 2021 to December 2023. A total of 1,925 AGE stool samples, collected from several states from children and adults, were used to investigate HAdV DNA presence. Quantitative multiplex TaqMan ®-based PCR was used to detect non-enteric and enteric HAdVs. Fecal suspensions (10% w/v) prepared with Tris-calcium buffer (pH 7.2), was used for DNA extraction using a QIAamp Viral RNA Mini kit according to the manufacturer's instructions. purification using Qiagen automatic system. HAdV was detected in 15% (292/1925) of samples, in which the highest HAdV detections occurred in 2023 (25%). The viral load, genomic copies per gram of feces (gc/g), of all HAdV were quantified, with a higher viral load observed in age groups <60 months (4.8×10^3 gc/ g). Despite the greater number of samples positive for non-enteric HAdV (n=235), HAdV-F (n = 57) presented the highest viral loads, we observed a significant difference of HAdV-F (5.7×10^7 gc/g) when compared to non-enteric (1.0×10^4 gc/g). The data elucidated above reveal the need for greater surveillance in HAdV-F research, evidencing this as one of the main current causes of AGE.

Financial Suport: FAPERJ, CAPES

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DETECTION AND CHARACTERIZATION OF CIRCULATING ARBOVIRUSES IN MOSQUITOES CAPTURED WITHIN THE CITY OF RIO DE JANEIRO

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During 2024, Brazil faces a new dengue epidemic. With the highest number of probable cases registered since 1986, the raise this year was 344,5% higher in comparison to 2023. In the southeast region, during the 12th epidemiological week, there has been an incidence rate of 4739,8 cases by one hundred thousand inhabitants, according to Brazil's Ministry of Health. In this context, the goal of the present study was the detection and characterization of arboviruses in mosquitoes captured within the city of Rio de Janeiro. Three neighborhoods were chosen as points of capture: Olaria, Vila Isabel and Cidade Universitaria. For the capture of the mosquitoes, a mosquito trap and a mouth aspirator were used. The mosquitoes were macerated with ceramic beads and their genetic material was extracted using the QIAMP Viral RNA kit. Afterwards, the samples were submitted to RT-qPCR targeting *Orthoflavivirus denguei*, *Orthoflavivirus zikaense* and *Alphavirus chikungunya*. During the capture period, a total of 499 mosquitoes were obtained and identified. From the total, there were 186 (37,27%) *Aedes aegypti* and 104 (20,84%) *Aedes albopictus*, with 134 (26,85%) *Aedes* spp.. One (0,2%) *Aedes fluviatilis* and 1 (0,2%) *Aedes taeniorhynchus* were identified. *Culex* species were also captured, with 20 (4,01%) mosquitoes identified as *Culex quinquefasciatus* and 53 (10,62%) as *Culex* spp.. However, none of the processed mosquitoes were positive for the studied arboviruses. The methods used are being optimized and other viral species will be included in further studies.

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Differential Inflammatory Responses in Centenarians and Young Adults Reveal Key Insights into COVID-19 Severity

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Although the oldest individuals have been at the highest risk of developing critical COVID-19, there have been reports of unvaccinated centenarians worldwide who experienced asymptomatic infections, contrasting with young adults who died from the disease. An exacerbated inflammatory response is a major factor contributing to the severity and negative outcomes of COVID-19. This study examines the differences in cellular SARS-CoV-2 infection rates and cytokine production in neurons, cardiomyocytes, endothelial cells, and alveolar type II cells derived from induced pluripotent stem cells (iPSCs) of unvaccinated Brazilian centenarians who recovered from COVID-19 and young adults who had critical COVID-19. Notably, the centenarian cohort includes the oldest woman (114 years) and man (110 years) to recover from COVID-19 in South America. The cells were infected with SARS-CoV-2 (Wuhan strain and Gamma P1 variant) at a MOI of 1.0 for 48 hours. Cellular infection was assessed using flow cytometry, immunofluorescence, and RT-PCR. Cytokine production was quantified using cytometric bead array (CBA). Our data showed that the rate of cell infection by SARS-CoV-2 was similar in both groups under the same conditions. However, cells from young adults exhibited significantly higher levels of IL-6, CCL2, and CXCL8 compared to cells from centenarians, with the P1 variant inducing even more inflammation. These results suggest that centenarians exhibit a more controlled inflammatory response across various cell lineages compared to young adults, which may contribute to the less severe progression of COVID-19 observed in this group, despite their advanced age.

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PT.240

Antiviral activity of organochalcogens against Zika and Chikungunya

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Arthropod-borne diseases such as Zika and Chikungunya fever are neglected tropical diseases responsible for thousands of cases annually. Organochalcogens are synthetic compounds with multiple biological activities reported, such as antimicrobial, anti-inflammatory and antiviral. Currently, there are no drugs approved against these viruses, therefore, the aim of this work was to evaluate the cytotoxicity and the antiviral activity of 6 organochalcogens derivatives against Zika virus (ZIKV) and Chikungunya virus (CHIKV) in Vero cells. The compounds had CC_{50} varying from 303µM to 125µM. At 20µM concentration compounds 1, 4, 5 and 6 were able to inhibit ZIKV replication while 2, 3 and 4 inhibit CHIKV. Next, different concentrations of these compounds were added to evaluate the EC_{50}. The results varied from 1.65µM to 0.83µM for ZIKV and 1.2µM to 0.72µM for CHIKV. Compounds 5 and 3 had the lower EC_{50} for ZIKV and CHIKV, respectively. In time-of-drug-addition assay all compounds showed high activity at the early phase of infection, compound 1 also was able to inhibit ZIKV when pre-treatment was performed and compound 3 had activity against CHIKV in pre- and post-treatment until 12h.p.i. In adsorption assay, compounds 1 and 3 were able to inhibit ZIKV and CHIKV adsorption, respectively. Compounds 6 and 3 presented virucidal profile for ZIKV and CHIKV, respectively. The low cytotoxicity and the high activity against ZIKV and CHIKV of these organochalcogens makes them promising candidates for the development of drugs against ZIKV and CHIKV.

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PT.241

EXPERIMENTAL EVOLUTION OF ZIKA VIRUS FOR ADAPTATION IN DIFFERENTIATED GLIOBLASTOMA CELLS AND ITS PHENOTYPIC AND GENOTYPIC CACTERIZATION

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Glioblastoma multiforme (GBM) is the most aggressive among all primary tumors and it is resistant to current therapeutic approaches. A significant contributor to the resistance is the microenvironment composed of undifferentiated (stem cells) and differentiated GBM cells. Recent studies have demonstrated that Zika Virus (ZIKV) has been shown to have oncolytic activity against GBM stem cells, presenting it as a promising therapy due to its selectivity. The main goal of this project is to focus on the experimental evolution of the Zika virus to enhance its oncolytic efficacy against differentiated glioblastoma cells *in vitro*. The primary objectives include characterizing the phenotypic traits of the parental ZIKV strain and the adapted virus, in particular, the fitness in GBM cell line T98G. Other objectives include the genotypic characterization of the parental and adapted strains and associated mutations with the phenotypic traits. The phenotypic characterization of the parental and adapted ZIKV strains focuses on the analysis of the production of infectious viral particles through plaque assay and evaluation of RNA replication through RT-qPCR. The sequencing was performed using the NextSeq1000 platform. After two passages of the virus, a consistent reduction of the production of infectious particles and replication of viral RNA was observed. This decrease in viral replication suggests that the adaptation is still ongoing. Also, no mutations were identified compared to the parental lineage. Characterizing these and future adaptations might give perspectives for novel, targeted treatments for GBM.

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PT.242

MULTIPLEX PCR METHOD FOR SEQUENCING *ORTHOPOXVIRUS VACCINIA* DIRECTLY FROM CLINICAL SAMPLES AND ISOLATES

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Genome sequencing is a powerful tool for studying viruses, including *Orthopoxvirus.* However, sequencing directly from clinical samples often results in a high number of host reads, which increases reagent costs and reduces sequencing throughput. Additionally, isolating and amplifying the virus can introduce mutations after successive passages in cell culture. We present a protocol for generating complete genomes of *Orthopoxvirus vaccinia* (VACV) from Brazilian group I, using a multiplex PCR enrichment method that can be applied to various types of samples, such as swabs, crusts, and primary isolates. The protocol incorporates 105 pairs of primers that amplify approximately 2000 bp amplicons. Skin crusts were macerated with a BeadBeater, while swabs and exudates were washed in water, and the viral culture was scraped and collected. Genetic material extraction was performed using a MAGNA 96 extractor, followed by amplification via multiplex PCR with sequencing primers divided into two sets. We employed Q5 High-Fidelity DNA Polymerase for amplification. The amplicons were purified using 1x AMPureXP magnetic beads and quantified on Qubit fluorometer. The genomic library was prepared using the NEBNext Prep Set and sequenced on the Ion Torrent platform. The genomes were assembled using a hybrid approach with Geneious Prime and Minimap2. The protocol was tested on 14 samples from humans and bovines from different Brazilian regions (North, Southeast, Midwest, and Northeast), yielding 10 complete genomes with coverage ranging from 90% to 99% and depth from 670x to 1520x. Total viral reads accounted for 86% to 97% of all reads, demonstrating the protocol's efficiency.

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PT.243

A NANOPARTICULATE ADJUVANT DOES NOT INTERFERE WITH THE IN VITRO INFECTIVE CAPACITY OF *MODIFIED VACCINIA VIRUS ANKARA*

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The Orthopoxviruses are DNA viruses that cause diseases in humans and animals, with variola virus being the most notorious, responsible for smallpox. Studies using Modified Vaccinia virus Ankara (MVA) have demonstrated good safety and immunogenicity profiles in preclinical/clinical trials. However, the restrictive replication of MVA is a factor that impacts the anti-poxviral response and the response against the antigen delivered by recombinant MVA. Therefore, alternatives need to be developed to enhance immunogenicity, such as heterologous dose/boost protocols, use of co-stimulatory molecules, increasing the replication capacity of the vector, and the use of adjuvants. It is known that BSA nanoparticles associated with poly (I:C) have adjuvant and immunostimulatory potential and can consequently be used in vaccine formulations. Therefore, this study aims to evaluate if these nanoparticles can interfere if the infectivity of MVA, since this nanoparticle could be used as adjuvant in vaccine formulations. MVA was incubated with NPPI for 1 h and subsequently added to BHK-21 cells. After 48 hours post-infection, an immunostaining with anti-VACV antibody was performed and the numbers of focus forming units (FFU) were counted. The results revealed no statistically significant difference in the number of FFU between cells infected with MVA and cells infected with NPPI+MVA. These results indicate that NPPI could be used as a potential adjuvant in vaccine formulation for immunizations using MVA, as they do not interfere with the infectivity of MVA viral particles.

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PT.244

MOLECULAR EPIDEMIOLOGY OF THE CHIKUNGUNYA VIRUS IN ALAGOAS

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In recent years, the state of Alagoas has recorded an increase in Chikungunya fever cases, highlighting the need for studies to understand viral dispersion within the state. Genomic surveillance contributes to understanding viral spread, as well as to predicting and controlling new epidemics, especially in regions with epidemic potential. Thus, we conducted a retrospective and current molecular epidemiological investigation on samples positive for Chikungunya virus by RT-qPCR, diagnosed by the Central Public Health Laboratory of Alagoas. Samples from 2016 (n=5), 2022 (n=39), and 2024 (n=22) were sequenced using the MinION Nanopore (Long reads) and Illumina MiSeq (Short reads) platforms. Data analyses were performed using the Genome Detective web platform (MinION) and the ViralFlow 1.0 tool (Illumina). Both tools perform steps including raw data quality control, reference-guided genome assembly, and variant calling. We obtained 66 partial genomes, with an average coverage of 87% (Nanopore) and 77% (Illumina), and a coverage depth of 3,783x (Nanopore) and 435x (Illumina). Phylogenetic analysis demonstrated that all genomes belong to the East-Central-South-African (ECSA) lineage. The samples clustered into four phylogenetically distinct clusters. Samples from 2016 and 2024 clustered into unique distinct groups, while those from 2022 formed two clusters. For 2022, the formation of two clusters may suggest two different introductions of Chikungunya virus in that year. Our study contributes to the epidemiology and genomic surveillance of Chikungunya virus in Alagoas, as well as the generation of new sequences that may support studies at the national level.

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PT.245

REVISITING THE INTRODUCTION OF THE CHIKUNGUNYA VIRUS IN ALAGOAS, NORTHEAST BRAZIL: A NEW HISTORY?

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The first infection by Chikungunya virus (CHIKV) in the Americas was reported in 2013, followed by the initial cases in Brazil in 2014, specifically in Pará and Bahia. In Alagoas, the first reported case occurred in late 2015, though earlier cases may have been underestimated. This study aimed to reassess the introduction of CHIKV in Alagoas, providing new evidence on its entry. The study was approved by the Research Ethics Committee (No. 2.896.643/2018). We retrospectively evaluated samples from a serological survey for Flavivirus to detect CHIKV. One sample collected in August 2014 tested positive for IgM, RT-PCR, and was confirmed through virus isolation in cell culture. The viral isolate was sequenced using short reads on the Illumina MiSeq platform. Data analysis was performed with the ViralFlow 1.0 tool, and the assembled sequence was used for variant calling, genotype identification, and phylogenetic analysis. The genome had 93.9% horizontal coverage and a depth of 1341x, classifying it as the ECSA genotype. Phylogenetic analysis showed that the CHIKV sequence clustered with sequences from Bahia, suggesting that the virus was introduced to Alagoas from this state. The A226V and L210Q mutations, associated with the E1 and E2 proteins, were absent. This study provides new evidence that CHIKV entered Alagoas earlier than previously reported, underscoring the critical role of genomic surveillance in tracking viral introduction, spread, and evolution.

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PT.247

POSITIVE IMPACT OF SILYMARIN ON ALPHAVIRUS-INDUCED ARTHRITIS AND MYOSITIS IN ANIMAL MODEL: MAYARO AND CHIKUNGUNYA VIRUSES

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Mayaro virus (MAYV) and *Chikungunya virus* (CHIKV) are members of the *Togaviridae* family and Alphavirus genus, causing similar diseases such as severe joint pain and fever. Our research group is focused on investigating factors contributing to the pathogenesis of MAYV and CHIKV and identifying natural substances with potential antiviral activity. Given that joint and muscle pain are prominent symptoms and silymarin has shown potent activity against MAYV and *in-vitro* antiviral activity against CHIKV in the literature, this study aims to evaluate the effect of silymarin in an animal model of arthritis and myositis induced by these viruses post-infection. To achieve this, 6-week-old BALB/c mice were infected with CHIKV and MAYV in the right hind paw pad (10^6 PFU). Preliminary results from infected animals showed paw oedema and inflammation, both of which were reduced in those treated with silymarin. Euthanasia was performed on day 7 and 12 post-infection, and various tissues including the liver, spleen, brain, paw, quadriceps, tibial, soleus, and extensor digitorum muscles were collected. CHIKV presence was assessed by qRT-PCR, revealing no virus in the quadriceps, tibial, and extensor digitorum muscles; however, viral load was reduced in the spleen, liver and brain at both 7 and 12 days post-infection in the treated group. Viral load in the paw was diminished on day 7, while that in the soleus was reduced on day 12 post-infection, in the treated groups. The qRT-PCR results for MAYV are currently being processed by our study group. These findings support further investigation into the potential benefits of silymarin in treating MAYV and CHIKV infections.

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PT.248

PHYLODYNAMICS AND DEEP-LEARNING MODELING: DE NOVO DESIGN OF PROTEINS FOR DIAGNOSTIC DIFFERENTIATION OF DENGUE 1-4 AND ZIKA VIRUSES

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A major challenge in distinguishing among the endemic and highly circulating Dengue virus 1-4 serotypes (DENVs) and Zika virus (ZIKV) in Brazil lies in their genomic similarity, which leads to controversial and imprecise results from commercially available diagnostic kits. This cross-reactivity phenomenon directly impacts the accurate generation of epidemiological data resulting in non-specific therapies. Aiming to address this issue, we initially retrieved representative viral sequences from specialized public databases and performed deep-clustering analysis followed by phylodynamic modeling, which allowed us to identify virus-specific peptides sampled from the non-structural (NS1) and Envelope (Env) proteins of DENVs and ZIKV. Through high-Performance computing, ten peptides were submitted to de novo protein design using deep-learning algorithms and subsequently to molecular modeling through epitope-scaffolding technique, generating 30,000 backbone structures derived from RFDiffusion software. Scaffolds successfully designed to contain our target peptides were then filtered based on the minimum radius of gyration and the maximum number of contacts per residue. Sequences for these scaffolds were generated using ProteinMPNN, and their structures were modeled using AlphaFold 3 software. The final selection was based on RMSD values and the number of secondary structures. Ongoing work will focus on conducting in vitro assays to validate the potential of these scaffolds for developing target-specific and more accurate diagnostic tools, as well as exploring their potential as therapeutic candidates. If successful, these advancements could significantly improve the management and control of infections caused by Dengue and Zika viruses, reducing false positives and enhancing patient outcomes.

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PT.249

CHARACTERIZATION OF CASES OF NEUROLOGICAL DISORDERS DUE TO CHIKUNGUNYA VIRUS (CHIKV) INFECTION DURING THE FIRST MAJOR EPIDEMIC IN PARAGUAY.

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At the end of 2022 and the beginning of 2023, Paraguay faced its largest chikungunya epidemic, marked by an unusual increase in reports of neurological complications associated to chikungunya virus (CHIKV) infection. This study aimed to characterize patients with arboviral neurological infections during this epidemic. It included 69 patients with neurological disorders from a high-complexity hospital in the capital city, of which 66 were suspected of CHIKV neuroinfection, 2 of dengue virus (DENV), and 1 of both CHIKV and DENV. Given that CHIKV, DENV, and Zika virus (ZIKV) can produce similar clinical manifestations, multiplex rRT-PCR was employed to detect these viruses in cerebrospinal fluid (CSF) samples. CHIKV neuroinfection was confirmed in 48 patients, with a median viral load of 5.336 Log_{10} copies/mL. No samples tested positive for DENV or ZIKV. Most patients were children under 12 years old (23/48) and adults over 60 years old (17/48), with the latter group comprising the majority of fatalities (10/17). The most frequent diagnosis was encephalitis (31/48), with predominant clinical manifestations including fever, altered consciousness and seizures. In general, no alterations were observed in the CSF cytochemical parameters of the patients. Whole-genome sequencing using the Oxford Nanopore platform confirmed that the detected CHIKV strains belonged to the East-Central-South-African (ECSA) genotype circulating in the region. These findings reinforce CHIKV as a significant cause of encephalitis, highlighting the importance of surveillance and appropriate clinical management of these infections, especially in older adults.

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PT.250

UMT MICE AS A MODEL OF ALPHAVIRUS MAYARO INFECTION TO ELUCIDATE PATHOGENESIS

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The Alphavirus mayaro is the etiological agent of the Mayaro Fever, an acute febrile illness with symptoms of arthritis, arthralgia, and myalgia which, in some cases, can become chronic and cause sequelae. Thus, Mayaro is an emerging virus and its disease has a great capacity to cause an epidemic with major impacts on an unimmunized population, so it is crucial to understand the viral pathogenesis. To achieve this, we partially evaluated the B cells response during infection. We obtained uMT mice as a model, we infected it, and we evaluated the tropism, survival rates, and gain or loss of mice weight at different time points after Mayaro infection. Our results show that the Mayaro infection in the uMT mice did not lead to death, but caused a slight decrease in their initial weight. In earlier time points, we observed an increase in paw swelling. In addition, we carried out a rt PCR also titration essays on the tissues collected, and we found viral load in the liver, spleen, inguinal lymph nodes, heart, spinal cord, and brain. We observed a viral load peak in seven days post-infection. In later time points, it was no longer possible to find high viral rates, and the paws slightly decreased throughout fourteen days post infection. Our preliminary results documented the infection of uMT model, and implies a necessity of further investigation in humoral response against Mayaro virus, and also in the importance of other types of cells in controlling Mayaro pathogenesis.

PT.251

EVALUATION OF INHIBITION OF HEPATITIS B VIRUS, VIA SMALL INTERFERING RNA

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Introduction: Chronic hepatitis B virus (HBV) infection causes about one million deaths annually. HBV clearence is challenging due to HBV DNA persistence as covalently closed circular DNA (cccDNA), allowing viral reactivation. Interfering RNA (RNAi) emerges as alternative for chronic hepatitis treatment by gene silencing. This study aimed to design and evaluate the efficacy of RNAi lentiviral vectors targeting key HBV proteins (HBsAg, HBcAg, HBeAg) and pre-genomic RNA (pgRNA) for silencing. ***Methodology***: Three silencing vector candidates were designed to target overlapping Open Reading Frames (ORFs) from HBV genome. The candidates were constructed and tested in silico to prevent off-target effects. HEPG-2 cells were transfected with 1 µg of purified HBV genotype A1 circular monomers, followed by infection with the first lentiviral candidate (siHBV-1), targeting S/Pol genes of HBV (108 TU/mL), threedays later. HBV proteins were quantified using chemiluminescence, and HBV DNA was measured using quantitative PCR (qPCR). ***Results***: An effective silencing of HBsAg expression in siHBV-1-infected cells, with undetectable levels from the third day post-infection compared to untreated controls ($p<0.002$). Furthermore, HBV DNA was undetected by qPCR, indicating successful silencing of HBV genotype A1 in vitro. The other two lentiviral RNAi candidates (siHBV-2/siHBV-3) are being trained in HEK293T cells for future infections and their efficacy will be evaluated. Lentiviral vector-mediated RNA interference offers a promising approach for sustained suppression of HBV replication and gene expression, potentially promoting HBV clearance in chronic carriers.

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PT.252

CHARACTERIZATION OF SMALL AND LARGE-PLAQUE SIZE VARIANTS OF MAYARO VIRUS ISOLATE

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Mayaro virus (MAYV) is an arbovirus with a single-stranded positive sense RNA virus belonging to the genus Alphavirus and family Togaviridae. The ability of MAYV to recombine with other strains and a lack of RNA-dependent RNA polimerase proofreading activities may arise in diverse viral populations with heterogeneity in plaque size. Plaque morphology reflects viral replication, cell-to-cell spread, and virulence of the virus, with larger plaques often associated with increased virulence, while small plaque variants are commonly used as a selection criterion for attenuation due to their reduced virulence. Thus, this study aims to characterize two isolates of MAYV with different size plaque. Aliquots of thawed patient plasma PCR-positive for MAYV (PAC169) were inoculated in C6/36 cell culture diluted 1:10 and incubated for two hours at 28 ºC. Blind passages were done at 7, 14, 21 and 28 dpi. Plaque assay revealed a mix of small and large viral plaques. The small and large plaque variants were isolated by plaque purification and designated as MAYV-LP and MAYV-SP. Both were characterized *in vitro* to compare their virus production by kinetics growth assay in BHK-21 and Vero cells. Preliminary results showed statistically difference in MAYV-SP yield progenies up to 7×10^6 and 6×10^6 PFU/mL in BHK-21 over Vero cells after 48 and 72 hours, respectively. In conclusion, MAYV-PAC169 passaged in C6/36 cells for 4 passages generated a potential attenuated virus with a small-plaque size in a mixed population, which was isolated and had significantly delayed replication in Vero cells.

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PT.254

Arboviruses outbreak reveals co-infection of Dengue virus and Chikungunya virus in the southeast region of Minas Gerais

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Dengue virus, Zika virus and Chikungunya virus are etiologic agents of dengue, zika and chikungunya that are considered a public health problem in tropical zones. In this study, we aimed to investigate the incidence of Dengue virus, Zika virus and Chikungunya virus in the first half of 2024 throughout the southeast region of Minas Gerais. For this work, we used a quantitative research approach based on information obtained from the datasus and the Centro colaborador/UFV in the southeast region of Minas Gerais in the first half of 2024, obtained from RT-qPCR test results. During the evaluated period, 16,470 tests were performed to detect Dengue virus, Zika virus and Chikungunya virus. As a result, 1,735 and 1,654 positive results were obtained for Chikungunya virus and Dengue virus, respectively; however, no Zika virus was detected. For the positive results for Dengue virus, 1,157 were for Dengue virus serotype 1; 457 for Dengue virus serotype 2 and 37 presented co-infection for Dengue virus serotypes 1 and 2. In addition, 179 cases of co-infection were identified for Chikungunya virus and Dengue virus serotype 1, 63 cases for Chikungunya virus and Dengue virus serotype 2 and 4 cases for Chikungunya, Dengue virus serotypes 1 and 2. Here, we report the incidence of Dengue virus and Chikungunya virus in the southeast region of Minas Gerais in the first semester of 2024, providing relevant epidemiological information to develop strategies for control and prevention.

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PT.255

IN VITRO EVALUATION OF THE ANTIVIRAL EFFICACY OF OXIDIAZOLES AGAINST MAYARO VIRUS

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The Mayaro virus (MAYV) from the Togaviridae family, cause severe complications, including debilitating arthralgia and neurological disorders such as encephalitis. Its wide geographical distribution and high morbidity associated with these infections highlight the urgent need for effective antiviral treatments to mitigate their impact on global public health. In this study, we evaluated the toxicity and antiviral activity of oxadiazoles compounds against MAYV in vitro tests using Vero cells. Additionally, we sought to understand mechanisms that may contribute to the characterized inhibition profile. Among the tested oxadiazoles, YAT-02 showed promising results and was selected for further testing. The cytotoxicity (CC_{50}) of YAT-02 was determined to be 790±22 µM by the MTT assay, indicating low toxicity. Its antiviral efficacy (EC_{50}) was 0.62±0.03 µM, reducing the replication of MAYV by 50% using the Plaque Forming Units (PFU) method. The selectivity index (CC_{50}/EC_{50}) was 1274. The compound YAT-02 demonstrated a significant virucidal effect against the Mayaro virus (MAYV), inhibiting its replication in a dose-dependent manner. Notably, an extremely low concentration of 0.65 µM resulted in more than 50% inhibition, highlighting the compound's efficacy. This impressive result underscores YAT-02's ability to inactivate the viral particle at minimal concentrations, achieving over 80% inhibition at 5 µM. The in vitro results of the synthetic compound YAT-02 show its potential as a safe and effective candidate for the treatment of MAYV. Currently, there is no specific treatment available to reduce the morbidity and mortality of this disease.

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PT.256

BOVINE SERUM ALBUMIN NANOPARTICLES CONTAINING POLY (I:C) CAN ENHANCE THE NEUTRALIZING ANTIBODY RESPONSE INDUCED BY ENVELOPE PROTEIN OF *Orthoflavivirus zikaense*

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Since the *Orthoflavivirus zikaense* (ZIKV) has been considered a risk for Zika congenital syndrome development, developing a safe and effective vaccine has become a high priority. Research groups have targeted the domain III of the ZIKV envelope protein (zEDIII) as promising. Subunit antigens like zEDIII are often poorly immunogenic, needing adjuvants or delivery systems to boost immune responses. Nanotechnology has expanded substantially, offering nanoparticles as drug delivery systems to enhance antigen immunogenicity and stability. This work aims to characterize and validate the potential of a vaccine formulation composed of domain zEDIII and bovine serum albumin nanoparticles containing polyinosinic-polycytidylic acid (NPPI). NPPI were uptake in vitro by immature bone marrow dendritic cells and histological analysis of the skin of mice treated with NPPI showed an increase in cellularity. Immunization assay showed that mice immunized with zEDIII in the presence of NPPI produced neutralizing antibodies. Through the passive transfer of sera from immunized mice to ZIKV-infected neonatal mice, it was demonstrated that these antibodies provide protection, mitigating weight loss, clinical or neurological signs induced by infection, and significantly increased survival rates. Protection was further substantiated by the reduction in the number of viable infectious ZIKV, as well as a decrease in inflammatory cytokines and tissue alterations in the brains of infected mice. Taken together, data presented in this study shows that NPPI + zEDIII is a promising vaccine candidate for ZIKV.

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PT.257

MICRONEUTRALIZATION-ELISA ASSAY FOR DETECTION OF INFLUENZA A NEUTRALIZING ANTIBODIES: STANDARDIZATION AND VALIDATION

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The influenza A virus (IAV) is an important global pathogen, recognized for its role in pandemics in the last century and for its impact on public health due to seasonal epidemics. The Hemagglutination Inhibition (HI) test is used to evaluate the immune response in clinical and pre-clinical trials of influenza vaccines. However, the test shows high variability between results, hindering analysis. This study aimed to standardize and validate a microneutralization test coupled to an ELISA to complement the HI assay. Briefly, antisera against the IAV(H1N1) strain and a fixed concentration of the respective virus were diluted and incubated. After, the mix is added to a MDCKCCL-34 cells grown in 96-well plates for 24h and incubated again for 24h. The cells were fixed and subjected to ELISA assay, using anti-NP-IAV and anti-mouse HRP as primary and secondary antibody, respectively. After adding the substrate (OPD/H_2O_2), the reaction is stopped and read at 492 nm. For the standardization we studied the cell concentration, manutenance medium, diluents and the primary and secondary antibodies and its concentration. Performance for seletivity, linearity, precision and the respective acceptance criterion were stablished. In addition, the correlation between the HI and Microneuitralization/ELISA assays were also studied. Results showed a good correlation between both tests, seletivity for IAH1 virus, when analised with heterologous and homologous antiserum, linearity ($r = 0.887$), and precision intra and inter-test with CV% <20%. The test was stablished and could be used as complementary of HI assay for evaluation of the imune response to Influenza virus.

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PT.258

Evaluation of the antioxidant activity of different doses of fullerol in cell infections by Zika virus and Chikungunya virus

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The Zika virus (ZIKV) and Chikungunya virus (CHKV) are arboviruses of significant public health importance due to their high dissemination potential and associations with severe diseases. Currently, there are no approved vaccines or treatments for these infections, emphasizing the need for new therapeutic strategies. Reactive oxygen species (ROS) activation during viral infections is well-documented, prompting investigation into antioxidant compounds as potential therapies. The fulerol (C60OH [22-24]), a nanomaterial with high ROS-neutralizing capacity, has demonstrated antioxidant activity in arbovirus-infected cells.
In this study, we analyze fulerol antioxidant capacity at various doses and application intervals (concentration of [3,125nM]) in ZIKV and CHKV infections in cell models. Fulerol cytotoxicity was evaluated in VERO and MRC-5 cells, and antioxidant activity was measured using an intracellular ROS assay post-infection. Our findings revealed that fulerol showed no cytotoxic effects at tested doses and reduced ROS levels in cells infected with both viruses across all experimental groups.
While no significant difference was observed between single and multiple doses of fulerol in ZIKV-infected cells, multiple applications in CHKV-infected cells led to reduced ROS production. These results suggest that a single application of fulerol can restore redox homeostasis in tested cellular models, while multiple doses may be more beneficial in cases of pronounced redox imbalance.
Therefore, our study demonstrates fulerol robust antioxidant activity, indicating its potential as a therapeutic agent for mitigating oxidative damage associated with ZIKV and CHKV infections.

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THERAPEUTIC EFFICACY AND SYNERGISM OF CAULERPIN AND RIBAVIRIN AGAINST CHIKUNGUNYA VIRUS

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The Chikungunya virus (CHIKV), from the Togaviridae family, is primarily transmitted by the *Aedes aegypti* and *Aedes albopictus* mosquitoes. It causes high fever and severe arthralgia, with symptoms that can persist for months, leading to chronic and debilitating conditions. Currently, there are no specific antiviral treatments for CHIKV, highlighting the urgent need for new therapeutic approaches. This study investigated the efficacy of caulerpin, a natural substance isolated from algae of the genus *Caulerpa*, both alone and in combination with ribavirin, in experimental models of CHIKV infection. The objective was to evaluate the synergistic effect of caulerpin with ribavirin and the therapeutic efficacy of caulerpin. In *in vitro* experiments, suboptimal (0.5 µg/mL of caulerpin and 0.5 µM of ribavirin) and high concentrations (10 µg/mL of caulerpin and 10 µM of ribavirin) were used. The combination of the subdoses inhibited up to 99% of viral replication, demonstrating a significant synergistic effect. In *in vivo* tests, infected C57BL/6 mice were divided into groups and treated with caulerpin (9 mg/kg), ribavirin (15 mg/kg), DMSO (1%) vehicle control, and an uninfected group. After 14 days of treatment, caulerpin reduced paw edema and a histological analysis of the muscle demonstrated rare inflammatory cells in capillaries and around small caliber vessels, and it did not cause mortality. The DMSO 1% group died on the 10th day and presented inflammatory infiltration in the muscle. The results indicate that caulerpine is effective, showing promising therapeutic potential for the treatment of chikungunya, especially when combined with ribavirin.

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PT.260

Poliovirus Immunity in Barreiras, Bahia: Assessing the Impact of Vaccination Across Age Groups

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Poliovirus, the causative agent of poliomyelitis, is a highly contagious virus primarily affecting children and can lead to paralysis or death. Global vaccination efforts with the Oral Polio Vaccine (OPV) and Inactivated Polio Vaccine (IPV) have significantly reduced polio incidence. Monitoring population immunity levels is essential for ensuring vaccine effectiveness and guiding public health policies. This study aimed to evaluate the immunity against poliovirus in the population of Barreiras, Bahia, Brazil, to assess the adequacy of the current vaccination regimen and inform public health strategies. Blood samples were collected from 388 volunteers aged 0 to 87 years, along with demographic data. Serum obtained from the blood samples was analyzed using ELISA to measure specific antibodies against poliovirus type 3 (anti-PV3 IgG). The data were then statistically analyzed. The analysis revealed no significant differences in serum levels of anti-PV3 IgG concerning gender, vaccination regimen, or time since the last vaccination. However, elderly individuals exhibited significantly higher serum antibody levels compared to adults, adolescents, and children, except for those aged 0 to 5 years. The results underscore the effectiveness of the current vaccination strategy in maintaining poliovirus immunity across different age groups in Barreiras. The elevated antibody levels in the elderly suggest potential booster effects or previous exposure. These findings highlight the need for ongoing surveillance and further research to understand the immune landscape, guiding future vaccination policies and ensuring sustained poliovirus control.

Keywords: Poliovirus immunity, Vaccination, Public health policy, ELISA, Age-related immunity

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PT.262

Trends in Dengue Serological Profile During the 2024 Epidemic: Observational Study of Hospitalized Patients in Belo Horizonte, Minas Gerais - Brazil

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Dengue is an arbovirus transmitted mainly by mosquitoes and manifests as a significant global health concern, affecting millions annually. Amid the 2024 epidemic in Brazil, where over 2,500,000 probable cases and 2,000 confirmed deaths have been recorded by May, Minas Gerais emerges as the second most affected state. This work investigated the serological profile (IgM and IgG) of dengue patients with warning signs who were hospitalized at the Eduardo de Menezes Hospital during the infection. Over the period from March to May 2024, blood samples were collected daily from 1,019 suspected dengue fever cases. These samples underwent processing, followed by simultaneous analysis utilizing Panbio™ Dengue IgM Capture ELISA (Abbott) and Anti-Dengue Virus IgG ELISA (Euroimmum). Subsequently, indices were computed, and data were subjected to descriptive statistical analysis alongside chi-square testing. Of the analyzed samples, 80% tested positive for IgM, with 18% yielding negative results, while 92% tested positive for IgG, with 6% negative. Ongoing investigations include the kinetics of patient antibodies and a comparative analysis between hospitalized and non-hospitalized cohorts. Given the ongoing nature of the epidemic, sample collection remains underway. This study underscores robust patient immune responses to dengue infection, as expected. Furthermore, this study presents trends in the presentation of dengue during the 2024 epidemic, which may help to understand this disease better and improve future disease management strategies.

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PT.263

Covid-19 Pandemic Shifts Clinical and Epidemiological Patterns of Acute respiratory distress syndrome in the State of Bahia, Brazil: A comparative analysis pre- and post-Covid-19 Pandemic

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In 2019, the SARS-CoV-2 emerged, rapidly spreading worldwide, and posing an unprecedented public health threat. Moreover, there is evidence that the Covid-19 pandemic has impacted community respiratory virus activity. In this context, in order to analyze the profile of ARDS and severe respiratory disease before and during Covid-19, we collected and analyzed data from all ARDS/severe respiratory disease cases (with positivity for viral infections by PCR) in Bahia State during 2019 and 2020 available on the Epidemiological Surveillance Information System of respiratory viral agents (SIVEP-Gripe). ARDS/severe respiratory disease cases were categorized into four groups: Influenza group (FLUAV), respiratory syncytial virus group (RSV), Covid-19 group, and other respiratory viruses (positive for all other respiratory virus tested, OV). In relation to the number of notifications, it was found that in 2020 the total number was 23 times higher than in 2019. The ARDS/severe respiratory disease cases reported in 2020 were typically older than those in 2019 ($p<0.0001$). Circulation of SARS-CoV-2 also shifts clinical characteristics of ARDS caused by FLUV and RSV. With the exception of diarrhea, we observed an elevated odds ratio for other symptoms reported in 2019 compared to 2020. Furthermore, notified cases before Covid-19 pandemic were more likely to need ventilatory support than those during Covid-19 pandemic: FLUV (OR 2,942; $p<0.0001$), RSV (OR 3.147; $p=0.0086$), and OV (OR 2.641; $p=0.0003$). Together, our data suggest that SARS-CoV-2 circulation shifted the clinic-epidemiological profile of other respiratory viruses in Bahia State, Brazil, especially for FLUV and RSV.

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PT.264

Efficacy of Hyperimmune Equine Anti-SARS-CoV-2 Serum in Immunocompetent and Immunosuppressed Hamsters: Evaluation of Monotherapy and Combination Therapy

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Introduction: In the context of COVID-19, alternative therapies remain crucial, especially for immunosuppressed individuals. Previous work produced industrial-scale hyperimmune heterologous anti-SARS-CoV-2 serum through horse immunization with inactivated virus. This process yielded a bulk concentrated serum containing the F(ab')2 fragment, which demonstrated effective viral neutralization in vitro assays.
Objective: This study aims to evaluate the efficacy of this serum in preclinical trials to determine its potential as a therapeutic agent.
Methods: Cytotoxicity and efficacy tests were performed using the plaque reduction neutralization test (PRNT), along with safety and pharmacokinetic assessments in both immunocompetent and immunosuppressed hamsters, including combination with corticosteroids.
Results: The serum exhibited potent in vitro neutralization capacity (PRNT), successfully halting viral replication. Compliance with Brazilian Pharmacopoeia standards was confirmed through pyrogen tests on the anti-SARS-CoV-2 serum in rabbits. Safety and decay kinetics studies in hamsters supported a recommended dose of 10 mg/kg. ELISA tests detected anti-SARS-CoV-2 antibodies up to 96 hours after a single serum dose. Efficacy evaluation in hamsters showed significant benefits, particularly in immunosuppressed animals, with viral RNA load quantifications by qRT-PCR and immunohistochemical analyses of lung tissue corroborating these data. Administration of methylprednisolone as monotherapy or combined with the serum showed benefits regarding lung histopathological scores.
Conclusion: The findings demonstrate that anti-SARS-CoV-2 serum can significantly inhibit viral replication and offers a promising therapeutic option, particularly under immunosuppressive conditions. These results suggest the potential for broader application in human medicine, pending further clinical trials to validate efficacy and safety in human patients.

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DEMOGRAPHIC AND PHYLOGEOGRAPHIC DYNAMICS OF SARS-COV-2 XBB-FLIP VARIANTS PREDOMINANT IN BRAZIL DURING THE SECOND HALF OF 2023

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The SARS-CoV-2 Omicron XBB lineages carrying mutations S:F455P and S:L456P comprises highly immune-evading viral variants, defined as XBB-FLip, that disseminated globally throughout 2023. In this study, we analyzed 14.068 SARS-CoV-2 complete genome sequences collected in Brazil between January 2023 and March 2024 to reconstruct the space-time dynamics and epidemiologic impact of XBB-Flip dissemination in the country. Our findings indicated that XBB-FLip outcompeted other XBB variants, becoming the predominant lineages between July and December 2023, a pattern observed across all Brazilian regions. In May, some XBB-FLip acquired an additional amino acid substitution in the Spike (S:A475V), which provides greater immune escape and restores ACE-2-binding receptor affinity. The main XBB-FLip+S:A475V sub-lineages were JD.1.1 and all its descendants, as well some GK.1* descendant lineages such as GK.1.2.1, GK.1.8, GK.1.10*, GK.1.11* and JD.1.1.8. A notable presence of genomes carrying mutations in the SD1/SD2 region of the Spike protein was also observed. The study emphasizes the relevance of reconstructing the phylogeographic and demographic dynamics aiming SARS-CoV-2 genomic surveillance to monitor its continuously changing composition across national territory.

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PT.266

The effectiveness of multiplex assay viral detection in identifying SARS-CoV-2 Variants of Concern in 2021 in the city of Guarulhos, Sao Paulo.

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The COVID-19 pandemic was initiated by the rapid spread of the etiological agent SARS-CoV-2. Highly pathogenic coronavirus, like SARS-CoV-2, can develop into severe acute respiratory illness in adults, compromising lung tissue and, in some individuals, progressing to death. Throughout the COVID-19 pandemic, new variants of interest (VOI) and concern (VOC) of SARS-CoV-2 emerged, identified, and deposited in GISAID, Nextstrain, and Pango. Many variant mutations were present in the Spike (S) structural protein that affect both tropism and immune evasion and are, therefore, the primary gene for epidemiological surveillance and diagnosis. In this context, this study compared the viral load in nasal swab samples and investigated the frequency of these variants during the pandemic period of SARS-CoV-2. For this purpose, 138 samples were collected between January and October 2021 in Guarulhos City, Sao Paulo state, Brazil. The samples were amplified for the N and RdRp targets and the TaqMan SARS-CoV-2 Mutation Assays multiplex kit for variant detection. Our results show that a local outbreak was initiated and sustained by the frequent importation of a wide diversity of SARS-CoV-2 lineages and variants. These results demonstrate that 90% of the viral lineage during this period was the VOC Gamma in Guarulhos, Sao Paulo. Surprisingly, the data indicates differences in viral load among individuals and variation in the SARS-CoV-2 targets studied, but a small proportion of variability.

