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Abstracts selected for

Oral Presentation

CO.01 to CO.40

SBV 40 ANOS

CO.01

WIDESPREAD OF NON-RETROVIRAL RNA VIRUS ENDOGENIZATION IN PHYTOPATHOGENIC ASCOMYCOTA GENOMES

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Among fungi of the Ascomycota phylum, several phytopathogenic species are responsible for significant agricultural losses. These pathogenic mechanisms are associated with their remarkable genomic plasticity, which is influenced by transposable elements (TE). Among the molecular records of these interactions are Endogenous Viral Elements (EVEs), viral-derived sequences integrated into the host genome. Although EVEs derived from non-retroviral RNA viruses have been described in different organisms, their diversity in most phytopathogenic fungi remains poorly understood. In this study, we investigated the occurrence of EVEs in 47 Ascomycota genomes, focusing on phytopathogenic fungi. Bioinformatic analyses identified 376 EVEs heterogeneously distributed across the analysed genomes, occurring in the genera *Ceratocystis*, *Colletotrichum*, and *Sclerotinia*. The detected elements showed similarity to viruses belonging to the families *Mitoviridae*, *Curvulaviridae*, *Endornaviridae*, *Pseudototiviridae*, *Mymonaviridae*, and genus *Ambivirus*. In *Ceratocystis*, EVEs related to *Mitoviridae* were frequently associated with TE regions, exhibiting intragenomic redundancy probably by transposon-mediated expansion. Phylogenetic analyses indicated that several EVEs were vertically inherited from ancient endogenisation events, followed by lineage-specific diversification within their fungal hosts. Additionally, some elements related to the family *Curvulaviridae* displayed conserved genomic context, suggesting ancient insertion within potentially functional genomic regions. Collectively, the data support the existence of an ancient and dynamic co-evolutionary relationship between fungi and RNA viruses, indicating that EVEs act as molecular records of past viral relevant to understanding the genomic evolution and virome diversity. Furthermore, this study expands current knowledge of fungal EVEs and establishes a foundation for functional investigations into the possible roles of these elements.

Financial Support

CAPES

CO.02

BIOLOGICAL CHARACTERIZATION AND EVALUATION OF ANTIBIOFILM ACTIVITY OF A *STAPHYLOCOCCUS GALLINARUM* BACTERIOPHAGE WITH POTENTIAL FOR THE BIOLOGICAL CONTROL OF *EUSCHISTUS HEROS*

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Soybean (*Glycine max* L.) is one of the most economically important crops in Brazil, but its productivity is substantially affected by insect pests such as the Neotropical brown stink bug, *Euschistus heros*. Given the limitations of chemical control, alternative approaches have been explored, including manipulation of insect-associated microbiota using bacteriophages. In this study, we characterized a phage isolated from urban wastewater that infects *Staphylococcus gallinarum*, a bacterium isolated from *E. heros*. The phage was isolated by liquid enrichment, purified using the double-layer agar method, and propagated in its bacterial host. Stability was evaluated under different temperature, pH, and organic solvent conditions. The bacterial receptor involved in adsorption was investigated by thermal treatment of host cells, and biofilm inhibition was assessed at MOIs of 0.01 and 0.1. The phage remained stable from 4 to 50 °C, showed reduced infectivity at 60 °C, and was completely inactivated at 70 °C. Infectivity was maintained between pH 3 and 9, with greatest stability at neutral pH. Chloroform moderately reduced phage titers, whereas ethyl ether had no significant effect. Maintenance of adsorption after thermal treatment suggests the involvement of a thermostable receptor, potentially of polysaccharide nature. The phage significantly inhibited biofilm formation when applied before bacterial growth at both MOIs but showed no effect on 24-h preformed biofilms. These findings demonstrate the physicochemical stability and preventive antibiofilm activity of this bacteriophage and support further investigation of its potential to manipulate *E. heros*-associated bacterial populations for sustainable pest management.

Financial Support: CNPq, Promip, FAP-DF, CAPES, Instituto Serrapilheira.

CO.03

ADAPTIVE EVOLUTION AS A STRATEGY TO EXPAND THE HOST RANGE OF BACTERIOPHAGES AGAINST *RALSTONIA SOLANACEARUM* SPECIES COMPLEX (RSSC)

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Bacterial wilt, caused by bacteria belonging to the *Ralstonia solanacearum* species complex (RSSC), is one of the most difficult plant diseases to control.. Recently, the occurrence of *Ralstonia syzygii* (phylotype IV), previously considered restricted to the Asian continent, was reported in Brazil, reinforcing concerns regarding the emergence and spread of RSSC lineages. Current control measures remain limited, and bacteriophages have emerged as a promising alternative for the biological control of bacterial wilt. This study investigated the adaptive evolution of the bacteriophage L25 against the BR19, a Brazilian strain of RSSC phylotype IV. Initially, L25 did not exhibit lytic activity against this bacterial strain, and the objective was to expand its host range to achieve effective bacterial lysis. For this purpose, the phage was subjected to successive adaptive evolution passages using the BR19 strain as a selective host to promote the selection of mutations associated with improved infectivity and lytic activity. The evolved phage was subsequently evaluated by spot assay to assess susceptibility and lytic activity against strain BR19. Viral identity was confirmed by PCR, followed by genomic library preparation, next-generation sequencing using the Illumina MiSeq platform, and bioinformatic analyses to identify mutations potentially associated with the evolved lytic phenotype. After serial passages, the evolved phage exhibited lytic activity against the BR19 strain, demonstrating the adaptive potential of bacteriophages under selective pressure. These findings contribute to the development of phage-based biocontrol strategies for bacterial wilt and provide insights into the evolutionary dynamics of host-range expansion in bacteriophages.

Financial Support: CAPES, CNPq e Fapemig

CO.04

NEW ISOLATES OF BETACHLOROVIRUSES SHED LIGHT ON THE DIVERSITY AND BIOLOGICAL COMPLEXITY OF AN UNEXPLORED GROUP OF GIANT ALGAL VIRUSES

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Within the family Phycodnaviridae, the genus Chlorovirus is the most studied, with three recognized subgenera based on genomics and host range, named Alphachlorovirus, Gammachlorovirus, and Betachlorovirus. Chloroviruses have double-stranded DNA and can encode up to 450 ORFs and 18 tRNAs, with some variation between groups and isolates. These viruses infect freshwater algae and have been isolated worldwide, with more than 130 viruses with genome sequences publicly available, unevenly distributed among the subgenera. In this study, we focus on betachloroviruses, a poorly explored group that infects the alga Micractinium conductrix Pbi. We describe the isolation and genomic analysis of 11 new betachloroviruses from water samples collected in Nebraska, USA, and the genomic analysis of 14 isolates previously available in a public database. With 25 fully sequenced genomes, we assessed the genomic diversity of these viruses using multiple bioinformatics analyses. They have genomes ranging from 295 to 374 kbp, encoding an average of 378 ORFs, of which 42% have no known function. Comparative genomics and phylogenetic analyses revealed three species of Betachlorovirus, some of them with high intra-species