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PT.268

THE GENETIC INFLUENCE OF THE IFNL4 POLYMORPHISM IN HBV AND HDV INFECTIONS

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Viral hepatitis is a group of infectious diseases that represent a significant public health problem worldwide. Hepatitis B is more widely distributed than hepatitis Delta, but both are endemic in the Brazilian Western Amazon. Co-infection with the Delta hepatitis virus (HDV) results in more severe conditions, progressing to cirrhosis and hepatocellular carcinoma (HCC), where human genetics may be associated with the clinical progression of the disease. Using human genotyping by qPCR (rs12979860 of the IFN-λ4 gene. The groups were stratified into: monoinfected (HBV) and coinfected (HDV) from patients followed up by a clinical team in Rondônia, with 23 control individuals without liver disease,109 HBV infected and 27 HDV. The study comprised 80.14% (109/136) monoinfected and 19.85% (27/136) coinfected. Analysis of the genotype frequency showed that the CT genotype was representative in the control group (52.18% (12/23)), HBV monoinfected patients (46.79% (51/109)) and HDV co-infected patients. The frequency of genotype distribution correlated with the degree of fibrosis in HBV patients and the CC genotype was the most predominant (F3/F4), unlike the HDV cohort for CT and TT genotypes. When the allele frequency was analyzed, the T genotype was more prevalent in advanced patients (F3/F4) with HDV. The study found a predominance of the heterozygous genotype, with the T allele being the most identified in HDV patients with severe fibrosis.

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PT.269

ANALYSIS OF NEUROLOGICAL AND ENDOCRINAL COMPLICATIONS IN PATIENTS WITH VIRAL HEPATITIS IN THE WESTERN AMAZON OF BRAZIL

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Viral hepatitis is related to inflammation of the liver and is considered a global public health problem that affects millions of people, causing significant morbidity and mortality, as well as contributing to extrahepatic alterations such as hepatic encephalopathy. We analyzed 1,089 medical records of patients with viral hepatitis (1993 to 2024) among 8,103 patients registered and followed up. The study population included 2.20% (24/1,089) of infected with HAV, 61.89% (674/1,089) HBV, 27.73% (302/1,089) HCV, 5.23% (57/1,089) HDV, 0.64% (7/1,089) HAV/HBV, 2.11% (23/1,089) HBV/HCV, 0.18% (2/1,089) HDV/HCV. The neurological alterations found were hepatic encephalopathy, anxiety, depression, panic disorder, bipolar disorder, as well type 2 diabetes mellitus, the main endocrine disease. Among HAV-infected patients, 4.16% (1/24) had neurological disorders with no signs of endocrine alterations, followed by HBV monoinfected patients, where 7.41% (50/674) had neurological disorders and 7.71% (52/674) endocrine disorders. Among HCV patients, 8.94% (27/302) had neurological disorders and 12.91% (39/302) endocrine disorders; HBV/HCV 13.04% (3/23) had neurological disorders and 8.69% (2/23) endocrine disorders, and finally HBV/HDV 22.80% (13/57) had neurological disorders and 7.01% (4/57) endocrine disorders. The results suggest that patients with HDV had a higher prevalence of neurological disorders 22.80%. Endocrine disorders were observed in 12.91% of patients with HCV. These data highlight the importance of evaluating the extra-hepatic manifestations mentioned for a better understanding and scientific contribution to the cases in the Brazilian Western Amazon.

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PT.270

EVALUATION OF THE SUSCEPTIBILITY OF RESPIRATORY SYNCYTIAL VIRUS ESCAPE MUTANTS TO PALIVIZUMAB USING “IN VITRO” MICRONEUTRALIZATION TEST.

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Respiratory Syncytial Virus (HRSV) is a pathogen of great importance for children, being the main cause of acute respiratory infections and one of the main causes of hospitalization and death in children under 5 years of age. The only available treatment is prevention with the monoclonal antibody Palivizumab. HRSV strains with mutations in the Palivizumab binding site have shown resistance to the drug. Little is known about the prevalence of these mutations in clinical samples, despite their potential importance in viral pathogenesis. The present study selected 430 clinical samples from children not treated with Palivizumab and who were positive for HRSV. Circulating Strains with mutations in F protein, more specifically related to potential resistance in the Palivizumab binding epitopes, were identified. Among these, 11 samples were separated that presented the N276S mutation, being all from genotype A_ON1. Using in vitro microneutralization tests, the capacity of the mutation found to generate resistance to the drug was evaluated. As a result of this research, all the samples tested were neutralized by Palivizumab, concluding that the mutations found do not confer resistance to the monoclonal antibody.

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VACCINATION INDUCES CROSS-NEUTRALIZATION RESPONSE AND HIGHER NEUTRALIZATION TITERS IN SARS COV-2 GAMMA-INFECTED INDIVIDUALS

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The worldwide circulation of SARS-CoV-2 represents a threat to public health since new variants could emerge by escaping the populational-acquired immune response. Understanding the dynamics of neutralizing humoral response in distinct scenarios will contribute to vaccination strategies that potentially interrupt the continuous circulation of SARS-CoV-2. We have characterized by VSV-based pseudovirus neutralization assays the neutralization response of individuals infected during the Gamma wave in Rio de Janeiro-Brazil against the main VoCs (Gamma, Delta and Omicron-Ba.1) and the Wuhan virus. Our results demonstrate that 13 of 14 individuals infected without previous vaccination responded against Wuhan, Gamma and Delta with intermediary neutralization titers (>log-2 </= log-3). Besides, Gamma infection was enough to produce a similar response against Delta and establish a medium to long-term response against these variants, except for BA.1. In both groups of infected individuals previous to vaccination and vaccinated individuals previous to infection, the neutralizing responses against Wuhan, Gamma, and Delta increased 1-2 log10 (3 of 4 individuals in both groups). However, for individuals that were infected prior to vaccination, BA.1 neutralization responses were at the same levels as Gamma and Delta, but it could not do so when vaccinated first. In conclusion, Gamma infection usually does not elicit neutralizing response against BA.1, but it can lead to equivalent levels to Delta. Also, hybrid immunization made by vaccination before or after infection showed a wider response against Wuhan, Gamma and Delta variants and was more likely to respond against Ba.1.

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PT.273

SYNTHETIC ACRIDONES AGAINST MAYARO VIRUS

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Mayaro fever is caused by the Mayaro virus (MAYV), which belongs to the Alphavirus genus within the Togaviridae family. This arboviral disease presents various nonspecific clinical symptoms that complicate the acquisition of a definitive diagnosis. Currently, there is no licensed vaccine or drug available for the treatment of this disease. In an effort to develop a treatment alternative for Mayaro fever, this study investigates the antiviral activity of two synthetic molecules derived from acridones (DAC1 and DAC2) as potential antiviral agents against MAYV. To achieve this, *in vitro* assays to assess the cytotoxic activity and anti-MAYV activity of these molecules were carried out in Vero cells using the MTT colorimetric method. DAC1 exhibited a Selectivity Index (SI) of 3.1, whereas DAC2 showed an SI of 23. To understand the antiviral mechanism of these molecules, pre-infection, post-infection, simultaneous, and virucidal assays were performed using the plaque reduction method. Neither molecule demonstrated virucidal activity or protective effects prior to MAYV infection. However, DAC2 exhibited a viral replication inhibition rate of approximately 90% in both simultaneous and post-infection treatments within a 24-hour period. Additionally, within a 48-hour period, both molecules reduced MAYV replication by around 70% in both simultaneous and post-infection treatments. Our data suggest that the antiviral action of acridones DAC1 and DAC2 occurs during the replication stages after MAYV entry into the host cell. Further studies are ongoing to confirm the precise site of action of these molecules.

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HEPATITIS D VIRUS GENETIC VARIABILITY AND SPATIO-TEMPORAL ANALYSIS IN BRAZILIAN WESTERN AMAZON REGION

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Brazilian Amazon region is highly endemic area for Delta hepatitis and has a complex territory and areas that are difficult to access for certain populations. In this scenario, the dynamics of HDV transmission among individuals living in these locations is extremely important for establishing the route of transmission. The aim of this study was to carry out a molecular analysis of hepatitis D virus (HDV) to determine the spatio-temporal dynamics of the cases. From 2010 to 2023, 35 patients were recruited from the Viral Hepatitis Outpatient Clinic of the Rondônia Tropical Medicine Research Center. They gave serum samples that were submitted to conventional PCR to amplify the complete HDV genome followed by nucleotide Sanger method. HDV genotype was determined using maximum likelihood phylogenetic reconstruction. A Skygrid coalescent approach with a Relaxed Random Walks phylogeographic model was used for the spatio-temporal analysis. As results, most of individuals were males (21/35) and a median age of population was 39 years. HDV-3 was identified in 100% (35/35) of the samples. The tMRCA was estimated in 1824 and a rate of 8.2×10^{-4} substitutions/site/year was found. The analyses showed that HDV possibly entered Brazil in 1820, in the state of Amazonas, and then spread initially to other Brazilian States, such as, Acre and Rondônia. Major migration events were found from 2010 onwards, from the state of Acre to other locations. This study suggests more than two centuries of HDV-3 evolutionary history, involving complex transmission routes in different locations in Brazil.

Financial support: Fiocruz/Rondônia; FAPERO; INCT-EpiAmo/CNPq.

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EPIDEMIOLOGICAL EVALUATION OF HEPATITIS E IN PATIENTS MONOINFECTED WITH HBV AND CO-INFECTED WITH HDV IN WESTERN AMAZON

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Viral hepatitis is a global public health problem. Hepatitis E virus (HEV) is described as an acute, self-limiting viral hepatitis that can become chronic in immunocompromised patients. However, HEV still remains neglected and underreported. Data from the Ministry of Health show that, between 2000 and 2021, there were 1,705 reported cases of EVH in Brazil, with 216 occurring in the northern region. HEV, when present as co-infection or super-infection in patients monoinfected with hepatitis B virus (HBV) or co-infection with hepatitis Delta (HDV), can influence the progression of the disease. This study evaluated 91 samples from HBV monoinfected and HDV co-infected patients selected between 2018 and 2023, for the evaluation of total anti-HEV antibodies, using the HEV Ab ULTRA kit. Of the 91 patients selected, 45/91 (49%) were monoinfected with HBV, 28/45 (62.2%) were male and 17/45 (37.8%) female, with a mean age of 46 years. Those co-infected with HBV/HDV were 46/91 (51%), 29/46 (63%) male and 17/46 (37%) female, with an average age of 42 years. Among the results obtained, 9 (9.9%) were positive for total antibodies, of which 7 (77.7%) HBV and 2 (22.2%) HBV/HDV. This study highlights the importance of epidemiological surveillance and the need for further research into co-infection between HEV, HBV and HDV, as well as the molecular characterization of cases to detect the viral genome.

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Phylogenetic analysis and genotyping of Dengue virus serotypes 1 and 2 (DENV-1 and DENV-2) in Brazil: a 32-year study using GenBank database complete genome sequences

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The aim of this study is to perform the phylogenetic analysis of DENV-1 and DENV-2, based on complete genomic Brazilian sequences available in GenBank. Forty-five DENV-1 sequences and 80 DENV-2 sequences (1990- 2022) were submitted to phylogenetic analysis using MEGAX program and genotyped using DENV Typing Tool. Two main clades of DENV-1 were observed, one containing sequences from 1990-2010, and other from 2000-2014. The largest sub-branch, with 17 sequences, had no lineages specified. The second sub-branch is subdivided into two lineages, F (only 1 sequence from 2008) and E (2010 and 2011) of genotype V. The second main branch contains only lineage A. A temporal correlation was observed between the sequences which grouped, even within the same lineage, notably two sub-branches of lineage A (one between 2000 and 2007, and another between 2006 and 2014). Additionally, between 2010-2011, DENV-1 lineages A and E circulated concomitantly. The DENV-2 analysis demonstrates two main branches, correlated to the two DENV-2 genotypes found in Brazil: genotype III, which has been circulating in the country since the 1990s, and genotype II. Within genotype III, a temporal grouping is observed: between 1995-2002, genotype III lineage B circulated; sequences between 2000-2006 formed another branch, between lineages C and B, but without a defined lineage. Between 2017-2019, the C1.1 genotype circulated. The remaining sequences did not have a determined lineage, which suggests the need for a thorough review of genotyping tools. The only sequence of genotype II dates from 2022, demonstrating its recent introduction and circulation in Brazil.

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NEW SYNTHETIC AMINOADAMANTANE DERIVATIVES INHIBIT MONKEYPOX VIRUS INFECTION in vitro

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The Orthopoxvirus monkeypox (MPV) re-emerged on a global scale in 2022, causing an outbreak classified as a public health emergency of international concern by the WHO. The antivirals used against MPV are scarce, highlighting the need for new therapeutic approaches, including the evaluation of the repositioning of already established drugs. In this context, this study evaluated the antiviral activity of Aminoadamantane (AMA) and five new synthetic derivatives as potential candidates for treatment of MPV infection. Here, the cell viability and antiviral activity against MPV (including the main mechanisms of action) of AMA and its derivatives were evaluated. The compounds evaluated showed low toxicity in BSC-40 cells and exhibited in vitro activity against MPV. The IS values were higher than 100, indicating the selectivity of the compounds tested, specially LV1 (IS >14084). Concerning the mechanism of action, LV4 has acted in the stage of viral adsorption to celullar receptors, and the LV5 derivative proved to be effective in the stage of viral penetration into the host cell. Finally, all the compounds tested acted in the early and late stages of the viral multiplication cycle, with emphasis on derivatives LV1, LV2 and LV5, which reduced the viral load up to 100 times. All in all, AMA and synthetic derivatives (mainly LV1) proved to be potential antiviral candidates against MPV, making them putative hits for antiviral therapeutic development.

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DEVELOPMENT OF A MELTING TEMPERATURE-BASED MULTIPLEX REAL-TIME RT-PCR (SYBR GREEN) FOR DETECTION OF SARS-COV-2 AND INFLUENZA A VIRUSES, INCLUDING RNASE P AMPLIFICATION

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Most of the multiplex diagnostic assays described for simultaneous and/or differential detection of severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) and influenza A virus (IAV) are probe-based real-time RT-PCR, which limits their accessibility to many laboratories or diagnostic institutions. Herein, we developed a melting temperature (Tm)-based multiplex real-time RT-PCR assay (one-step reaction, stained by SYBR Green) targeting SARS-CoV-2 and IAV detection, including endogenous control (RNAse P) amplification, in order to facilitate access to differential diagnostic strategies. Initially, we analyzed different and well-established primers sets for amplification of SARS-CoV-2, IAV and RNAse P whose amplicons could be reliably differentiated by Tm. The multiplex was then standardized by optimizing the reaction mix and cycling conditions performed in a final volume of 10µL, using the Power SYBR™ Green RNA-to-CT™ 1-Step kit. The performance of our assay was evaluated by testing different clinical samples previously identified as negative (n = 20) or positive for SARS-CoV-2 (n = 30) and IAV (n = 15). In our multiplex, the different Tm for SARS-CoV-2 (77.19-77.4°C), IAV (80-80.38°C) and RNAse P (81.13-82.17°C) allowed the differentiation of all assay targets. In addition, all samples previously identified as positive or negative for SARS-CoV-2 and IAV maintained their status after being analyzed in our multiplex assay. In conclusion, we developed a Tm-based multiplex real-time RT-PCR assay for SARS-CoV-2 and IAV, including RNAse P detection, to expand access to differential diagnosis in resource-limited laboratories.

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CHARACTERIZATION OF IMMUNODOMINANT HLA-A*02-RESTRICTED EPITOPES IN HTLV-1 TAX PROTEIN: NEW FINDINGS FROM N-TERMINAL, CENTRAL, AND C-TERMINAL REGIONS

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Human T-lymphotropic virus (HTLV-1) is a globally spread retrovirus that causes HAM/TSP and ATL diseases, with no effective vaccine or immunotherapy available. Understanding the immunological role of Tax is crucial for developing preventive and therapeutic strategies against HTLV-1-associated diseases mentioned. In this sense, the presente study sought to verify the immunodominance of the N-terminal, Central, and C-terminal regions of HTLV-1 Tax using novel, cutting-edge peptide microarray. In silico predictions conducted identified successfully 9mer peptides along Tax restricted to the HLA-A*02 haplotype, assessing their potential to induce pro-inflammatory (IFN-γ) and regulatory (IL-4) cytokines. Results showed significant dose-dependent reactivity for HLA-A*02 haplotype across all Tax regions, with specific hotspots. Peptides from all three regions were identified and exhibited up to 1000 times more reactivity than Tax^{11-19} immunodominant peptide at lower HLA-A*02 concentration. However, at higher concentrations, Tax^{11-19} showed similar or superior reactivity. The in silico analysis revealed a higher frequency of IFN-γ-inducing peptides in the N-terminal portion, while the C-terminal portion had more IL-4 inducers. These findings highlight the potential of new Tax immunodominant epitopes beyond the canonical Tax^{11-19}, providing valuable insights for designing immunotherapeutic strategies for HTLV-1 chronic diseases.

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FREQUENCY DYNAMICS OF HIV-1 DRUG RESISTANCE MUTATIONS: A DECADE-LONG OVERVIEW USING DATA FROM THE BRAZILIAN NATIONAL GENOTYPING NETWORK

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A remarkable strategy for managing HIV infection is through antiretroviral therapy (ART). However, a significant barrier to the success of ART is the emergence of drug resistance mutations (DRMs), which can be monitored through genotyping tests. Brazil represents an important epidemic region of HIV and has one of the biggest public ART programs globally. Our study aims to analyze the frequency dynamics of DRMs over 10 years of the public genotyping service in Brazil and to understand populational factors that might impact DRM emergence. We analyzed 43,480 sequences from individuals undergoing treatment (pol gene of interest in ART), provided by the Brazilian National Genotyping Network, sampled between 2008-2017 across all regions of Brazil. The mutations were identified using the Stanford HIVdb Program tool and the output data analyzed with R software. Our results show that 82.4% of population study presented at list one class of DRM and 65.5% presented multidrug resistance. We also observed a decline in the overall DRM frequencies over the years, except for an increase in the K65R and S68G reverse transcriptase (RT) mutations. Additionally, the years 2013 and 2015 marked significant declines in common RT mutations, such as M184V/MV (74.4% to 43.9%) and M41L (31.7% to 6.1%). These dynamics correspond with updates to ART guidelines over the years. Our results highlight the benefits of updating treatment protocols in reducing the populational overall frequency of DRMs, but also underscore the need for careful monitoring of specific DRMs that could affect the effectiveness of ART in treatment baselines.

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EARLY DETECTION OF IGM AGAINST CHIKV IN HUMAN SERUM SAMPLES NEGATIVE VIRAL RNA FROM EPIDEMIC REGION OF ALAGOAS STATE, BRAZIL

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Chikungunya virus (CHIKV) is an arthritogenic Alphavirus belonging to the Togaviridae family that infects humans through vectors of the genus Stegomyia. It is an emerging arboviral disease in Brazil with characteristics similar to dengue-like viruses, causing febrile illness. The first cases were detected in 2014, and the virus has become endemic in the country in recent years. Molecular diagnosis is performed using RT-qPCR for viral RNA detection. However, factors such as sample quality, low viral load, and the presence of co-infections can lead to non-detectable results. The objective of this study was to conduct serological research for IgM Anti-CHIKV antibodies in samples from patients with up to five days from the onset of symptoms, negative for RT-qPCR, and from regions with CHIKV incidence in Alagoas. A screening of serum samples obtained from LACEN-AL between January and July 2024 was performed using the ELISA assay with the commercial Anti-Chikv IgM kit (EUROIMMUN®), following the manufacturer's recommendations. Out of the 174 samples analyzed, 114 were negative, 31 were borderline, and 29 (16.66%) tested positive, from the municipalities of Atalaia (16) and Viçosa (10). The identification of positive IgM samples in patients with symptoms of CHIKV confirms infection in the acute phase of the disease, especially after the peak of viremia where RT-qPCR may fail. Furthermore, it demonstrates the importance of epidemiological surveillance and different diagnostic strategies for monitoring cases and distinguishing between other arboviral diseases.

Keywords: Chikungunya, IgM antibodies, Alagoas

Financial Support: CNPq, FAPEAL, CAPES.

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PREVALENCE AND VULNERABILITY IN THE CONTEXT OF HEPATITIS A INFECTION AMONG MEN WHO HAVE SEX WITH MEN, BELÉM, PARÁ, BRAZIL

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Hepatitis A is an infectious disease caused by the Hepatitis A virus (HAV). Although this virus is transmitted mainly through the fecal-oral route, the disease is considered a sexually transmitted infection in men who have sex with men (MSM). The objective of the study is to evaluate the prevalence of hepatitis A and vulnerability among MSM in the city of Belém, Pará, Brazil. This is a cross-sectional, descriptive and analytical study, using a qualitative and quantitative approach, which aimed to evaluate individuals (MSM) between the years 2021 and 2022. 177 individuals were interviewed and samples were collected from 98 individuals for testing for total anti-HAV and anti-HAV IgM. The predominant age group was 25 to 35 years (39.54%), with 76.83% black and mixed race, 76.83% had completed high school, 81.92% were single, 44.7% declared to be homosexual and family support was reported by 43.6% of participants. Regarding the type of anal sex, it was revealed that 67.23% were active and passive, 10.73% claimed to be only active and 16.38% revealed to be only passive. More than half of the population evaluated (62.71%) stated to perform oroanal sex and 48.58% digit-oroanal sex. Of the 98 individuals, 61 (prevalence of 62.24%) tested positive for total anti-HAV marker, with 37.76% of the population being vulnerable to HAV infection. It is concluded that the susceptibility of the MSM subgroup and the understanding of their particularities still constitute a great challenge to be faced in all spaces where risk conditions for HAV acquisition are present.

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VIRAL GENOMIC SURVEILLANCE IN BRAZIL INTO THE ALERT-EARLY SYSTEM OF OUTBREAKS WITH PANDEMIC POTENTIAL (AESOP)

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Effective and innovative surveillance strategies for the early detection of pathogens are essential for preparedness and guiding control measures. This study aims to molecularly determine viral and bacterial outbreaks. Based on an alert signal of increase of Influenza-like illness (ILI) from the local epidemiological team in a specific location without an associated virus, we began collecting 100 samples from sentinel units for influenza-like illness over the course of a week. Real-time RT-PCR was performed for 9-pathogen respiratory panel (Influenza A/H1, A/H3, and B; RSV-A and B; SARS-CoV-2; Rhinovirus; Adenovirus; and Metapneumovirus), followed by targeted enrichment high-throughput sequencing using the Respiratory Pathogen ID/AMR Enrichment Panel (RPIP). Three alert signals (AS1-AS3) were actioned: two in Rio de Janeiro (EW29/2023, AS1, and EW10/2024, AS3) and one in Aracaju, Sergipe (EW31/2023, AS2). The real-time RT-PCR provided for the epidemiological team a rapid initial report to understand the scenario. Pathogens were identified in 48%, 22%, and 60% of cases, respectively. Nine pathogens were recovered in single detection, with 23% Rhinovirus and 7% SARS-CoV-2 prevalence. Co-detections were 2% of cases. However, 40% to 78% of cases were negative by RT-PCR. In general, the RPIP corroborated the detection by RT-PCR, Rhinovirus A and Adenovirus C (AS1); human Cytomegalovirus, Herpes simplex virus, and RSV (AS2); and Rhinovirus C (AS3) were identified. Among bacterial pathogens, *Haemophilus* parainfluenzae, Streptococcus pneumoniae, and Staphylococcus aureus were prevalent. Early detection of pathogens is crucial for effective outbreak response, highlighting the need for robust surveillance systems and increased investment in Brazilian public health policy.

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RE-EMERGENCE OF DENGUE VIRUS SEROTYPE 3 (DENV-3) IN NORTHWESTERN SÃO PAULO, BRAZIL

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In dengue hyperendemic regions, the evolution of the virus is marked by frequent virus introduction/reintroduction and clade replacement events, occasionally linked to an epidemic outbreak. Thus, molecular and genomic surveillance of circulating dengue viruses (DENV-1-4) strains is crucial for public health preparedness and response efforts for the disease. We conduct active DENV molecular surveillance on samples from patients with acute febrile illness combined with next-generation sequencing and phylogenetic analyses. Since late 2023, we have characterized twelve autochthonous dengue virus serotype 3 (DENV-3) cases in São José do Rio Preto (SJRP), Northwestern São Paulo, Brazil. The genomes clustered within genotype III closely related to DENV-3 genomes recently (2022-2023) identified in Puerto Rico, Florida, and the Caribbean. The last outbreak of DENV-3 in Brazil and SJRP occurred more than 15 years ago (2007). The DENV3 genomes identified in 2023-2024 belonged to a new lineage of GIII, in a different monophyletic clade from those DENV3 strains circulating during the 2000s in Brazil. Therefore, the introduction of a new lineage of DENV-3 genotype III in São Paulo is worrying, as the state has high human mobility and population density combined with a naïve population to this serotype. Our results demonstrate the resurgence of DENV-3 in the region since 2009, raising concerns about a potential epidemic. We stress the importance of continuing active surveillance of arboviruses to detect new virus introductions and advocate for sustainable control strategies to mitigate outbreaks in regions with a high epidemic potential.

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NEUTRALIZING ANTIBODYS AND IGG TITERS IN INDIVIDUALS VACCINATED WITH BOOSTER DOSES AGAINST SARS-COV-2

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The levels of neutralizing antibodies (nAb) induced by the SARS-CoV-2 vaccination decreases over time, so boosters was required to restore the nAb levels. The aim of this study was to quantify nAb and IgG titers after boosters doses. The population studied consisted of 25 healthcare workers (CoronaVac/ChAdOx1) who received BNT162b2 (first booster) and BNT162b2 or Ad26.COV2.S (second booster). nAb titers were determined with SARS-CoV-2 nAb ELISA kit (≥ 20% inhibition) and IgG titer with a in-house ELISA (absorbance < 0.1). The mean age of the participants was 63,0±4,4 years, the majority were female (68%) and reported no prior Sars-CoV-2 infection (53%). Those vaccinated with ChAdOx1+BNT162b2 (n=9) had a shorter period between vaccination and recruitment (mean: 28±3.5 weeks) compared to CoronaVac+BNT162b2 (n=16) (mean: 32±2.9 weeks) ($p<0.05$). After the first booster vaccination, 100% of participants had detectable nAb [geometric mean titer (GMT): 223.1±1.0] and IgG titers (mean: 1785±2705). Those vaccinated with CoronaVac+BNT162b2 (GMT: 281.0±1.0) had higher nAb values than those vaccinated with ChAdOx1+BNT162b2 (GMT: 126.0±1.6) ($p<0.05$); the same was observed for IgG titers (mean: 2040±2336 vs. mean: 640±762) ($p<0.05$). After the second booster, the nAb levels (GMT: 460.4±4.3) increased compared to the first booster ($p<0.05$). No differences were found for IgG. In summary, the second booster vaccination significantly increases the nAb levels. Individuals who were first vaccinated with CoronaVac and then with BNT162b2 had higher nAb and IgG titers than individuals who received ChAdOx1.

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SUBPOPULATIONS OF MEMORY T LYMPHOCYTES SPECIFIC TO SARS-COV-2 VARIANTS IN INDIVIDUALS VACCINATED WITH BOOSTER DOSES

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In addition to humoral immunity, vaccination also triggers a cellular immune response. We characterized the phenotype and function of memory T lymphocytes from volunteers vaccinated against SARS-CoV-2. Twenty-five volunteers who had received CoronaVac or ChAdOx1 and a first (BNT162b2) and a second (BNT162b2/Ad26.COV2.S) booster vaccination were included. Peripheral blood mononuclear cells were stimulated with a pool of peptides from the spike (S) of the reference virus (Wuhan), P.1 (Gamma), B.1.617.2 (Delta), B.1.1.529 (Omicron), BA.4-5 and XBB.1 (Omicron subvariants). Cells were labeled with antibodies (CD3, CD4, CD8, CD45RA, CD27, CCR7, IFN-γ, TNF-α) and acquired with a flow cytometer (Fortessa-BD). The data were analyzed using FlowJo software. So far nine volunteers were tested after the first and second booster. After the first booster, the frequency of IFN-γ- producing CD8+ T lymphocytes in response to Wuhan (0.05%; IQR: 0.02-0.12%) was higher than that of the negative control (0.01%; IQR: 0.00-0.06%) ($p<0.05$). After the second booster, a higher frequency of CD4+ central memory T lymphocytes (TCM) was observed in response to Delta (1.54%; IQR: 0.95-2.88%), BA.4-5 (1.97%; IQR: 0.88-2.22%) and XBB.1 (2.15%; IQR: 0.82-3.09%) compared to the negative control (0.87%; IQR: 0.55-1.95%) ($p<0.05$). Higher frequencies of TCM CD8+ T lymphocytes were observed in response to Omicron (0.62%; IQR: 0.24-0.81%) and BA.4-5 (0.81%; IQR: 0.39-0.98%) compared to the negative control (0.36%; IQR: 0.22-0.47%) ($p<0.05$). In conclusion, these results suggest that booster vaccinations of SARS-CoV-2 vaccines increase the number of TCM lymphocytes.

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SEROLOGICAL EVALUATION REVEALED A HIGH DETECTION OF ANTIBODIES AGAINST CHIKUNGUNYA VIRUS IN RESIDENTS FROM IMPOVERISHED AREAS OF MACEIÓ CITY, ALAGOAS

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The Chikungunya virus (CHIKV) is the cause of Chikungunya fever. Given the importance of monitoring viral distribution, the aim of this study was to evaluate the circulation of the virus in two neighborhoods in the city of Maceió, which were Vergel do Lago and Vale do Reginaldo. Regions were chosen for being peripheral, with poor sanitary conditions and exposure to factors that increase the mosquito vector proliferation. A total of 90 samples collected between 2021-2023 were selected, using a non-probabilistic sampling criterion alongside the absence of clinical history for arboviruses. The samples were subjected to the ELISA indirect test (Euroimmun®) in order to detect IgM/IgG antibodies. The 45 samples from the Vergel do Lago region showed a prevalence of 48.89% (n=22). The positivity for IgM detection was 31.11% (14/45) and 24.44% (11/45) for IgG detection. Three samples showed simultaneous positivity for IgM and IgG. The 45 samples from the Vale do Reginaldo region showed 40% (n=18) positivity for IgG antibodies and none positive for IgM. The sample collection period was characterized by a CHIKV epidemic in the city of Maceió with 9,875 confirmed cases between 2021-2023, which explains the high seroprevalence found in this study. Additionally, recent research indicates that impoverished communities have a greater serologic frequency of arbovirus infection. Our results may indicate increased virus circulation in these regions marked by poverty conditions and inadequate infrastructure, highlighting the epidemiological relevance of the situation.

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ANALYZING THE SPATIAL-TEMPORAL SPREAD OF SARS-COV-2 LINEAGE B.1.1.33 DURING THE SOCIAL DISTANCING PHASE

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Introduction: The COVID-19 pandemic has caused more than 7 million deaths worldwide, including over 700,000 in Brazil, with more than 37 million cases. This study analyzed the dispersion of the SARS-CoV-2 B.1.1.33 lineage in the state of Espírito Santo (ES), which has a strategic location and economy. Methods: A total of 140 nasopharyngeal samples positive for SARS-CoV-2 (collected between 07/09/2020 and 03/29/2021), with RT-PCR results showing CT <27, were sequenced using Oxford Nanopore technology (Ethics Committee 9.173.702). Local sequences were combined with all GISAID sequences up to 03/05/2024 and aligned using MUSCLE to construct the phylogenetic tree in IQ-TREE. The spatial and temporal diffusion patterns of the clusters were estimated using a Bayesian approach in BEAST. Results: Among the 140 sequenced samples, 30% were identified as the B.1.1.33 lineage. Analysis of 3,921 B.1.1.33 sequences obtained from sequencing and GISAID showed that ES, despite having the smallest population in the Southeast region, recorded 3.2 B.1.1.33 sequences deposited per 100,000 inhabitants, one of the highest among Brazilian states. Phylogenetic analysis revealed 4 clusters predominantly composed of samples from ES, indicating extensive intraregional circulation and virus exportation to other regions of Brazil (South, Northeast, North) and internationally (United States and Paraguay). Conclusion: This study presents the molecular epidemiology of the SARS-CoV-2 B.1.1.33 lineage. The dispersion was influenced by movement restrictions and economic and geographical characteristics, resulting in the predominance of local circulation and virus exportation to other states and countries.

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NEUROPSYCHIATRIC SEQUELAE IN AN EXPERIMENTAL MODEL OF POST-COVID SYNDROME IN MICE

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The world is facing a new challenge known as post-COVID syndrome (PCS). Current estimates suggest that more than 65 million people are grappling with PCS, which includes musculoskeletal and neuropsychiatric sequelae. The mechanisms underlying PCS remain unclear. Here, we aimed to: (i) comprehensively characterize the acute effects of pulmonary inoculation of the betacoronavirus MHV-A59 in immunocompetent mice at clinical, cellular, and molecular levels; (ii) examine potential acute and long-term pulmonary, musculoskeletal, and neuropsychiatric sequelae induced by MHV-A59; (iii) assess sex-specific differences. Male and female C57Bl/6 mice were inoculated with 3×10^4 PFU/30 μL of MHV-A59 via the intranasal route. Mice exhibited an altered neutrophil/lymphocyte ratio in the blood on the 2nd and 5th day post-infection (dpi). Marked lung lesions included hyperplasia of the alveolar walls, infiltration of polymorphonuclear leukocytes (PMN) and mononuclear leukocytes, hemorrhage, increased concentrations of CCL2, CCL3, CCL5, and CXCL1 chemokines, and high viral titers until the 5th dpi. While these lung inflammatory signs resolved, other manifestations persisted up to 60 dpi, including mild brain lesions with gliosis and hyperemic blood vessels, neuromuscular dysfunctions, anhedonic-like behavior, deficits in spatial working memory, and short-term aversive memory. These musculoskeletal and neuropsychiatric complications were exclusive to female mice and associated with reduced testosterone and increased estradiol levels. Interestingly, they were fully prevented after ovariectomy. In summary, our study describes a novel sex-dependent model of PCS focused on neuropsychiatric and musculoskeletal disorders, providing a unique platform for future investigations regarding the effects of acute therapeutic interventions on long-term sequelae from betacoronavirus infection.

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GENOMIC VARIABILITY OF HEPATITIS B VIRUS ACCORDING TO THE INFECTION CLINICAL PROFILE IN BRAZIL

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INTRODUCTION: It is estimated that 296 million people are living with chronic hepatitis B virus (HBV) infection. The disease has different clinical profiles: acute, chronic with active replication (HBeAg+), or not (HBeAg-), leading to variable outcomes. Factors such as host and virus genomic variability influence it. OBJECTIVE: To evaluate the variability of HBV in Brazil according to disease profile. METHODOLOGY: Sixty-five monoinfected patients had their serum extracted. HBV genotyping was performed by direct sequencing of partial surface (HBsAg)/polymerase region (~ 900 bp). The clinical profile was obtained by serological testing and liver biomarkers. RESULTS: 7.7% (5/65) individuals were classified as acute and 92.3% as chronic carriers. Of these, 10% (6/60) were classified as HBeAg-positive chronic hepatitis (EPH), while 90% (54/60) were classified as HBeAg-negative chronic infection (ENI). The most prevalent genotype was A (75,4%, 49/65) followed by D (16,9%,11/65) and F (7,7%, 5/65). On acute carriers, seventeen amino acid substititions were located within the major hydrophilic region (MHR) of HBsAg, such as T113S (5/5) and S143T (4/5). On EPH, fourty-two mutations were found in SHB domain (such as T113S, A194V, and V209L (5/6)). From them, 39 occurred within MHR. Regarding viral polymerase, 32 mutations were observed on reverse transcriptase domain. Lastly, on ENI group, there were 51 on MHR (such as S143T (33/54) and M129L (29/54)). Conclusion: HBeAg- clinical profile and genotype A were the most prevalent in Brazil. Although some mutations appeared in all profiles, their prevalence changed within the groups, modeling infection.

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PRELIMINARY ANALYSES OF HUMAN PAPILLOMAVIRUS (HPV) CO-INFECTION IN PATIENTS FROM SOUTHERN BRAZIL.

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Human Papillomavirus (HPV) is a significant public health concern due to its association with various cancers, notably cervical cancer. Advances in bioinformatics have greatly enhanced HPV research, enabling detailed genomic analyses and deepening our understanding of viral evolution, genetic diversity, and pathogenesis. This study aimed to detect HPV and characterize its genotypes in cervical swab samples using high-throughput sequencing (HTS) technology. Four cervical swab samples collected from female patients between June and September 2022 were analyzed. HPV genotype detection was performed using quantitative Polymerase Chain Reaction (qPCR). One sample tested positive for HPV16 (Ct value of 22.6), while HPV18 was not detected. Other HPV types were present in all samples, with Ct values ranging from 18.14 to 23.3. Genome sequencing yielded 9 complete HPV genomes, identifying multiple genotypes: HPV51, HPV66, HPV70, HPV16, HPV68, HPV39, and HPV56. Coverage rates ranged from 51.6% to 92.9%, with identities between 91.8% and 99.9%. Coinfections were present in all samples. Phylogenetic analysis classified all genotypes within the Alphapapillomavirus genus, with two HPV68 samples showing microevolution compared to the reference subtype. This study underscores the genetic diversity of HPV in Southern Brazil and reveals a complex landscape of co-infections. These findings highlight the necessity for ongoing surveillance and further research to explore the implications of HPV diversity and co-infections on disease risk and treatment. Future research should expand the sample size and assess the clinical impact of these findings.

Key Words: Human Papillomavirus (HPV), Southern Brazil, Co-infections, Multiple HPV genotypes, High-Throughput Sequencing (HTS).

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Gene expression of the cytosolic sensors SAMHD-1 and TREX-1 in COVID-19 convalescent patients randomized to Bacillus Calmette-Guérin (BCG) or Placebo

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Individuals convalescing from COVID-19 may have sequelae and persistence of symptoms in a condition called Long COVID. Thus, the *Bacillus Calmette Guérin* (BCG) vaccine can be used as a therapeutic intervention, with its effectiveness already proven against other pulmonary viral infections. This study evaluated the gene expression levels of *SAMHD1* (viral restriction sensor) and *TREX1* (viral permittivity sensor) in individuals convalescent from COVID-19 randomized to BCG or placebo up to 14 days of COVID diagnosis. The study included 40 patients who received a BCG intradermal vaccine booster and 40 who received a placebo. These individuals were evaluated at three times: T0 (admission to the study), T45D (45 days after vaccines) and T6M (6 months after vaccines). Blood samples were collected from all individuals and their RNA was subsequently extracted. *SAMDH1* and *TREX1* gene expression levels were quantified by real-time PCR. For statistical analyses, the Friedman and Mann-Whitney tests were used. There was no significant difference in the expression levels of *SAMHD1* and *TREX1* between the three time points evaluated in both the BCG group and those who received placebo. In the comparison by period, individuals revaccinated with BCG showed higher levels of *SAMHD1* expression at T6M ($p = 0.0463$) when compared to the placebo group, but not *TREX1*. At times T0 and T45 there was no significant difference between the groups in the evaluation with *SAMHD1* and *TREX1*. The results suggest that the BCG vaccine does not appear to influence the expression levels of *SAMHD1* and *TREX1* in individuals recovering from COVID-19.

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Higher expression levels of *FAS* and *FASL* and IFN- γ are associated with the severity of COVID-19

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The severity of COVID-19 may be associated with factors such as an exacerbated immune response and lymphopenia. In individuals with COVID-19, expression of the apoptosis molecule Fas was correlated with T-cell lymphopenia. Another characteristic of SARS-CoV-2 infection is an inflammatory cytokine storm. Thus, this work evaluated the gene expression levels of *FAS* and *FASL* and the plasma levels of the cytokines IFN-γ, TNF-α and IL-10 with the severity of COVID-19. 370 individuals with COVID-19 participated in the study, of which 116 had severe COVID-19 and 254 with non-severe clinical manifestations. Gene expression of *FAS* and *FASL* was quantified by real-time PCR. Plasma quantification of the cytokines IFN-γ, TNF-α and IL-10 was measured using the flow cytometry technique. For statistical analyses, the Mann-Whitney, Kruskal-Wallis and Spearman correlation tests were performed. Higher gene expression levels of *FAS* (p= 0.0029), *FASL* (p= 0.0013) and plasma IFN-γ (p= 0.0209) were identified in individuals with severe clinical manifestations compared to those with non-severe manifestations, while TNF-α and IL -10 had no association with severity. A slight positive correlation between the levels of *FAS* and *FASL* (r= 0.392; p= 0.0257), *FAS* and *IFN-γ* (r= 0.4603; p= 0.0006) and the levels of *FASL* and *IFN-γ* (r= 0.2624; p= 0.0602) was observed. The results suggest that increased expression levels of the *FAS* and *FASL* genes contribute to the intense activation of the apoptosis process and inflammatory response, leading to the development of the severe form of COVID-19.

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The *TLR3* rs5743305T/A Polymorphism does not influence the severity of COVID-19

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COVID-19 has a broad clinical spectrum, ranging from asymptomatic infections to serious and fatal conditions. The manifestations of the disease are mainly related to the intensity of the immune response. TLR3 is a receptor that recognizes dsRNA and activates the synthesis of IFN-I and inflammatory cytokines to control infection. Genetic changes in the *TLR3* gene can influence the receptor's gene expression and cytokine production, compromising the immune response against viral infections. The study included 368 blood samples from individuals diagnosed with COVID-19, divided into two groups: severe (n = 116) and non-severe (n = 252). All samples were subjected to DNA extraction and genotyping for the *TLR3* rs5743305T/A polymorphism was performed by real-time PCR. The measurement of the cytokines TNF-α and IL-6 was carried out using the flow cytometry technique. For statistical analyses, the chi-square, Mann-Whitney and Kruskal-Wallis tests were performed. The frequency of genotypes and alleles for *TLR3* rs5743305T/A was not different between the groups with severe and non-severe COVID-19. TNF-α levels were not different between the two groups. Patients with severe COVID-19 had higher IL-6 levels compared to non-severe patients (p= 0.0137). The levels of TNF-α and IL-6 were not different between carriers of the *TLR3* rs5743305T/A genotypes in both groups. The *TLR3* rs5743305T/A polymorphism was not associated with the severity of COVID-19 or with changes in the levels of inflammatory cytokines of innate immunity. Elevated IL-6 levels have been associated with the severity of COVID-19.

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Uncovering Zooanthroponosis: Human Norovirus in Domestic Dogs and Cats in Brazil

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Recent increases in zoonotic diseases highlight the transmission risks of integrating companion animals into urban environments, emphasizing the need for One Health approaches, as viruses can be transmitted between humans and animals. This study assessed the circulation of human noroviruses (NoV), adenoviruses (HAdV), enteroviruses (EV), parechoviruses (PeV-A), bocaviruses (HBoV), hepatitis A (HAV) and E (HEV), Influenza A and B (Flu A/B), respiratory syncytial virus (RSV) and SARS-CoV-2 in domestic dogs and cats in Brazil to understand potential zooanthroponosis risks. Between 2012 and 2021, 600 fecal samples (516 dog and 84 cats) were collected at small animal clinics in São Paulo state. The specimens underwent in-house (RT)-qPCRs for HBoV, HAdV, EV, PeV-A, NoV and HEV, and commercial RT-qPCR for SARS-CoV-2, Flu A/B and RSV. HAV detection used conventional nested (RT)-PCR. Positive samples were sequenced for molecular characterization and phylogenetic analysis. NoV was detected in 0.2% (1/600), while all other viruses tested negative. The NoV-positive sample, collected in 2012 from a pet dog, was identified as genotype GII.4_Sydney[P31]. The Dog/BRA/2012/GII.4_Sydney[P31]/IAL-M21 strain showed close genetic similarity to Brazilian human and environmental GII.4_Sydney[P31] strains, with 98.1-99.2% nucleotide similarity in ORF1 and 99.2-99.6% in ORF2 sequences, suggesting interspecies transmission. Pet dogs are often exposed to human fecal-borne viruses, underlining the potential for zooanthroponotic transmission due to close interaction with people. Enhanced surveillance in companion animals is urgently needed to understand the implications of detecting human NoV strains in pets, as NoV could act as a reverse zoonotic disease in households, animal hospitals or shelters worldwide.

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DETECTION OF A PREVIOUSLY UNIDENTIFIED POLYOMAVIRUS IN THE TONSILS OF WATER BUFFALO (*BUBALUS BUBALIS*)

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The domestic buffalo, *Bubalus bubalis*, is highly valued in agriculture due to its high fertility and lower replacement costs compared to cattle, besides its high yield of milk and meat. Tonsils, essential lymphoid tissues, play a crucial role in pathogen defense, making them key targets for diagnosing infectious diseases. Tonsil fragments were collected from 293 clinically healthy bubalines in an abattoir from Marajó Island (Pará state, Brazil). Tonsils from 60 animals were randomly selected for virus detection by metagenomics. Briefly, after tissue thawing, nucleic acids were extracted, and randomly amplified by *phi29*. The products were sequenced by high throughput sequencing. Genomes were assembled using SPAdes 3.10 Genome Assembler and Genome Detective Virus Tool. Generated contigs were analyzed by BLAST tool against NCBI nucleotide data bank. Sequences were aligned using MAFFT online and phylogenetic analysis was performed by maximum likelihood using software MEGA v7. Among the genomes detected, it was identified a polyomavirus, which had not been previously reported in buffalos. This genome showed more than 15% divergence in the large T antigen compared to other species described in the family. As non-native animals in Brazil, water buffaloes can serve as reservoirs for pathogens that might affect other species in the same ecological niche. Although no pathology was associated with the viruses identified, this study highlights the importance of a sanitary management approach and a One Health strategy when farming these animals.

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Probe-based screening and sequencing of the equine parvovirus-hepatitis in Rio de Janeiro State

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Equine parvovirus-hepatitis (EqPV-H) has been identified as the likely causative agent of Theiler's Disease, with or without the administration of equine-derived biological products. In Brazil, to the best of our knowledge, Theiler's Disease was only reported in an adult horse in 2015, prior to the description of the virus and the availability of diagnostic tests in the country, in 2018 and 2021, respectively. The aim of this study was to investigate the prevalence of the EqPV-H in farmed horses from Rio de Janeiro State using a probe-based Real Time PCR directed to the NS1 region, designed with available nucleotide sequences. Positive animals were confirmed by Nested PCR and sequencing. After screening of 92 animals, 14 (15.2%) presented Cq values ranging from 31.7-37.6, and mean ± standard deviation of 34.29 ± 2.2. A group of 8 samples, previously confirmed as EqPV-H positive, presented mean Cq of 35.02 ± 2.3. A synthetic DNA was included in each run. Positivity was found in 21.1% (11/52) and 7.5% (3/40) of animals dedicated to sports and reproduction, respectively; in 13.4% (9/67) of female and in 20% (5/25) of male population; the average age was 10.9 years old (yo), without positive under 5 yo. Nucleotide analysis with 13 partial (354bp) NS1 sequences obtained presented 3.1% between Brazilian isolates and other available EqPV-H isolates. In conclusion, the probe-based screening assay can be a fast and reliable alternative for EqPV-H diagnostic with serum samples. Other biological samples must be evaluated.

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DETECTION OF A BETACORONAVIRUS IN A MOLOSSID BAT CAPTURED IN SOUTHERN BRAZIL

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In 2012, a novel coronavirus, MERS-CoV, caused another epidemic in human populations with a mortality rate of 35%. This virus emerged in the Middle East and spread to other regions and countries, leading to isolated infections and occasionally outbreaks of moderate size. Bats are recognized as natural hosts for a variety of emerging viral agents, as well as bridge hosts for viral agents such as the betacoronaviruses (β-CoV) MERS-CoV, SARS-CoV and SARS-CoV-2, which may cause serious illness in humans. In this way, the present study evaluated the presence of coronaviruses in samples of *Molossus molossus* bats. Samples of oral and rectal swabs from 17 bats were collected in Southern Brazil. One sample tested positive in a pan-specific RT-nested PCR for detecting RpRd polymerase of coronaviruses. Following DNA sequencing and phylogenetic analyses, positive sample was classified in *Betacoronavirus* genus. This sample was classified into *Merbecovirus* subgenus and presented 89.08% of nucleotide identity with MERS-CoV and high identity with other betacoronavirus detected previously in bats from southeastern Brazil. These results complement the circulation of β-CoVs genetic close related to human pathogens in molossid bats from South America. Thus, the findings contribute to improving information about the viral diversity of betacoronavirus and show the importance of better investigating bats, considering that they harbor a variety of viruses that may favor zoonotic events.

Keywords: Coronavirus; *Molossus molossus*; Chiroptera; MERS-CoV.
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PT.299

A PUTATIVE NEW GENOTYPE WITHIN A PREVIOUS UNCLASSIFIED RODENT HEPACIVIRUS IN WILD GUINEA PIG

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The *Hepacivirus* genus comprises RNA viruses within the *Flaviviridae* family, which hepatitis C virus (HCV) is the prototype. Many hepaciviruses have been identified in different mammals, including rodents of multiple species and families. Each rodent hepacivirus species has been described in rodents into the same species heretofore. In this study, we report the found and characterization of a putative new genotype of a previous unclassified rodent hepacivirus species that was reported in *Proechimys semispinosus*, in a wild cavy (*Cavia aperea aperea*). This viral agent was detected in one of 14 (7.14%) of prea sera tested by RT-PCR. The complete genome was successfully obtained by high throughput sequencing using Illumina MiSeq plataform. Considering that this is the first report of hepacivirus in Cavidae rodents, our findings suggested that different rodent species and families may host rodent hepacivirus of same species, but different genotypes. The identification and characterization of novel hepaciviruses might towards the identification of lurking reservoirs of human genetic-related pathogens, and can make it easier to elucidate the evolutionary origins of HCV and other hepaciviruses.

Keywords: Prea; wild guinea pigs; *Flaviviridae*; hepacivirus; Rodentia.
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PT.300

DETECTION AND QUANTIFICATION OF VIRUSES RELATED TO NEONATAL DIARRHEA IN CALVES FROM THE CENTRAL-WEST REGION OF BRAZIL

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INTRODUCTION: Brazilian agribusiness is vital to the national economy, yet it faces significant challenges, notably the high neonatal calf mortality rate, which can reach up to 75%. This high mortality results in severe economic losses due to treatment costs, decreased production, and even animal death. Bovine neonatal diarrhea (BND) is a primary cause of this high mortality rate, characterized by liquid, abundant diarrhea, sometimes with blood. Bovine coronavirus (BCV) and bovine rotavirus (BRV) are key pathogens responsible for BND. **MATERIAL AND METHODS:** This study aimed to examine the presence of BCV and BRV in fecal samples from calves aged 1 to 30 days, both diarrheic and non-diarrheic, collected from various municipalities in Goiás from May 2019 to April 2024. A total of 269 fecal samples were processed. RT-qPCR was used to detect BCV and BRV, and the viral loads in positive samples were quantified. **RESULTS:** The overall positivity rate for BRV was 52% (141/269), while for BCV it was 1.12% (3/269). The average viral loads ranged from $2.4x10^2$ to $6.20x10^{13}$ for BRV and $6.05x10^4$ to $1.63x10^6$ CG/g of feces for BCV. Notably, two samples from 1-day-old neonatal calves, which had been separated from their mother before receiving colostrum, tested positive for both viruses. **CONCLUSIONS:** This study underscores the importance of BRV and BCV in BND cases in Goiás and highlights the need for active virus surveillance in veterinary settings. Such research is crucial for enhancing herd management practices and reducing economic losses associated with BND in the region.

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PT.301

INVESTIGATING THE FECAL VIROME OF PASSERINE BIRD FROM SERRA DO MAR STATE PARK, SÃO PAULO, BRAZIL

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Wild birds can be key reservoirs of avian viruses, affecting poultry and wildlife conservation. An unusual 10-segment dsRNA pattern was detected by polyacrylamide gel electrophoresis in one fecal sample from *Troglodytes musculus* (Curruíra) during a virological survey of passerines in Serra do Mar State Park, São Paulo, 2022-2024. In an attempt to identify this virus, a fecal virome investigation was conducted. Total nucleic acid was extracted using the AllPrep PowerViral DNA/RNA Kit, followed by reverse transcription and dsDNA synthesis. Library preparation was performed and sequenced on Illumina NextSeq 500. Data was preprocessed, assembled with SPAdes, classified using CAT_pack, and genetically analyzed with BLASTx and IQ-TREE. Of 41.669 contigs *de novo* assembled, 166 were classified as viruses. About 43% were unclassified at any taxonomic level, while 95 contigs (>200bp) were classified at the order or family level, including ssDNA viruses *Genomoviridae* (410bp–2161bp) and *Parvoviridae* (225bp–601bp); +ssRNA viruses *Iflaviridae* (230bp–805bp) and *Astroviridae* (5663bp–6706bp); and dsRNA viruses *Reovirales* (4201bp–4298bp). We recovered two potential novel viruses: an *Avastrovirus* with 6706bp that remained in a divergent clade in phylogenetic analysis and showed 47% amino acid identity in ORF2 with wild bird isolate (MW735969.1); and a dsRNA segment of 4298bp encoding a reovirus RdRp (possibly the virus initially identified in the sample) with 26% identity to the Rice ragged stunt virus RdRp (O92604.1). These findings from a single sample highlights the unknown viral diversity in wild birds and underscores the importance of virome studies.

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PT.302

EVALUATION OF THE INFLUENCE OF INTRA-HOST VARIANTS ON HEMATOLOGICAL PARAMETERS IN FELV-INFECTED ANIMALS USING HIGH-THROUGHPUT SEQUENCING

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Feline leukemia virus (FeLV) is a Gammaretrovirus that affects cats. Its replication occurs in the bone marrow and lymphoid cells, and the clinical course of the disease can be abortive, regressive, progressive or focal. FeLV's high mutation rate generates a high number of viral variants in the infected individual. High-throughput sequencing (HTS) can be used to identify and track genetic variants within a viral population. Understanding the intra-host genetic diversity of FeLV is important to comprehend the evolutionary dynamics of the virus and its impact on disease development. Phylogenetic analysis of the env gene has emerged as an approach to differentiate virus variants. These variants are an attempt by the virus to gain adaptive advantages under selective pressures. Here, HTS was used to identify intra-host FeLV variants and assess their impact on hematological parameters in FeLV-infected animals. Erythrograms of 18 FeLV-infected cats were analyzed. DNA was extracted from blood samples, followed by PCR targeting the entire env gene. Gel bands were extracted, purified and prepared for HTS. The obtained reads were aligned with the env SU region reference sequence (NC_001940.1) and haplotypes were identified. No significant correlation was detected between age or blood changes in FeLV-positive cats and the number of intra-host quasispecies or between the number of haplotypes and the presence of anemia. However, a statistical difference was identified when comparing the number of haplotypes between cats with altered blood count and those within the reference range. The impact of variants on FeLV clinical outcomes requires more research.

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PT.303

EVOLUTIONARY INSIGHTS INTO WESTERN EQUINE ENCEPHALITIS VIRUS: IMPLICATIONS FOR OUTBREAK PREPAREDNESS

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The Western Equine Encephalitis virus (WEEV) is an arbovirus mainly transmitted by mosquitoes of the genus Culex, affecting both equines and humans. WEEV infections range from asymptomatic cases to severe encephalitis, which can be fatal. From 2023 to 2024, cases were reported in South American countries, including Argentina, Uruguay, and Brazil, with occurrences confined to Rio Grande do Sul. This study investigates the virus's evolutionary trajectory to understand its historical spread, period of inactivity, and significant resurgence at the end of 2023, which raises concerns about a new outbreak. We analyzed all available complete WEEV genomes (N = 61) from GenBank, aligning sequences with MAFFT (v7.490). Phylogenetic trees were constructed using the maximum likelihood method in IQTREE v1.6.12, with 1,000 UFBOOT replicates for branch support. The optimal evolutionary model was selected by MODELFINDER. Treetime inferred the divergence times of the main WEEV lineages. Trees were visualized using FigTree v1.3.1. Phylogenetic analysis revealed a distinct separation between South American and North American WEEV lineages, indicating separate evolutionary paths dating back to the 1910s. The South American clade includes sequences primarily from horses or humans, whereas the North American clade comprises samples from diverse hosts like mosquitoes, wild animals, and mammals. This genetic division may reflect specific adaptations to different hosts and vectors, explaining the increased virulence of the South American lineage. This study provides insights into WEEV's recent evolution and is crucial for developing strategies to control and prevent future outbreaks.

Financial Support: CAPES, CNPq

PT.304

EXPLORING RNA VIRAL DIVERSITY THROUGH METAGENOMIC ANALYSIS OF BATS IN SÃO PAULO AND BAHIA STATES, BRAZIL

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Studies on viral diversity originating from animal reservoirs such as bats are essential for comprehensive surveillance in identifying and tracking zoonoses. In this study, a metagenomic approach was used to elucidate the viral diversity in bats captured in the rural areas of São José do Rio Preto-SP and Barreira-BA. A total of 75 bats from five different species (*Desmodus rotundus*, *Phyllostomus discolor*, *Molossus molossus*, *Molossus rufus*, *and Artibeus lituratus*) were sampled, with RNA extracted from organ macerates (lung, liver, intestine), resulting in seven cDNA libraries (Zimo-RNA library kit). The libraries were deep-sequenced using the MiSeq Illumina platform with paired-end applications. Reads were trimmed, and the contigs were assembled de novo (RiboDetector, SPAdes software). The taxonomic classification of generated contigs was performed using the Kaiju pipeline. Notably, the total number of classified contigs varied between 2,814 and 42,121, with a low percentage of viral contigs (0.04%). Viral sequences belonging to more than nine different virus families were identified. The most abundant families were *Retroviridae*, *Poxviridae*, *Herpesviridae*, *Baculoviridae*, *Hepeviridae*. The prominent viral genera included *Alpharetrovirus*, *Betaretrovirus*, *Gammaretrovirus*, *Orthopoxvirus*, *Avipoxvirus*, *Molluscipoxvirus*, *Pestivirus*, *Proboscivirus*, *Cytomegalovirus*, *Rhadinovirus*. Initially, species associated with poxviruses deserve special attention due to their frequency rates (Fowlpox, Turkeypox, Orf, and Yokapox viruses). Nevertheless, continued analyses will be important to determine the depth and coverage of viral contigs for the classification of viral species. Our preliminary NGS results showed reduced virome detection from tissues of apparently healthy bats; however, the study underscores the importance of continued surveillance to monitor potential emerging zoonoses.

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PT.305

NEW EVIDENCE HIGHLIGHTS HPAI H5N1 AS AN IMPORTANT THREAT TO SOUTH AMERICAN PINNIPEDS BIODIVERSITY AND PUBLIC HEALTH

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Influenza A viruses (IAV) pose significant risks to biodiversity and public health, since they are constantly evolving genetically, facilitating infections to new hosts. The recent emergence of highly pathogenic avian influenza (HPAI) H5N1 in pinnipeds from South America caused mass mortality mainly of South American sea lions (SASL) (*Otaria flavescens*), South American fur seals (SAFS) (*Arctocephalus australis*) and Southern elephant seals (*Mirounga leonina*) (SES). Here, we describe the detection of influenza A H5N1 virus in SASL and SAFS new samples collected during systematic beach surveys along 103 km of coastline in southern Brazil from September to December 2023. Forty-seven (nasal and anal swabs) samples of pinnipeds carcasses (n = 10 SAFS and n = 19 SASL) were tested to influenza A virus by RT-qPCR protocol. Half of nasal samples from SAFS tested positive for IAV. While the positivity in SASL samples was lower, with only 21% of nasal positive samples. Metagenomics approach confirmed the AIV H5N1 reads in all nasal positive samples from SAFS, and in one nasal sample from SASL. Coverage from sequenced samples varied from 50.1% to 85.5% and 50.8% to 77%, respectively, for N1 and H5 genes. These new cases of H5N1 in Brazilian territory associated to the high mortality, highlights the importance of genomic surveillance of this virus, which became an important issue on public health and one of the most important threats to South American pinnipeds.

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PT.306

SUBMITTING BIOLOGICAL SAMPLES IN FILTER PAPER: A RISK FOR VIRUS SPREAD

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Spotting serum samples on filter paper has become a popular means of submitting samples for serological testing, yet it may also provide means for pathogen dissemination. Herein, we investigated the viability of six bovine viruses spotted on filter paper and kept at room temperature for several days. Viral suspensions ($10^{6,5}$-10^{7} $TCID_{50}$) of bovine viral diarrhea virus (BVDV), bovine alphaherpesvirus 1 (BoHV-1), bovine enterovirus (BEV), vaccinia virus (VACV), bovine parainfluenza 3 virus (bPI-3V) and vesicular stomatitis virus (VSV) were diluted 1:5 in fetal bovine serum, spotted on protein saver paper and maintained at room temperature for 10 days. Daily, virus suspensions were eluted from the membranes overnight in culture medium and virus infectivity was quantitated by limiting dilution. In general, the spotting and elution steps resulted in a ~ 1.5 to 2 log_{10} reduction in virus titers. A progressive reduction in virus titers was observed for all viruses. Infectivity was detected up to day 3 for BVDV and BEV, day 5 for VACV, day 6 for BoHV-1 and VSV, and day 7 for bPI-3V. No infectious virus was detected beyond day 7. Microwaving the virus-spotted membranes at maximum energy for 1 min completely inactivated the viruses, but also reduced virus-neutralizing titers to BVDV and BoHV-1. In summary, our results show that the infectivity of some viruses may be maintained for up 7 days in paper filters. Thus, this method of sample submission for serology represent some risk for pathogen spread and, therefore, should be carefully evaluated as to eliminate this risk.

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PT.307

MOLECULAR EPIDEMIOLOGY OF *SENECAVIRUS A*: A PHYLOGENETIC ANALYSIS OF 280 SEQUENCES

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Senecavirus A (SVA) infection in pigs causes a vesicular disease that is clinically indistinguishable from foot and mouth disease and is commonly detected in swine slaughter establishments as healing or healed lesions. This implies both in direct losses, such as decreased productivity due to clinical signs and neonatal deaths, and indirect losses, including commercial restrictions and resources expended for investigation by the official veterinary service due to clinical similarities with foot and mouth disease. A viral sample collected during a SVA outbreak in Minas Gerais, Brazil, in 2015, was isolated in cell culture (NCI-H1299) and sequenced by *MiSeq* Sequencing System (*Illumina* Inc., EUA). Bioinformatics tools were employed to assemble the sequenced genome, named LPVA4, and to conduct phylogenetic analysis among it and 279 SVA sequences available in databases. Our results corroborate the phylogenetic findings of other research groups, showing a tendency for sequences to cluster based on the year of isolation and geographic region. The phylogenetic relationship between lineages from different countries was also observed, reinforcing the hypothesis of viral transfer between different geographic areas. The 15 Brazilian samples grouped together, close to the American samples from 2015. The sample MZ456812.1, isolated in Santa Catarina in 2020, exhibited a notably larger branch than the other Brazilian sequences, prompting distance matrix analysis. The results demonstrate that all Brazilian sequences available in the databases are genetically closed, but comparatively, the sample sequenced in 2020 is more distant from the others.

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PT.308

Identification of new bat hosts for Paramyxovirus in the Atlantic and Amazon Forests in Brazil

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Zoonotic viruses have been responsible for outbreaks and epidemics in recent decades. Bats are recognized as large hosts of potentially zoonotic viruses, such as those from the *Paramyxoviridae* (PMX) family, and they are considered the main hosts for PMX. In Brazil, PMX descriptions were done in bats from different parts of the country in the last years. Here, we analyzed tissue samples from bats from both Amazon and Atlantic Forests*, aiming to identify and classify PMV diversity found in these mammals, in a partnership with* the National Virus Surveillance Network in Wild Animals (PREVIR). In total, we analyzed tissue samples (lungs, intestines, kidney,s heart, liver, and embryos/placenta, when available) and oral/rectal swabs from 297 individuals, in which a semi-nested PCR assay targeting the Large (L) gene was done for each sample. PMX was detected in 14 (4.7%) of all sampled bats, represented by the species *Glossophaga soricina (*n=1), *Molossus molossus* (n=1), *Rhynchonycteris naso* (n=7), *Cynomops planirostris* (n=1), *Nyctinomops laticaudatus* (n=2), *Trachops cirrhosus* (n=1), and *Vampyriscus bidens* (n=1), with the last five species being their first PMV detection. The 14 obtained sequences were all grouped into the *Jeilongvirus* genus of the *Orthoparamyxovirinae* subfamily, wih amino acid (aa) identities ranging from 91.8% to 100%. The identification of PMX in new bat host species highlights the lack of knowledge of PMX hosts in Brazilian wildlife and underscores the need to continue and expand wildlife surveillance programs.

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PT.309

FIRST DETECTION OF BOVINE ALPHAHERPESVIRUS 5 IN WATER BUFFALOES (*Bubalus bubalis*) IN PARÁ STATE, BRAZIL

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Herpesviruses are significant ruminant pathogens. In water buffaloes, however, herpesviruses have not been thoroughly studied. Although bubaline alphaherpesvirus 1 (BuAHV1) and bovine alphaherpesvirus 1 (BoAHV1) have already been recovered from water buffaloes, to date, there are no reports on the occurrence of bovine alphaherpesvirus 5 (BoAHV5) in these animals. Therefore, the aim of this study was to search for BuAHV1, BoAHV1, and BoAHV5 in palatine tonsils of apparently healthy water buffaloes from the Pará state (Brazil). Tonsil tissue samples (n=293) were screened by nested PCR targeting a UL44 (gC gene) region, followed by sequencing, to detect and differentiate between the viral types. Viral genome segments were detected in 18 out of 293 (6.1%) of the samples. Two animals carried genomes of BoAHV1 only, 11 animals carried BoAHV5 genomes only, and four animals carried BuAHV1 only. Another animal had both BoAHV1 and BoAHV5 genomes in its tonsils. No infectious virus could be recovered from any sample. The BuAHV1 identified here were more closely related to Indian BuAHV1. Phylogenetic analyses suggested a closer relationship between the recovered BoAHV5 and BuAHV1 genomes. Therefore, evidence is provided here to confirm that not only BoAHV1 and BuAHV1, but also BoAHV5, can infect water buffaloes. This is the first report of BoAHV5 in water buffaloes and of the occurrence of coinfections with BoAHV1 and BoAHV5 in that species. Such findings and the similarity of BoAHV5 to Indian BuAHV1 suggest that BoAHV5 origin may be linked to recombinations between BoAHV1 and BuAHV1 within bubalines.

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PT.310

DETECTION OF BOVINE GAMMAHERPESVIRUS 4 IN PALATINE TONSILS OF WATER BUFFALOES (*Bubalus bubalis*)

Paredes-Galarza BS[1], Stone NV[1], Gasparetto R[1], Oliveira MT[1], Violet-Lozano L[1], Melgarejo AS[1], Timm FCB[1], Teixeira MAS[2], Rivero GRC[3], Cerqueira VD[3], Bezerra Júnior PS[3], Campos FS[1], Franco AC[1], Roehe PM[1] - [1]Universidade Federal do Rio Grande do Sul - Laboratório de Virologia/ICBS, [2]Agência Estadual de Defesa Agropecuária do Estado do Pará, [3]Universidade Federal do Pará - Laboratório de Patologia Animal

The Bovine gammaherpesvirus 4 (Rhadinovirus bovinegamma 4; BoHV-4) is a pathogen that can infect bovine, buffaloes and other species. BoHV-4 infection can cause clinical manifestations, including abortion, endometritis and respiratory signs. Like other herpesviruses, BoHV-4 remains latent in the host, specially in lymphoid tissues, reactivating in cases of immunosuppression. However, the pathogenesis of BoHV-4 in buffaloes is still poorly understood. The aim of this study was to determine if palatine tonsils could be a BoHV-4 latency site in water buffaloes (Bubalus bubalis). Tonsil samples (n=50) of apparently healthy water buffalos were analyzed by nested PCR targeting BoHV-4 glycoprotein B (gB). For all positive samples, virus isolation was attempted in MDBK cells: 40 mg of minced tissue were suspended in 1 mL of DMEM and 200 uL was used as inoculum. Seven days post-infection, cells were examined, and compared with mock infection, daily for cytopathic effects (CPE). Up to five blind passages were performed. Four samples (4/50, 8%) were PCR positive for gB BoHV-4 DNA; however, no infectious virus could be recovered. Although detection of BoHV-4 in water buffalo has already been reported in other samples, this is the first study in Brazil reporting BoHV-4 detection in palatine tonsils. These results suggest palatine tonsils as a site of latency for BoHV-4 in buffalos. Therefore, buffalos can serve as potential transmitters of BoHV-4 and require monitoring in regions where there is interaction between buffalo and cattle.

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PT.311

DETECTION AND PHYLOGENETIC ANALYSIS OF HUMAN -ASSOCIATED GEMYKIBIVIRUS-2 FOUND IN BATS CIRCULATING IN BRAZIL.

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Bats harbor various zoonotic viruses, making them crucial for studying their virome and identifying potential public health threats. In this study, we collected oral and rectal swab samples from different bat families and species in the State of Ceará, Northeastern Brazil. Using RNA-Seq with a sequencing depth of at least 40 million 150 bp paired-end reads, we identified four contigs (8,129 reads, with a depth of coverage of 675.8x) representing 17.1% of the genome of *Human Gemykibivirus 2* (HuGkV-2), a circular 2 kb DNA virus from the *Genomoviridae* family. Three contigs resulted in 100% nucleotide identity to the Gemycircularvirus (GemyCV) SL1, SL2, and SL3 lineages (Genbank Accession codes NC_026818.1, KP133075.1, KP133076.1 and KP133077.1), designated as HuGkV-2, which has been linked to unexplained cases of human encephalitis and diarrhea. Phylogenetic analysis using the maximum-likelihood method on concatenated regions placed our sequence into a well-supported clade with the aforementionated GemyCV lineages. HuGkV-2 is an emerging virus, and its clinical impact remains to be elucidated. This emerging human virus was detected through metagenomics in parenterally infected blood donors from the Brazilian Amazon in 2020. Its detection in bats marks the first reported instance, and its association with human infections raises a public health concern. These results highlight the need for ongoing surveillance and research to better understand the clinical and epidemiological impact of this virus. Monitoring HuGkV-2 is essential to elucidate potential spillback events, its role in human infections, and its broader public health implications.

Financial Support: FAPESP

PT.312

CORONAVIRUS CRYPTIC LANDSCAPE AND DRAFT GENOME OF A NOVEL COV CLADE-RELATED TO MERS FROM BATS CIRCULATING IN NORTHEASTERN BRAZIL.

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Coronaviruses (CoVs) are a group of viruses that cause disease in both humans and animals, with bats serving as important reservoirs. This study aimed to screen CoVs in bat swabs using broad-spectrum PCR, followed by RNA-Seq and evolutionary analysis. We identified seven distinct CoVs. A first set of sequences related to Merbecovirus was detected in *Molossus molossus*, with 82.8% genome coverage (79,252 reads, 318.5x coverage depth, covering 24,948 nucleotides from de novo genome assembly) and 71.9% nucleotide identity to MERS-related CoV. The spike-encoding protein gene is being explored. *Tylonycteris bat CoV HKU4* and *Pipistrellus bat CoV HKU5* were also identified, each covering 5.5% of the genome, yielding 4,913 (with 61.7% nucleotide identity) and 3,657 reads (with 72.0% nucleotide identity), respectively. CoV sequences from *Artibeus lituratus* were classified as SARS-related CoV ("subtype: outgroup") with 30% genomic coverage. Additionally, a contig with 3.8% genomic coverage (63.9% nucleotide identity) was related to *Hedgehog-CoV-1*, while another contig was related to *BatCoV HKU9*, with 5% genomic coverage (62.1% nucleotide identity). A small contig related to Alpha-CoV was also retrieved, with 100% similarity to a 229E-related bat CoV, covering 0.5% of the genome with 78.6% nucleotide identity. Highly supported phylogenetic trees corroborate our results, with a few exceptions being inferred as unclassified CoVs. Our study constitutes the first report of a CoV clade related to MERS, as well as several Merbecovirus, in bats in South America. It provides insights into genetic diversity and global footprint of CoVs in bat populations.

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PT.313

EVIDENCE OF VERTICAL TRANSMISSION OF RABIES IN THE VAMPIRE BAT *DESMODUS ROTUNDUS* (CHIROPTERA, PHYLLOSTOMIDAE) IN NORTHEASTERN BRAZIL.

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Bats have garnered significant attention for harboring zoonotic viral pathogens. In this context, evolutionary approaches involving vertical transmission can be crucial for understanding intra-host evolutionary dynamics and viral dissemination over time. Here, we empirically demonstrated the vertical transmission of rabies virus (RABV) in bats. Eleven individuals and their fetuses sampled from Northeastern Brazil were analyzed using a broad-spectrum PCR pan-assay focusing on rhabdoviruses (RhabV, polymerase) and lyssaviruses (LYSSAV, nucleoprotein), coronavirus, paramyxovirus, and influenza A. Samples were negative for the latter three, while five individuals tested positive for RhabV and LYSSAV. Among those positive, one sample is from *Eumops perotis* and the others are from *Molossus molossus*. Additionally, *Desmodus rotundus* tested positive for RhabV and LYSSAV in the central nervous system (CNS), as well as in the fetal and kidney compartments, with LYSSAV present in both. Maximum-likelihood-based phylogenetic analysis using concatenated nucleoprotein and polymerase sequences placed our samples into two distinct and well-supported RABVs clades. The first set of laboratory-produced sequences from *Eumops perotis* and *M. molossus* clustered with RABV detected in *Nyctinomops laticaudatus*. The RABV-positive sequences from the CNS and fetus of *D. rotundus* clustered with a rabies sample from a dog host and were closely related to other bat-hosted samples. Notably, a difference in branch length was also observed between the RABV detected in the fetus and that in the CNS. Our study provide the first evidence of vertical transmission of rabies in bats in Brazil, underscoring an important mechanism in zoonotic virus evolution and rabies transmission dynamics.

Financial Support: FAPESP, CAPES and French Embassy in Brazil – TerrEE Scholarship

PT.314

IN VITRO CHARACTERIZATION OF THE ANTIVIRAL ACTIVITY OF BAT INTERFERON-INDUCED PROTEINS WITH TETRATRICOPEPTIDE REPEATS 5 (BAT IFIT5) AGAINST BAT-ASSOCIATED RABIES VIRUS

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Bats are acknowledged as significant reservoirs of zoonotic viruses, mainly due their sophisticated immune system. In Brazil, rabies virus (RABV) has been detected in various bat species, playing a crucial role in maintaining the disease. Interferon-induced protein with tetratricopeptide repeats 5 (IFIT5) belongs to a group of interferon-stimulated genes (ISGs) known for their antiviral activity, particularly against RNA viruses. Therefore, this study aimed to investigate the interaction of bat IFIT5 protein with distinct genetic lineages of RABV related to insectivorous bats, as from *Eptesicus furinalis* and *Tadarida brasiliensis*. The HEK-293T cells were transfected with a plasmid containing bat IFIT5 (wtbatIFIT5-FLAG). Subsequently, the cells were inoculated with 1 MOI of RABV genetic lineage related to *Eptesicus furinalis* (IP 964/06) and *Tadarida brasiliensis* (IP 3214/19) and revealed at 24 and 48 hours by direct immunofluorescence followed by fluorescent foci counting. The results indicated similar behavior between the two samples tested. At 1 MOI, a modest decrease in infection rates was observed for both samples over the duration of the experiment. However, sample IP 3214/19 showed a slightly greater reduction of around 35% after 24 hours when compared to IP 964/06 and at 48 hours IP 3214/19 showed a reduction of 25% while IP 964/06 around 15%. The results indicate the antiviral role of IFIT5 against RABV infection.

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PT.315

SURVEILLANCE OF RESPIRATORY VIRUSES IN ARAGUAIAN RIVER DOLPHIN (*Inia araguaiensis*) AND WATER SAMPLES FROM TOCANTINS RIVER IN MOCAJUBA, PARÁ.

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Respiratory viruses are commonly found in cetaceans worldwide, including those exposed to wastewater from anthropogenic sources. These viruses threaten animal and human health, especially in areas where these species interact. This study aimed to evaluate the presence of Sars-CoV-2, Influenza A and B, Human Respiratory Syncytial Virus (HRSV), Respiratory Enterovirus and Parainfluenza 1 in samples from Araguaian river dolphin and water from Tocantins river in Mocajuba-PA. Samples were collected from respiratory and enteric tracts of dolphin populations at a tourist interaction site with humans, Mirante do Boto - Mocajuba - Pará. Additionally, water samples were collected from three points along the river: at the Mirante do Boto, from sewage deposited in the river, and at an intermediate point distant from the others. Water samples were filtered, followed by filtration with amicon ultra-15 tubes. All biological and environmental samples were extracted using Biogene DNA/RNA Viral Extraction kit and analyzed by RT-qPCR using Allplex Sars-CoV-2/FluA/FluB/RSV Assay and GoTaq qPCR Promega kit. None of the samples tested positive for the viruses of interest. Other respiratory viruses will be analyzed, and a metagenomic approach will also be performed for the investigation of new viruses. This study reinforces the need for ongoing monitoring of both water and animals due to the environmental vulnerability to which they are exposed. This research is important for preventing potential outbreaks of zoonotic diseases caused by respiratory viruses, contributing to the preservation of I. araguaiensis and the maintenance of aquatic ecosystem health.

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PT.316

Molecular diagnosis of equine hepacivirus (*Hepacivirus equi*): comparison between probe-based and nested methodologies

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Equine hepacivirus (*Flaviviridae* family, *Hepacivirus equi,* EqHV) is associated with hepatitis in horses. Diagnosis is performed by molecular detection of the viral genome in serum/plasma or liver tissue samples, but there is no standard method. In Brazil, EqHV infection was demonstrated in Rio de Janeiro, Mato Grosso do Sul, Espírito Santo, and Pará States. In Rio de Janeiro, positive animals were detected in most farms and municipalities investigated, suggesting it may be broadly distributed in the country. The aim of this study was to compare the EqHV detection rates using two molecular methods: (i) Probe-based One-step Real Time targeting viral 5'NC and (ii) Nested RT-PCR targeting viral NS5b. A total of 382 equids slaughtered for meat exports, from Bahia, Goiás, and Piauí, were screened with both methods revealing detection rates of 5 (1.3%) using method (i) (mean Cq 32.6 ± 0.4) and 8 (2.1%) using method (ii). Three samples 062, 287 and 310 presenting discordant results were repeated with method (i) and evaluated with a third method using Probe-based assay in a Two-step test (using cDNA). Samples 287 and 310 turned positive with repeated method (i) (Cq287 37.6 and Cq310 37.5) and with two-step method (Cq287 36.9 and Cq310 32.2), may be due to low viral loads, but sample 062 remained negative. According to these findings, method (i) presented one false negative result, compared with method (ii), then should be used with 2 or 3 replicates. The method of choice for the diagnosis of EqHV should be carefully evaluated.

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PT.317

EVOLUTION OF INFLUENZA A VIRUSES COCIRCULATING IN BRAZILIAN SWINE: INSIGHTS FROM 2020-2023 ANALYSES

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Influenza A viruses H1N1, H1N2, and H3N2, enzootic in Brazilian swine, harbor an extensive genetic diversity that has been shaped mainly by the incursions of gene segments derived from human seasonal viruses, followed by antigenic shift and drift. Pre-2009 human seasonal viruses were introduced at least eight times in swine in Brazil, and multiple introductions have been observed for the pandemic lineage (H1N1pdm). This study aimed to provide an update on the circulation of IAV in Brazilian swine herds. For this purpose, pig samples collected in nine states of Brazil from 2020 to 2023 were prepared for genome sequencing by NGS. Datasets containing the 100 most similar sequences to these isolates, along with reference HA sequences from BV-BRC and GISAID were constructed. Nucleotide alignments were generated using MAFFT, and maximum-likelihood phylogenetic trees were inferred using IQTREE2 with automatic model selection. Branch support was assessed using the UFBoot method (1,000 replicates). Out of 95 HA sequences generated herein, 43 corresponded to H1pdm, 42 to H1 (1B lineage) and 10 were of H3 subtype. We found five additional H1N1pdm09 virus spillovers into swine, and in two of these events, the virus seems to be sustainedly transmitted among pigs. Although no novel clades were detected in the H1 (1B lineage) and H3 phylogenetic trees, the expansion of the genetic diversity within the previously known clades - also observed in the H1pdm tree - indicates these lineages are well adapted to swine and are continuously being transmitted among pigs in different regions of Brazil.

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PT.318

Concerns about bat virome of the city of Campinas - SP

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Bats are widely regarded as special virus reservoirs due to their association with several important zoonosis, including lyssaviruses, paramyxoviruses, and filoviruses as well as the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Although there is constant contact between humans and bats in some world regions, especially in urban areas, most viral families that appear regularly in bat virome analysis have not yet spilled over into human populations. Besides, transmission to humans usually involves an intermediate animal host. Therefore, constant surveillance about the ecology of the potential reservoir species and main routes of interspecies transmission is central to any preventive campaign. The present study aims to analyze the virome of the most prevalent bat species in the city of Campinas, São Paulo, Brazil. For this purpose, swab samples were obtained from bats collected by the municipal department of zoonosis control. The animal presented atypical behavior such as appearance at daytime, floor standing, injuries caused by other animal attack, and even found dead. All animals were euthanized and tested by direct immunofluorescence for rabies virus. Results obtained by molecular techniques mentioned above indicated the presence of viruses such as coronavirus and paramyxovirus. Correlated analysis with viral occurrence and animal historical, considering its health status and contact with other species may provide a valuable data for the epidemiological study regarding these animals.

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PT.319

RETROSPECTIVE OF ORF VIRUS INFECTION IN SHEEP IN RIO GRANDE DO SUL, BRAZIL.

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The Orf virus (ORFV) belongs to the genus Parapoxvirus (PPV) and is associated with the infection of sheep and goats, causing ORF or Ecthyma Contagiosum. This study aimed to demonstrate the presence of ORFV and to clinically and epidemiologically characterize cases of natural infection in sheep in Rio Grande do Sul (RS), Brazil. To this end, we collected clinical, epidemiological, and virological data from ORF outbreaks between 2011 and 2024 in the southwestern region of RS. The data was obtained from producers or visits to affected herds. The presence of ORFV was confirmed by electron microscopy or partial amplification of the B2L gene by PCR. During the period analyzed, 31 outbreaks were identified, which occurred in extensive herds (87.1%) or confined animals (13%). The disease was diagnosed in all years and during all months. Different breeds were affected, and, considering the animal category, the disease affected only lambs (29%), only adults (22.6%), both lambs and ewes (35.5%), and for 12.9%, information was not available. Crusted lesions were identified on the nostrils (32.2%), lips (32.2%), limbs (18.6%), oral mucosa (8.5%), udder and teats (5.1%), and genital region (1.7%), with varying degrees of severity. The infection was considered endemic in at least 35.5% of the properties, and only two properties vaccinated their herds. Vaccinating the herd is not a regular practice due to the unavailability of the vaccine in Brazil. Therefore, ORF is an endemic disease in sheep, with weaknesses in diagnosis and prevention.

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PT.320

Molecular characterization of Feline Immunodeficiency Virus in Cuiabá, Mato Grosso

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Feline immunodeficiency virus (FIV) is a retrovirus of great importance to felines, as it causes persistent infection and chronic depletion of the immune system. It has several subtypes that vary in pathogenesis and their occurrences are well characterized in the world. However, there is no information on the subtypes that occur in the feline population in Cuiabá-MT. Therefore, the objective of this study was to determine which subtype of FIV is active in domestic cats in the city of Cuiabá. Twenty-nine samples from FIV-positive cats were evaluated by amplification and nucleotide sequencing of the *env* and *gag* genes. Ten samples presented amplified products of 800 base pairs of the *en*v gene and nine presented amplification products of 260 bp of the *gag* gene. Nine amplified samples of the *env* gene and six of the *gag* gene were sent for Sanger sequencing. The sequencing showed that the cats were infected with subtype B and presented similarity between them ranging from 86.2 to 100% for the *env* gene and 86.8 to 100% for *gag*. The *env* gene sequences presented similarity ranging from 94.9 to 98.3% compared to different sequences available in GenBank, while the *gag* gene presented similarity ranging from 93.1 to 99%. Although subtype B has already been detected in other states, the confirmation of this subtype in Cuiabá expands the etiological and epidemiological knowledge about FIV, since Brazil is a country with a large territorial extension and data regarding the diversity of this retrovirus in different states are still scarce.

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PT.321

First detection of influenza A H5N2 LPAI virus in the Pantanal of Mato Grosso State

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Influenza A virus (AIV) causes significant infections in wild birds. Interspecies spillover can occur especially in migratory bird sites. The Pantanal has several locations of migratory sites for birds using the "Brazil-Central" and "Central Amazon/Pantanal" routes. The aim of this study was to investigate the activity of AIV in the wild avifauna of the Pantanal. Samples of cloacal and orotracheal swabs from birds captured in the Pantanal of Mato Grosso during 2021 and 2023 were evaluated. A total of 1,138 birds were analyzed by TaqMan RT-qPCR targeting the M protein gene of influenza virus A. When positive, the sample was processed for amplification of the eight genomic segments for subsequent NGS sequencing. Mixed swab (pooled tracheal and cloacal swabs) and tracheal swabs samples from the species *Charadrius collaris* identified as MT1403 were positive in RT-qPCR (Cts = 29 and 33, respectively). The complete genome of the virus was obtained by sequencing the amplified segments, which confirmed the presence of influenza A H5N2 virus. All other samples were negative. The sequences of the HA and NA genes showed 93% to 94% similarity with sequences detected in wild anatids in Colombia in 2011. Additional PCRs for influenza A H5 HPAI virus were also negative, and after preliminary evaluation of the cleavage sites, it was observed that this is a LPAI virus. This is the first detection of influenza A virus in the Pantanal of Mato Grosso, demonstrating the importance of monitoring this virus in migratory sites of wild birds in Brazil.

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PT.322

Neurological syndrome in horses infected with Equine Herpesvirus types 5 and 2 in Mato Grosso, MT

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Equine herpesviruses (EHV) are important for horses and responsible for respiratory, reproductive and neurological manifestations. EHV can establish lifelong latency and infected horses disseminate the virus after situations of stress. EHV-2 and EHV-5 belong to the subfamily *Gammaherpesvirinae* and genus *Percavirus* and are associated with respiratory manifestations. We report two fatal cases of neurological disease associated with EHV types 2 and 5 in horses from Mato Grosso State. The cases occurred in February 2024, in the municipality of Brasnorte with horses (B10 and B11) presenting history of acute clinical evolution with apathy, lowered head, recumbency, paresis of hind limbs, absence of pain reflexes. The horses died and only blood samples were collected in EDTA. Genomic material extracted from the samples was tested by Taqman qPCR for EHV-1, EHV-4, EEEV, WEEV, VEEV, WNV and rabies viruses and by a PanHerpesvirus nested PCR based on the detection of a conserved sequence of DNA polymerase gene present in all herpesvirus. All qPCR analyzes showed negative results. However, nested PCR amplified products of 220 base pairs. The products were purified and subjected to Sanger sequencing. The sequences of the amplified products presented 140 nucleotides that were 99% similar to different EHV-5 sequences and 100% identical to different EHV-2 sequences in GenBank, respectively. EHV-2 and EHV-5 infections are endemic in different horse's herds, with type 2 generally being more prevalent than type 5. Although neurological manifestations due to these types are rare, they should be considered in the diagnosis of equine viral encephalitis.

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PT.323

CO-INFECTION OF PORCINE *CIRCOVIRUS TYPE 2* AND *MYCOPLASMA HYOPNEUMONIE* IN LUNG SAMPLES FROM PIGS WITH PNEUMONIA LESION.

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Brazil's pig production stands out in the world meat market, being the fourth largest producer and exporter of pork in the world. However, respiratory diseases continue to challenge pig production. Viruses of great respiratory importance such as *porcine circovirus type 2* (PCV2) have already been identified with several co-infections such as *M. hyopneumoniae*, considered the main causative agent of porcine enzootic pneumonia (PES). A total of 40 pig lungs were macroscopically analyzed at slaughter for the presence of lung consolidation lesions, indicative of pneumonia. They were separated into four distinct groups according to the pneumonia lesion at slaughter, and the positive detection of *M. hyopneumoniae* by PCR. High-performance sequencing was performed using the Illumina MiSeq Platform, and phylogenetic analyzes were inferred using Geneious Prime and Mega X software. After sequencing, the Porcine Circovirus was detected in the different groups, according to nucleotide identity and phylogenetic analyses. The sequences were classified as PCV2 subtype 2b, which has already been reported in pigs that showed decreased weight gain, progressive weight loss, enlarged lymph nodes and other non-specific clinical signs, such as dyspnea and diarrhea. Bacterial and viral respiratory diseases are an important health problem in species raised in confined conditions in large groups. *M. hyopneumoniae* is a pathogen that induces a chronic respiratory disease and can influence the outcome of a subsequent viral infection such as Circovirus. A bacterial-viral co-infection induces worsening of lung lesions and greater inflammation and immune response, with increased production of pro-inflammatory cytokines.

Keywords: Circovirus, pneumonia, respiratory diseases, swine.

Financial Support: CAPES, CNPq, FAPERGS, PROSUC, FEEVALE.

PT.324

IDENTIFICATION OF THE STRONG DRUG RESISTANCE MUTATION H155Y IN H5N1 DETECTED IN *SULA LEUCOGASTER* IN BRAZIL, 2023

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The highly pathogenic avian influenza (HPAI) subtype has been spreading worldwide, exploiting the routes of migratory wild birds, which serve as its reservoir. This virus has the capability to infect mammals and domestic birds, a prevalence that has increased in recent years, raising concerns about its potential to infect humans. This study focused on the diagnosis and genomic evaluation of a brain sample collected from a *Sula leucogaster* seabird found dead in Matinhos, PR, in July 2023. The RT-qPCR technique for Influenza A was used to diagnose the sample, which was then sequenced using Next-Generation Sequencing, analyzed with bioinformatics tools, and evaluated in silico on GISAID. The H155Y amino acid substitution, associated with reduced inhibition by Oseltamivir and Zanamivir, was identified among the antiviral resistance mutations. Additionally, mutations conferring antigenic drift and changes in virulence were observed. The mutation potential of the HPAI influenza virus necessitates special attention and surveillance to monitor its evolution, as it may adapt to humans as more than just accidental hosts, thereby increasing the risk to public health.

Keywords: H155Y; Surveillance; H5N1; HPAI.

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PT.325

DETECTION OF GI-23 INFECTIOUS BRONCHITIS VIRUS IN VACCINATED BROILER FLOCKS WITH RESPIRATORY DISEASE IN PARANÁ, BRAZIL

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Infectious Bronchitis Virus (IBV) is an avian coronavirus that significantly impacts poultry production worldwide. Analysis of the complete S1 gene or its hypervariable regions (HVR1, HVR2, and HVR3) has classified IBV into genotypes GI to GIX and 38 lineages to date. This study aimed to investigate whether a new IBV variant was involved in respiratory disease outbreaks observed in 2021 in commercial broiler flocks vaccinated against IBV in the state of Paraná, Brazil. Day-old chicks in all affected flocks had received mass IBV vaccination at the hatchery with a combination of two live-attenuated vaccines, specifically the predominant serotypes Mass (GI-1) and BR-I (GI-11). Organ samples (air sac, trachea, lung, cecal tonsils, and kidney) were collected from twelve commercial broiler flocks belonging to four integrated poultry companies, with birds' ages ranging from 9 to 38 days. Using conventional semi-nested PCR with universal primers targeting the HVR3 region of the S1 gene, IBV was identified in all 12 flocks, with 80.4% (37/46) of samples testing positive. IBV was detected in all types of organs studied, with a 100% detection rate in kidneys and trachea, and 90.9% in cecal tonsils. Eighteen amplicons, representative of each flock, were sequenced. The nucleotide identities ranged from 99.2% to 100% among them and demonstrated high homology with IBV Var-2 strain sequences (GI-23 lineage), prevalent in the Middle East and Europe. The detection of the exotic GI-23 lineage underscores the importance of continued surveillance of circulating IBV strains to refine disease control strategies.

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PT.326

EPIDEMIOLOGICAL EVALUATION OF BOVINE HERPESVIRUS 1 IN CATTLE IN THE STATE OF ALAGOAS

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Bovine herpesvirus 1 (BHV-1) is the causative agent of respiratory, genital clinical symptoms and reproductive failure. In Brazil, BHV-1 was discovered in 1978, and since then, the virus has been spreading through herds around the country. This work is pioneering in Alagoas state. The study aimed to evaluate the presence of antibodies against the BHV-1 in dairy farms in Alagoas state, Brazil. For this purpose, we performed an *in vitro* serum neutralization assay using the MDBK cell line (Madin-Darby Bovine Kidney). MDBK cells were infected with BHV-1 strain LA and $TCID_{50}$ assay was performed for the viral titration. In a preliminary result, 212 samples from 5 farms in the state were tested. The samples were collected from 08/2022 to 12/2023. Regarding these samples, 168 (79.24%) were positive for antibodies against BHV-1. The farms analyzed were from Viçosa (54/68), Pão de Açúcar (42/42), Santana do Ipanema (2/6), Chã Preta (50/67) and Olho D'água Grande (20/29). This study may indicate recent virus outbreaks on the farms since the high antibody titers were found and the farms had signs of reproductive problems. Furthermore, the circulation of the virus through the herds may impact dairy cattle farming, since it is associated with reproductive failure.

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SEROLOGICAL SURVEY OF EHV-1 IN EQUIDS FROM ALAGOAS STATE, BRAZIL

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Infection of horses with Equine herpesvirus 1 (EHV-1) is characterized by neurological, abortive and respiratory symptoms. EHV-1 is part of the *Orthoherpesviridae* family. There are no recent surveys regarding viral circulation in Alagoas state. In this context, we used serum neutralization techniques to identify antibodies anti-EHV-1 in Alagoas state, Brazil. Non-vaccinated equids were selected for whole blood collection and subsequent serum collection. The sera were tested to evaluate the existence of anti-EHV-1 antibodies using VERO cells. We evaluated 295 samples from five municipalities in Alagoas state. Regarding these, 172 showed antibodies against EHV-1. There was 59% overall positivity. The animals from Mar Vermelho showed the highest positivity (67/75), indicating their presence of antibodies in 89.3% of samples. In animals from Rio Largo, 60.7% (62/102) of the tests were positive. In Quebrangulo, the percentage was 58.3% (14 positives in 24 samples). A lower proportion was observed in Viçosa, which showed the positivity of 32.9% (28/85). Six samples were collected from Maceió and only one was positive. It can be concluded that there is a significant prevalence of anti-EHV-1 antibodies among equids from various regions of Alagoas state. Furthermore, the study highlights the importance of obtaining epidemiological data to track infections and control diseases in farmed animals in Alagoas.

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MOLECULAR INVESTIGATION OF SARS-CoV-2 IN COATIS FROM SERRA DO CURRAL, BELO HORIZONTE, MINAS GERAIS

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Emerging infectious diseases (EIDs) are linked to socioeconomic, ecological, and environmental factors, characterized as imminent threats to human health. The emergence of SARS-CoV-2 and the COVID-19 pandemic reinforce the role of viral pathogens in this global context of human and animal health, with monitoring spillover and spillback events in synanthropic mammals in urban areas being an important approach of the One Health initiative. This project aims to prospect SARS-CoV-2 in biological samples from procyonids of the species *Nasua nasua* inhabiting parks of Serra do Curral in Belo Horizonte, Minas Gerais, Brazil. The collection in this study comprises samples of oral and anal swabs from seventy-three coatis that were captured using Tomahawk traps. All collections were carried out during 2023, under the approval of the Animal Ethics Committee. The samples were processed and RNA was extracted using the QIAamp Viral RNA Mini Kit (QIAGEN®), followed by endogenous control tests for murine beta-actin. Subsequently, RT-qPCR assays were performed using specific primers and probes for SARS-CoV N1 and N2 targets. No positivity for the viral genome was observed in the coati samples tested, indicating the absence of viral circulation in these procyonids during the collection period in the sampled parks. Continuous monitoring of EIDs is important for understanding zoonotic transmission dynamics and preventing future public health emergencies, especially in sylvatic-urban interface areas such as the parks of Serra do Curral in Minas Gerais.

Financial Support: FAPEMIG; CNPq; Capes; PRPq.

PT.329

MOLECULAR DETECTION AND PHYLOGENETIC ANALYSIS OF CIRCOVIRUS IN DOMESTIC BIRDS FROM BRAZIL

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Avian circovirus, particularly Beak and Feather Disease Virus (PBFD) in psittacines, causes significant ecological and economic losses due to its high infectious potential. This study aimed to investigate the frequency of circovirus in domestic birds and analyze the circulating strains in Brazil. A total of 719 Brazilian domestic birds were tested for *Circovirus sp* using broad-spectrum Nested-PCR to detect circovirus Rep gene fragment. Positive samples were sequenced using both Sanger sequencing, for species identification, and Next Generation Sequencing (NGS) for complete genome analysis of the circulating strains. Phylogenetic inferences were performed using maximum likelihood. Results showed a 33.4% [95% CI; 22.3%-45.3%] frequency of *Circovirus sp* infection in non-psittacines and 25.3% [95% CI; 22%-28.7%] in psittacines. Most positive samples originated from ring-necked parakeets, followed by cockatiels and parrots from Southeastern Brazil. Statistical tests showed no association between the taxonomic order and positive results. Sequencing results indicated a predominance of BFDV [identity: 98.4%-100%; cover: 99%-100%; accession: MN175611.1] in domestic birds belonging to the Psittaciformes order, except for one individual infected with a canary circovirus strain (CaCV) [identity: 99%; cover: 100%; accession: MG266165.1]. Phylogenetic analyses of the Rep and Cap genes and the complete genomes of BFDV strains, compared with sequences deposited in GenBank, showed different topographies but homogeneous clustering of those identified in Brazil. Here, we conclude that circovirus is quite widespread in Brazilian domestic birds, with BFDV strains likely introduced through the exotic bird trade.

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PT.330

Monitoring Infectious Bronchitis Virus (IBV) in Vaccinated and Non-vaccinated Broiler Chickens in Brazil

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Infectious bronchitis virus (IBV) is a widely distributed pathogen that causes respiratory, renal, and reproductive issues in chickens. There are various antigenic types of IBV co-circulating globally, with some being prevalent worldwide and others being limited to specific regions. This study aimed to conduct IBV surveillance in Brazil, understand potential viral escapes, and identify new variant strains in the poultry population. A total of 1,000 nasotracheal swabs from 100 batches were collected from poultry carcasses in six Brazilian states (Rio Grande do Sul, Santa Catarina, Paraná, São Paulo, Minas Gerais, and Ceará). Screening for the presence of IBV was conducted using Reverse Transcription Polymerase Chain Reaction (RT-PCR) targeting the hypervariable region of the IBV S1 gene. Three different lineages of IBV were identified, with 2.19% being the Massachusetts strain, 30.76% being BR-I/BR-II strain, and 8.79% testing positive for both BR-I/BR-II and Massachusetts strains. Additionally, 7.69% were positive for BR-I/BR-II and Variant-2 strains, 3.29% for BR-I/BR-II, Massachusetts, and Variant-2 strains, 3.29% for Massachusetts and Variant-2 strains, and 6.59% as Variant-2 strains, with many samples showing the co-occurrence of more than one lineage. This study identified the GI-23 lineage in 2021, suggesting prior circulation in Paraná and Rio Grande do Sul. It underscores the importance of genomics and molecular epidemiology in understanding and managing infectious diseases in poultry and vaccination programs.

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PT.331

Detection of Coronaviridae and Paramyxoviridae in bats from the Atlantic Forest – Brazil

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Approximately 70% of Brazil's population lives in the Atlantic Forest, where human disturbances and biodiversity loss have increased animal-human contact and the risk of new diseases. According to the most recent data, the Atlantic Forest inhabits 9 families of bats, which are associated with several viruses of public health concern, such as the Coronaviridae and Paramyxoviridae families. This study aims to increase knowledge about the diversity of the viral population in Atlantic Forest bats to improve our preparedness for the emergence of new diseases. Collections were done in Santa Catarina state, Brazil (2021 to 2023). The bats were sampled (oral and rectal swabs pooled) and morphologically classified at the species level. Viral identification was done using RT-PCR targeting the polymerase gene of each family. Positive samples were sequenced by the Sanger method for the phylogenetic analysis. We sampled 583 bats from 16 genera, comprising the Phyllostomidae family (91.3%), Vespertillionidae (7%), and Molossidae (1.5%) families. The positive samples were sequenced, and the phylogenetic analysis was performed using obtained sequences and available sequences in GenBank. Thirty (5.1%) of the 583 samples were positive for the Orthocoronavirinae subfamily, with twenty-nine sequences grouped within the Alphacoronavirus and one within the Betacoronavirus genera. Regarding the Paramyxoviridae family, 11 (1.9%) samples of the 583 tested were positive, with nine of the obtained sequences grouped within the genus Jeilongvirus, and two others were grouped within the genus Morbillivirus. Further studies for molecular and biological characterization are ongoing.

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PT.332

A 13 - YEAR RETROSPECTIVE REPORT OF SEROLOGIC DIAGNOSIS OF BOVINE LEUKEMIA VIRUS IN THE VIROLOGY SECTION - UFSM

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Bovine leukemia virus (BLV) is a widespread oncogenic *Deltaretrovirus* associated with important economic losses to the dairy cattle industry worldwide and is endemic among Brazilian cattle. We herein present a 13-year (2011 – 2023) retrospective report of serologic BLV diagnosis performed at the Virology Section – Universidade Federal de Santa Maria (UFSM). The study encompassed samples from 11727 cattle from 1086 herds (mostly dairies) located in 189 counties in three states that comprise the second dairy basin in Brazil, Rio Grande do Sul (RS), Santa Catarina (SC) and Paraná (PR). The samples were tested by agar-gel immunodiffusion (AGID) or immunoenzymatic assay (ELISA). Overall, the animal seroprevalence was 38.1% (4467/11727) and herd prevalence 64.5% (700/1086). The three states presented similar animal prevalences (39.1%, 37.6% and 36.9%, respectively). Herd prevalences were 63.5 (RS), 65.2 (SC) and 67.2% (PR). As a whole, the animal and herd seroprevalence showed an increasing trend from 2011 (19.6% and 57.5%, respectively) to 2023 (60.4% and 73.8%). These results demonstrate that BLV continues circulating widely in cattle herds in the three Southern states and indicate the need of official and/or voluntary measures to control/reduce the prevalence and the losses associated with the infection.

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KEY WORDS: *bovine leukosis, persistent infection, serologic diagnostic*, ELISA, IDGA.

PT.333

DETECTION OF ANTIBODIES AGAINST RABIES IN CATTLE

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Rabies is a neurotropic zoonosis caused by the *Lyssavirus rabies* (RABV) and can affect all mammals. The rural cycle of this disease causes serious losses to national livestock, as it is generally identified in cattle and horses, which are the preferred food source for hematophagous bats (*Desmodus rotundus*). The *D. rotundus* are reservoirs of RABV and responsible for maintaining this cycle. Despite of not mandatory, the vaccination is the best way to control rabies in cattle. The present study aimed to detect anti-rabies antibodies in cattle after prophylactic pre-exposure vaccination. For this purpose, 219 serum samples from vaccinated cattle were processed using the Rapid Fluorescent Focus Inhibition Test (RFFIT). As results, 52 samples (24%) tested negative, and 167 samples (76%) tested positive, showing antibodies with titers starting from 0.5 IU/ml. This study proves the effectiveness of vaccination in cattle, contributing to rabies control. It is important to highlight that in addition to the economic loss caused by rabies in herbivores, the risks posed by the disease to other animals and human health must also be considered.

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PT.334

DEVELOPMENT OF AN ELISA ASSAY FOR THE DETECTION OF ANTI-EQUINE INFECTIOUS ANEMIA VIRUS ANTIBODIES USING SENSITIZATION AND CONJUGATION WITH VIRAL RECOMBINANT ANTIGEN

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Equine Infectious Anemia (EIA) is a disease caused by *Lentivirus equinfane*, previously known as Equine infectious anemia virus (EIAV), a member of the *Retroviridae* family within the genus *Lentivirus*. EIA affects equids, including horses, donkeys, and mules. Economic activity generated by the equine industry in Brazil, totals approximately R$16.15 billion annually and supports around 3 million jobs. Unfortunately, there is no treatment or vaccine available for EIA, and the primary method of controlling the disease involves diagnosing infected animals and implementing euthanasia as a measure of control. Therefore, there is an increasing need to develop and refine more specific diagnostic tests to effectively control the spread of EIA. One method used for identifying anti-EIAV IgG is ELISA. This study aimed to standardize a test utilizing Equine Infectious Anemia Virus (EIAV) capsid protein (p26) for both sensitization and conjugation, thereby enhancing the assay's specificity. For plaque coating, a concentration of 50 µg/mL of recombinant p26 protein was utilized along with commercial blocking agents. Test samples were combined with labeled recombinant p26 conjugate, and the reaction was visualized using 3,3’,5,5’-tetramethylbenzidine (TMB). Preliminary analysis of the developing ELISA results revealed significant differences in optical density values between a known positive equine sample (OD = 2.047) and a known negative equine sample (OD = 0.034), using a 1:10 conjugate dilution. This test is advantageous for use in different species of equids that can be infected with EIAV because it does not rely on species-specific conjugates.

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PT.335

MONITORING SARS-CoV-2 IN BELO HORIZONTE'S ANIMALS: INSIGHTS FROM MUNICIPAL PARK STRAY CATS AND ZOO MAMMALS

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SARS-CoV-2, the virus that causes COVID-19, has had a major impact on human and animal health worldwide. To find out a possible viral infection in animals, two important sites were investigated in Belo Horizonte, Brazil: Americo Rene Giannetti Municipal Park, located in downtown Belo Horizonte, near to hospitals and health facilities, and the Foundation Zoo-Botany of Belo Horizonte (FPMZB), which is significant for animal conservation. The municipal park survey sampled 78 stray cats from February to September 2021, while the FPMZB study assessed SARS-CoV-2 exposure in 47 captive mammals from November 2021 to March 2023. Swab samples from both locations were tested for SARS-CoV-2 RNA using RT-PCR, and positive samples underwent whole-genome sequencing on the MiSeq platform. Genomes were analyzed using Nextclade and Pangolin systems for phylogenetic classification and compared with public genomes from the GISAID EpiCoV database. In the Municipal Park, six cats tested positive, with four sequences identified as VOC Delta. At FPMZB, nine mammals tested positive, with two sequences identified as VOC Alpha in a Maned Wolf and a Fallow Deer, and VOC Omicron in a Gorilla. Phylogenetic and phylogeographic reconstructions grouped the SARS-CoV-2 sequences from animals with human SARS-CoV-2 sequences from the same areas and dates, suggesting human-to-animal transmission. These results show the significance of monitoring SARS-CoV-2 in wild and domestic animals to track viral adaptations in new hosts and the emergence of new lineages, enhancing our understanding of virus transmission dynamics.

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PT.336

DETECTION OF ROTAVIRUS A IN A NATURALLY INFECTED FREE-LIVING COUGAR (PUMA CONCOLOR) FROM MATO GROSSO DO SUL, BRAZIL.

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Rotavirus species A (RVA), a segmented double stranded RNA (dsRNA) virus, is frequently identified as a causative agent of gastroenteritis in children and young animals worldwide. However, although RVA is found in a diverse range of animal species, reports on rotaviruses infections in wild felids are lacking. The objective of this study was to report the first detection of RVA in the feces from a free-living cougar (*Puma concolor*) from Mato Grosso do Sul state. The animal was a young cougar, male, that was found after its mother's death by roadkill and was kept in a separate fauna maintenance unit enclosure. Nucleic acid extraction from the stool sample was performed using a combination of phenol/chloroform/isoamyl alcohol and silica/guanidinium isothiocyanate methods. The extracted nucleic acid was submitted to 7.5% polyacrylamide gel electrophoresis followed by silver staining (ss-PAGE) and RT-PCR with specific primers that amplifies a fragment with 1,062bp of the VP7 protein gene of RVA. The amplicon obtained was sequenced and the nucleotide (nt) sequence was analyzed using MEGA and BioEdit softwares. The analysis of the 11 segments of dsRNA in ss-PAGE displayed a characteristic RVA pattern (4:2:3:2). Due to the low sensitivity of the ss-PAGE technique, positive result indicates high viral excretion at the time of sampling. RT-PCR and nt sequencing analysis confirmed the presence of RVA, showing 84% nt identity with the RVA strain from alpaca (RVA/Alpaca-wt/PER/Alp11B/2010/G35P[50]). This report confirms a natural infection of RVA in *Puma concolor*; however, the clinical and epidemiological significance in wild felids remains to be determined.

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PT.337

INVESTIGATION OF PARAMYXOVIRUSES IN FREE-LIVING WILD MAMMALS

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The *Paramyxoviridae* family comprises a large group of viruses that includes four subfamilies, 17 genera, and 78 species, according to the current ICTV Taxonomy release. Members of this family can infect a wide range of hosts, primarily mammals, in which a diversity of novel paramyxoviruses has been identified. This study aimed to investigate whether wildlife mammals could act as hosts for new paramyxoviruses. Eleven urine samples from nine species of free-living wild mammals were collected during their treatment at Veterinary Teaching Hospitals in Santa Catarina and Paraná. The species included South American Coati (*Nasua nasua*, n=1), Crab-Eating Fox (*Cerdocyon thous*, n=1), Common Marmoset (*Callithrix jacchus*, n=1), White-Eared Opossum (*Didelphis albiventris*, n=1), Capuchin Monkey (*Sapajus sp.,* n=1), Maned Wolf (*Chrysocyon brachyurus*, n=2), Southern Tiger Cat (*Leopardus guttulus,* n=1), Margay (*Leopardus wiedii*, n=1), and Northern Tiger Cat (*Leopardus tigrinus*, n=2). A semi-nested RT-PCR (sn RT-PCR) was performed using primers targeting a conserved region of the L gene of diverse paramyxoviruses. Paramyxovirus RNA was detected exclusively in a Southern Tiger Cat (*Leopardus guttulus*), a small wild cat species native to Brazil, Argentina, and Paraguay. Following the initial positive result, two additional urine samples from this animal were collected and also tested positive by sn RT-PCR, with an approximate interval of two weeks between collections. DNA sequence analysis demonstrated a 94.21% nucleotide identity with a paramyxovirus strain from the genus *Jeilongvirus* (Genbank accession number MN850340.1). Our results suggest that *Leopardus guttulus* may serve as a natural reservoir for *Jeilongvirus* and contribute to expanding our understanding of the distribution of these viruses.

Financial support: CAPES

PT.338

A NEW PICORNA-LIKE VIRUS IDENTIFIED IN SNAKES WITH NEUROLOGICAL SYMPTOMS

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In spite of the prophylactic measures adopted by snake breeding facilities to maintain the animals' sanitary conditions, during the routine handling of a *Bothrops jararaca* we noticed the absence of proprioception and paralysis without other clinical alteration. The snake was submitted to euthanasia, and samples were collected from various organs for viral and histopathological analysis. No gross lesions were observed. As we suspected of virus infection the brain and liver were disrupted an submitted to acid nucleic extraction using kit MagMax Core nucleic acid purification followed by Next Generation Sequencing Library Preparation. Raw data generated from sequencing was submitted to Varsmetagen, an online platform for bioinformatics analyses and interpretation of NGS data for infecctious diseases. According to analyses in Varsmetagen, a total of 8.9 million paired-end reads were generated to the sample. About 96% of reads passed quality control filters and 18% were not mapped to host. An RNA virus (PhageMS2) was spike-in as internal control to validate the reaction and 652 reads were sucessfully identified by the pipeline. No sequences from any virus other than the spike-in control was initially found, but after the extension of bioinformatics analyses to include a more sensitive search using HMMs proteins profiles a full genome sequence of a novel picorna-like virus was found. Although the causal relationship between the virus and the disease was not proven, it was detected in another 10 snakes with the same symptoms of the same facilitie. Further studies are needed to understand the significance of this finding.

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PT.339

CO-INFECTION CANINE PAPILLOMAVIRUS TYPE 8 AND 9 IN A DOG

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Papillomaviruses (PV) are known to infect mammals, birds, and reptiles. Intensely studied in humans, they are known to cause pre-neoplastic and neoplastic lesions in different species. Co-infections have been described, and can be associated with the severity of the lesions. *Canis familiaris papillomaviruses* (CPVs) are classified into the Papillomaviridae family, with three genus: *Lambdapapilomavirus*, *Taupapillomavirus* and *Chipapillomavirus*. They have been associated with different lesions, such as oral and cutaneous papillomatosis, pigmented plaques, and squamous cell carcinomas (SCCs). The aim of this study was to sequence the complete genome of CPV in pigmented papillomatosis plaques in a dog, using high throughput sequencing with Illumina MiSeq platform. Based on nucleotide identity (Blastn) and complete genome phylogenetic analysis, CPV-8 and CPV-9, belonging to the *Chipapilomavirus* genus, were identified. Therefore, this study presents a CPV co-infection in dog papillomatous lesions, which its importance lying in predicting prognosis and aiming to improve the quality of life for both dogs and their guardians.

Keywords: co-infection, papillomavirus, veterinary virology, bioinformatics analysis
Finantial Support: Capes, CNPq, FAPERGS, Feevale.

PT.340

DETECTION OF EQUINE INFECTIOUS ANEMIA VIRUS PROVIRAL DNA IN SERONEGATIVE HORSES AFTER THE SANITATION OF AN OUTBREAK

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The equine infectious anemia virus (EIAV) is a lentivirus belonging to the *Retroviridae* family, with an RNA genome that incorporates proviral DNA into the host cell upon infection. The diagnosis of Equine Infectious Anemia (EIA) is made by AGID, and positive animals must be isolated or euthanized. In Brazil, the infection occurs in all regions with different prevalence levels, and each state has a control program. This study aimed to characterize an outbreak of EIA and investigate the presence of proviral DNA in seronegative horses. To this end, official forms and whole blood samples collected during the last sanitation were analyzed for EIAV proviral DNA. The herd consisted of 77 horses, and one animal was seropositive. Eight sanitary inspections were conducted, and the property was closed for 372 days. Another 11 horses were seropositive in the AGID; two births, six deaths, two entries, nine exits, and 12 animals were discarded. Ultimately, the herd consisted of 42 horses that tested negative for AGID on two consecutive occasions. Five of these animals were PCR-positive for the LTR-gag region of the EIAV provirus. The products were sequenced, and genetic analysis confirmed their identity with EIAV. Controlling an EIA outbreak can be time-consuming, and even in seronegative animals, DNA regions of the EIAV provirus can be detected. Finally, the role and relevance of seronegative carrier animals in the epidemiology of the infection needs to be investigated.

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PT.341

FIV AND FELV DETECTION IN JAGUARS (*PANTHERA ONCA*) IN THE STATE OF MATO GROSSO DO SUL, BRAZIL

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Feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) are retroviruses that affect felines of various species, targeting the animals' immune systems and predisposing them to opportunistic infections and neoplasms. In Brazil, both viruses occur in domestic cats, which often have access to the streets, favoring infection. Despite their presence in domestic cats, the viruses are also described in wild felids, such as jaguars (Panthera onca). Accordingly, this study aimed to investigate the presence of FIV and FeLV infection in both free-ranging and wild-caught jaguars (Panthera onca) in the state of Mato Grosso do Sul. Eighty-four blood samples from jaguars collected from July 2017 to February 2024 were analyzed. The detection of retroviruses was performed using the real-time polymerase chain reaction (qPCR) technique to identify proviral DNA present in the samples. Proviral DNA extractions were performed using the phenol-chloroform extraction method, a standard technique for obtaining high-quality genetic material. Out of the samples analyzed, seven were positive for FeLV, while none was positive for FIV. Three jaguars were wild-caught (1 male and 2 females), while four were free-ranging felids (3 females and 1 male). These results indicate that jaguars are exposed to FeLV, suggesting the need for further studies to evaluate the circulation and effects of these retroviruses in wild felid populations in Brazil, especially concerning conservation programs.

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PT.342

RABIES OUTBREAKS IN FEEDLOT CATTLE IN MATO GROSSO DO SUL, BRAZIL

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Rabies is a zoonotic disease of worldwide concern. In Brazil, the main reservoir to herbivores is the hematophagous bat (Desmodus rotundus). In Mato Grosso do Sul, rabies cases are endemic in livestock, favored by the climate, topography, abundance of food, and the availability of bat shelters, ecological imbalance resulting from deforestation for the implementation of new crops, forcing bats to move to other areas. Cattle previously raised extensively were confined to make room for agriculture, altering the relationship between hematophagous bats and their food source, cattle. Accordingly, this study aimed to analyze rabies cases in feedlot cattle from 2020 to 2023. During the analyzed period, 28 outbreaks of rabies in confined and semi-confined cattle tested positive for rabies in 21 municipalities of Mato Grosso do Sul, including Inocência (3), Bonito (3), Sidrolândia (2), Bandeirantes (2), and Cassilândia (2). Regarding rabies vaccination, 33.3% (12) of the animals were vaccinated, 30.6% (11) were not vaccinated, and in 36.1% (13) the vaccination status of the animals was not informed. Regarding death, 63.9% (23) died naturally and 36.1% (13) were euthanized. Rabies is rarely described affecting confined cattle due to the conditions and the period that the animals are kept under confinement. Thus, it is essential that cattle from extensive properties, upon entering confinement systems, receive an additional vaccination protocol in which the initial doses act as a booster vaccination. This highlights the importance of health education and implementation of prophylactic and control measures against rabies, as the disease also occurs within livestock system.

PT.343

A FAST AND CHEAP MAGNETIC BEAD BASED PROVIRAL DNA EXTRACTION FOR FeLV AND FIV MOLECULAR DIAGNOSIS

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Retroviruses that cause feline immunodeficiency (FIV) and feline leukemia (FeLV) are common persistent infections in feline medicine worldwide. Anemia, immunosuppression, neoplasia, and death represent some of the clinical signs. Molecular diagnosis has become an important tool for FIV and FeLV detection. Different protocols for RNA and, mostly used, proviral DNA purification can be applied. Accordingly, a total of 203 whole blood samples were analyzed from felines received at the Virology Laboratory. The proviral DNA from the samples were obtained using an in-house magnetic bead extraction protocol adapted from the Bio-On-Magnetic-Beads (BOMB) platform, originally developed for the diagnosis of SARS-CoV-2. During the process of standardization and validation of the FIV and FeLV tests, the bead-based protocol underwent adaptations. In general, the protocol includes cell lysis steps with guanidine isothiocyanate and proteinase K, followed by the addition of a magnetic bead solution, separation of nucleic acids with a magnetic rack, purifications with 100% isopropanol and 75% ethanol, and elution of nucleic acids using a DNA-specific solution. In qPCR, of the 203 samples, 37 were positive for FIV and 71 were positive for FeLV. The implementation of this protocol in the laboratory routine made the extraction process quick, practical, and less harmful to the environment. Furthermore, the use of common reagents allows a considerable reduction in exam costs, making the diagnosis of FIV and FeLV more accessible for feline owners.

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PT.344

INFECTIOUS BRONCHITIS VIRUS CIRCULATION IN BACKYARD POULTRY IN SÃO PAULO, BRAZIL: PREVALENCE AND LINEAGE VARIABILITY

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Infectious bronchitis virus (IBV), a member of the family Coronaviridae, causes respiratory and reproductive signs in chickens. The present study evaluates the circulation of IBV in backyard poultry in São Paulo state from December 2022 to April 2024. Active surveillance was carried out in 140 farms from 15 municipalities, including 742 birds (718 chickens, 17 ducks, and seven geese) with no mortality or vaccination record. Molecular screening was performed on 304 cloacal and tracheal swab samples using RT-qPCR, targeting the 5′ untranslated region (5′UTR) of the IBV genome. Forty-three samples (14.15%) were IBV positive, with 42 cases in chickens (97.6%) and a duck (2.4%). Among the municipalities, 86.6% (13 out of 15) had at least one positive case, with Ilha Comprida and São Sebastião showing the highest incidence rates at 39.53% and 13.95%, respectively. Virus isolation was performed on three high-load IBV samples using 10-day-old SPF eggs, followed by molecular analysis of allantoic fluid for confirmation. DNA sequencing using primers targeting the S1 gene of GI-1 and GI-11 lineages, the most common in Brazil, failed to amplify these strains in the positive samples. Further molecular tests of DNA sequencing will be performed to better characterize the IBV circulation. The preliminary findings indicate a high circulation of atypical IBV lineages in the São Paulo state backyard poultry. Our data highlights the importance of biosecurity measures in the poultry industry to ensure sanitary status due to the continuous threats of viruses.

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Keywords: Infectious Bronchitis Virus, avian, Backyard poultry, Coronavirus

PT.345

ANTIBODIES DETECTION SPECIFIC TO AVIAN INFLUENZA VIRUS FROM WILD BIRDS COLLECTED ON THE SANTA CATARINA COAST, BRAZIL

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The avian influenza virus, a member of the Orthomyxovirus family, causes a highly contagious disease that affects both domestic and wild animals. Waterfowl are the main reservoir of the sixteen AIV subtypes; however, little is known about their circulation in Brazil. The study aims to identify the antibodies specific to nucleoprotein (NP), a conserved AIV protein among AIV subtypes, in 166 sera from 147 wild birds captured in Santa Catarina between July 2023 and July 2024. Samples were tested using a competitive ELISA kit specific to avian influenza nucleoprotein for multispecies. Birds belonged to the eight species from five orders: Sphenisciformes (47.5%), Charadriiformes (41.4%), Suliformes (9.9%), Procellariiformes (0.6%), and Pelecaniformes (0.6%). Hundred-forty-four samples (86%) were collected from Spheniscus magellanicus (n=77) and Larus dominicanus (n=77). Results showed that 26.5% of tested samples were positive for NP AIV. Surprisingly, 93% of Larus dominicanus tested positive for AIV, while antibodies were detected in only 7% of Spheniscus magellanicus samples. Specific tests for other subtypes will be performed soon. The present study provides valuable insights into the disease AIV dynamics in wildlife from the southern hemisphere, which can help improve the current epidemiological surveillance program.

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PT.346

Detection of *Avipoxvirus* in yellow-bellied seedeater (*Sporophila nigricollis*) and blue-black grassquit (*Volatinia jacarina*)

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Avian pox is a viral disease of the epithelial tissue, with a nodular and proliferative character and occurring worldwide, caused by members of *Avipoxvirus*. In the genus there is a diversity of viral species, occurring in more than two hundred species of birds. Morbidity and mortality vary depending on the viral strain and the immune and health status of the hosts. Some strains found in canaries and quails can produce infection with high mortality. In industrial poultry farming, infection can result in a reduction in the feed conversion rate and a drop in egg laying. In conservation medicine, avian pox can be a serious threat to species in remote and isolated areas, as described for Passeriformes from the Galapagos Islands. *Avipoxvirus* infection is here reported in wild specimens of *Sporophila nigricollis* and *Volatinia jacarina* received at the poultry disease laboratory. For the laboratory analyzes and confirmation of infection, the complete necropsies were carried out, with the detection of a large, elevated and firm nodule in the periocular region (1.5cm Ø) in *S. nigricollis*, with samples taken for viral isolation and molecular detection (PCR). By PCR, the detection of the viral core protein 4b gene of *Avipoxvirus* was confirmed in both birds. The PCR product of *V. jacarina* was sequenced and a phylogenetic tree was prepared (Maximum Parsimony, MEGA11), with the sample analyzed grouping with an *Avipoxvirus* described in crows from Japan. The detection of *Avipoxvirus* in birds, especially in endangered species in triage, can be a serious impediment to rehabilitation.

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PT.347

ENVIRONMENTAL FACTORS ASSOCIATED WITH THE OCCURRENCE OF WESTERN EQUINE ENCEPHALITIS IN HORSES IN THE STATE OF RIO GRANDE DO SUL, BRAZIL.

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Alphaviruses are arboviruses with a sylvatic cycle that eventually causes neurological diseases in horses and humans. In 2023, the circulation of the Western Equine Encephalitis (WEE) virus was identified in the west of Rio Grande do Sul. This study aimed to describe cases of neurological disease in horses and investigate the relationship between weather and environmental conditions. To this end, the official notifications recorded in Barra do Quaraí and Uruguaiana municipalities were analyzed. Meteorological information and environmental characteristics were obtained from INMET and IBGE, respectively, and the period analyzed covered July/2023 to March/2024. Ten suspected cases were reported during this period: three on November/23, six on December/23, and one on March/24. The diagnosis of WEE was confirmed in two outbreaks, and one animal had a co-infection with the rabies virus. In five cases, neurological signs were confirmed without determining the cause. Three cases were discarded due to the absence of compatible signs. In the analyzed months, the minimum and maximum temperatures and the average humidity were above the historical averages. There was a severe drought in the first two months of the period analyzed, followed by a rainy period. Flooding of the River Uruguay for more than 90 days was also recorded. The cases were recorded in regions characterized by the cultivation of irrigated rice. The emergence of WEEV in horses may be associated with the meteorological variations observed and landscape use. In addition, the influence of other factors cannot be disregarded.

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PT.348

COMPLETE GENOME SEQUENCING OF BOVINE PAPILLOMAVIRUS IN EQUINE SARCOID LESION

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Papillomaviruses (PV) are important agents with the capacity to induce simple, multiple and/or proliferative lesions in the dermis and epidermis of animals, known as cutaneous papillomatosis. These lesions can be benign or neoplastic in nature, and have already been identified in a series of hosts, such as mammals and birds. Bovine PV belongs to the Papillomaviridae family, composed of the Deltapapillomavirus (BPV-1, 2,13 and 14), Xipapillomavirus (BPV-3, 4, 6, 9, 10, 11, 12 and 15.), Epsilonpapillomavirus (BPV- 5 and 8), Dyoxipapillomavirus (BPV-7) and unclassified (BPV- 16 to 43) genus. BPV types 1, 2, and, probably BPV13, are related to the pathogenesis of sarcoid, the most common equine skin tumors, that could be can be locally aggressive and cause significant clinical symptoms in affected horses. Therefore, the main focus of the present study was to perform papillomavirus sequencing, using the high-throughput, Illumina MiSeq platform, of an equine sarcoid with histopathological diagnosis and papillomavirus positive PCR. Through sequencing, 387,923 reads and 17 contigs were obtained, generating a complete BPV genome, with 99% coverage. The nucleotide identity analysis, carried out through the Blastn platform on GenBank, presented 99% of identity with Deltapapillomavirus 4 (BPV1), classification corroborated by phylogenetic analysis, based on the L1 region. This study confirms the presence of BPV1 DNA in sarcoid lesions in horses, in addition to enriching the papillomavirus genomic databases.

Keywords: virology, veterinary, papillomavirus, bioinformatics, phylogenetic analysis.

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PT.349

SURVEILLANCE OF AVIAN INFLUENZA VIRUS IN *HAEMATOPUS PALLIATUS* (AMERICAN OYSTERCATCHER) ON THE COAST OF TORRES, RIO GRANDE DO SUL, BRAZIL

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Avian influenza virus (AIV) is a real threat to human health, poultry and wildlife. Shorebirds are well known to spread the virus and, depending on the viral subtype, possibly develop the disease. The American Oystercatcher (*Haematopus palliatus*) is a shorebird that remains on the coast year-round; it encounters large flocks of migratory birds at different times of the year. This study was conducted, in collaboration with Projeto Piru-Piru (Uergs-GEMARS) to monitor the presence of AIV in the American Oystercatchers, at Torres, Rio Grande do Sul state, southern Brazil. Sampling was conducted during the breeding season, from November to December 2023. Thirteen birds were sampled, including four adults and nine chicks. The birds were captured using a handheld bird net at night with the help of flashlights to disorientate the animals. Each one was banded and measured. Cloacal and orotracheal swabs were obtained to screen for the presence of AIV RNA. Additionally, feathers and blood were collected. Total nucleic acid extraction was performed using the Kingfisher® automated extractor, using the MagMAX CORE Nucleic Acid Purification Kit. RT-qPCR targeting the matrix (M) gene was employed for AIV RNA detection. All samples collected (cloacal and orotracheal swabs) from the thirteen birds were negative in the RT-qPCR. Although no Influenza genomes were detected this season, monitoring will continue, since AIV has a great range of hosts (including different species of birds and mammals) and shorebird flocks gathering at this area changes along the seasons, potentially allowing its spread.

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PT.350

DETECTION OF INFLUENZA A VIRUS IN PORCINE FECAL AND ENVIRONMENTAL SAMPLES

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Influenza A viruses (IAV) cause acute respiratory infections in swine, leading to significant economic losses in production. Experimental studies in pigs show that only the H3N2 subtype can be detected in feces post-intranasal infection. The objective is to investigate the presence of IAV in porcine fecal and environmental samples using molecular detection techniques, aiming to assess the potential for fecal-oral transmission and environmental contamination risks. This study occurred on commercial pig farm in southeastern Brazil with recurrent respiratory issues. In 2023, 244 nasal and 245 rectal swabs were collected from symptomatic pig. Additionally, 13 environmental samples were collected. RT-qPCR was performed for IAV detection, and positives were subtyped using RT-PCR for Brazilian swine lineages. IAV was detected in 27% (67/244) of nasal swabs and 22% (54/245) of rectal swabs. Among 121 positive samples, 15% were positive in both swabs. Of the 82 (32 nasal swabs and 34 rectal swabs) subtyped samples, the most detected lineage in nasal swabs was H3 (13/32), followed by pH1 (6/32) and huH1 (5/32) complex. In rectal swabs, pH1 was more prevalent, with no huH1 detected. Co-infections were observed in 6% (5/82) of samples. Four environmental samples detected IAV, notably in reused water and raw effluent, with successful viral isolation suggesting potential fecal-oral transmission. The study highlights significant swIAV lineage co-circulation, genetic diversity in the herd, and the role of fecal-oral transmission. IAV presence in feces and environmental samples emphasizes the need for continuous monitoring and effective control strategies due to the significance for One Health.

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PT.351

AN END-POINT MULTIPLEX PCR/RT-PCR FOR DETECTION OF FIVE AGENTS OF BOVINE RESPIRATORY DISEASE

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Bovine respiratory disease (BRD) is a clinic-pathological syndrome associated with interaction among host and environmental factors, viral and bacterial pathogens, with negative impacts on beef and dairy cattle worldwide. The potential multiagent etiology makes the laboratory diagnosis of BRD challenging, as different pathogens must be investigated to ensure an adequate diagnosis. To improve and facilitate laboratory diagnosis of BRD, we describe an end-point multiplex PCR/RT-PCR for the differential and simultaneous detection of five important BRD agents: bovine herpesvirus 1 (BoHV-1), bovine viral diarrhea virus (BVDV), bovine parainfluenza virus 3 (BPIV-3), bovine respiratory syncytial virus (BRSV) and bovine coronavirus (BCoV). We designed target-specific primers that may be combined in a multiplex PCR/RT-PCR for the detection of the five viruses described above. The multiplex assay was optimized for reaction conditions and mix. Furthermore, we evaluated the limit of detection (LoD) for each multiplex target, as well as the specificity of the assay against different bacteria potentially related to BRD. Herein, the amplicons of the five multiplex targets were easily differentiated, including in simultaneous detection. The LoD of our multiplex was 38 TCID50 of BoHV-1, BoHV-5 and BPIV-3, 25 TCID50 of BVDV-1, BVDV-2 and HoBi-PeV, 13 TCID50 of BRSV and a 10^{-2} dilution of a pool of two BCoV positive samples. No nonspecific amplification of potential bacterial agents associated with BRD was observed. Overall, our multiplex PCR/RT-PCR assay may be an alternative for easier, faster and more practical laboratory diagnosis of BRD.

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PT.352

FIRST REPORT OF BRAZILIAN PORCUPINEPOX VIRUS IN Coendou spinosus IN PARANÁ, BRAZIL

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In 2019, a novel poxvirus was described in *Coendou longicaudatus* (previously *C. prehensilis*), named Brazilian porcupinepox virus (BPoPV), in Uberlândia, Minas Gerais. This virus caused crusted and vesicular skin lesions on the nose, limbs, and genital and perianal regions. This study aimed to investigate the presence of BPoPV in a free-ranging porcupine (*C. spinosus*) in Paraná (PR), Brazil. In March 2024, a young female porcupine was referred for veterinary care in Telêmaco Borba-PR, presenting with dehydration, eye and nasal discharge, edema, and crusted lesions on the nose, plus vesicles in the genital region. Nasal crusts were collected, prepared, and submitted for DNA extraction. The sample was subjected to PCR for GAPDH and Pan-Poxvirus, testing positive in both assays. The poxvirus amplicon was purified and submitted to Sanger sequencing, showing 100% identity with the BPoPV sequence. This study marks the first detection of BPoPV in Paraná, expanding its known geographic range. The virus was previously described in the same porcupine species in São Paulo, indicating the susceptibility of *C. spinosus* across different regions. With the discovery of new porcupine species in Brazil, BPoPV could negatively impact the conservation of species with unknown vulnerability status. This research highlights the expanding range of BPoPV and emphasizes the need for ongoing monitoring of porcupine species in Brazil.

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PT.353

PHOTODYNAMIC ACTIVITY IN VITRO OF PORPHYRINS AGAINST *VACCINIA VIRUS*

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Porphyrins are derivatives of tetrapyrrolic compounds used in photodynamic therapies with antiviral or virucidal potential. *Monkeypox virus* (MPXV), family *Poxviridae,* is an emergent human pathogen to which no specific treatment is available. Brazil is the second country with the highest number of MPXV cases. In this context, we evaluated the *in vitro* photodynamic effect of porphyrins 3-PtTPyP, 4-PtTPyP, 3-H2TMe, and 4-H2TMe against VACV as a model for MPXV. The porphyrins were analyzed for cytotoxicity by MTT with and without light exposure, and maximal non-cytotoxic concentrations were used in virucidal assays. For this VACV suspensions ($10^{6,8}$) amplified in VERO cells were incubated with porphyrins at concentrations: 320 μM (3-H2TMe), 40 μM (4-H2TMe), 20 μM (3-PtTPyP), and 10 μM (4-PtTPyP), exposed (ou not) to white light for 0, 5, 15, 30 and 45 min. At each time point, virus suspensions were quantitated and virus titers were compared with titers of the negative control (no porphyrin). The 3-PtTPyP porphyrin inactivated the virus even in the absence of light. In contrast, the porphyrins 4-PtTPyP, 3-H2TMe and 4-H2TMe showed photodynamic activity against VACV, resulting in progressive virus inactivation in the presence of light and completely inactivating the virus with 5, 30 and 45 min of light exposure, respectively. These results indicate photodynamic activity of porphyrins 4-H2TMe, 3-H2TMe and 4-H2TMe and open the way for their use as poxvirus virucidal for surfaces and biologicals as well. Studies are underway to investigate the activity of these porphyrins against MPXV.

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PT.354

EPIDEMIOLOGICAL SURVEILLANCE OF CIRCOVIRUSES IN AVIAN SPECIES ON THE COAST OF PARANÁ

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Members of the Circoviridae family are recognized for being associated with avian species and for their highly infectious nature, resulting from their rapid replication, easy dissemination and great environmental resistance. In this study, we investigated the presence of Circovirus in biological samples (oral, anal, pancreas or brain) of avian species collected on the coast of Paraná in the period between 2022-2024. The study involved DNA extraction using the magnetic beads method (UniXtractor), followed by Nested-Polymerase Chain Reaction (nPCR) for amplification and Sanger method for identification and later for phylogenetic analysis of the subtypes found. Regarding the results, a total of 350 samples were screened, of which 3 (0.86%) were positive, from the species *Sterna sp.* and *Larus dominicanus*. Nucleotide similarity with sequences from the GenBank database was assessed using the BLAST tool. Comparison of the available sequences revealed significant homology with gull circoviruses ranging from 88.84% to 85.59%. The data obtained are consistent with the fact that the treated virus commonly affects psittacines and columbids and is considered a disease in Australia. Therefore, continuing the study is extremely important since the migratory routes of wild birds allow the widespread dissemination of the virus and contact between humans and animals has been implicated in the emergence of several emerging pathogens.

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PT.355

EVOLUTIONARY DYNAMICS AND RECOMBINATION OF ALPHAVIRUSES IN SOUTH AMERICA

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Alphaviruses are primarily transmitted by mosquitoes of the *Aedes*, *Culiseta*, and *Culex* genera, infecting several mammalian and avian species. Outbreaks in both South and North America raise public health concerns due to the severe neurological diseases they can cause. This study aims to understand the evolutionary history of recombination events driving the emergence of alphaviruses that circulates in South America. Complete genome reference sequences of 16 alphaviruses from South America were obtained from GenBank database and aligned using MAFFT v7.490. Recombination detection were performed by the BootScan method implemented in SimPlot software v3.5.1. Maximum likelihood trees were reconstructed in IQTree v2.0.7. The analysis revealed that four of the 16 genomes had similar recombination profiles. The Western Equine Encephalitis Virus (WEEV) has a non-structural genomic region (nsp1, nsp2, and nsp3) derived from Eastern Equine Encephalitis Virus (EEEV), while the whole 3‘-region, including E1, E2, and E3, are derived from Sindbis Virus (SINV). The breakpoint is located around nucleotide 8,207 within the E3 gene. Similar patterns were observed in the Fort Morgan Virus (FMV) and Highlands J Virus (HJV), with breakpoint around position 8,349. Venezuelan Equine Encephalitis Virus (VEEV) shows a non-structural region derived from SINV and a 3‘from EEEV (breakpoint around 8.229 within gene C). These viruses co-circulate in the same geographical region and likely originated from the co-infection of different alphaviruses within the same host. For WEEV, FMV, and HJV, the structural proteins-encoding are derived from SINV, suggesting an adaptive advantage. Ongoing analyses aim to further understand these mechanisms.

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PT.356

MONITORING OF CORONAVIRUSES IN MIGRATORY BIRDS ON THE COAST OF RIO GRANDE DO SUL STATE, BRAZIL

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Coronaviruses are positive-sense RNA, enveloped viruses that can cause many diseases. Infectious bronchitis virus (*Gammacoronavirus galli,* IBV) is a pathogen of chickens and other birds that is highly contagious. IBV is one of the leading causes of mortality and morbidity in chickens, causing respiratory, renal, and gastrointestinal signs. The transmission occurs, mainly, by respiratory aerosols and feces. The aim of this study was to investigate the presence of coronavirus in migratory birds passing through the Lagoa do Peixe National Park, in Rio Grande do Sul state by molecular analysis of fresh fecal samples. Ninety eight fecal samples were collected between May 2023 and February 2024 in collaboration with Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA). RNA was extracted using magnetic beads, cDNA was synthesized, and a nested PCR targeting a segment of *Orthocoronavirinae* RNA-dependent RNA polymerase was performed. Coronavirus genomes were not detected in any samples. Despite the negative results, it is important to maintain regular surveillance of the sanity of migratory birds, since they can be a source of infection to poultry, as wild species can be a reservoir of IBV and other viruses. IBV outbreaks can have several severe consequences to poultry farming, including weight loss, decrease in egg production, and even death. Therefore, monitoring of viral diseases circulating in migratory birds is a critical tool to guide disease control measures and to avoid the entry of pathogens into production animals.

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PT.357

FELINE CALICIVIRUS CAUSING HIGH MORTALITY IN CATS IN SOUTHERN BRAZIL: CASE REPORT

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Feline calicivirus (FCV) is one of the most important pathogens of the upper respiratory tract in cats. It causes respiratory, ocular, and oral signs. Transmission occurs vertically or by contact with either infected animals or fomites. FCV is prevented by vaccination, although antigenic diversity leads to decreased vaccine efficacy. Eleven felines, without vaccination history, presented clinical signs of lethargy, fever, limb edema, respiratory signs and skin lesions, with rapid progression. Five cats died within two days of disease development. Furthermore, two vaccinated animals that had contact with the sick animals showed clinical signs compatible with FCV infection. The aim of this study was to determine if FCV was the causative agent of this high mortality and high morbidity disease. One of the five animals was necropsied and tissue fragments (lung, liver, nasal turbinates and skin) and fluids (chest blood and urine) were collected. A nasal swab and whole blood were collected from one of the surviving vaccinated animals. RNA was extracted by Trizol and RT-PCR was performed targeting capsid protein gene. Virus isolation in CRFK cells was attempted. Lung, nasal swab, and nasal turbinates samples showed cytopathic effect (CPE) in the first passage in cell culture; skin and nasal swab showed CPE in the second passage. Interestingly, by RT-PCR, only the lung sample (necropsied animal) and the blood sample (vaccinated animal) were positive. This case suggests a possible antigenic diversity of the agent causing serious infection, showing the importance of vaccination in animals to reduce clinical signs and death toll.

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PT.358

MONITORING OF NEWCASTLE DISEASE IN PENGUINS (*Spheniscus magellanicus*) ON THE COAST OF RIO GRANDE DO SUL STATE, BRAZIL

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Wild birds are an important reservoir of many viral diseases, including Newcastle Disease. Newcastle Disease is caused by the avian paramyxovirus 1 (*Orthoavulavirus javaense*, NDV), a virus belonging to the *Paramyxoviridae* family and *Orthoavulavirus* genus. Newcastle disease is extremely contagious, causing important economic losses especially in poultry production. The main signs of infection in birds are respiratory, neurological and gastrointestinal. NDV can also infect humans, causing conjunctivitis. Penguins are important reservoirs to NDV and the virus has already been isolated from some penguins species. During the period of July and August of 2024, a high mortality event of penguins was observed on the coast of Rio Grande do Sul state around the Lagoa do Peixe National Park. The aim of this study was to verify the presence of NDV from dead penguins (*Spheniscus magellanicus*) found in this coastal region. Oropharyngeal and cloacal swabs of 16 animals were collected in collaboration with Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA). RNA was extracted by Trizol, cDNA was synthesized and RT-PCR was performed targeting fusion protein gene. Samples from all animals tested negative for NDV by RT-PCR. Due to its zoonotic potential, monitoring of NDV in penguins that arrive at the coast must be conducted continuously. Animals that migrate from other locations, such as penguins, can bring new strains of the virus along their route, disseminating them to local wild birds, and posing a risk even to previously vaccinated animals.

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PT.359

GENETIC DIVERSITY AND EVOLUTIONARY DYNAMICS OF SENECAVIRUS A IN SWINE IN BRAZIL

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Senecavirus A (SVA) is a picornavirus that was first isolated in the U.S in 2002, though it had been circulating in the US swine herds undetected since 1988. Despite frequent reports of vesicular disease outbreaks caused by SVA infection in Brazilian swine since 2014, there is a lack of data on the genetic variability and evolution of SVA in Brazil. This study analyzed 501 SVA isolates, obtained from swine herds from 57 cities located in eight Brazilian states, collected between 2018 and 2022, using Sanger and Oxford Nanopore sequencing. Phylogenetic analysis of the complete CDS revealed that Brazilian sequences are distinct from sequences from other countries, and form a monophyletic clade, suggesting a single introduction of this virus in Brazil. Also, at least two main subclades with sequences from the midwest and southern regions were detected, suggesting that SVA is evolving independently in the swine population of the country. Pairwise sequence comparisons allowed us to identify seven unique mutations with high frequency in the Brazilian SVA sequences. We also detected amino acids undergoing evolutionary changes based on the year of isolation. Notably, some mutations were identified in B-cell epitopes and in specific regions of capsid proteins that interact with host cell receptors, suggesting potential evolutionary advantages. Recombination analysis provided strong evidence of SVA recombination in Brazilian sequences. These findings offer new insights into the evolution of SVA. Further analysis is required to fully understand the impact of these mutations on SVA infection, pathogenesis, and virulence.

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PT.360

Immortalized Brazilian Bat Cell Lines as Tools for the Study of Rabies Virus.

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Bat lyssavirus genotype 1 (family Rhabdoviridae) is still considered the most important zoonotic bat-borne virus in Brazil. In a previous work we showed the first immortalized cell lines from Brazilian bat species and we also evaluated their permissiveness to viruses from different families. This time, our BKC cell lines (Bat Kidney Cells) of Artibeus lituratus, Artibeus planirostris and Carollia perspicillata were infected with the rabies virus to infer their permissiveness to this viral agent. Viral titer assays in cell culture and molecular markers by real-time PCR allowed us to analyze the level of viral infection and cell sensitivity to infection. RNA-Seq analysis is being carried out to measure the effect of infection on the expression of different genes and to compare the behavior of different bat species in the face of viral infections and their possible evolutionary relationship. One thing is certain, rabies remains a serious public health issue of fatal prognosis especially in developing countries, where many cases are underreported, making it difficult to account for a precise prevalence of the disease. To attain broader control of the virus and minimize the rabies threat from bats on human populations, broader sampling efforts along molecular characterizations and transmission mechanistic studies of the virus are not only essential but will further elucidate the epidemiology and dynamics of rabies. In this sense, novel immortalized cell lines come out as an indispensable tool to resolve possible gaps in the study of rabies virus.

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PT.361

REFINING THE GENOMIC TARGET FOR PROPER PHYLOGENETIC CLASSIFICATION OF CANINE DISTEMPER VIRUS (*MORBILLIVIRUS CANIS*)

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Canine distemper virus (CDV, *Morbillivirus canis*) displays significant genetic variability due to high mutation rate and recombination events. This variability highlights the need for genomic analyses to accurately classify the CDV lineages. Whole-genome sequencing (WGS) is the most appropriate strategy for identifying CDV lineages, however, for practical and biological reasons, CDV variants have been classified by analyzing the hemagglutinin (H) gene, whose suitability for proper genotypic identification has been questioned. Herein, we propose an alternative genomic target for phylogenetic classification of CDV in scenarios where WGS is not feasible. Initially, we designed primers for the P/V/C gene, previously suggested as potential target for CDV classification. The primers were based on 138 whole-genomes of different CDV lineages available in GenBank, targeting a 568 bp region. The amplicon obtained with these primers was then evaluated *in silico* for its ability to reproduce the genetic classification obtained by WGS. After concordance confirmation, we optimized the RT-PCR assay and tested its performance on different CDV positive samples. *In-silico* analysis confirmed that classification based on this amplicon reproduces the classification based on CDV whole genome. After this validation, we demonstrated the primers successfully amplified CDV RNA from different samples. The amplicons were also easily sequenced by Sanger method, allowing CDV classification. Finally, we propose a novel genomic target for proper phylogenetic CDV classification, which may be a viable alternative when WGS is not feasible.

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PT.362

MOLECULAR SURVEY OF ROTAVIRUS AND PICOBIRNAVIRUS IN WILD BOARS (SUS SCROFA) FROM SÃO FRANCISCO FOREST STATE PARK, PARANÁ, BRAZIL.

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Wild boar (*Sus scrofa*) is an exotic invasive species in Brazil and may serve as a reservoir for various pathogens. The spread of infectious microorganisms from wild species to domestic animals and humans has significant implications for public health, livestock, and biodiversity conservation. Rotavirus (RV) and picobirnavirus (PBV) have been detected in a wide range of host species; however little is known about the prevalence of these viruses in wild boars. This study aimed to investigate the occurrence of RV and PBV in free-living wild boars. From August 2021 to July 2022, fecal samples were collected from 32 wild boars in São Francisco Forest State Park, located in Paraná state, Brazil, after capture and humane euthanasia. Fecal samples were submitted to nucleic acid extraction by the combination of phenol chloroform-isoamyl alcohol and silica/guanidine isothiocyanate. RV screening was performed using polyacrylamide gel electrophoresis followed by silver staining (ss-PAGE), while PBV was screened using both PAGE and RT-PCR with primers PicoB25 and PicoB43 that amplify a 201 bp fragment of the RdRp gene of PBV genogroup I (PBV-GI). No electrophoretic migration profile characteristic of RV or PBV was identified in the ss-PAGE. However, PBV-GI was detected in 40.6% (13/32) of samples by RT-PCR. This is the first report of PBV detection in free-living wild boars. These findings expand our understanding of the host species affected by PBV and underscore the importance of monitoring its circulation in wild populations, given the potential for interspecies transmission.

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PT.363

CONSTRUCTION OF A RECOMBINANT VACCINIA VIRUS ABLE TO DRIVE THE EXPRESSION OF EQUINE CHORIONIC GONADOTROPHIN IN EUKARYOTIC CELLS.

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Equine chorionic gonadotrophin (eCG) is a highly glycosylated hormone produced by mares during pregnancy. Studies have shown that, in non-equine species, eCG exhibits an activity similar to that of LH and FSH, due to its affinity for the receptors of these hormones. Currently, in Brazil, eCG is obtained by purifying serum from pregnant mares. The amount of hormone obtained through this technique is influenced by a number of factors, such as the size of the mare, genetic factors, number of pregnancies and health condition. The production process is prone to the presence of biological contaminants or partial loss of biological activity, resulting in a reduced final yield. In addition, there are ethical concerns regarding the risks faced by the animals, especially given the conditions the mares are subjected to in order to increase eCG production. In this context, the heterologous production of this protein is an important alternative. Due to the presence of post-translational modifications, eCG production must take place in a eukaryotic system. Therefore, in view of the ability of poxviruses to introduce heterologous genes into eukaryotic cells, a recombinant Vaccinia virus Western Reserve capable of expressing the eCG protein in infected cells was constructed. The presence of the DNA of interest in the virus and the transcription of the mRNA containing the relevant genes were confirmed. The expression of the protein was confirmed using serological methods and SDS-PAGE and Western blotting assays. However, the biological activity of the heterologous protein remains to be verified.

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PT.364

IMMORTALIZED CHICKEN EMBRYO CELLS AS TOOLS FOR STUDYING HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUSES

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In 2023, the Brazilian poultry meat production reached a historic mark of 14.8 million tons with a record projection in 2024 of 15.1 million tons. A sum that guarantees Brazil the 2nd position among the world's largest producers of chicken meat. This scenario occurs under the threat of highly pathogenic avian influenza caused by the H5N1 virus first detected in Brazil in May 2023. The panzootic in wild birds was followed by outbreaks in commercial and backyard flocks and most recently begun to infect land and marine mammals. Human infections are still sporadic and associated with close contact with infected animals. However, the rapid adaptation and spread of the virus has led to the alert of the main surveillance bodies. Understanding the complex pathogen-host interactions is crucial for developing control strategies, determining the role of chicken in the emergence of zoonoses and can also protect the poultry industry. This project aimed to evaluate the permissibility of immortalized Chicken Embryo Kidney Cells (CKC) and Chicken Embryo Liver Cells (CLC) previously established by our group to highly pathogenic avian influenza virus - H5N1. Viral infection was determined by titration of viral stocks in cell culture, flow cytometry and fluorescence microscopy. In addition, molecular markers were used along real-time PCR to determine susceptibility of the cells to the virus. CKC and CLC cell lines were susceptible to infection by the H5N1 avian virus, making them a versatile and practical tool that can facilitate the understanding of the virus and its fluctuations over time.

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PT.365

DEVELOPMENT OF A MULTIVALENT VLP VACCINE FOR RESPIRATORY DISEASES IN CHICKENS

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Avian Metapneumovirus (aMPV) is considered a threat to the poultry industry due to its highly contagious nature and wide geographic distribution throughout the world. The virus is primarily associated with upper respiratory infections that lead to clumping and loss of cilia, predisposing birds to secondary viral and bacterial pathogens that cause severe bronchiolitis and pneumonia. Virus-like particles (VLPs) are nanoparticles composed of one or more structural viral proteins that do not contain an infectious viral genome. Therefore, they are potentially safer alternatives than live-attenuated and inactivated vaccines. In this project, a retrovirus-derived VLP was developed that combines the insertion of the nucleoprotein from the Avian Metapneumovirus and the Infectious Bronchitis Virus. VLPs were characterized by molecular biology techniques, nanoparticle tracking analysis (NTA) and flow cytometry. *In ovo* immunization with VLPs demonstrated that there was an immunological stimulation in T cells and B cells in the spleen of the immunized animals. Furthermore, serum neutralization assays for the aMPV virus estimate the induction of neutralizing antibodies to the virus. Therefore, these multivalent VLPs proved to be a potential alternative vaccine which combined with immunostimulatory molecules or adjuvants may provide broad vaccination protection against viral infections that lead to economic losses in industrial poultry farming.

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PT.366

MOLECULAR DIAGNOSIS OF MURINE NOROVIRUS (MNV), MURINE REOVIRUS (MuReV) AND MURINE ASTROVIRUS (MuAstV) IN MICE FACILITIES FROM BRAZIL.

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Murine Norovirus (MNV) and Murine Reovirus (MuReoV) are frequent viruses in mice while Murine Astrovirus (MuAstV) was recently described as a potential emerging virus. The diagnosis of MNV/MuReoV is routinely performed by serological methods and the high genetic variability of these viruses and host-related factors make this technique less sensitive and error-prone. The aim of this study was to develop a diagnostic panel through qPCR targeting MNV, MuReoV and MuAstV for subsequent implantation in sanitary monitoring of Brazilian facilities. A total of 297 fecal samples from sentinel mice (*Mus musculus*) from SCQA–Fiocruz (2022 to October 2023) were tested. The targets of qPCR (TAQman system) were: MNV (ORF1), MuAstV, (ORF1b) and MuReoV (VP2). MuAst was detected in 55/297 tested samples (18.5%) followed by MNV (41/297 or 13.8%) and MuReoV (11/297 or 4.1%). Coinfections were detected in 16 samples (MNV+MuAstV in 14 and MNV+MuReoV in two samples). Most of the MNV and MAstV positive samples were from conventional facilities (47.7% and 29.8% respectively). For MuReoV, all nine positive samples were from specific pathogen free (SPF) mice. MuAstV were more frequent in younger mice (1-3 months) (42.6%), MuReoV in 4-6 months old mice and MNV in 7-10 months old mice. No association between mice gender/genetic status and viruses detection was observed. In conclusion, the detection of the three viral agents in mice from different sanitary status (even in SPF animals) and different ages demonstrates the importance of adopting more sensitive techniques such as qPCR in the health monitoring of rodents facilities.

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PT.367

GENETIC ANALYSIS OF THE PARVOVIRUS FULL-LENGTH VP2 GENE IN DOMESTIC CATS IN BRAZIL

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Feline parvovirus (FPV) and canine parvovirus (CPV) are over 98% identical in their DNA sequences, and the new variants of CPV (2a/2b/2c) have gained the ability to infect and replicate in cats. The aim of this study was to determine the genetic diversity in the VP2 gene of parvovirus strains circulating in domestic cats during 2008–2017. A total of 25 PCR-positive samples, obtained from kittens with diarrhea, were submitted for full-length VP2 sequencing (1755bp). Sequence editing and multiple alignments were performed with BioEdit. Phylogenetic reconstruction was performed using the maximum likelihood method in the IQtree/BioAider software and by Bayesian Inference using the Bayesian MCMC statistical framework implemented in the BEAST package. Recombination analysis was performed using the RDP4 Program. Based on the key VP2 amino acid (aa) residues, eight sequences were characterized as FPV, seven as CPV-2a, and nine as CPV-2b. These sequences displayed aa changes previously described for FPV (A14T, A91S, I101T, N564S, and A568G) from cats and CPV-2a/2b (S297N and Y324L) from dogs. However, the Y324L mutation has not yet been reported in any CPV-2a/2b strains from cats. Phylogenetic analysis supported the division of these sequences into two well-defined clades, clade 1 for FPV and clade 2 for CPV2a/2b. One sequence (RJ1085/11) grouped separately and two recombination breakpoints were detected by Bootscan and 3Seq methods. This study is the first report of CPV-2a/2b in cats in Brazil. The detection of FPV strains with mutations characteristic of CPV indicates that Brazilian FPV strains have undergone genetic changes.

PT.368

INVESTIGATING THE INTERACTIONS BETWEEN RECEPTOR BINDING DOMAINS AND SU PROTEINS ACROSS VARIOUS FELV SUBGROUPS

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The Feline Leukemia Virus (FeLV) is a retrovirus that affects cats and had been classified into different subgroups (FeLV-A, FeLV-B, FeLV-C, and FeLV-T). New infectious viruses are produced in cats from mutations and/or recombination between the exogenous virus and endogenous sequences of FeLV. Through interference analyses, it is suggested that each of the subgroups uses a specific receptor for entry into target cells; however, genomic and phylogenetic analyses of the target receptor-binding protein (SU) have not been able to explain the genetic origin of the specificity of the interaction of these variants. Therefore, the interaction between the SU proteins of the different FeLV subgroups and feline cellular receptors was analyzed *in silico*. The three-dimensional structures of SU and the receptors were obtained from the PDB when available and otherwise modeled with AlphaFold2 using its machine learning approach. The four SU proteins were docked to the receptors using ClusPro, and the binding energies were re-calculated with FoldX and compared for the four subgroups. Partial results suggest that binding energies can only explain the interaction between FeLV-C and its feline receptor, FLVCR1. Current inconsistencies in predicted affinity for SU-receptor pairs may reflect sub-optimal models and/or knowledge gaps in the annotation of SU interaction regions.

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PT.369

EVALUATION OF DIFFERENT RNA EXTRACTION AND REVERSE TRANSCRIPTION PROTOCOLS FOR MOLECULAR DETECTION OF AVIAN REOVIRUS

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Avian reovirus (ARV), a member of the genus *Orthoreovirus* and family *Spinareoviridae*, is associated with tendinitis/arthritis and runting stunting syndrome in commercial poultry farms worldwide. This study aimed to evaluate different RNA extraction and reverse transcription (RT) protocols for the molecular diagnosis of ARV by conventional RT-PCR targeting S4 gene. A modified live virus vaccine containing S1133 strain of ARV (Vaxxon® REO L, Vaxxinova) was used for comparative analysis. We tested two commercial kits, Quick-Zol (TriZol, Ludwig Biotec) and QIAamp Viral RNA Kit (Qiagen), as well as an in-house protocol based on phenol/chloroform and guanidinium isothiocyanate for RNA extraction. For cDNA synthesis, we evaluated the GoScript™ Reverse Transcription Mix with Random Primers (Promega) and three different RT mixes using specific S4 gene AVR primers (forward primer only, reverse primer only, and both primers). The resulting cDNA was subjected to PCR using GoTaq® G2 Hot Start Master Mixes (Promega) according to the manufacturer's instructions. All RNA extraction methods were efficient when combined with RT using random primers, yielding the expected amplicon of 747 bp. However, among the RT reactions with specific primers, only the cDNAs obtained from the reverse and both primers RT reactions were successfully amplified. Based on these results, we concluded that RNA extraction using Quick-Zol and RT with a master mix containing random primers were efficient and suitable for routine ARV diagnosis. Key factors in determining the best method included cost per sample, amplicon intensity, analysis time, and absence of non-specific amplicons.

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PT.370

DETECTION OF MORBILLIVIRUS IN SOTALIA GUIANENSIS

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The Sotalia Guianensis have estuarine and coastal habits and inhabit regions of the Western Atlantic, being part of the marine fauna in the regions of South America in southern Brazil and possibly in the region of Honduras. In recent years, with the occurrence of unusual mass mortality events or even undetermined deaths of these animals in different areas of their distribution, concerns have increased about the conservation of the species, which is currently on the species threatened with extinction list, being classified as vulnerable. Morbilliviruses are negative single-stranded RNA viruses and are responsible for promoting high rates of infection and mortality in cetaceans, representing a very significant biological threat. So, the present work aimed to detect Sotalia Guianensis morbillivirus-GDMV (family Paramyxoviridae)in samples of brain and lung from Sotalia Guianensis, which died without a determined cause on the coast of Paraná, collected by researchers from the Ecology and Conservation Laboratory of the Federal University of Paraná., The total RNA was extracted, using TRIzol methodology, from 134 tissue samples (26 brains and 25 lungs) from 27 individuals. After, the cDNAsynthesis was performed , and finally the samples were submitted to RT-qPCR to detect GDMV, in which 8 tested positive for GDMV, thus characterizing the circulation of morbillivirus in Sotalia guianensis in Brazil. These results provide relevant information to contribute to epidemiological surveillance in the region, as well as promoting the development of public policies about the conservation of these animals.

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PT.371

ACTIVE SURVEILLANCE OF AVIAN INFLUENZA VIRUS IN WILD ANIMALS OF BRAZILIAN COAST, 2024

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The highly pathogenic avian influenza (HPAI) H5N1 virus was first introduced in Brazil in 2023 from South American countries by wild birds. The PREVIR-MCTI network characterized the virus detected in wild birds and mammals in Brazil through collaboration with the Santos Basin Beach Monitoring Project (PMP/BS) group of Santa Catarina and researchers from Brazilian Institutions. Seven-hundred and seven wildlife samples (swab and carcass) were collected from January to August 2024 in Santa Catarina (n=248), São Paulo (n=191), Espírito Santo (n=169), and Bahia (n=99) states. Samples were collected from healthy and rehabilitation animals, and carcasses in fourteen orders as follows: Charadriiformes (20.8%), Passeriformes (18.7%), Anseriformes (17.7%), Suliformes (13.6%), Sphenisciformes (11.3%), Columbiformes (6.3%), Phaethontiformes (5.6%), and Carnivora, Pelecaniformes, Caprimulgiformes, Coraciiformes, Cuculiformes, Gruiformes, Hirundinidae (below 1% each). Carcass samples of two seagulls and one sea lion found with an unknown cause were collected on the Northern coast of Santa Catarina from February to May. Nevertheless, all samples were tested negative for the RT-qPCR specific to the avian influenza virus. Next-generation sequencing analysis will be conducted in carcasses samples to identify the pathogen that might cause any infection. The data suggest a decline in HPAI in wildlife from the Brazilian coast during the first semester of 2024 despite the continuous outbreaks of HPAI H5N1 worldwide.

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Development of indirect ELISA to detect antibodies to SARS-CoV-2 in coatis (*Nasua nasua*)

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Interspecies transmission of SARS-CoV-2 has been documented in various animal species. Concerns about SARS-CoV-2 spreading to animals' stem from its impact on animal health and the potential for animals to be virus reservoirs, possibly reintroducing it to humans. Understanding infection dynamics is crucial. In this study, a standard Plaque Reduction Neutralization (PRNT) was conducted, and an indirect ELISA serological test was developed to detect anti-SARS-CoV-2 antibodies in free-living coatis (*Nasua nasua*) inhabiting urban parks in anthropized areas of Belo Horizonte due to the potential for them to serve as reservoirs. Blood samples from 166 coatis were collected between 2021 and 2024 and evaluated. The receptor-binding domain (RBD) and the nucleocapsid (N) protein are antigens representing the most immunogenic regions of the virus. Many studies have demonstrated that humans and mammals produce antibodies against these antigens after virus infection. Therefore, the recombinant RBD and N proteins were used in an indirect ELISA to detect specific anti-SARS-CoV-2 (IgG) antibodies in sera of coatis. After ELISA standardization the better conditions for reagents dilutions and concentration were set up. Initial results showed that some animals exhibited high reactivity (OD values), out of 166 animals tested, 36 were found reactive in the ELISA, indicating that coatis are susceptible to SARS-CoV-2 infection, with implications for disease epidemiology that needs further investigation.

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PT.374

IN VITRO ANTIVIRAL EFFICACY OF Wet Wipe with A PHTHALOCYANINE DERIVATIVE AGAINST SARS-CoV-2 to clean folds and paws After Dog Walk

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Phitta Petcare Virucidal Wet Wipes are the ideal solution to keep your pet's hygiene up to date, whether after a walk, when cleaning folds, paws or during a dry bath. With the powerful Phitta cream, these wipes effectively combat viruses and bacteria, while the moisturizing active ingredients ensure soft and protected skin. To perform the test, quadrangular fragments of the fabric measuring 2 x 2 cm were cut out. The cutouts were soaked in 500 µL of a topical formulation containing the phthalocyanine derivative at concentrations of 0.1, 0.05 and 0.03% (Phitta cream). The embedded tissues were exposed to 500 µL of viral suspension (3x10^6 TCID50/mL) of SARS-CoV-2 JN.1 and incubated for 15 minutes at 37ºC. Afterwards, the contents were serially diluted and inoculated into a 96-well plate (VERO CCL-81, 2x10^4 cells/well) to be titrated by TCID50/mL. The monolayers were observed daily to record the occurrence of cytopathic effect, with the final reading performed after 72 hrs. A titer of 1.5x10^6 TCID50/mL was observed in the untreated control, while concentrations of 0.1, 0.05 and 0.03 presented titers of 3.16x10^2, 5.16x10^2 and 3x10^2 TCID50/mL, respectively. Thus, a reduction of 99.97, 99.65 and 99.98 in viral titer inhibition was observed.

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PT.375

Phylogenetic Analysis of the Glycoprotein and Phosphoprotein of Rabies Viruses Isolated from Cattle in the Southern Region of Brazil

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Rabies is a viral zoonosis that is almost always fatal affects all mammals. In the Southern region of Brazil cattle rabies is endemic, causing negative impacts on animal health and the local economy. The etiological agent of this disease is the *Lyssavirus rabies* (RABV), an RNA single strand virus. The RABV is one of the most stable among RNA viruses and encodes the following proteins: nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), and RNA polymerase (L). Among these proteins, G and P are the most variable and target of this study that aims to conduct a phylogenetic analysis of these proteins from samples of RABV isolated from cattle in Soutern of Brazil. For this purpose, 76 samples of RABV isolated from central nervous system of cattle were submitted to RNA extraction, reverse transcription followed polymerase chain reaction (RT-PCR), and genetic sequencing targeting the G and P genes (rescpectivelly *primers Ga3222_40, Gb4119-39*, *Panti*, and *Psense*). After editing the obtained genetic sequences, phylogenetic trees were constructed for the respective genes. Preliminary analyses demonstrated that the RABV samples exhibit a genetic lineage characteristic of the hematophagous bat *Desmodus rotundus*. These bats are the main transmitters of rabies to cattle, so this result is not surprising. However, the phylogeny based on the more variable genes of RABV and the identification of sublineages can help understand the virus dynamics within bat colonies and its transmission to cattle**.**

Financial Support: Secretaria da Saúde.

PT.376

SEROLOGICAL ANALYSIS FOR HEPATITIS A (HAV) IN FREE-LIVING RODENTS OF THE SPECIES *PROECHIMYS GUYANNENSIS* IN THE STATE OF PARÁ

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The hepatitis A virus (HAV) is classified as Hepatovirus and causes acute hepatitis with enteric transmission of the oro-fecal type. Non-human primates are the only animals that contract HAV naturally, and do not present clinical signs. However, the presence of HAV has been reported in fecal samples from ferrets, suggesting other possible hosts for the virus. It is important to emphasize that sanitation and hygiene conditions are essential for the proliferation of the virus and its pathogenesis consists of infection by the agent that is directed to the bloodstream and subsequently lodged in the hepatocytes, where it replicates and is deposited in the bile ducts, small intestine, and finally, excreted in the feces. In this study, 75 blood samples from rodents of the species Proechimys guyannenis collected between 1977 and 1981 and stored in a research institute in the North region were tested for the serological markers anti-HAV IgM and anti-HAV IgG by enzyme-linked immunosorbent assay (ELISA). One sample from 1977 presented a reactive result for the anti-HAV IgG marker. The presence of human Hepatitis A virus in free-living rodents highlights a possible new reservoir for the etiological agent, as well as a source of environmental contamination, and reinforces the need for further research to investigate the presence of the virus in both animal reservoirs and environmental samples.

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PT.377

MOLECULAR INVESTIGATION OF ORTHOPOXVIRUS IN DOMESTIC ANIMALS FROM URBAN AREAS

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The increasing process of urbanization promotes more interaction between domestic and wild animals and, therefore, favors the sharing of zoonotic pathogens. Given this context, viruses from the genus *Orthopoxvirus* have been emerging in urban areas all around the world, including in Brazil, with a *Orthopoxvirus monkeypox* outbreak in 2022 and recurrent *Orthopoxvirus vaccinia* (VACV) outbreaks since 1999 being remarkable examples. Serological and molecular data from recent studies show a possible VACV circulation in Brazilian urban environments, between wild and domestic animals, such as cats and dogs. In this study, we aimed to detect orthopoxvirus DNA in serum samples from 30 cats and 3 dogs from Belo Horizonte (n=33), capital of the state of Minas Gerais, Brazil, collected in the year of 2023. The samples were diluted in a phosphate saline buffer in a proportion of 1:10 and then submitted to real time PCR targeting the C11R and A56R genes. None of the samples was positive for C11R and tests for A56R are underway. Despite not finding viral DNA in the samples analyzed, as evidence of *Orthopoxvirus* circulation in urban environments has been found, we stress the importance of epidemiologic surveillance over alternative transmission routes to these pathogens. Since urban areas concentrate a large number of people susceptible to orthopoxviruses, more studies are needed to better understand viruses ecoepidemiology.

Financial Support: FAPEMIG, CAPES, CNPq, INCT-POX, Câmara Pox - MCTI

Keywords: vaccinia virus, disease ecology, urbanization, ecoepidemiology

PT.378

MAPPING POTENTIAL TRANSMISSION RISKS OF AVIAN INFLUENZA VIRUS BETWEEN WATERFOWL AND BACKYARD POULTRY IN SÃO PAULO

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The study aimed to identify high-risk areas for avian influenza virus (AIV) transmission between waterfowl and backyard birds in São Paulo state to improve the AIV surveillance plan. From December 2022 to April 2024, the Secretary of Agriculture and the Poultry Health State Programme collected 304 cloacal and tracheal swab samples and 228 sera samples from 742 birds across 140 backyard poultry farms, including chickens, ducks, and geese. A comprehensive database was developed, incorporating geographical coordinates, taxonomic classification of migratory waterfowl, municipalities of sightings, years, and 54 reported H5N1 cases from CEMAVE/ICMBio and official veterinary services. The data were analyzed using the Kernel Density Estimator, classifying them into five relative density categories. The analysis did not find AIV in swab samples. However, antibodies against AIV were detected in eight chickens (3.5%): three from Ithanhaém, two from Santos in 2023, and a total of three from Ilha Comprida, Peruíbe, and Praia Grande in 2024 using a competitive ELISA specific for the AIV nucleoprotein. No specific antibodies against H5, H7, and H9 subtypes were found, indicating the presence of other AIV subtypes. The study identified two mesoregions, the São Paulo Metropolitan area and Paraíba Valley, as hotspots for AIV transmission. Additionally, the municipalities of Bertioga, Itanhaém, Santos, and São Sebastião were pinpointed as high-risk areas. These hotspots are recommended as priority areas for inclusion in the AIV surveillance plan for São Paulo state.

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PT.379

POTENCIAL NEW AVIAN SPECIES AS CARRIERS OF THE CIRCOVIRUS SUBTYPES

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The diseases associated with porcine circovirus type 2 (PCV2), psittacine beak and feather disease virus (BFDV), Culex circovirus-like virus (MosACV1), pigeon circovirus (PiCV) and raven circovirus (RaCV), can be classified as members of the genus Circovirus (CV) of the family Circoviridae. These viruses are responsible for painful and long chronic illness and several deaths of avian species and mammals. Therefore, the main objective of the work is to analyze bird oral and cloacal swab samples from the São José do Rio Preto-SP region collected at the Municipal Zoo for the presence of CV. The samples are initially submitted to DNA extraction, followed by Nested-PCR and the electrophoresis run on a 1.5% agarose gel. Then, in cases considered positive for CV, the samples are sent for sequencing using the Sanger technique. In total, of the 482 samples, 18 (3.7%) samples were considered positive and submitted to Sanger Sequencing. The fragments with the greatest identity are those related to viral subtypes PiCV (27.7%) mainly, as well as PCV2 (11.1%), BFDV (5.5%), MosACV1 (5.5%), and RaCV (5.5%). However, in contrast to what is pointed in the literature, the avian families that had positive samples are different of what were already considered hosts of CV, implying that it could possibly represent new oders of avian species as carriers of the virus, such as: accipitriformes, falconiformes, nyctibiiformes and catartiformes.

PT.380

BIRDS AND MAMMAL POSITIVE IN REAL-TIME PCR FOR INFLUENZA A VIRUS

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The global Influenza A virus (AIV) has several infections outbreaks of the highly pathogenic (HPAIV), mainly in the Americas. Mostly caused by H5N1, which is responsible for the high morbidity and mortality of wild birds, domestic birds, and mammals, representing a potential threat to humans due to its zoonotic nature. Recently, H5N1 infection in dairy cows in the United States of America has been highlighted, including transmissions cow-humans and high virus levels detected in unpasteurized milk. Considering the current situation, many birds are natural reservoirs of the virus and can contribute to the evolution, maintenance and spread of AIV. So, the objective of this research is to analyze the presence of AIV virus in oral, anal and other organs swabs such as lungs, pancreas and genitals from birds of São José do Rio Preto-SP region and birds and mammals from the Coast of Paraná-SP. The samples analyzed for the presence of AIV were initially subjected to RNA extraction, followed by real-time PCR for the presence of AIV and for the endogenous control beta-actin. In positive cases for AIV, the sample is submitted to Next Generation Sequencing. In relation to the results, of the total of 1.179 samples, eight samples from the coast of Paraná were positive in real-time PCR for AIV, related to an Atobá-pardo (Sula leucogaster), Leão-marinho-da-patagônia (Otaria flavescens) and Trinta-réis-de-bando (Thalasseus acuflavidus).

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PT.381

Acanthamoeba sp. AS HOSTS FOR GIANT VIRUSES FROM THE ARCTIC AND THE POTENTIAL PRODUCION OF ANTIMICROBIALS DURING THE INFECTION

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Giant viruses are part of the nucleocytoplasmic large DNA viruses (NCLDVs) group. They were first described in 2003, leading to a revolution in microbiology. Recent metagenomic research revealed that giant viruses are ubiquitous, and their genome size can reach 2.5Mb. Such characteristics lead to a remarkable biotechnology potential coded by their still mysterious genomes. The arctic region have been described as hotspots for finding giant viruses. Herein, we aimed to collect different samples above the arctic circle and use them to isolate giant viruses using *Acanthamoeba* sp. as hosts, and further explore different compounds produced during infection. So far, we collected over 250 samples around the north of Norway and Nordic Sea, including: marine and freshwater environments, urban, sewage and deep-sea vent samples. All of them were prepared and used for virus isolation by mixing samples and cells, following culturing steps until cytopathic effect (CPE) appeared. Six different samples presented CPE and the presence of a giant virus was confirmed by Transmission Electron Microscopy images. Virus species are still to be confirmed through sequencing analysis, yet morphological features suggest viruses to be part of the marseilleviridae and mimiviridae families. Preliminary results from metabolomics analysis pointed to different compounds being produced during a Norwegian Marseillevirus infection in amoeba, although the infection by 5 of our viruses' isolates did not produce antibiotics against four different bacteria. Subsequent steps are virus species identification and characterization, and the description of these diverse compounds produced during the infection.

Financial Support: Centre for New Antibacterial Strategies (CANS) of the Arctic University of Norway (project #2520855).

PT.382

RESEARCH ON ORTHOFLAVIVIRUSES IN WILD MOSQUITOES (DIPTERA: CULICIDAE) DURING THE YELLOW FEVER OUTBREAK IN RIO GRANDE DO SUL, BRAZIL, BETWEEN 2021 AND 2022

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The Yellow Fever (*Orthoflavivirus flavi*) (YFV) returned to Rio Grande do Sul (RS) state at the beginning of 2021, after more than ten years without detection. The spread of the virus was marked by deaths among non-human primates. In an effort to identify YFV and other circulating arboviruses in wild mosquitoes in RS, an entomological survey was conducted in areas affected by YFV where primate deaths had been recorded, as well as in other areas of interest within the state. For viral detection, a pan-*Orthoflavivirus* RT-qPCR assay was standardized. To determine the sensitivity of the RT-qPCR, ten-fold serial dilutions of the TOPO TA plasmid containing a fragment of the NS5 gene from the YFV vaccine strain were tested, revealing a detection limit of 1 to 10 copies per reaction. A total of 1,210 individuals from the Culicidae family were collected, and 203 mosquito pools were formed, grouped by species, location, and collection date. The most abundant mosquitoes were *Psorophora ferox*, followed by *Culex* (*Culex*) spp., comprising 27% and 12% of the collected mosquitoes, respectively. The main vector of YFY in RS, *Haemagogus leucocelaenus*, was among the most abundant species and was sampled in nearly half of the municipalities. When subjected to RT-qPCR for YFV and pan-*Orthoflavivirus*, no genome was detected. Despite being collected at an opportune time with active YFV circulation, the results of the present study support the theory that the circulation of arboviruses in mosquitoes in nature is low and highly influenced by environmental factors.

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PT.383

VIROME OF WILD MOSQUITOES (DIPTERA: CULICIDAE) FROM THE NORTHWEST REGION OF RIO GRANDE DO SUL STATE, BRAZIL

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Mosquitoes (Diptera: Culicidae) are vectors of various arboviruses of public health importance and harbor a wide diversity of Insect-Specific Viruses (ISVs). However, there is a need for further studies to comprehend and identify the viral diversity present in these insects. In this context, the present project aims to detect viruses through metagenomics of wild mosquitoes collected in the northwest region of Rio Grande do Sul state, Brazil. For this purpose, 489 mosquitoes of different species were collected in Derrubadas and Santo Antônio das Missões. A sample size of 90 individuals was used, belonging either the *Psorophora ferox* species or *Culex* spp. The mosquitoes were pooled (n=30) and then clarified, filtrated, and ultracentrifugated. Nucleic acids were extracted, and, subsequently, the samples underwent various processes of amplification and enrichment. High-throughput sequencing was performed with Illumina MiSeq, generating an average of 342,782 reads, resulting in the assembly of 787 contigs. The pools exhibited an average of 82% of unclassified reads, 14% bacterial reads, 4% eukaryotic reads, and 1.5% viral reads. Regarding viral reads, besides an approximately 80% of uncategorized viral sequences, a notable abundance of bacteriophages was observed. The average of viral sequences related to mosquitoes identified among the pools was 1.5%, encompassing eight viral families(*Dicistroviridae, Microviridae, Narnaviridae, Phenuiviridae, Hypoviridae, Mitoviridae, Nairoviridae, Pospiviroidae*) and the Order Reovirales. This study enhances knowledge about viral diversity in mosquitoes,highlighting both the opportunities and limitations of metagenomics in this context.

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PT.384

ASSESSMENT OF CRASSPHAGE AS ENDOGENOUS CONTROL IN ENVIRONMENTAL SURVEILLANCE

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Monitoring viral pathogens in environmental surveillance requires quality control of molecular assays, due to the complexity of matrices. Different international regulatory agencies suggest using crAssphage as an endogenous process control. This study aimed to quantify crAssphage in wastewater, evaluating its role as endogenous control in a country with a tropical climate. For this purpose, 1-year monitoring was carried out in eight wastewater treatment plant (WWTPs A-G) in Niterói, Rio de Janeiro, Brazil, during 2020/2021. One hour composite samples were collected biweekly. Ultracentrifugation was used for viral concentration. Nucleic acid was extracted using an automatic QiAcube (QIAGEN) and qPCR based on TaqMan protocol. Annual sewage samples showed 100% detection (188) with quantifications ranging from 8.29 to 10.43 $\log_{10}$ genomic copies per liter (gc l^{-1}). A spatial analysis demonstrated statistically significant differences between absolute crAssphage concentrations detected in the analyzed WWTP with lower crAssphage values at WWTP G compared to the others WWTPs. A temporal analysis revealed a non-normal distribution, with median (Mdn) values ranging from 11.54 to 12.34 $\log_{10}$ gc pcpd, from the results of the and 45th, 32nd, and 34th, epidemiological weeks, identified as deviations by the multiple Mdns comparison (Dunn's test). This study reports the concentration of crAssphage in a country with a tropical climate, where climatic factors are considered critical, influencing the dispersion of this endogenous control in environmental samples. The findings align with previously reported concentrations in different temperate countries, stating that crAssphage can be considered an indicator of contamination.

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PT.385

ASSESSMENT OF SURFACE WATER AS A COMPLEMENTARY TOOL FOR WASTEWATER-BASED EPIDEMIOLOGICAL SURVEILLANCE USING A NEXT-GENERATION SEQUENCING PROTOCOL TO DETERMINE THE SARS-COV-2 VARIANT

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Several studies suggest monitoring urban streams as a complementary tool for viruses' environmental surveillance in countries where sanitary sewer infrastructure is inefficient or nonexistent. This study, conducted in three watersheds and two sewage treatment plants in Niterói, Brazil, over four weeks in 2021, aimed to characterize SARS-CoV-2 lineages detected in those matrices during the pandemic period. Two viral concentration methodologies were used: adsorption-elution and ultracentrifugation for surface water (streams and seawater) and sewage samples, respectively. Nucleic acid was extracted using an automated QiAcube system (QIAGEN, CA, USA), and qPCR based on the TaqMan protocol targeted the N1 and N2 genes. Molecular characterization and classification were done using complete genome sequencing, following the MinION Oxford Technologies protocol. N1 gene SARS-Cov_2 was detected in 92.5% (37/40) and 31.25% (10/32) of wastewater and stream water, respectively. Virus concentration ranged from 3.75 to 6.41 $\log_{10}$ genomic copies per liter (gc l^{-1}) and 3.60 to 6.22 $\log_{10}$ gc l^{-1} in those matrices. SARS-CoV-2 was not detected in seawater samples. CrAssphage was detected in 100% (40/40) of wastewater, 95% (30/36) of stream water, and two out of four seawater samples. As a marker of human fecal contamination, crAssphage validated the results obtained for SAR-Cov-2 in all matrices. Molecular characterization identified the Gamma variant of concern during the study period in both wastewater and surface waters, revealing widespread contamination in those watersheds. This study highlights the use of methodological controls to reduce uncertainties in environmental findings.

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PT.386

CRASSPHAGE AS NORMALIZER OF SARS-COV-2 DATA DETECTED IN WASTEWATER FOR CORRELATION WITH CLINICAL CASES

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Sewage- based epidemiology studies have recommended the use of endogenous controls for correlation studies of clinical and environmental data. This study aimed to evaluate crAssphage as a normalizer for SARS-CoV-2 environmental data. For this purpose, both viruses were quantified in 188 composite raw sewage samples collected biweekly at eight wastewater treatment plant during pandemic period (September 2020 to August 2021). Nucleic acid extraction was performed using the automated QIACUBE system (QIAGEN), and (RT)qPCR was performed based on the TaqMan protocol. SARS-CoV-2 RNA was detected in 94% (177) and 90% (170) of the samples using the N1 and N2 target regions, respectively, with a median global concentration of 5.00 $\log_{10}$ genomic copies per liter (gc l^{-1}). CrAssphage was present in all samples, ranging in concentration from 8.29 to 10.43 $\log_{10}$ gc l^{-1}, and a median of 9.46 ± 0.40 $\log_{10}$ gc l^{-1}. The correlation between wastewater surveillance data and reported SARS-CoV-2 cases per epidemiological week (20 834 cases) was initially weak, falling within the moderate threshold when SARS-CoV-2 data were normalized by crAssphage. However, this correlation strengthened when SARS-CoV-2 data were normalized using crAssphage per capita wastewater flow concentrations, exceeding the moderate classification index ($r = 0.5637$). When using the average of reported cases obtained one week after the wastewater collection date, a smoother correlation was observed in both normalizations. The greater adherence to environmental and clinical data correlations for SARS-CoV-2 employing pcpd-normalized crAssphage data points to the importance of normalizing crAssphage data.

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PT.387

MOLECULAR INVESTIGATION OF YELLOW FEVER VIRUS IN GONADS OF NON-HUMAN PRIMATES COLLECTED IN THE STATE OF MINAS GERAIS IN 2017 AND 2018

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Yellow fever (YF), caused by the yellow fever virus (YFV), affects humans and non-human primates (NHPs). In Brazil, YFV is transmitted to NHPs by mosquitoes in the sylvatic cycle. As part of the Brazilian YF surveillance program, it is mandatory to investigate the presence of YFV in the carcasses of free-living wild NHPs during epizootic events. Liver samples are commonly used for viral investigation, but YFV can also be detected in other organs, such as the kidneys, brain, and lungs. This study aimed to investigate the presence of YFV in the gonads of NHPs collected in the state of Minas Gerais during the YF outbreak (2017-18). The gonads of 39 NHPs with confirmed YFV detection in the liver were analyzed. The NHPs belonged to the genera Callithrix (n=19), Alouatta (n=14), and Callicebus (n=6). Total RNA from the samples was extracted, and RT-qPCR was performed targeting the β-actin gene and the YFV 5'UTR. Of the 39 animals tested, 28 (71.8%) were positive for YFV, primarily from the Alouatta and Callithrix genera (42.8%), followed by Callicebus (14.4%), with Cq values ranging from 17 to 37. Quantitative PCR on these YFV-positive samples revealed a range of 1.8x10E1 to 5.0x10E7 RNA copies/mg of tissue. These data demonstrate the presence of YFV RNA in gonads, which warrants further investigation, as these organs are important in the pathogenesis of other orthoflaviviruses. Additionally, this study highlights the importance of understanding YFV infection in gonads and employing multi-organ testing strategies to detect the circulation of YFV.

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PT.388

NGS AS A TOOL FOR ASSESSING SURFACE WATER USED FOR HUMAN CONSUMPTION IN ENVIRONMENTAL PROTECTION AREAS

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This study aimed to analyze viral diversity in raw surface water used for human consumption in a conservation unit. In February 2023, water samples were collected from streams located in three areas on the Marambaia Island, Rio de Janeiro state (R1-R3). The samples were concentrated using the negatively charged membrane filtration method, followed by ultrafiltration. The nucleic acids were extracted using the Biopur Nucleomag Virus kit (Mobius Life Science, BR) and quantified by Qubit 4.0. The construction of the library and massive sequencing were conducted on the Fiocruz Sequencing Platform with NextSeq 2000 sequencer (Illumina, USA). The data analysis was performed with Genome Detective software. Of the 55.960.252 reads generated, 0.54% (284.097) were classified as viral. The R1 region showed the highest number of contigs and diversity of viral families, including 11 unique families in the sample without anthropogenic impact. The Bacteria were the most common hosts, followed by fungi, arthropods, and insects. In terms of viruses that infect mammals and humans, they were identified in both impacted (n=1) and non-impacted (n=2) R1 regions, including Venezuelan equine encephalitis virus, Rotavirus A, and Human endogenous retrovirus K113. The regions R2 and R3 did not present viruses of public health importance but was observed the presence of zoonotic potential virus family (*Parvoviridae*). The virome analysis suggests that anthropogenic activity has an impact on the viral diversity of the R1 region in surface waters when compared to other areas.

Keywords: viral diversity; surface water; water assessment
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PT.389

UNRAVELING THE HIDDEN DIVERSITY OF CHLORELLA HELIOZOAE-INFECTING GIANT VIRUSES

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Chlorovirus is a genus of algae-infecting viruses found in the most diverse aquatic environments divided into three proposed subgenera. The gammachloroviruses infect *Chlorella heliozoae*, have large and complex linear dsDNA genomes, encoding more than 300 proteins and up to 13 tRNA. Isolating and characterizing new viruses will provide better comprehension on the diversity and biology of these giant viruses. Here we describe and compare the genomic features of 37 gammachloroviruses, including 24 novel isolates. Water samples were used to isolate viruses through plaque assay. New viruses were further purified and sequenced using PacBio technology. New genomes were assembled using Canu and included in a final dataset containing 37 gammachloroviruses for genomic analysis. Genes were predicted using GeneMarkS, tRNAScan-SE and Aragorn, and annotated using HHpred, BLASTp, and InterProScan. *Nucleocytoviricota* hallmark genes were aligned using Muscle and the phylogenetic tree built through IQ-TREE2. Average Nucleotide Identity (ANI) and Pangenome analysis were performed using FastANI and OrthoFinder. New isolates have genomes ranging from 283 to 385 kbp. The phylogenetic reconstruction suggests 2 main groups of isolates, also reflected in statistical analysis of their genomic features. Using ANI>94% as cutoff for species identification, we observed a total of 10 gammachlorovirus species, being the most diverse group of chloroviruses to date. Our analysis indicates an open pangenome composed of 791 COGs, of which ~50% cluster uncharacterized genes. Our results reveal the great diversity within *Gammachlorovirus* subgenera and reinforce the importance of continuous efforts for isolating and characterizing giant algae viruses.

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PT.390

PROSPECTION AND IDENTIFICATION OF GIANT VIRUSES IN BRAZILIAN BIOMES

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Since the discovery of the first giant virus infecting amoebas in 2003, Acanthamoeba polyphaga mimivirus, research has been conducted on this group with the aim of understanding its ecological importance, phylogenetics, biology, and its role in the virosphere. It has been found that these viruses exhibit unique characteristics, such as the size of Tupanvirus particles, which can reach 2.6 µm, and extensive double-stranded DNA genomes reaching 2,700 kb in pandoravirus. The work aims to isolate new giant viruses to better understand their richness and diversity in Brazilian territory. The collections used consist of 308 samples collected from rivers, reservoirs, tree hollows, and water puddles in the north and areas of the Jequitinhonha River basin in the state of Minas Gerais between 2020 and 2022; and in the Paraguay River, in the Mato Grosso Pantanal region in 2023. For viral isolation, free-living amoebas of the species *Acanthamoeba castellanii* cultured in peptone-yeast-glucose medium inoculated with environmental samples and incubated for up to 21 days were used. Additionally, samples showing cytopathic effects were sent for analysis by transmission electron microscopy (TEM). Among the processed samples, seven induced cell rounding with subsequent lysis in amoebas. TEM analysis revealed the isolation of two marseilleviruses and 2 mimiviruses (one of them associated with a virophage). The remaining three samples await analysis. Finally, this work demonstrated the presence of giant virus isolates in different natural environments in Brazil, contributing to the expansion of the study of the biodiversity of this newly discovered group of viruses.

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PT.391

MOLECULAR EPIDEMIOLOGICAL SURVEILLANCE OF RESPIRATORY VIRUSES IN WASTEWATER FROM SÃO JOSÉ DO RIO PRETO

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Respiratory infections affect the upper and lower respiratory tract, representing the leading cause of mortality in children under five, the elderly, and immunocompromised individuals. Monitoring pathogens through wastewater has been a valuable tool for epidemiological surveillance since municipal sewage reflects the composition of all individuals in the locality, allowing the estimation of infection outbreaks and the identification of present pathogens. Therefore, this project aims to investigate the presence of respiratory viruses, such as *Orthopneumovirus hominis*, *Metapneumovirus hominis*, SARS-CoV-2, *Alphainfluenzavirus influenzae* (Influenza A virus), and *Betainfluenzavirus influenzae*, using RNA extraction techniques and identification by RT-qPCR in untreated wastewater samples from the municipality of São José do Rio Preto - SP, collected weekly over two years (2022-2024). In total, 87 samples were analyzed, corresponding to the period from July 2022 to March 2024. Of these, 20 samples were positive for Influenza A virus (23%), 22 positive for SARS-CoV-2 (25%), 1 for *O. hominis* (1.15%) and 1 for *M. hominis* (1.15%). The highest positivity period for Influenza A virus was from May to September 2023, coinciding with the seasonal period during the colder and rainier months. SARS-CoV-2 was mainly detected at the end of October and early December 2022, and additionally, for several consecutive weeks in February and early March 2024, due to the increase in the number of cases during these periods. Wastewater monitoring includes asymptomatic individuals, making Wastewater-Based Epidemiology crucial for complementing public surveillance.

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VIRAL DIVERSITY IN URBAN SOLID WASTE: COMPARISON BETWEEN FRESH TRUCK AND LANDFILL LEACHATE

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Municipal solid waste (MSW) leachate is a complex effluent, with variable microbiology and physicochemical characteristics, generated during waste collection in truck waste basins, and landfills. MSW such as toilet paper, disposable diapers, human and domestic animal excreta could carry microorganisms to the leachate. The study aimed to describe and compare the viral genetic diversity of MSW in fresh truck and landfill leachate in the city of Rio de Janeiro, Brazil. Twenty-two truck leachate samples were collected from March to December 2019, nine from waste truck basins in a waste transfer station, and thirteen raw leachates from the equalization tank of an MSW landfill. All samples were ultracentrifugated, a pool of each leachate type was treated with DNAse, and nucleic acid extracted by QIAamp Fast DNA Stool Mini kit®. Viral enrichment was performed, genomic libraries were prepared with Nextera XT®, and metagenomics analysis developed by Illumina® NextSeq 550. The analysis with Genome Detective Virus Tool detected 66 viral families in the leachates. Viral abundance reveals a significant difference between both leachates (Wilcoxon test, ρ value <0.0001). The most prevalent viral families and abundance were detected in truck leachate as *Tymoviridae, Herelleviridae, Secoviridae, Solemoviridae, Parvoviridae, Alphaflexiviridae, and Virgaviridae.* Related human and animal public health importance included *Adenoviridae*, *Astroviridae*, *Caliciviridae*, *Circoviridae*, *Picobirnaviridae*, and *Picornaviridae*. The viral metagenomics in solid waste allowed the detection of fecal-oral transmission virus sequences in fresh truck leachate and reinforced the importance of personal protective equipment for MSW collection workers.

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PT.393

HUMAN MASTADENOVIRUS GEOGRAPHIC DISTRIBUTION IN SOLID WASTE LEACHATE FROM RIO DE JANEIRO CITY

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Municipal solid waste (MSW) leachate is a wastewater formed at final waste disposal sites and in collection trucks, containing organic and inorganic compounds as well as microorganisms, from human an animal's excretions. Wastewater-based epidemiology (WBE) is a useful tool for monitoring pathogens circulating in populations. While sanitary sewage is considered the gold standard for WBE, solid waste fresh truck leachate can be an alternative matrix in areas without sewage collection system. This study aimed to establish geographic distribution and quantification of human mastadenovirus (HAdV) based on fresh truck leachate in Rio de Janeiro city. Between January and February 2024, 19 samples of fresh leachate were collected on basins of waste collection trucks, from 16 different neighborhoods, in three MSW transfer stations that receive around 7,100 t/day. Samples were ultracentrifuged, nucleic acid extracted (Viral MagBead – Zymo Research, USA), Taqman® Real-Time qPCR applied for HAdV quantification at 10^{-1} and 10^{-2} dilution, and data analyzed in neighborhood level. 89.47% (17/19) of samples were positive for HAdV, with a mean quantification of 3.34×10^{6} genomic copies (GC) per 100 mL (1.3×10^{4} - 5.9×10^{7} GC/100 mL). To date, the study has shown the presence of HAdV in leachate from all evaluated administrative regions of the municipality, with no indication of geographical influence on the detected viral load. For future, we seek to establish methodologies for the detection, quantification, molecular characterization and distribution of other enteric virus based on fresh truck leachate.

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PT.394

DETECTION OF ENTERIC VIRUSES AND EVALUATION OF HUMAN FECAL CRASSPHAGE AS A MARKER OF HUMAN VIRAL CONTAMINATION IN COMMERCIALIZED BIVALVE MOLLUSKS IN RIO DE JANEIRO, 2022.

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CrAssphage is a bacteriophage of human gut commensal *Bacteroides* spp. and has demonstrated a potential indicator for wastewater contamination. This study investigated the prevalence and the genetic diversity of enteric viruses in commercialized bivalve samples in three touristic cities in Rio de Janeiro state, between January-December 2022, and the performance of crAssphage as a marker of human viral contamination. The screening of 134 samples was performed according to ISO/TS 15216:2017 and revealed the detection of norovirus GII (37.3%), norovirus GI (20.1%), human astrovirus (HAstV; 14.2%), sapovirus (SaV; 12.7%), human mastadenovirus (HAdV; 7.5%), rotavirus A (RVA; 5.9%), hepatitis A virus (HAV; 3.7%), and crAssphage (60.5%). There was no detection of hepatitis E virus. In total, 48.5% of samples tested positive for one or more viruses. Norovirus GII showed the highest mean viral load (3.4×10^5GC/g), followed by crAssphage (1.2×10^5GC/g), HAstV (4.2×10^4GC/g), SaV (1.4×10^4GC/g), RVA (1.1×10^4GC/g), HAdV (3.9×10^3GC/g), HAV (8.7×10^2GC/g), and norovirus GI (6.7×10^2GC/ g). The molecular characterization revealed that the norovirus strains belonged to genotypes GII.2, GII.6, GII.9, GII.17, and the rare GII.27; HAstV-1; SaV to GI.1 and GIV.1; RVA to G6, G8, P[8]-III, HAdV to F40/F41, and HAV-IA. The specificity and sensitivity values of crAssphage as a viral marker were calculated for all viruses, with an average specificity value of 0.63 and a high sensitivity value of 1.0 for HAstV and HAV, showed a moderate correlation with HAstV (r:0.424, $p<0.001$). This study demonstrates the dissemination and diversity of enteric viruses in commercialized bivalves and, highlights crAssphage as a reliable indicator to monitor foodborne virus contamination.

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PT.395

INVESTIGATION OF OSTREID HERPESVIRUS-1 AND HUMAN ENTERIC VIRUSES IN A SCALLOP PRODUCTION AREA IN BRAZIL

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Bivalve mollusks may be affected by numerous infectious diseases, which cause high mortality rates and economic burdens for producers. Another challenge for bivalve aquaculture is the protection of farms from human contamination, such as sewage and stormwater discharges. Ilha Grande Bay (IGB), located in Rio de Janeiro state, is the largest Brazilian producer of scallops (Nodipecten nodosus). This region has recently suffered a mass mortality of mollusks, and several environmental contaminants have been reported in the area. To determine the reason for scallop collapse and better characterize the human impacts, this study assessed the circulation of mollusk (Ostreid Herpesvirus-1 [OsHV-1]) and human (Norovirus GII and adenovirus [HAdV]) viral pathogens in waters and animals produced at IGB. Neither water nor animals were positive for OsHV-1. However, of the 7 points analyzed, 5 points showed contamination by HAdV or norovirus. Norovirus and HAdV were detected in 6.9 and 5.5% of the analyzed water samples, respectively, in concentrations ranging from 2.39×10^3 to 1×10^5 genome copies/L. One scallop sample was positive for norovirus (4.5%). These results demonstrate human contamination in the region, presenting a risk of consumer contamination, and a non-association between OsHV-1 and the mass mortality described in scallops.

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PT.396

PRESENCE OF INFECTIOUS GASTROINTESTINAL AND RESPIRATORY VIRUSES ON FOMITES IN PUBLIC RESTROOMS OF EDUCATIONAL AND HEALTH FACILITIES IN NITERÓI, RIO DE JANEIRO

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Contaminated restroom fomites have been implicated as sources of respiratory and enteric viral infections, especially under improper hygiene and disinfection procedures. This study aims at detecting human mastadenovirus (HAdV) and SARS-CoV-2 on fomites in public restrooms of educational and healthcare facilities in the city of Niterói, Rio de Janeiro, before and after cleaning procedures. From July to December 2022, samples from toilet seat, flush, side wall, internal and external door handles, tap and soap dispenser were obtained using a swab soaked in DMEM, before and after cleaning two bathrooms located in teaching and health units of an educational institution. Eighty-eight samples were obtained, being 31.8% positive for HAdV by qPCR, in concentrations ranging from 2.4E+02 to 2.3E+04 GC/fomite. HAdV were found on all fomites, except on the external door handle of the health unit, and before (26.7%) and after (43.3%) cleaning procedures. Infectious HAdV were detected by ICC-qPCR in toilet seat, flush, side wall, and door handles, being flush and door handle after cleaning procedures. Molecular characterization by sequencing of the hexon gene (301bp) revealed the presence of *Mastadenovirus faecale* on fomites. SARS- CoV-2 was detected by RT-qPCR in door handle and tap before cleaning. Results demonstrated viral contaminations on fomites in the public restrooms and that cleaning procedures did not completely remove viral particles from these environments, which represents a possible source of infection for users.

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PT.397

COMPARISON OF DIFFERENT METHODS FOR VIRAL CONCENTRATION IN DAIRY AND MEAT PRODUCTS

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This study aimed to compare the viral recovery of different methods for viral concentration in complex matrices using murine norovirus (MNV-1) and murine hepatitis virus (MHV-3) as process control and models for enveloped and non-enveloped viruses. In dairy products, pasteurized milk and mozzarella cheese were tested using PEG and TGBE for both matrices and organic flocculation solely for milk. The meat products tested were deli meats (colonial salami, Italian salami, and coppa) and swine and poultry chilled meats. Three different cuts from different regions of the animal were tested for a better representation, considering their different composition. Three concentration methods were tested for meats, (i) lysis buffer+B-mercaptoethanol (ii) PBS and glycine elution (iii) PEG. Magnetic beads (Kit RNAdvanceViralXP BeckmanCoulter) were used for nucleic acid extraction. For dairy products, TGBE showed better viral recuperation, in milk 1.46% and 4.31%, and in cheese 5.78% and 15.14% for MNV-1 and MHV-3 respectively. For deli meats, the best mean recovery for MNV-1 was achieved by method (i) 0,13%, and for MHV-3 (ii) 0.29%. The highest recovery for swine-chilled cuts was achieved with method (i) for MNV-1 and MHV-3 with 2.36% and 0.22% respectively. For poultry-chilled meats, the best protocol for MNV-1 recovery was (i) 29.45%, and for MHV-3 (iii) 2.78%.

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PT.398

DETERMINING THE PHYLOGENETIC RELATIONSHIP BETWEEN TWO MYCOVIRAL ORPHAN FAMILIES.

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The multiple origins of viruses are expressed in different realms established by the ICTV. Realm *Riboviria* contains all RNA viruses. They share a common ancestor, and the conserved palm domain in RdRp or RT protein represents their phylogenetic relationship. Recent studies have led to a quick increase in our virosphere knowledge, so continuous work is required to organize the taxonomy of novel viral groups. For example, the mycoviral families *Polymycoviridae* and *Hadakaviridae* were recently established. Both families share standard biological features and are phylogenetic-related. However, *Polymycoviridae* has unassigned order-kingdom taxa, while *Hadakaviridae* is a member of the phylum *Pisuviricota* without order and class established. To determine their phylogenetic relationship, we search on NCBI for the RNA segment that encodes their RdRp protein and download the nucleotide and amino acid sequence. We also downloaded sequences of other viruses inside *Pisuviricota* and *Lenarviricota*. Then, we use mafft to multiple-align the sequences, modeltest to calculate their best evolutive model, and RAxML to create the phylogenetic tree. We observe a well-supported monophyletic clade harboring these two families. They seem more related to each other than other viruses in *Pisuviricota*. Therefore, we suggest creating a novel class, *Adfinisviricetes*, composed of two novel orders, *Adopertavirales* and *Nudavirales*, harboring the *Polymycoviridae* and *Hadakaviridae* families, respectively.

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PT.399

ISOLATION AND CHARACTERIZATION OF THE FIRST BRAZILIAN CHLOROVIRUS FROM INLAND WATERS OF MINAS GERAIS

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Chloroviruses are large dsDNA viruses from the family *Phycodnaviridae*, infecting chlorella-like green algae. Those viruses circulate in continental waters worldwide, and genomic sequences have been detected in marine environments, including the Arctic. Found in waters mainly from the North hemisphere, searching for chloroviruses in waters from the South hemisphere and Antarctic Ocean is a fascinating opportunity to find those viruses and evaluate for possible differences in morphology and replication cycle compared to Northern chloroviruses. This work aims to isolate chloroviruses from water samples from multiple regions from Brazil, the Atlantic Ocean, and the Antarctic continent and characterize them by morphology, replication, and biology. Until now, we screened 63 samples using *Chlorella variabilis* as cell support. In a river sample from Guanhães City, Minas Gerais, we observed cytopathic effects characterized by cell lysis and complete clearance of the cell culture. After plaque assays, we observed two different plaque phenotypes. After plaque purification, the titre of the viruses was 1.0 x 10^9 PFU/ mL. Both viruses were submitted to resistance assays, showing good temperature stability, remaining viable from –80ºC to 56°C, but low UV radiation resistance, being inactivated after 3 minutes of exposition. The host spectrum tests showed no sign of infection in other four tested algal species, suggesting a restricted host range of these viruses. Negative staining electron microscopic analysis revealed icosahedral particles with diameters of approximately 160 nm. Here, we report the first chlorovirus isolated in Brazil, paving the way for important advances in the Brazilian virology.

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PT.400

EMERGENCE OF THE OROPOUCHE VIRUS IN THE STATE OF RIO DE JANEIRO

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According to the epidemiological bulletin from the brazilian Ministry of Health, in 2024, the number of suspected cases of dengue in Brazil have increased almost 4 times when compared to the same period of the previous year. However, the overall laboratory positivity rate for dengue in this period was only 36.4%. To expand the number of etiological agents researched, in addition to testing for Dengue, Zika and Chikungunya, the Central Public Health Laboratory of Rio de Janeiro included the research of Mayaro and Oropouche viruses. The Oropouche Virus was detected in patients from the mid-Paraíba region and autochthonous circulation in the state was confirmed in april. Given the number of suspected cases of unconfirmed arboviruses at the beginning of the dengue epidemic in 2023, our work aimed to retrospectively investigate the presence of Oropouche virus in suspected cases of dengue with an inconclusive diagnosis and support studies on the dispersion of this vírus. A total of 3208 negative samples for Dengue, Zika and Chikungunya, from september 2023 to July 2024 were selected for detection of Mayaro and Oropouche viruses by Real time RT-PCR. A total of 118 samples were positive for Oropouche virus. The earliest positive sample was collected in march 13th 2024 and the most affected county was Piraí with 60.1% of all positive cases. Samples with lower Ct values were selected for nucleotide sequencing. These findings highlight the importance of arbovirus surveilance.

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PT.401

Exploration of Antioxidant and Antiviral Potential of Compounds Extracted from Microalgae

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Microalgae are rich in bioactive compounds with substantial potential for medical and health applications, showing antibacterial, antioxidant, and antiviral properties. The genus Dunaliella salina, a unicellular eukaryotic microalga, is particularly known for producing β-carotene, which is crucial for immune response and intracellular communication and offers benefits against diseases. Chlorella vulgaris, which is abundant in chlorophyll and carotenoids, provides significant antioxidant properties that aid in disease prevention. Haematococcus pluvialis, a commercially cultivated microalga, yields β-carotene and astaxanthin, known for their antioxidant, anti-inflammatory, and immunomodulatory effects, and shows promise in treating viral infections, including COVID-19.This project investigates the antiviral and antioxidant potential of microalgae, focusing on identifying and characterizing bioactive compounds with antiviral activity and exploring their applications in combating infections. The experimental approach was divided into two parts: the first assessed the cytotoxicity of the compounds in Vero CCL-81 cell cultures, and the second evaluated the cytopathic effects on cells infected with SARS-CoV-2 in the presence of these microalgae extracts. Dilutions of microalgae extracts from Aquasmart were tested in duplicate using a 96-well plate. Results showed that a 1/4 dilution adversely affected cell visibility, while dilutions between 1/8 and 1/64 achieved 100% viral reduction. Higher dilutions exhibited no cytotoxicity, with viral reduction ranging from 66% to 75%. Compounds derived from microalgae, such as astaxanthin and β-carotene, may be essential in future treatments by inhibiting viral replication and enhancing the host's immune response.

PT.402

Genomic analysis of a new *Ghunavirus* infecting Plant Pathogenic *Pseudomonas*

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Pseudomonas syringae is a plant pathogen responsible for economic losses in important crops worldwide. The specie is divided in pathovars according to its host range, from which the *P. syringae* pv tomato, responsible for speck disease in tomato, is a model for the study of plant-pathogen interactions. This work describes the isolation and sequencing of a new phage that infects *P. syringae*, isolated from a polluted stream in Minas Gerais. Four phage isolates, named Pst1 to 4, were obtained from water samples by using the strain CCRMPST01 of *P. syringae* pv tomato (from the Collection Culture Rosa Mariano of Universidade Federal Rural de Pernambuco) as host. The isolate Pst4 was selected for genomic characterization. Its DNA was extracted by standard procedures and the complete genome was sequenced using the MiSeq platform and assembled using SPAdes software. It has 41,074 pb and 50 ORFs predicted by Prokka annotation software. The comparison of the sequence with the GenBank database indicated it is close to phages of the *Ghunavirus* genus, at the family *Autographiviridae*. These are tailed phages with a podovirus-like morphology that infects plant associated *Pseudomonas*. To classify Pst4, the sequences of all ghunaviruses accepted by ICTV were compared with Pst4 by VIRIDIC web served. The results revealed a similarity between Pst4 and the analyzed sequences below to 91%, which implies it is a new specie on this genus. Further studies are needed to better characterize Pst4 and to evaluate its potential to control plant diseases caused by *Pseudomonas*. Financial support: FAPEMIG

Key words: Biologic control, phage therapy, *Pseudomonas syringae* pv tomato

PT.403

CHARACTERIZATION OF vB_MC_KP1 PHAGE SPECIFIC FOR *Klebsiella pneumoniae* COLLECTED FROM CONTAMINATED STREAM WATER IN MINAS GERAIS

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Bacteriophages are viruses that specifically target and lyse bacteria, making them promising alternatives for treating bacterial infections, especially those caused by multi-resistant bacteria like *Klebsiella pneumoniae*. This study focused on the genomic characterization of a bacteriophage which infects *K. penumoniae,* called vB_MC_KP1, isolated from the Corredor Stream in Mário Campos, MG. Its DNA was extracted and sequenced using the Illumina MiSeq platform, trimmed with trimmomatic, assembled using SPAdes and annotated with the Prokka software. A genomic map was constructed using SnapGene. The genomic sequences closest to vB_MC_KP1 found at GenBank database were compared with it by using VIRIDIC web server, and a phylogenetic tree with the same dataset was build using the software VipTree. vB_MC_KP1 genome has dsDNA, consisting of 116.524 base pairs and encoding 289 ORFs, most of which code for hypothetical proteins. It also includes proteins involved in structure, DNA metabolism, lysis, superinfection control, defense evasion, translation, transcription and 16 tRNAs. Sequence comparison with the GenBank database by BLASTN algorithm showed that vB_MC_KP1 is close to the *Jiaodavirus* genus. Comparison with multiple sequences using VIRIDIC webserver revealed it has less than 95% of similarity with all analyzed sequences, including the ones of the jiaodavirueses species accepted by ICTV. This indicates vB_MC_KP1 is a novel species not previously described. Previous assays from our group have demonstrated its specificity and lytic capability and indicate its potential as an antimicrobial agent against *K. pneumoniae.* Further studies are ongoing to explore its potential in treating multi-resistant bacterial infections.

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PT.404

MOLECULAR SURVEILLANCE OF AVIAN INFLUENZA VIRUS IN MIGRATORY BIRDS WINTERING ON THE COAST OF RIO GRANDE DO SUL STATE, BRAZIL

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Avian influenza viruses (AIV) represent a persistent threat to animal and human health, particularly in regions where migratory birds congregate. This study aimed to investigate the prevalence of influenza A virus among migratory birds wintering along the Lagoa do Peixe National Park, in Rio Grande do Sul state. In collaboration with Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA), we conducted field visits twice a month to collect fecal samples near to stopover sites. Viral RNA was isolated using magnetic beads-based methods, and a RT-qPCR targeting the *matrix* (M) gene was employed for detection. In order to differentiate the *hemagglutinin* (HA) gene subtype of positive samples, a PCR amplifying the whole gene was performed and products were sequenced by Sanger. To date, 610 samples were collected and screened for influenza A virus. Seven fecal samples, collected in December, tested positive for influenza A virus, all isolated from *Larus dominicanus* (kelp gull). One sample was further analyzed, revealing it to be of the H13 subtype. This study illustrates the necessity of continuous surveillance of migratory bird populations in order to monitor the dynamics of AIV and assess the potential risks of transmission to domestic birds and human populations. Migratory bird populations share their environment with a multitude of species of birds and mammals, including humans. The gathering of such diversity of potential hosts may facilitate the transmission and spread of influenza A virus, underscoring the need for targeted surveillance and control measures to mitigate public health risks.

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PT.405

Investigating Epizootics of Non-Human Primates in Urban Environments: Detection of Human Alphaherpesvirus-1/2 infection

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Human activity in sylvatic environments and contact with wildlife, such as non-human primates (NHP), can lead to the spillover or spillback of various infectious and zoonotic agents. Both NHP and humans host a variety of specific herpesviruses. While these viruses typically cause asymptomatic infections in their natural hosts, they can lead to severe disease when transmitted to different species. Cross-infection by herpesviruses can result in severe illness or even death for both NHP and humans. During the first half of 2024, epizootics involving *Callithrix penicillata* were reported in an urban park in Belo Horizonte, MG, Brazil. The epizootic was investigated in collaboration with CETAS/IBAMA and the Zoonoses Secretary of Belo Horizonte. A total of 4 sick animals, presenting seizure and other clinical signs, were forwarded to CETAS-BH IBAMA/IEF and died. At the Laboratory of Zoonosis, carcasses were tested for rabies and were all negative. Four carcass samples were collected (lung, kidney, liver, testicle, and tongue with lesion) and used for nucleic acid extraction using the QIAamp cador Pathogen Mini Kit (Qiagen). Subsequently, qPCR for human alphaherpesviruses (1/2, and 3) was performed using primers targeting the DNA polymerase region. All tissue samples tested negative for HSV-3 while being positive for HSV-1-2. Further analysis for genotyping will be conducted. Monitoring NHP infections provides indicators of transmission dynamics, including maintenance and potential spillover/spillback in disease cycles. It is crucial to continue investigating these events and promoting educational initiatives to foster responsible behavior toward wildlife and prevent viral spillback events and future epizootics.

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PT.406

EVALUATION OF ENTERIC VIRUSES IN BIVALVE MOLLUSCS FROM MANGROVE REGION OF SÃO LUÍS ISLAND, MARANHÃO

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During the period from August 2023 to March 2024, 46 samples of mussels (*Mytella falcata*) and 17 oysters (*Crassostrea rhizophorae*) were acquired monthly from shellfish farming communities that sell these bivalves in the metropolitan region, São Luís Island, Maranhão. The viral analysis were performed according to ISO/TS 15216-1:2017. The internal control used mengovirus (MgV vMC0) was detected in 100% of the samples, with a recovery rate of 37.79% ± 35.70 (mean ± SD). The qPCR and RT-qPCR enterics virus assays showed norovirus genogroup II (GII) detected in 28.6% (18/63) of the samples, norovirus genogroup I (GI) 12.7% (8/63), rotavirus A (RVA, 4.8%, 3/63), hepatitis A virus (HAV, 4.8%, 3/63), and human mastadenovirus 1.6% (1/63). In total, 34.9% (22/63) of the samples tested positive for one or more virus, with 68.1% (15/22) for a single viral agent, 18.1% (4/22) for two viral agents, 9.1% (2/22) for three viral agents, and 4.5% (1/22) for all four viral agents tested. RVA had the highest viral load (3.48 x 10^4 GC/g), followed by norovirus GII (2.93 x 10^4 GC/g), norovirus GI (6.14 x 10^3 GC/g), human mastadenovirus (2.72 x 10^3 GC/g), and HAV (9.74 x 10^2 GC/g). The hepatitis A virus has been characterized as genotype HAV-IA. The study highlights the first description of enteric viruses circulation and dissemination in bivalves from natural banks in a mangrove region of São Luís Island, Maranhão, emphasizing the importance of viral monitoring to aid in the prevention of outbreaks among consumers of these foods and improvements in the quality of the estuarine environment of this region.

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PT.407

WASTEWATER-BASED EPIDEMIOLOGICAL INVESTIGATION OF SEVERE ACUTE RESPIRATORY SYNDROME (SARS-COV-2) IN EFFLUENTS FROM PORTO ALEGRE

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Monitoring environmental wastewater is a widely used method for gathering data on the spread of infectious agents within populations. This approach enables the identification of outbreak trends and the implementation of prevention and control measures. Viral particles of SARS-CoV-2 are excreted in feces. The particles enter the sewage network, and eventually reach wastewater treatment plants (WWTPs), where they can be detected in effluents. This study aims to conduct epidemiological surveillance of SARS-CoV-2 by monitoring and quantifying wastewater samples collected from the Serraria WWTP in Porto Alegre from January to March 2024. Composite wastewater samples collected over a 12-hour period were concentrated through ultracentrifugation at 110,000 x g for 1 hour. They were then subjected to glycine incubation at pH 9.5, followed by centrifugation at 3,000 x g for 20 minutes. A second cycle of ultracentrifugation was performed under the same conditions. RNA extraction was carried out using the Total Viral RNA Maxwell kit in the Maxwell® automatic extractor, followed by RT-qPCR, targeting the N1 coding region. Twenty-three samples were processed, of which 22 were positive with a threshold cycle between 33 and 37 and with a genomic load per liter between 85,097 and 849,099. Since most infected people are neither presenting symptoms nor reporting infection cases, the results highlight the importance of epidemiological surveillance of SARS-COV-2 in effluents, enabling the prediction of outbreak trends and development of prevention and control measures.

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PT.408

ENVIRONMENTAL MONITORING OF NOROVIRUS IN EFFLUENTS IN PORTO ALEGRE

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Norovirus (NoV) is the main etiological agent causing outbreaks of acute gastroenteritis worldwide, mainly due to its great genetic diversity. The Norwalk virus belongs to the *Caliciviridae* family and the *Norovirus* genus, which contains six genogroups (GI to GVI), of which GI, GII and GIV infect humans. Considering that people excrete the virus in their feces and that it can be found in untreated effluents, surveillance of untreated effluent becomes an important epidemiological tool for indirect mass testing. The present study aims to monitor Norovirus GI in samples of urban effluents in Porto Alegre city. Raw sewage samples were collected at the Serraria Wastewater Treatment Plants in Porto Alegre between January and March 2024. The samples were concentrated by ultracentrifugation and RNA extraction was performed using the Maxwell Total Viral Nucleic Acid Purification Kit. The analysis of the presence of NoV was carried out using RT-qPCR targeting the ORF 1. This technique allows the amplification and specific detection of the virus genetic material in environmental samples. Twenty-three samples of raw sewage were processed, and Norovirus RNA was detected in sixteen samples, seven of which were negative. Based on these results, these findings confirm the presence of Norovirus GI in urban effluents in the region, highlighting the importance of continuous surveillance and environmental monitoring as essential tools for early detection and control of outbreaks of acute gastroenteritis.

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PT.409

NEW VIRUSES INFECTING Micractinium conductrix PBI REVEALS THREE NEW CHLOROVIRUS SPECIES

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Pbi-viruses are large dsDNA viruses of the genus *Chlorovirus* associated with the green algae *Micractinium conductrix* Pbi, recently named Betachloroviruses. Pbi-viruses are elements of an intricate environmental network, involving several species, including the virus host and one of their possible symbionts, *Hydra viridis*, a freshwater cnidarian. In contrast to their ecological relevance, there is little knowledge about its diversity, genomics and evolution, since there are only 11 registered isolates to date. In this work 14 new Pbi-viruses were isolated from Nebraska alkaline lakes (USA). The genomes were sequenced using PacBio technology, assembled de novo with the software Canu and used for comparative genomics and phylogenomics analyses. Genome size ranged from 295 to 403 kbp, with 45% GC content (mean). The average nucleotide identity (ANI) ranged from 83 to 99% indicating a high level of genomic conservation among the 25 isolates. Nevertheless, it was possible to distinguish three well-marked groups, forming three new viral species. In phylogenetic analyses, we had some topological variation depending on the hallmark gene, but concatenating six *Nucleocytoviricota* core genes (VLTF3, PolB, A32, SNF2, TFIIB and TopoII) resulted in the groups found in ANI analysis, reinforcing the species demarcation. Our data suggest that Pbi-viruses exhibit the lowest genetic diversity among chloroviruses, which is impressive, considering that with only 25 isolates we defined 3 viral species. Further studies will be able to reveal the biological traits of the Betachloroviruses species.

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PT.410

VIRAL DIVERSITY IN MARINE WATERS OF COSTA VERDE IN THE STATE OF RIO DE JANEIRO, BRAZIL

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The Restinga of Marambaia, belonging to the Costa Verde (Rio de Janeiro), constitutes a strip of sand a few meters above sea level, isolating the waters of Sepetiba Bay from the ocean. This study characterizes the viral community in water samples collected at Sepetiba Bay and the coast region of Marambaia. Three water samples from each area were obtained in January 2023, concentrated and grouped into a pool representing each region. Samples were filtered through a 0.45µm PVDF membrane, treated with DNAse and nucleic acid were extracted by the Biopur Nucleomag Virus Kit (Mobius Life Science, BR). After viral enrichment, the samples were purified by AMpure XP beads (Beckman Coulter, USA), quantified by Qubit 4.0, sequenced using NextSeq 2000 (Illumina, USA), and analyzed with Genome Detective software (version 2.59, BE). Among the 571 genomic sequences obtained, there was a predominance of dsDNA virus (51.66%), followed by ssRNA (27.49%), ssDNA (16.63%) and dsRNA (4.72%). The main families found were *Microviridae (*8.58%), *Autographiviridae* (7.35%), and *Kyanoviridae* (5.1%). Fifty-four percent of viruses had no taxonomic family classification. Among the hosts, bacteriophages were the majority found (40.80%), followed by invertebrates (27.14%). Only 0.70% were found to infect vertebrates. The pool of samples from Sepetiba Bay showed more than twice genomic sequences as that obtained from the coast of Marambaia samples. The results point to the diversity of viruses in marine ecosystems with different anthropogenic impacts, providing clues about the behavior and health of the oceans.

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PT.411

SEQUENCING OF DENV-1 IN WASTEWATER SAMPLES FROM RIO GRANDE DO SUL IN 2024: A KEY TOOL FOR PUBLIC HEALTH SURVEILLANCE

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The dengue epidemic in Brazil has been a significant public health challenge, particularly in recent years and the state of Rio Grande do Sul (RS) has also experienced a surge in dengue cases. Factors such as climate change, increased urbanization, and the mobility of people have contributed to the spread of the Aedes aegypti in regions where it was previously less common. Dengue surveillance in wastewater is becoming increasingly important as a tool for public health monitoring and prevention. Wastewater surveillance involves analyzing sewage systems to detect the presence of the dengue virus genome, providing an early indication of virus circulation in a community. The main goal of this study was to perform a genomic surveillance of DENV serotypes in sewage samples from a wastewater treatment plant (WWTP) located in the metropolitan region of RS. 15 composite samples were collected weekly (January to March/2024) and concentrated by ultracentrifugation. DENV detection and serotyping was performed by RT-PCR and positive samples were selected for sequencing. The library was prepared using the COVIDSeq kit and primers designed for DENV-1/-2. Sequencing was implemented on the Illumina MiSeq platform. All samples were positive for DENV-1. A total of 15 positive samples were sequenced and the DENV genomes were recovered confirming the DENV-1 serotype. Molecular characterization is ongoing. Overall, continuous DENV genome surveillance in wastewater is a valuable tool that can help track changes in the DENV genome, providing insights into viral evolution and the emergence of new strains.

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PT.412

Standardization of Virome Panel for Epidemiological Surveillance of Wastewater

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Wastewater- Based Epidemiology (WBE) is a promising tool that has been successfully used for monitoring and tracking pathogens in wastewater. The wastewater virome, i. e. a set of viruses or viral genetic material excreted in feces by individuals in a population, provides critical information about the circulation of viruses, the introduction of emerging viruses, and how they are transmitted among the community. In view of the consequences caused by the SARS-CoV-2 pandemic, the study of the virome focused on specific viral groups is extremely important to predict possible new viral outbreaks and anticipate decision-making to reduce social and economic impacts. Eleven wastewater sampling points are being established in the city of Foz do Iguaçu, in south Brazil. The genomic library will be constructed with Twist Comprehensive Viral Research Panel, which contains 3.153 viral probes, allowing the identification of up to 15,488 different viral strains. The panel broadly covers all viral families that contain at least one virus known to affect humans, including arboviruses, adenoviruses, coronaviruses, flaviviruses, noroviruses, influenza viruses, among others. The present study is an ongoing work that aims to establish standardized wastewater virome panel protocols so them can be used in other cities as an epidemiological monitoring tool.

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PT.413

WASTEWATER-BASED EPIDEMIOLOGICAL INVESTIGATION OF HUMAN HEPATITIS E VIRUS IN EFFLUENTS FROM PORTO ALEGRE

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Wastewater- based epidemiological investigation is essential to identify circulating pathogens in a specific population. The genetic material of human hepatitis E virus (Paslahepevirus balayani, HEV) can be found in the feces, being directed to sewage systems and, consequently, to wastewater treatment plants (WWTP). Sewage-based genomic surveillance enables confirmation of the presence and virus circulation in the population. This research performed epidemiological surveillance of HEV by analyzing wastewater samples taken from the Serraria WWTP in Porto Alegre between January and March 2024. Each composite sewage sample was collected over a period of 12 hours at Serraria WWTP, concentrated by ultracentrifugation at 110,000 x g for 1 hour. Next, a glycine incubation at pH 9.5 was performed, followed by a centrifugation at 3,000 x g for 20 minutes. A second ultracentrifugation cycle was performed with the previous setting to reconcentrate the sample. RNA extraction was conducted using the Total Viral RNA kit (Maxwell) in the Maxwell automatic extractor, followed by RT-PCR targeting the genomic region ORF3 of each virus. A total of 23 samples were processed, all of which tested negative for HEV. This method allows a rapid assessment of virus circulation, enabling prediction of new outbreaks and fast execution of preventive and controlling measures.

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PT.414

THE YELLOW FEVER VIRUS-VECTOR-HOST NETWORK IN THE METROPOLITAN REGION OF MINAS GERAIS/BRAZIL, 2023: EVIDENCE OF PERSISTENCE AND RECENT CIRCULATION OF YELLOW FEVER VIRUS

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From late 2016-18, Brazil experienced a yellow fever outbreak, notably in Minas Gerais state (MG). Since then, yellow fever virus (YFV) has been detected in non-human primates (NHP) in the state, including the Metropolitan region of Belo Horizonte (BH). We performed NHP and mosquito surveillance in six urban parks of BH. A total of 6,487 mosquitoes have been collected and 70% of individuals have been identified to genus level. Sera from live NHP captured or rescued in 2023 (n=63) tested negative for YFV, ZIKV, and CHIKV by RT-qPCR, indicating that none of the animals were actively infected. So far, 36 NHP sera have been screened via PRNT against YFV showing 20 positives (PRNT 50% at 1:20 dilution). These samples were from *Callithrix penicillata* - 11 adults, 6 young, and 3 infants - sampled in BH (n=19), and southwest MG (n=1). Four out of 20 NHP positive by PRNT had IgM anti-YFV (3 adults, 1 young). Although it is known that IgM can persist after orthoflavirus infection, these NHP were sampled more than 4 years since the end of MG's yellow fever outbreaks in 2018. The detection of neutralizing and IgM against YFV in NHP reinforces the circulation of YFV in urban areas of MG. Urban parks in BH present the host-vector network, which could contribute to the maintenance and transmission of YFV. Continued surveillance and studies are vital to public health in Southern Brazil due to ongoing YFV circulation.

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PT.415

COMBINING CERVICAL SAMPLING AND WASTEWATER-BASED EPIDEMIOLOGY FOR COMPREHENSIVE ANALYSIS OF HUMAN PAPILLOMAVIRUS INFECTIONS.

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Introduction: Human papillomavirus (HPV) is responsible for a variety of clinical conditions, ranging from common warts to cervical cancer (CC). HPV genotypes are classified as high-risk (HR) and low-risk (LR) based on their association with cancer development. HR genotypes, such as 16 and 18, are known to cause CC, while LR genotypes, like 6 and 11, lead to anogenital warts without a significant cancer risk. HPV has been detected in wastewater samples from sanitation facilities in several countries to provide a snapshot of the circulating genotypes within those populations. This study aimed to determine the circulating HPV genotypes in both the female population (via cervix swabs) and wastewater from the city of Salto, Uruguay, to evaluate the efficacy of the wastewater-based epidemiology approach for this virus. Material and Methods: Samples underwent PCR, and genotypes were identified through DNA sequencing. Results: In clinical samples, HPV 6 and 16 were the most prevalent, which was consistent with the results from wastewater samples where these genotypes, along with 31, 66, and 88, were frequently detected. Both clinical and environmental samples showed a great diversity of genotypes. Conclusion: The wastewater-based epidemiology approach effectively represents the circulating HPV genotypes in the population, proving to be an important tool for molecular epidemiologic studies, especially in developing countries like Uruguay, where information on genotype circulation is limited.

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PT.417

FIRST DETECTION OF ALPHACORONAVIRUS IN BATS IN MATO GROSSO, BRAZIL

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Small wild mammals are potential hosts and reservoirs of viruses that can cause zoonotic diseases, among them, chiropterans are reservoirs of viral pathogens, such as viruses from the Coronaviridae family. Coronaviruses are capable of infecting a variety of hosts and causing large epidemics. In Brazil, the state of Mato Grosso encompasses three biomes rich in biodiversity, the Pantanal, Cerrado and the Amazon. The order Chiroptera represents 40% of the mammal species in the Pantanal, among the 178 species recorded in Brazil, 118 occur in the Cerrado biome. Considering the diversity of bats in the state and given the recent SARS-COV-2 pandemic, the objective was to detect the presence of coronavirus in bats from different municipalities in the state of Mato Grosso. 345 samples were collected in 2021, in the Pantanal and Cerrado biomes. Swab samples were analyzed by nested-PCR for molecular detection of Orthocoronaviridae, according to the PREVIR/MCTI protocol, considering the RNA-dependent RNA-polymerase (RdRp) gene. Around 20% of the samples were suspicious, and ten samples were sequenced using the Sanger method, being confirmed as belonging to the Alphacoronavirus group. The bat species positive for Alphacoronavirus were: *Carollia perspicillata* (4); *Desmodus rotundus* (1); *Molossus rufus* (1); *Myotis cf. nigricans* (1); *Phyllostomus discolor* (1); *Phyllostomus discolor cf.* (1); and *Phyllostomus hastatus* (1). This is the first description of Alphacoronavirus in bats from the Pantanal of Mato Grosso. Bats are natural reservoirs of coronaviruses, constant monitoring of these animals is important to understand the epidemiology and the emergence of new emerging viruses.

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PT.418

EPIDEMIOLOGICAL SURVEILLANCE OF VIRAL LOAD IN FLOODWATERS OF PORTO ALEGRE AFTER HISTORICAL FLOODING

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The state of Rio Grande do Sul experienced a historic flood in May 2024, in which rainwater ended up mixing with the city's sewage system and exposing the population to contaminated water. Here, genomic epidemiological surveillance is being used to detect pathogens in these waters, which is essential for the health system to anticipate infection outbreaks and mobilize action plans to contain health threats. Water samples are being periodically collected from water bodies of flooded regions in the Metropolitan Area of Porto Alegre, from May 2024 to May 2025. Sample processing involves floodwater concentration through two cycles of ultracentrifugation (110.000xg). Viral RNA is extracted by Maxwell® Viral Total Nucleic Acid Purification Kit. Specific primers and probes for *Norovirus GI* (Noro 1) and *Norovirus GII* (Noro 2) NSP region and for *Betacoronavirus pandemicum* (SARS-CoV-2) N1 region are used to detect sample genomic viral load through RT-qPCR. Thus far, only the first three collect time points (between May and June) were analyzed. In the first group, one out of 17 samples was positive for Noro 1, one for Noro 2, and two for SARS-CoV-2. In the second group (n=20), two samples were positives for Noro 2 and two for SARS-CoV-2. In the third group (n=20), four samples were positive for SARS-CoV-2, whereas Noro 1 and 2 genomes weren't detected. Samples are still being processed and analyzed. The results highlight the relevance of pathogen monitoring as a tool to provide informative data about virus circulation in the population.

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PT.419

IDENTIFICATION AND QUANTIFICATION OF DENGUE AND CHIKUNGUNYA VIRUS IN WASTEWATER OF SÃO JOSÉ DO RIO PRETO

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Arboviruses are viruses transmitted by arthropod vectors and their infections are highly prevalent in São José do Rio Preto, being chikungunya fever and dengue the most frequent infections. There was an increase in these diseases in the city in 2023 and 2024, and, therefore, there is a need to complement clinical surveillance in the city, to track the diseases and prevent possible outbreaks. Thus, wastewater-based surveillance is an alternative, as it covers the entire city and, according to studies, is capable of detecting viruses even before symptoms develop, acting as an early warning tool. The project aims to identify and quantify the viruses that cause Dengue and Chikungunya in sewage samples from the city of São José do Rio Preto. So, samples were collected weekly from the first week of January 2023 until July 2024 and viral concentration in these samples was carried out, followed by RNA extraction and subsequent real-time PCR for the two viruses, using an exogenous control. To date, PCR for CHIKV and DENV was performed in 26 samples from march 2023 to 2023 to may 2024, and 15 samples were positive for CHIKV for CHIKV and none for DENV, representing that this surveillance can act as an early warning tool for outbreaks, complementing the clinical surveillance.

Financial Support: CAPES

PT.420

HUMAN MASTADENOVIRUS AND JC POLYOMAVIRUS SURVEILLANCE IN AQUATIC ECOSYSTEMS IN NITERÓI, RIO DE JANEIRO

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This study aimed to evaluate virological indicators for assessing the quality of environmental waters in a municipality with over 500,000 inhabitants, in the metropolitan region of Rio de Janeiro. Twenty-four raw sewage samples from two Wastewater Treatment Plants (WWTP) and ninety-seven samples from drainage channels in urban areas of Icaraí, Jacaré, Jurujuba, and São Francisco watersheds were collected between September 2020 and May 2021. Samples from WWTP were concentrated by ultracentrifugation and those from drainage channels by negatively charged membrane filtration followed by ultrafiltration. Nucleic acids were extracted using Quick-DNA/RNA MagBeads (ZYMO, USA), and qPCR with TaqMan™ system was applied for human mastadenovirus (HAdV) and JC polyomavirus (JCPyV) quantification. HAdV was detected in 79,17% (19/24) of the WWTP samples, with concentration ranging from 1.07×10^4 to 6.35×10^8 genomic copies (GC)/L, and in 35% (34/97) of drainage channels samples with concentrations ranging from 2.13×10^5 to 6.35×10^8 GC/L. JCPyV was detected in 70,83% (17/24) of the sewage samples (1.32×10^3 to 1.14×10^6 GC/L, and in 68% (66/97) of drainage channels samples (1.11×10^2 to 9.76×10^5 GC/L). HAdV positive samples were sequenced using the Sanger method. Nucleotide sequences showed 98% identity with *Human mastadenovirus D*. JCPyV are being processed for molecular characterization. The high percentage of detection of both viruses researched, as well as the similar concentrations in sewage and drainage channels samples, reveal the anthropogenic impact of these densely populated regions.

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PT.421

DISINFECTION AND NUTRITIONAL EVALUATION OF *MAGALLANA GIGAS* OYSTERS TREATED WITH NANOENCAPSULATED OREGANO OIL

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The Santa Catarina state, particularly Florianópolis, is the largest Brazilian market for Magallana gigas oysters, generating millions of revenue annually. Although a gastronomic delicacy, these oysters farmed near urban areas prone to contamination, especially by enteric pathogens like human norovirus (HuNoV). This virus, transmitted via the fecal-oral route, is found in the excreta of infected individuals, making sewage a potential environmental contamination source. Essential oils, such as from Origanum vulgare (oregano), have well-documented antimicrobial properties, but their volatility and oxidation sensitivity limit their use. Nanoencapsulation offers a possible solution, providing greater stability and increased bioavailability of the active compounds. The aim of this study is to evaluate the efficiency of nanoencapsulated oregano essential oil in disinfecting Magallana gigas contaminated with HuNoV. Developing a stable nanoencapsulated formulation could enhance oyster depuration systems, increasing the effectiveness of disinfection with a natural compound. By employing the ionotropic gelation technique, a formulation of nanoparticles incorporated with oregano essential oil was successfully developed. The formulation was characterized, and its stability was validated for up to six months using LUMiSizer® and Zetasizer (Malvern Panalytical) equipment. The formulation's efficacy in viral inactivation was evaluated in vitro through cell culture with murine norovirus type 1, a primary surrogate for human norovirus (HuNoV). The non-cytotoxic virucidal concentration was tested in aquariums to evaluate the toxicity for M. gigas oysters. With an efficient non-toxic concentration, the next steps will determine the efficiency of the supplementation of depuration systems with the nanoencapsulated essential oil.

Financial support: CAPES

PT.422

Description of two novel *Caudoviricetes* isolated from Brazilian soil that infect *Ralstonia solanacearum* and *Ralstonia pseudosolanacearum*

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This study reports the isolation and characterization of two new phages from Brazilian soil that infect *Ralstonia solanacearum* and *Ralstonia pseudosolanacearum* named RS-Phage-AB1 and RS-Phage-CA1. The phage genome was sequenced and described, revealing two novel *Ralstonia* phages. RS-Phage-AB1 is a novel virus species from the *Peduoviridae* family. While RS-Phage-CA1 was not classified as part of any described family. Thus, we propose a new viral family, “Anamaviridae”, with two subfamilies, “Kantovirinae” and “Mascarenevirinae”, the last one harboring RS-Phage-CA1. We propose the species names “Cocadavirus alagoinhas” and “Acarajevirus bahia” for RS-Phage-CA1 and RS-Phage-AB1, respectively. These findings increase the understanding of phage diversity infecting plant pathogens**.**

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PT.423

Viral infection can influence microbial niche occupation on plants

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A tubulavirus infecting an isolate of *Ralstonia solanacearum* was described and shown able to prevent this bacterium from developing wilt in plants, i.e., this phage becomes virulent *Ralstonia* in a hypovirulent bacterium. The phage was named Ralstonia Solanacearum Inovirus Brasil 1 (RSIB1). This work evaluated how viral infection can influence competition between infected and uninfected *Ralstonia solanacearum* isolates. We tested if the virus-infected hypovirulent isolate can colonize and exclude, by competition, avoiding the establishment of virulent *Ralstonia*. To do this, we inoculated *R. solanacearum* (UB) and *R. pseudosolanacearum* (G7) infected by RSIB1 on tomato plants. Subsequently, after four days, the same plants were infected with an aggressive isolate of (GMI). We observed that plants inoculated with UB and G7 showed small necrosis only at the point of leaf infiltration, which may indicate activation of bacterial recognition by the plant. In contrast, plants infiltrated only with GMI exhibited typical wilting symptoms.Furthermore, we observed that plants inoculated with virus-infected bacteria before GMI did not show wilting. When the virulent isolate (without the virus) is infiltrated first, and the one infected with the virus is placed four days later, we observe the development of the disease. These results support the hypothesis that niche occupancy is the primary competition mechanism between virus-infected and uninfected isolates.

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PT.424

GH18 CHITINASES HIGHLIGHTS DIFFERENCES BETWEEN CHLOROVIRUS AND THEIR SPECIFICITY TO THEIR HOSTS

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Chloroviruses are nucleocytoplasmic large DNA viruses classified in the family *Phycodnaviridae* that infect unicellular chlorella algae. They have extensive genomes that code for several enzymes, including many involved in carbohydrate metabolism. Chloroviruses are divided into three viral clades that infect different host species, exhibiting a very narrow host range. However, molecular mechanisms involved in this host-specificity are currently unknown. In this study, we characterized chitinases from the GH18 glycosyl hydrolase family encoded by chloroviruses. These enzymes can have one or two GH18 domains and a carbohydrate-binding type II domain (CBM2). Two structural differences of aspartate amino acid on the catalytic site resulted in different affinity scores to chitin tretramer, a polymer present in the chlorella cell wall, by docking analyzes with DOCKGOLD. We demonstrated by fluorescence microscopy that cell wall degrading enzymes appear to be involved in the infection mechanism, being important in the initial phase for PBCV-1, a chlorovirus that infects *Chlorella variabilis*, but not for CVM-1, which infects *Micractinium conductrix*. The increased amount of viral transcripts on qRT-PCR showed that the expression of chitinases occurs mainly in the final phase of the CVM-1 viral cycle (6 hpi) These data show that these viruses have phenotypical differences that affect interactions with the host cell. In vitro characterization of viral chitinases expressed during the virus cycle can provide valuable information about their role in viral biology. These analyses will be useful for the characterization of the proteins and the pathways they are involved.

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PT.425

VIRAL GENETIC DIVERSITY IN GROUND AND SURFACE WATER AT A CLOSED DUMPSITE

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Leachate is a subproduct of the decomposition of municipal solid waste (MSW) containing a diversity of microorganisms including viruses. This study aimed to describe the viral diversity in a non-operational solid waste dumpsite located in the Metropolitan Region of Rio de Janeiro, using *shotgun* metagenomic analysis. Between 2021 and 2023, 106 water samples (2 liters) from five monitoring wells installed to access groundwater and from three leachate lagoons were collected. Samples were filtered through 0.45µm PVDF (polyvinylidene difluoride) membrane and extracted using the Biopur Kit Nucleomag Virus (Mobius Life Science, BR). Viral enrichment, purification by AMpure XP beads (Beckman Coulter, US) and quantification by Qubit 4.0 were performed. The sequences were forwarded by NGS in NextSeq 2000 (Illumina, USA) and analysed by Genome Detective software (2.77 version, BE). The analysis using International Committee on Taxonomy of Viruses website and NCBI's BLAST identified 256 viral strains, covering 25 families. *Microviridae* family was the most frequently detected. DNAss viruses were identified more frequently in both ground (44.3%) and surface water (41.6%). The main hosts in both matrices were bacteria (45.1%; 41.7%), followed by invertebrates (29.8%; 34.8%) and vertebrates (13.7%; 12.1%). Three human viral strains were identified: *Cressdnaviricota, Gemykibivirus sewopo1 and Parvovirus NIH-CQV*. Our data contribute to the knowledge about viral diversity in areas of inadequate MSW disposal, especially in leachate accumulation ponds and underground aquifers.

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PT.426

PMMoV AND CRASSPHAGE AS INDICATORS OF HUMAN FECAL CONTAMINATION IN SURFACE WATERS.

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Microbiological Source Tracking (MST) approaches suggest the use of Pepper Mild Mottle Virus (PMMoV) and crAssphage as a potential indicator of human fecal contamination in aquatic matrices. This research aimed to quantify these bacteriophages to assess the anthropogenic impact on three basins in the Niterói municipality, Rio de Janeiro. Superficial water samples (2 liters) as river, stream and seawaters were collected from 2020 to 2021 in areas with different population densities. The samples were concentrated using the electronegatively charged filtering membrane method followed by ultrafiltration. Nucleic acid extraction was performed using the automated QiAcube system (QIAGEN, CA, USA) and qPCR was performed based on the TaqMan protocol. A total of 56.79% (46/81) of these samples were positive for PMMoV being: 10% (2/20) in river samples (mean quantification of 4.55 $\log_{10}$ gc l^{-1}); 79.59% (39/49) in stream water (mean of 5.21 $\log_{10}$ gc l^{-1}) and 41.67% (5/12) in seawater samples (mean of 5.22 $\log_{10}$ gc l^{-1}). For crAssphage the results revealed 60.49% (49/81) of positivity: 40% (8/20) in river samples (mean 3.87 $\log_{10}$ gc l^{-1}); 77.55% (38/49) in stream water (mean of 6.90 $\log_{10}$ gc l^{-1}) and 25% (3/12) in seawater samples (mean of 3.92 $\log_{10}$ gc l^{-1}). This result demonstrated the increase in the detection rate of both bacteriophages in the streams located in the most urbanized regions revealing the irregular discharge of domestic sewage in these areas, despite having sanitation coverage.

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PT.427

VIRAL LOAD ANALYSIS AND MOLECULAR CHARACTERIZATION OF *ENTEROVIRUS* IN WASTEWATER FROM SÃO JOSÉ DO RIO PRETO/SP REVEALS HFMD-ASSOCIATED SEROTYPES

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Enteroviruses, *Picornaviridae*, a potential cause of infection via the fecal-oral route, are remarkably persistent in water and difficult to inactivate. Despite being linked to various diseases, they often cause asymptomatic infections, making wastewater surveillance a sensitive method for detecting these viruses that circulate silently in communities. Therefore, this work aims to identify serotypes associated with diseases and determine the viral load of positive enterovirus samples. Samples (n=156) were collected weekly from three sewage processing points with SeMAE's (Serviço Autônomo Municipal de Água e Esgoto) help and previously analyzed by qPCR, detecting enterovirus in 54.5%. Using RD cell cultures and $TCID_{50}$ titration, 85 positive samples were quantified by qPCR in triplicate to estimate their viral load. Samples collected before treatment exhibited a significantly higher (2-way ANOVA, p-value= <0.05) log_{10} peak viral load (6.28 CG/mL) compared to those collected after treatment (4.45 CG/mL), while samples collected during treatment averaged 5.32 CG/mL. A seasonal pattern showed higher (p-value= <0.05) average quantification during spring (5.5 CG/mL) compared to other periods, ranging from 4.79 CG/mL in summer to 4.00 CG/mL in winter. Species were characterized using semi-nested PCR and gel electrophoresis. Amplicons were sequenced using Sanger methodology and sequenced positive samples were aligned with Coxsackievirus A5 and A16 sequences, both associated with hand-foot-and-mouth disease. The study seeks to provide valuable information on the circulation and prevalence of enteroviruses in the city, helping fill surveillance gaps and serve as an early warning of viral spread, particularly in vulnerable populations with limited sanitation access.

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PT.428

Exploring viral diversity in *Phakopsora pachyrhizi:* implications for asian soybean rust management.

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Asian soybean rust, caused by the pathogen *Phakopsora pachyrhizi*, has become one of the most significant challenges to food security due to its economic impact. This study analyzes the viral diversity associated with *Glycine max* using 47 short-read DNA libraries, 238 RNA libraries, and three genomes of *P. pachyrhizi* (K8108, MT2006, and UFV02) available at the National Center for Biotechnology Information. We aim to identify and classify possible giant virus sequences within the fungal genomes and DNA and RNA viruses in the different libraries. RNA libraries (159) were processed through trimming and quality filtering with TRIMMOMATIC. The filtered reads were assembled with SPAdes, and potential open reading frames (ORFs) were predicted using EMBOSS getORF. Redundant sequences were removed with CD-HIT, and potential viral sequences were identified with HMMsearch, resulting in 62 libraries containing 1,384 viral transcripts. These transcripts represent seven viral groups: *Secoviridae* (0.1%), *Dicistroviridae* (0.9%), *Chrysoviridae* (0.4%), *Partitiviridae* (4.1%), *Closteroviridae* (5.1%), *Narnaviridae* (24.4%), *Totiviridae* (56.0%) and unclassified (4.6%). Fifty unique ORFs of RNA-dependent RNA polymerase (RdRp) were obtained and filtered with parameters above 90%. Subsequently, transcripts similar to viral sequences were investigated for conserved domains using PHMMER and InterProScan. Mycoviruses, known for their impact on fungal biology, could be candidates for biocontrol strategies. This pioneering study in exploring the viral diversity of *P. pachyrhizi* will contribute to future research and the development of new agricultural management strategies.

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PT.429

A BASIC PROLINE-RICH PROTEIN 1 LIKE GENE IDENTIFIED IN COTTON AT THE COTTON BLUE VIRAL DISEASE RESISTANCE LOCUS IS MODULATED DURING VIRAL INFECTION

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Cotton (*Gossypium sp*) is the world's most important fiber crop, significantly affected by Cotton Blue Disease (CBD), which is caused by the *cotton leafroll dwarf virus* (CLRDV) and transmitted by *Aphis gossypii*. CLRDV, a non-enveloped virus from the *Solemoviridae* family and *Polerovirus* genus, has a positive single-stranded RNA genome with seven open reading frames (ORFs). This study identified two ORFs within the CBD resistance locus in *Gossypium hirsutum*, focusing on the characterization of one ORF, named CBD1. The CBD1 sequence and structural organization are almost identical to the *Arabidopsis thaliana*'s basic proline-rich protein 1 (BPP1). This structural analysis was carried out using Alphafold coordinates in DALI bioinformatics tool. CBD1 amino acid sequence revealed a peculiar composition rich in proline, serine, and threonine content, a fact that may explain its structural organization, showing an intrinsically disordered folding pattern. This set of constitutive characteristics suggests the protein's ability to form liquid-liquid separation, as confirmed by bioinformatic analysis using LLPSDB v2.0, which indicates CBD1's high potential for such separation. Quantitative real-time PCR (qRT-PCR) was used to assess GhCBD1 expression in CBD-resistant (Delta Opal and Cedro), moderately resistant (IMACD), and susceptible (FM966 and CNPA ITA90) cotton varieties. The results showed varying levels of CBD1 expression, with lower levels in susceptible cultivars in the first hours after CLRDV infection. In resistant plants, CBD1 expression decreased following infection but showed slight fluctuations afterward. These findings indicate a potential role for CBD1 in CLRDV resistance, warranting further investigation into its function.

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PT.430

EVALUATION OF OVEREXPRESSION OF ATE FOR INHIBITION OF POTATO X VIRUS (PVX) IN *NICOTHIANA BENTHAMIANA* AND ARABIDOPSIS PLANTS

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This study investigates the role of the ATE gene, identified in *Gossypium hirsutum* (cotton) and *Arabidopsis thaliana*, in inhibiting the replication and spread of potato virus X (PVX) in plants. The ATE gene encodes an arginyl-tRNA transferase, an enzyme that adds arginine to proteins with specific N-terminal amino acids, leading to their ubiquitination and subsequent degradation via the proteasome. Previous work showed that overexpression of ATE in *A. thaliana* inhibits the spread and synthesis of CLRDV (*cotton leafroll dwarf virus*), suggesting a possible broader antiviral role. In this study, *Nicotiana benthamiana* and *A. thaliana* plants were used to evaluate ATE's antiviral activity against PVX. *N. benthamiana* plants were co-agroinfiltrated with PVX-GFP and either the cotton ATE gene or an empty vector. Fluorescence analysis under UV light at 6 days post-infiltration (dpi) showed a significant reduction in the fluorescence area in plants expressing ATE, indicating reduced viral replication. For *A. thaliana*, both wild-type and 35S:ATE transgenic plants were agroinfiltrated with PVX-GFP. Analysis at 7 to 14 dpi revealed a significant decrease in PVX movement protein transcripts in systemic leaves of 35S:ATE plants, suggesting an inhibitory effect on viral spread. Ongoing quantification of GFP fluorescence aims to confirm whether there is a reduction in viral replication in the systemic and inoculated leaves of these transgenic plants. This study suggests that the ATE gene could have potential as a broad-spectrum antiviral agent in plants.

PT.431

Genetic variability and population structure of Macroptilium yellow vein virus (*Begomovirus macroptilivenae*) in *Macroptilium* spp. in the state of Alagoas

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Begomovirus (family *Geminiviridae*) is an important genus of viruses whose members infect cultivated plants, causing great economic losses in tropical and subtropical regions around the world. These viruses are also associated with a wide range of non-cultivated (wild/weed) plants, which may serve as alternative hosts or inoculum sources for epidemics in commercial crops. At least nine begomoviruses have been described in *Macroptilium* spp. (Fabaceae), highlighting this plant as a natural host of a diverse range of begomoviruses. Aiming to evaluate the genetic variability and population structure of Macroptilium yellow vein virus (*Begomovirus macroptilivenae*, MacYVV) in *Macroptilium* sp., leaf samples showing symptoms indicative of begomovirus infection were collected in different municipalities in the state of Alagoas in 2019 and 2020. Total DNA was extracted, amplified by rolling circle amplification, digested with endonucleases, cloned and Sanger-sequenced. Pairwise DNA-A sequence comparisons confirmed that all clones corresponded to MacYVV isolates. Phylogenetic analysis showed the division of MacYVV isolates into five subpopulations, with a low degree of genetic variability. Although DAPC and STRUCTURE analysis suggested the formation of a distinct number of subpopulations (6 and 4, respectively), in general they corroborated the results of the phylogenetic analysis. Recombination and crosslinking analyzes showed evidence of intraspecific recombination events among MacYVV isolates. Strong negative selection pressure acting on the MacYVV *CP* and *Rep* genes was also detected. Together, these results suggest low genetic variability in MacYVV subpopulations, possibly due to genetic bottlenecks and geographic structuring.

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PT.432

First report of tomato interveinal chlorosis virus (*Begomovirus solanumintervenae*) infecting *Rhynchosia minima* plants in Brazil

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Begomoviruses (family *Geminiviridae*) represent a serious problem for several crops of economic importance due to the diseases they cause. This genus is also commonly associated with a wide range of non-cultivated plants that can serve as a source of inoculum for epidemics in agricultural crops. The objective of this study was to identify a possible begomovirus infecting *Rhynchosia minima* plants collected in 2020 in the state of Alagoas, northeastern Brazil. Total DNA was extracted from *R. minima* plants showing typical symptoms of begomovirus infection (mosaic and interveinal chlorosis). Aliquots of the DNA were used to perform PCR-based detection at the genus level, using the universal primers PAL1v1978 and PAR1c496. PCR-positive samples were used to obtain complete viral genomes through rolling circle amplification, which were cloned into plasmid vectors and Sanger-sequenced. Four clones were obtained, and pairwise comparisons of DNA-A sequences indicated that the isolates had 94% nucleotide identity with isolates of the begomovirus tomato interveinal chlorosis virus (*Begomovirus solanumintervenae*, ToICV. Phylogenetic analysis showed that the isolates formed a monophyletic clade with ToICV, corroborating the results of pairwise comparisons. Evidence of interspecific recombination was observed among the new isolates and begomoviruses that infect *Macroptilium* spp., tomato (*Solanum lycopersicum*) and soybean (*Glycine max*). ToICV has already been described infecting tomato plants and *Macroptilium* sp. This is the first report of *R. minima* infection by a begomovirus in Brazil, although it has already been described as a host for several begomoviruses in North and Central America.

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PT.433

VIROME ANALYSIS REVEALS A NOVEL PICORNA-LIKE VIRUS IN THE GUT OF THE SUGARCANE WEEVIL (*Sphenophorus levis*)

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Picornaviruses are positive-sense single-stranded RNA viruses and have been reported in arthropods. This study aims to characterize a potential novel picorna-like virus, provisionally named *Sphenophorus levis associated virus 1* (SLAV-1), identified in the gut virome of the sugarcane weevil (*Sphenohorus levis*). Ten adult weevils were collected from a commercial sugarcane farm in São Joaquim da Barra, São Paulo state, in March 2023. After 72 hours of induced fasting, gut tissues were dissected, pooled and subjected to nucleic acid extraction using the Total RNA Purification Kit (Norgen). The library was constructed using the Zymo-Seq RiboFree Total RNA Library Kit (Zymo Research) and sequenced on Illumina NextSeq 500. Raw data was preprocessed, assembled with SPAdes and taxonomically classified using Read Annotation Tool. This was followed by manual curation that included the BLAST analysis, identification of open read frame (ORF) and conserved domains. Phylogenetic analyses were conducted with IQ-TREE. The viral genome retrieved has 12.414 bp with an average coverage of 408x and contains a single ORF (4,071 aa) with conserved RNA helicase, RdRp, protease, and picornavirus capsid protein domain-like. BLASTx analysis showed 32.62% identity (Query Cover: 68% and E.value: 0.0) with *Hypera postica associated virus 1* (MW676138), described in Alfalfa Weevil (*Hypera postica*). Phylogenetic analysis indicated that SLAV-1 formed an unclassified group closely related to members of the family *Solinviviridae*. These findings may suggest the presence of a novel virus infecting *S. levis* and future studies are essential to determine the natural incidence and pathogenicity of SLAV-1 in these arthropods.

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PT.434

Selection of common bean (*Phaseolus vulgaris*) lines with natural resistance to *Cowpea mild mottle virus* (CPMMV)

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Cowpea mild mottle virus (CPMMV) has caused significant yield losses in common beans. Variability in resistance to CPMMV has been identified in the IPR Celeiro cultivar. Celeiro lines derived from individual plants (IPL) mechanically inoculated with CPMMV and showing no virus symptoms were repeatedly selected after four generations. Our objective was to investigate the plant-virus-insect vector (*Bemisia tabaci*) interactions by performing mechanical and biological inoculations of four Brazilian CPMMV isolates (collected at Santo Antônio-GO, Luziânia-GO, Cambará-PR, and Sorriso-MT). The BRS FC401 RMD cultivar, susceptible to CPMMV, served both as control and CPPMV inoculum source. A preliminary mechanical inoculation test was conducted with one of the IPL (n=10), which showed no virus symptoms after 20 days. Next, we expanded the number of lines to five for the biological inoculation of the virus isolates. CPMMV-free adult insects were submitted to 2h virus acquisition and then transferred to caged IPL test plants for a 2h inoculation period (n=10). Plants were assessed 20 days post-inoculation for incidence and severity of symptoms, using a 1-4 scoring scale. The Celeiro lines, biologically inoculated with different virus isolates, did not exhibit any viral symptoms. In contrast, the controls showed a 100% incidence rate and an average severity of 3 (mild-strong symptoms). Viral load on the CPMMV-inoculated plants was quantified by qRT-PCR, showing that CPMMV did not replicate in the resistant lines. The four CPMMV isolates were sequenced to check their similarity. RNA-seq data analysis is in progress.

Financial support: Embrapa and CNPq.

PT.435

Activation of a critical component of the Ribosome-associated protein quality control by viral infection in *Arabidopsis thaliana*

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The Ribosome-associated Quality Control (RQC) pathway is a mechanism that rescues stalled ribosomes that failed to reach a stop codon during translation. Stalling can be caused by truncated mRNA that lacks stop codons or stable secondary structures in the mRNA sequence. A viral infection can induce both factors; the truncated mRNA species are by-products of mRNA degradation by the siRNA response, and the secondary structures are commonly present in viral transcripts. When stalling occurs, non-canonical termination factors dissociate the ribosomal subunits. However, this process leaves the 60S bound to an uncompleted protein, where RQC intervenes. Among the RQC components, LTN1, an E3 ligase, binds to the obstructed 60S subunit and targets the incomplete peptide for proteasomal degradation. Over the past fifteen years, RQC has been extensively studied in mammals and yeast. Our research group has identified orthologs of RQC components throughout the Viridiplantae clade, although experimental evidence of this pathway in plants is still lacking. This study aims to characterize AtLTN1's function in *Arabidopsis thaliana* under various abiotic and biotic stress conditions. Our results indicate slight differences in growth between wild-type and AtAtLTN1 knockout mutants, with the latter exhibiting a 30% reduction in biomass. Additionally, infiltration with a replicating vector containing the Potato virus X (PVX) increased AtLTN1 expression in systemic leaves by two-fold. These findings suggest that AtLTN1 is implicated in plant development and is responsive to viral infections.

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PT.436

Analysis of the apoptotic effects of ankyrin and protein tyrosine phosphatases from Cotesia flavipes bracovirus in insect cells.

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Bracoviruses are a genus of segmented dsDNA viruses that are symbiotically associated with the braconidae family of parasitoid wasps. The Cotesia flavipes wasp is widely used as a biological control agent of the Diatraea saccharalis larvae in sugar crops. During oviposition there is a release of a large amount of viral particles into the caterpillar body that will infect the host hemocytes. This leads to an induced immunosuppression in the host which increases fitness of the wasp`s progenies. In this work, we constructed plasmids containing several viral genes derived from the CfBV genome, protein tyrosine phosphatase (ptp) and ankyrin (ank), for transient expression in insect cells. The plasmids were transfected into Trichoplusia ni cells (Tn5B) and we checked at 48 hours post transfection for morphological changes, viability and effector caspase activity. Cells undergoing apoptosis were observed in transfections with ank-8, ptp-a, ptp-o, ptp-omega, ptp-q, ptp-w, ptp-n and ptp-t genes. Effector caspase activities were varied but positive compared to negative controls. These results demonstrate that the isolated ANK and PTP proteins are sufficient to induce apoptosis in vitro and affect cellular viability . The functional redundancy within this set of genes correlates well with the presence of conserved domains and topologies, but in contrast the low overall identities may point to distinct mechanisms of apoptosis induction by each protein which requires in-depth assessments.

Financial Support: CNPq, FAPDF, CAPES.

PT.437

Characterization of the chitinase/cathepsin locus in an alphabaculovirus that does not liquefy the integument of *Dione juno* larvae, a major passion fruit pest in Brazil

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Dione juno is a major pest in passion fruit crops, causing significant damage due to its gregarious habits and aggressive attacks. An alphabaculovirus infecting *D. juno* larvae, named *Dione juno* nucleopolyhedrovirus isolate Araguari (DijuNPV-A), was previously described as causing intense liquefaction in the larvae. This process is linked to two viral enzymes: cathepsin, which degrades proteins, and chitinase, which breaks down chitin, both thought to enhance virus spread. In this study, we aimed to characterize a novel virus, DijuNPV isolate Pató (P), which does not cause integument liquefaction in infected *D. juno* larvae. Symptomatic larvae were bled, and their hemolymph was observed under optical microscopy. The occlusion body (OB) purification method was used on infected larvae, and the OBs were observed under scanning and transmission electron microscopy (EM). Viral DNA was extracted from the OB-derived virus particles, subjected to PCR amplification of the *chitinase A* (*chiA*) and viral *cathepsin* (v-*cath*) gene regions, and sequenced. The sequencing results were aligned with DijuNPV-A using MAUVE alignment. OB-like structures and cells with hypertrophic intact nuclei and intranuclear OBs were found in the larvae's hemolymph. Alignment with DijuNPV-A revealed a 76 bp deletion in the v-*cath* gene of DijuNPV-P, likely causing cathepsin malfunction. These findings suggest that gregarious behavior might reduce the selective pressure to maintain functional v-*cath* homologs, favoring more efficient orofecal viral transmission. Similar trends have been observed in other baculoviruses from gregarious or semi-gregarious insects, which also lack functional cathepsin and chitinase.

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PT.438

GEOMETAGENOMICS REVEALS PLANT DNA VIRUS DIVERSITY IN AN AREA WITH LOW HUMAN INTERVENTION

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Research on the dynamics of plant viruses has typically focused on economically important plants, which are more prevalent in agricultural than in natural ecosystems. Therefore, studying viruses found in non-cultivated (wild) plants is crucial to obtain an unbiased understanding of plant virus diversity. Viral metagenomics allows the detection of both well-known and extremely divergent viral genomes in non-cultivated plants. Here we applied a geometagenomics approach to characterize the viral community in an ecosystem with a low level of human intervention. The study was carried out in the Parque Estadual da Serra do Brigadeiro (PESB), located in the state of Minas Gerais. A total of 335 plant samples were collected, consisting mostly of uncultivated plants, and grouped into five pools. Total DNA was extracted from each pool and sequenced using an Oxford Nanopore MinION device. Viruses present in the samples were initially identified using BlastN and BlastX, and classified using geNomad. We found viral sequences in all five pools, but only 14% of the sequences identified as belonging to viruses could be reliably classified (in the orders *Geplafuvirales*, *Herpesvirales*, *Ortevirales*, *Orthopolintovirales* and *Pimascovirales* and the class *Caudoviricetes*). The great percentage of unclassified sequences likely reflects the fact that most of the samples are from native, non-cultivated plants which were sampled for the first time. Other approaches are being tested to classify these viral sequences.

Financial support: CAPES, CNPq and FAPEMIG

PT.439

Picorna-like viruses found associated with transcriptomes of the spider mite, Tetranychus truncatus (Acari: Tetranychidae)

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Mites pose a significant threat to agriculture due to their ability to feed on various plants. Despite their importance, our understanding of viruses in mites remains limited like in the case of the Asian spider mite, *Tetranychus truncatus*, which has been relatively understudied in terms of viral presence. In this study, we identified three distinct picorna-like viruses associated with transcriptomes of *T. truncatus*. Tetranychus truncatus-associated iflavirus 1 (TtAIV-1) features a canonical Iflaviridae genome structure with a single ORF, marking it as the first iflavirus identified in the *Tetranychus* genus. Tetranychus truncatus-associated picorna-like virus 1 (TtAV-1) and 2 (TtAV-2) exhibit bicistronic arrangements similar to dicistroviruses. Phylogenetic analysis clustered TtAIV-1 as a novel species within the *Iflaviridae*, while TtAV-1 and TtAV-2 clustered among unclassified picorna-like viruses. These viruses are predominantly co-associated, with viral reads comprising up to 30% of total reads among different transcriptomes. Virus presence and abundance were influenced by mite treatment and origin, with no significant impact from *Wolbachia* infection or abamectin exposure. Temperature was a key factor affecting virus abundance, and variability was noted among Chinese mite populations. Our analysis of viral replication demonstrated that mite treatment, condition, location, and plant host presented both positive and negative correlations among the treatments. Overall, our findings enhance the understanding of the diversity of picorna-like viruses associated with *T. truncatus* and offer potential implications for managing this acarine pest in agriculture.

Financial support: FAPDF, CNPq, Instituto Serrapilheira.

PT.440

FIRST REPORT OF *TOPILEVIRUS SOLANI* ASSOCIATED TO *GLYCINE MAX* IN BRAZIL

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Topilevirus solani (*Geminiviridae*) was described in tomatoes in Brazil and Argentina. Herein, 94 symptomatic leaf samples were collected from 11 families growing near tomato crops in five Brazilian macro-regions. The total DNA was extracted, enriched by rolling circle amplification, and sequenced in an Illumina NovaSeq-6000 platform. The raw reads were trimmed and assembled using CLC Genomics workbench v.8.0 (Qiagen). The assembled contigs were analyzed via BLASTn against the viral RefSeq database at GenBank. A total of 153 plant-virus contigs were selected. The complete genomes were assembled (with Geneious® 11.1.5 program). Six contigs with 2.873 nucleotides were recovered with 3.388 reads. These contigs exhibited 97.91% identity and 100% coverage with *T. solani* (MT135209.1).
Bayesian phylogenetic analyses and PCR assays using virus-specific primers were employed to confirm the presence of *T. solani* in one soybean (*Glycine max*) sample. This is the first report of *T. solani* in soybean, indicating an increase in the host range and the need for monitoring this virus in this crop in Brazil.

Financial Support: Embrapa; UnB; FAPDF; CAPES

PT.441

COSMOS SULPHUREUS SPECIES FROM THE ASTERACEAE FAMILY AS A NEW NATURAL HOST OF *BEGOMOVIRUS BLAINVILLEAE*

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Begomovirus has a bipartite genome composed by single-stranded DNA and transmitted by whiteflies (*Bemisia tabaci* Middle East Asian Minor 1 – MEAM1). This group of viruses has a range of hosts, including plants belonging to the Asteraceae family. Here, ninety-four leaf symptomatic samples from plants of 11 botanical families in five Brazilian macro-regions were collected and the total DNA was extracted, enriched by Rolling Circle amplification (RCA), and sequenced using High-Throughput Sequencing (HTS). Of the 153 contigs recovered, two contig showed identity with *Begomovirus blainvilleae,* with identity 94.97% (Accession: MT626984.1) and with identity 96.53% (Acession: MT626983.1). Specific primers were used in PCR. This Begomovirus was detected in *Cosmos sulphureus* (Asteraceae family). This will be the first report of the occurrence of this virus in *C. sulphureus*. These findings expand the known host range of *B. blainvilleae* and highlight the importance of ongoing surveillance to understand the distribution of this *Begomovirus*.

Financial Support: Embrapa; UnB; FAPDF; CAPES

PT.442

MOLECULAR DETECTION AND IDENTIFICATION OF CULEX FLAVIVIRUS IN MOSQUITOES FROM PARAGUAY

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Insect- specific flaviviruses (ISFVs), including Culex flavivirus (CxFV), are viruses that are not capable of infecting vertebrates, vertically transmitted that belong to the Flavivirus genus, which also includes important human infecting arboviruses. CxFV has been isolated from various Culex species in countries such as Brazil and Argentina. In Paraguay, flaviviruses were found in mosquitoes of the Aedes and Culex genera, with Culex exhibiting the highest prevalence. Therefore, this study aimed to implement molecular techniques to detect and identify CxFV in Culex mosquitoes previously found to be positive for flavivirus and negative for specific medically significant viruses. Mosquitoes from three locations in 2018 were collected and sorted into 62 pools (932 mosquitoes) by species, location, collection date, and feeding status. CxFV screening was performed using CxFv-specific nested RT-PCR to amplify a fragment of NS5 gene (206pb). The implementation of PCR screening was successful, showing a high positivity in the samples tested (80,6%), indicating a wide distribution of these virus. Eight positive selected samples were subjected to three nested RT-PCRs for subsequent Sanger sequencing. Fragments of ~ 1275pb of NS5 gene were assembled and genotype 2 was identified in Paraguayan samples, that clustered together with Brazil and Mexico CxFv. Although ISFVs are not known to cause human diseases, they may have implications for the transmission of pathogenic arboviruses and vector control. The study of CxFv is important to understand the interactions between flaviviruses, ISFVs, and their vectors to better comprehend their roles in disease dynamics.

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PT.443

PRODUCTION OF CRYSTALIZED BACULOVIRUS ENHANCIN PROTEINS FOR THE BIOLOGICAL CONTROL OF AGRICULTURAL AND FOREST PESTS

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Enhancins are metalloproteases capable of facilitating baculoviral infections by degrading the insect's peritrophic matrix. The genera *Alphabaculovirus* and *Betabaculovirus* in the *Baculoviridae* family encode Enhancins. The aim of the present study is to produce baculovirus derived Enhancin proteins, utilizing the Bac-to-Bac expression system, under a novel crystallization strategy that allows a more stable formulation and use as an adjuvant for baculovirus based biopesticides. The genetic fusion of proteins with an alpha helix of Thyrinteina arnobia Cypovirus (TharCPV) polyhedrin gene, co-expressed with the full polyhedrin gene, allows for its crystalization. Primarily, we have extracted the genomic DNA of the Spodoptera frugiperda granulovirus (SfGV) from the occlusion bodies of a viral isolate. Specific oligonucleotides were designed and used to amplify and isolated the Enhancin 1 and 2 genes of SfGV by polymerase chain reaction (PCR). Purified interest genes are being cloned in a modified plasmid vector, genetically fused with the crystalization domain of TharCPV. The work aims to produce such proteins in insect cells through the construction of recombinant baculovirus vectors by the Bac-to-Bac expression system. Finally, we aim to produce and purify polyhedrin crystals containing SfGV Enhancins and conduct tests that measure their ability to enhance the lethality and efficacy of baculovirus against insects, attempting to increase their biopesticide capability.

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PT.444

DEVELOPMENT OF INTERACTIVE TAXONOMIC KEYS APPLIED TO ENTOMOVIROLOGY – SPEEDING UP THE IDENTIFICATION OF NEOTROPICAL MOSQUITOES VECTORING ARBOVIRUSES

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Arboviruses pose significant public health risks worldwide. Mosquitoes in the Culicidae family are particularly relevant for entomovirology due to their role in harboring and transmitting arboviruses to humans. Rapid identification of mosquito vectors under controlled low temperatures is imperative for preserving the integrity of potential viral genetic material. However, traditional morphological identification using taxonomic keys, followed by manual data recording, can be challenging and time-consuming. This study aimed to develop a user-friendly, interactive taxonomic key based on morphological characteristics for identifying neotropical mosquito vectors of arboviruses and to compare its efficiency with traditional methods. Morphological identification keys were used to create a program in Python 3.12 with associated libraries. The program includes a tube identification tab for biogeographic data input and a mosquito ID tab that navigates through subfamily, tribe, genus, and species keys with visual aids. All data is stored and automatically exported in SQL and CSV formats. The program's efficiency was assessed by comparing mosquito identification with and without it, considering the total time for data tabulation and identification. The program significantly expedited identification, which is particularly beneficial for projects focusing on genetic analysis. Additionally, automated data tabulation enhances standardization and usability, underscoring the utility of this tool in mosquito identification projects.

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PT.445

EVALUATION OF THE INFLUENCE OF CLIMATE VARIATIONS ON EGG INFESTATION IN A DENGUE HYPERENDEMIC CITY LOCATED IN THE NORTHWEST OF SÃO PAULO STATE, BRAZIL.

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São José do Rio Preto is a city located in the northwest region of São Paulo State. It is a hyperendemic dengue municipality and has experienced simultaneous transmission of Zika and chikungunya in the last decade. In accordance with the World Health Organization's guidelines, the city's Health Department conducted entomological surveys based on the collection of immature forms of mosquitoes responsible for arbovirus transmission. These surveys occurred four times a year, considering both epidemic and interepidemic periods of dengue occurrence. For this study, we selected six neighborhoods in São José do Rio Preto and chose 46 residences to install ovitraps. Each trap was placed in a residence for a maximum 7 days. Egg collection took place between October 2023 and April 2024. After the traps were removed, the collected eggs were sent to the Laboratório de Pesquisa em Virologia for drying, counting, and proper storage. Climate data, including rainfall and temperature, were obtained from the Integrated Agricultural Meteorological Information Center website and were used to analyze the influence of climate on mosquito egg infestation. 3,121 eggs were collected during the study period, with the highest numbers of eggs collected in February and April (723 and 765, respectively). The neighborhoods Vila Toninho (1,283) and Solo Sagrado (710) had the highest egg infestations. Climate data showed that the period from December to February experienced the highest rainfall (199 to 288.2 mm), while the temperature remained constant at 27°C throughout the study period. We concluded that egg infestation fluctuates according to climate variations.

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PT.446

THE FIRST REPORT OF BLAINVILLEA YELLOW SPOT VIRUS (BEGOMOVIRUS BLAINVILLEAE) IN RIO GRANDE DO NORTE STATE SHOWS HIGH GENETIC SIMILARITY TO ALAGOAS ISOLATES

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Many studies show that weeds can be natural reservoirs for viruses, including begomoviruses, which threaten agriculture. Blainvillea yellow spot virus (Begomovirus blainvilleae, BlYSV) has been reported infecting Blainvillea rhomboidea in Alagoas, Bahia, Minas Gerais, and Pernambuco. This study aimed to identify the virus infecting this weed in Rio Grande do Norte. Leaf samples with begomovirus symptoms were collected in Touros, RN. DNA was extracted following a CTAB-based protocol, amplified using rolling circle amplification, digested with XbaI, and ligated to the pBluescript II vector. The recombinant plasmids were transformed into E. coli DH5α and one clone was Sanger- sequenced. ORFfinder annotated the segment, and viral identity was determined via BlastN analysis. Six ORFs (Rep, REn, TrAP, CP, AC4, and AC5) and a typical begomovirus nonanucleotide (TAATATT//AC) were identified. BlastN analysis indicated that the clone corresponded to a BlYSV isolate, as the top 20 hits were isolates of this virus with >9X% identity. Pairwise sequence alignments using SDT v.1.2 indicated 98.66% identity with a BlYSV DNA-A from Alagoas (JX871392). A multiple sequence alignment, including the sequence from the RN isolate and 31 BlYSV sequences from GenBank, was obtained with MEGA X using the MUSCLE algorithm. Evolutionary analysis included model selection and maximum-likelihood phylogenetic inference with 1000 bootstrap replications. Phylogenetic analysis clustered the isolate with those from Rio Largo (AL). This marks the first BlYSV report in Rio Grande do Norte, showing high genetic similarity to isolates from Alagoas state.

Financial support: CAPES, CNPq and FAPEMIG

PT.447

EPIDEMIOLOGICAL AND MOLECULAR INSIGHTS INTO CHIKUNGUNYA VIRUS IN ALAGOAS, BRAZIL: PHYLOGENETIC ANALYSIS AND VECTOR SURVEILLANCE

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Chikungunya fever is an arboviral disease caused by Chikungunya virus (CHIKV) and transmitted by Stegomyia aegypti and S. albopictus. Although there are three genotypes of CHIKV circulating worldwide, the ECSA (East/Central/South African) is predominant in Brazil. It is important to highlight that each of these variants has unique characteristics and modifications related to virulence and vector affinity. This study aims to analyze the molecular epidemiology of CHIKV in humans and vectors, especially in the state of Alagoas, Brazil. First, we conducted a survey of complete CHIKV genomes obtained from human samples (817) and vectors (13) from around the world , available in GenBank from 2018 to 2023. The phylogenetic analysis (maximum likelihood approach) revealed that the genetic sequences of Brazilian samples from humans (691) or vectors (4) are closer to each other and are of the ECSA genotype. Considering the low number of sequences obtained from vectors in Brazil, we performed vector collection in the state of Alagoas, from february to july 2024, for real-time PCR identification of CHIKV. A total of 321 S. aegypti specimens were captured, of which 185 were females. Despite the high number of cases in the state of Alagoas from 2022 to 2024, we have not yet identified positive vectors for CHIKV. New genetic analyses will be performed, and vector collections will continue. Phylogenetic analyses identified that the ECSA genotype is predominant in Brazil and that the genotypes sequenced from vectors clustered with strains from humans, strengthening the hypothesis that the same genotype is circulating.

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PT.448

DIVERSITY OF TOMATO-INFESTING WHITEFLIES (*Bemisia tabaci*), THE VECTOR OF BEGOMOVIRUSES, IN THE REGION OF FLORESTAL, MG

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Begomoviruses (family *Geminiviridae*) are responsible for losses in crops of economic interest, including tomatoes. The presence of an efficient vector is a primary ecological factor driving the expansion of the host range of plant viruses transmitted by arthropods, with vectors playing an essential role in the emergence of diseases. Begomoviruses are transmitted by whiteflies of the *Bemisia tabaci* cryptic species complex, which is known for having a high degree of inter- and intraspecific diversity. It has been hypothesized that changes in the composition of *B. tabaci* populations may be the reason why begomoviruses, including tomato severe rugose virus (*Begomovirus solanumseverugosi*, ToSRV), have been declining in occurrence in recent years in Brazil. We assessed the diversity of whiteflies in tomato fields located in the region of Florestal, MG. Whitefly adults were collected from thirteen tomato fields. A total of 358 individuals were genotyped based on PCR amplification of a microsatellite locus that differentiates *B. tabaci* Middle East-Asia Minor 1 (MEAM1) and Mediterranean (MED). Out of the 358 individuals collected, 289 were identified as *B. tabaci* MED, and only 9 as *B. tabaci* MEAM1. Considering that *B. tabaci* MED has been reported as a poor vector of Brazilian begomoviruses, our results are consistent with the hypothesis that the high incidence of *B. tabaci* MED and low presence of *B*. tabaci MEAM1, could be responsible for the declining incidence of tomato begomoviruses in Minas Gerais state.

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PT.449

INCIDENCE AND MOLECULAR CHARACTERIZATION OF A NOVEL CRESSDNA VIRUS ASSOCIATED WITH COCONUT CROWN ATROPHY DISEASE

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Coconut crown atrophy disease (CCAD) affects coconut palm, reducing yield due to leaf shortening and fruit drop. While its etiology is unclear, evidence suggests an association with a cressdnavirus. This study evaluated the incidence of the putative DNA-R of this virus in coconut trees in the state of Pará, Brazil. We collected 268 samples of the Anão Verde cultivar, with (*n*=201) and without (*n*=67) CCAD symptoms, from fields around the cities of Santa Izabel, Moju, Capanema, Altamira, and Belém. Incidence was verified through RCA followed by PCR with universal primers for the nanovirus DNA-R. Three samples were Sanger-sequenced and the identity was verified using BLASTx. MUSCLE alignment was used to construct maximum likelihood and neighbor-joining phylogenetic trees with Rep amino acid datasets from *Cressdnaviricota*. The viral segment was detected in 194 symptomatic and 50 asymptomatic samples, an overall incidence of 91%. The sequences showed 77.91-97.16% nucleotide identity among each other. BLAST*n* found no similar sequences, but BLAST*x* indicated 38-39% identity with the deduced Rep protein of a *Cressdnaviricota* member (AXH73792) obtained from metagenomics, and ~37% identity with sophora yellow stunt virus (*Nanovirus sophorae*, WGT79592). In the ML tree, our isolates clustered separately from *Nanoviridae*. NJ-tree analysis suggested the virus is within the order *Mulpavirales*. These results suggest CCAD is strongly associated with a new virus in the *Mulpavirales* order, distinct from *Nanoviridae*. Further studies are needed to determine if this DNA-R-like segment is infectious by itself or requires additional segments and if it is the causal agent of CCAD.

Financial Support: SOCOCO S/A Agroindústrias da Amazônia

PT.450

Mapping the occurrence of the main common bean (*Phaseolus vulgaris*) viruses in several Brazilian producing regions

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Common bean (*Phaseolus vulgaris L.*) is an important source of protein in many tropical and subtropical countries, especially in Africa and Latin America, with emphasis to Brazil. Bean cultivation faces serious phytosanitary challenges due to the high incidence of viruses, the majority of which transmitted by whiteflies (Bemisia tabaci), such as the golden mosaic, which can cause losses of up to 100%. These viruses include Bean golden mosaic virus (BGMV), Cowpea mild mottle virus (CpMMV), Macroptilium yellow spot virus (MaYSV) and Bean-Associated Cytorhabdovirus (BaCV), which often occur in mixed infections, making it impossible to identify by symptoms, which can be confused or masked. For some regions, information is not yet available regarding some viruses. It is important to monitor the occurrence of viruses to prevent outbreaks from occurring and prepare management strategies. The objective of this study was to identify the presence of these viruses in bean plants in important producing regions of Brazil, during the years of 2022-2024. Samples were collected in 15 locations in the states of GO, BA, MG, MT, SP, AL and SE (<10 samples per area) and were analyzed by PCR with specific primers for each virus. Preliminary results identified BGMV and CPMMV in all states evaluated. BaCV was not identified in samples from MT, SP and AL. Analysis to identify the presence of MaYSV in the samples are still being processed. Altogether, this information will subsequently contribute to the development of a mathematical model for estimating virus dispersion considering soil and climate variables.

PT.451

Management of common bean viruses: are we using insecticides rationally?

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Despite the risk of damage to the environment and human health, the management of common bean (*Phaseolus vulgaris*) viruses is still carried out mainly with the intensive use of insecticides to reduce the population of the insect vector, the whitefly *Bemisia tabaci*, often without considering the Economic Threshold Level (ETL) of the insect or the tolerance/resistance level of the bean genotype. A field experiment (4 plots of 4 rows x 4m per treatment) was conducted in Itaberá-SP in January, when whiteflies usually cause greater damage. A randomized block experimental design (3x4 factorial) was adopted, comprising three insect management strategies 1- no spraying = control, 2- spraying at hypothetical ETL (5 adults/meter), 3- weekly sprays/ 7 weeks, evaluated on the productivity of four bean cultivars with different levels of tolerance/resistance to BGMV and CPMMV (1-IPR-Campos Gerais, 2-BRSFC401RMD, 3-CNFCT19119 and 4-IPR-Sabiá). The following variables were evaluated: initial/final stand, number of whitefly nymphs and adults (weekly), virus severity, productivity and pest management cost. Despite a mild whitefly infestation (average of 1.9 adults/m; 11.3 nymphs/leaf), there was no significant difference in the productivity of the cultivars among the three management strategies, except for the cultivar BRSFC401RMD, highly susceptible to CPMMV. Considering that the insecticide application cost in average R$ 120-300/ha, and returned no significant difference in productivity, insecticides may be unnecessary or reduced, depending on the tolerance level of the cultivar and the level of infestation of the insect vector. Our data also suggest an estimate for whitefly ETL, considering several factors.

PT.452

THE VIROME OF COCOA FERMENTATION-ASSOCIATED MICROORGANISMS

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Theobroma cacao plantations hold significant economic importance globally, primarily due to their role in chocolate production. Environmental microorganisms (spontaneous fermentation) or manually inserted cultures are responsible for the fermentation, significantly influencing the beans' flavor and color. Viruses may significantly influence this process by infecting fermenting organisms, potentially disrupting microbial diversity and affecting fermentation quality. This study utilized publicly available metatranscriptomic libraries from cocoa bean fermentation in Limón Province, Costa Rica, to identify viruses associated with fermenting microorganisms. Samples were collected at 7, 20, and 68 hours of fermentation, which correspond to yeast- and lactic acid bacteria-driven phases. Samples were collected at 7, 20, and 68 hours of fermentation, corresponding to the yeast- and lactic acid bacteria-driven phases. A comprehensive analysis identified 68 viral sequences, 62 of which were novel viral species, with the majority belonging to the Lenarviricota phylum. Among these, 44 sequences were associated with ssRNA phages (*Fiersviridae*) and fungal-infecting families (*Botourmiaviridae*, *Narnaviridae*, and *Mitoviridae*), highlighting complex evolutionary transitions from bacterial to fungal infections. Additionally, viruses from the *Totiviridae* and *Nodaviridae* families were identified. The per-sample viral abundance showed a general decline pattern, similar to the trend observed in some microbial genera during fermentation. Unexpectedly, potential RNA1 and RNA2 segments related to narnaviruses were identified and analyzed using Hidden Markov Models. This study underscores the complexity of the virome in cocoa bean fermentation, which could influence strain selection, metabolite production, and the sensory characteristics of cocoa beans.

Financial Support: CNPq

PT.453

COMPARATIVE TRANSCRIPTOME ANALYSIS REVEALS MOLECULAR RESPONSES IN SUSCEPTIBLE AND RESISTANT WHEAT CULTIVARS INFECTED WITH WHEAT STRIPE MOSAIC VIRUS

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Wheat stripe mosaic virus (WhSMV), a benyvirus transmitted by the soil-borne plasmodiophoromycete *Polymyxa graminis*, is the causal agent of wheat mosaic disease in Brazil, which is a serious threat to wheat production in the southern part of the country. Typical symptoms of wheat plants infected with WhSMV are yellow mosaic or irregular mottling and streaking on leaves and stems. In present study, comparative gene expression analysis using RNA-Seq was performed in susceptible and resistant wheat cultivars in response to WhSMV infection. The comparative transcriptome analysis showed that a total of 2244, 2505, 5172, and 3331 differentially expressed genes (DEGs) were identified in comparisons between Embrapa16 (resistant) with and without virus infection; Guamirim (susceptible) with and without virus infection; Embrapa16 vs. Guamirim with virus infection; Embrapa16 vs. Guamirim without virus infection, respectively. Genome ontology (GO) annotation revealed several DEGs associated with different biological processes, such as phytohormone metabolism, photosynthesis, DNA metabolic process, response to biotic stimuli, and defense response. DEGs related to photosynthesis and phytohormone metabolism were further enriched and analyzed. This suggests that genes related to hormone biosynthesis, and photosynthesis may play an important role in developing symptoms following WhSMV infection. Furthermore, several genes involved in various stress responses and plant defense mechanisms were enriched, such as CALM, WRKY33, PR1, RPM1, NBS-LRR, and genes related to protein kinases. These results highlight the intricate interaction between wheat and WhSMV at the transcriptomic level and shed light on the genetic and molecular mechanisms underlying infection that may influence disease resistance and susceptibility.

Financial Support: CAPES, CNPq, EMBRAPA, FAPESC.

PT.454

COMPLETE GENOME SEQUENCE OF banana streak bacilliform GF virus (*Badnavirus alphavirgamusae*) INFECTING *Alpinia purpurata* IN BRAZIL

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Badnavirus (*Caulimoviridae*) is a genus of pararetroviruses infecting economically important crops in tropical and subtropical regions of the world. Banana streak GF virus (BSGFV) causes streak disease in *Alpinia purpurata* (Zingiberaceae) in Hawaii. However, the genome of this badnavirus isolate has not been characterized. In 2021, an *A. purpurata* plant showing chlorotic streak symptom was collected from a commercial tropical flower plantation in Marimbondo, Alagoas, Brazil. Aiming to perform the molecular characterization of the possible badnavirus infecting *A. purpurata,* total DNA was extracted and used as a template to obtain complete viral genome through rolling circle amplification (RCA). Aliquot of the RCA were used to perform PCR-based detection using universal primers. After confirmation of episomal badnavirus infection, the RCA product was subjected to Illumina high-throughput sequencing (HTS). The complete genome of the 7.148 bp was assembled from 13.500 reads, with a coverage depth of 180 x. Based on the identity of pairwise nucleotide sequences of the reverse transcriptase (RT) and ribonuclease H (RNAse H) domains, the badnavirus from *A. purpurata* belongs to the species *Badnavirus alphavirgamusae* (BSGFV), with 97.1% nucleotide identity. In the Bayesian inference tree, BSGFV_BR_ALPÍNIA grouped with another BSGFV isolate from banana. The new BSGFV isolate is putative recombinant sequence, with recombination breakpoints at the nucleotide positions 1458 and 2349, which are located in ORFIII, and banana streak VN virus was identified as putative major parental. To our knowledge, this is the first complete badnavirus genome sequences characterized from *Alpinia* in Brazil.

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EPISSOMAL DETECTION AND COMPLETE GENOME SEQUENCE OF *Badnavirus alphavirgamusae* (BSGFV) IN BANANA PLANT, BRAZIL

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Banana streak disease, caused by nine different banana streak virus (BSV), has limited banana production in all growing areas of de world. In Brazil, BSOLV, BSGFV, BSUgBV e BSMYV cause episomal infection in banana, however, their genome has not been characterized. Aiming to characterize the circular DNA virome of banana leaf samples showing symptoms of streak, collected in northeastern Brazil from 2021 to 2023. Total DNA was extracted and used as a template to obtain complete viral genome through rolling circle amplification (RCA). Aliquot of the RCA were used to perform PCR-based detection using universal primers. After confirmation of episomal badnavirus infection, the RCA product of two representative samples (UP21 and SM15) was subjected to Illumina high-throughput sequencing (HTS). The complete genome of the 7.263 bp was assembled from 3.662 reads, with a coverage depth of 118 x. Based on the identity of pairwise nucleotide sequences of the reverse transcriptase (RT) and ribonuclease H (RNAse H) domains, the badnavirus from isolate UFAL2024 belongs to the species *Badnavirus alphavirgamusae* (banana streak GF virus, BSGFV), with 98,2% - 99,0% nucleotide identity. In the Bayesian inference tree, BSGFV-UFAL2024 grouped with another BSGFV isolate from banana. The BSGFV-UFAL2024 isolate is putative recombinant sequence, with recombination breakpoints at the nucleotide positions 954 and 2630, covering ORFs I, II and III. The badnavirus BSVNV and BSCAV were identified as putative major and minor parental. To our knowledge, this is the first complete badnavirus genome sequence characterized from banana in Brazil.

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PHYLOGENETIC ANALYSIS AND EVOLUTIONARY DYNAMICS OF RICE STRIPE NECROSIS VIRUS AND BEET NECROTIC YELLOW VEIN VIRUS: INSIGHTS FROM BRAZILIAN AND EUROPEAN SEQUENCES

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The *Benyviridae* family comprises viruses that affect agricultural crops such as rice and beet, crucial for food security, particularly in underdeveloped countries. This study reviews the evolutionary trajectory of the main *Benyviruses*: Rice Stripe Necrosis Virus (RSNV) and Beet Necrotic Yellow Vein Virus (BNYVV). We analyzed all sequences of the coat protein gene of BNYVV (N=371) and RSNV (N=41) viruses available on GenBank. Each dataset was individually aligned using MAFFT (v7.490) and phylogenetically analyzed using the Maximum Likelihood method with IQTREE v1.6.12, employing 1,000 UFBOOT replicates for branch support. The optimal evolutionary model was determined by MODELFINDER. The resulting trees were visualized with FigTree v1.3.1. Lineage identification was based on tree topology, according to previous phylogenetic studies of RSNV and BNYVV. The phylogenetic analysis revealed that RSNV had two separate introductions in Brazil and suggests that two lineages are cocirculating in the country. The minor introduction is related to one sequence isolated in the state of Santa Catarina (SC) while the major clade is composed of 24 sequences isolated in the states of Goiás, Roraima, SC, and Tocantins. On the BNYVV phylogeny, one single clade clustered all sequences from Brazil, sharing a direct common ancestor with sequences isolated in Europe. These findings highlight the complexity of viral dissemination and the need for management strategies that consider their phylogenetic characteristics, given that the mode of virus dissemination has yet to be conclusively determined. Bioinformatics tools identified key patterns in these pathogens, guiding future research and control strategies.

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A Structural Analysis of the Geminivirus Capsid: AlphaFold2 Predictions Versus Experimental Three-Dimensional Models

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The *Geminiviridae* is a numerous family of 522 different species of ssDNA plant viruses that infect significant crops. The family has a distinctive twinned geminated particle known in molecular details for only three species. We sought to explore the structural diversity of the capsid proteins of geminiviruses using alphafold2-derived structures. In this work, we used alphafold to generate capsid pentons, the capsomeres involved in geminivirus assembly. We used Alphafold multimer to generate the tridimensional structural models of pentamers from AYVV CP (AF_AYVVpent). The predicted contact interfaces between the CPs were analyzed using ChimeraX, and the accuracy of the predicted model was compared with the Cryo-EM-derived model of AYVV (6f2s_AYVVpent) using the Dockground server. Even though the interacting interfaces of the AF_AYVV5fold were rated as highly similar with the AYVVpent by Dockground metrics, we observed a rmsd of 0.676 for the overall structure of AF_AYVVpent and 6f2s_AYVVpent respectively. The residues involved in the subunit contacts were 82,9% similar between the predicted and the experimental structure. The predicted pentamer interfaces had a larger buried area, resulting in a smaller pentamer internal area and different contact angles between the subunits than the Cryo-EM structure. We noticed that four residues contacting the ordered segment of the DNA structure in 6f2s_AYVVpent were engaged in the subunit-subunit interface interaction in the alpha-fold model, which could be introducing the observed deviations. These findings indicate that the DNA should be accounted for when modeling virus capsomers and emphasize the role of the DNA as a molecular switch for geminivirus capsid assembly.

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PT.458

HIGH-THROUGPUT SEQUENCING AND RT-PCR IN SURVEY OF VIRUSES ASSOCIATED WITH APPLE TREES IN SOUTHERN BRAZIL

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Apple trees (*Malus domestica* Borkh) are the most important fruit species in Southern of Brazil, with 96% of production concentrated in the states of Rio Grande do Sul (RS) and Santa Catarina (SC). Among the barriers to the development of this crop are the apple chlorotic leaf spot virus (ACLSV), apple stem grooving virus (ASGV), apple stem pitting virus (ASPV), and apple mosaic virus (ApMV). Identifying affected areas is crucial for monitoring their spread and developing management strategies. This study aimed to investigate these viruses in SC and RS. Samples of scion and rootstock branches were collected from SC and RS. RNA was extracted using the RNeasy Plant Mini Kit and analyzed by High-Throughput Sequencing (HTS), which indicated the presence of ASGV and ASPV. Validation was performed with RT-PCR using specific primers for each virus. Nucleotide sequences were compared with GenBank data using BLAST. In addition, a survey using RT-PCR was conducted on 44 samples from SC and RS municipalities to detect ASGV, ASPV, ACLSV, and ApMV. HTS analysis yields complete genome sequences of ASGV and ASPV and identities of more than 96% for ASGV and 94% for ASPV. The survey data revealed ASGV in 31 samples, ASPV in 29, ACLSV in 4, and ApMV in 2 samples. Most samples (26) were infected with more than one virus. These findings highlight the widespread distribution of ASGV, ASPV, ACLSV, and ApMV in apple-growing regions of SC and RS, underscoring the need for continuous monitoring and virus-free propagation material, ensuring crop sustainability.

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PT.459

STUDY OF VIRAL AND MOLLICUTES SPECIES IN SYMPTOMATIC MAIZE PLANTS INDICATES MAIZE STRIATE MOSAIC VIRUS PREVALENCE IN BRAZIL

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Maize (*Zea mays* L.) is the most produced food in the world and its production is influenced by biotic and abiotic factors. Among the biotic factors, viruses and mollicutes are associated with a decrease inproductivity in the cultivated areas. The aim of this study was to investigate the viral and mollicutes species associated with the maize crop in Brazil (2023/2024 harvest). For this purpose,104 samples were collected in the states of Paraná, São Paulo, Maranhão, Mato Grosso, Rio Grande do Sul, Minas Gerais and Pernambuco. Samples were subjected to total nucleic acid extraction using the CTAB protocol. PCR was then performed to detect corn tunt spiroplasma (CSS), maize bushy stunt phytoplasma (MBSP) and maize striate mosaic virus (MSMV) and RT-PCR was performed to detect sugarcane mosaic virus (SCMV), maize rayado fino virus (MRFV) and maize yellow mosaic virus (MaYMV). Samples that had fragments of the expected size for each pathogen were considered positive. The incidence of each pathogen was calculated based on the number of positive samples in relation to the total samples analyzed. Single infection was observed in 38 samples (39.52%) and mixed infection in 45 samples (46.8%), with the combination CSS+MSMV being the most frequent, infecting 58 samples (60.32%).

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IN VITRO EVALUATION OF ANTI-HERPETIC ACTIVITY OF DITERPENES 7- HYDROXYFRUTICULIN A, DEMETHYLFRUTICULIN A AND FRUTICULIN A FROM ISOLATED FROM *SALVIA PROCURRENS*

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Herpes Simplex Virus type 2 is a widespread human pathogen, endemic in many regions, and can cause oral herpes, genital herpes, meningitis, and encephalitis. Medicinal plants are increasingly recognized as innovative alternatives for preventing and treating viral infections. The Lamiaceae family, which includes the genus Salvia, contains several species used medicinally for parasitic and viral infections. Notably, diterpenes in Salvia species are considered pharmacologically active. This study investigated the *in vitro* anti-herpetic activity of diterpenes 7-Hydroxyfruticulin A, Demethylfruticulin A, and Fruticulin, isolated from *Salvia procurrens*. Cell viability was assessed using AlamarBlue, and antiviral activity was evaluated in the Vero E6 cell line through direct virucidal assays, aciclovir was used as a parameter of antiherpetic activity and the data analyzed using ANOVA. 7-Hydroxyfruticulin A showed dose-dependent anti-herpetic activity, inhibiting 35.39% (±1.23) of viral growth at 5 µM, 62.07% (±0.98) at 12.5 µM, and 74.64% (±1.75) at 25 µM. Demethylfruticulin A also demonstrated dose-dependent inhibition, with 18.75% (±0.8) at 5 µM, 48.87% (±2.1) at 12.5 µM, 85.43% (±1.66) at 25 µM, and 93.75% (±3.02) at 50 µM. Fruticulin exhibited 52.5% (±1.61) inhibition at 12.5 µM and 100% (±4.97) at 25 µM. These findings suggest that diterpenes isolated from *Salvia procurrens* have significant anti- herpetic potential, but further research is necessary to confirm their therapeutic efficacy.

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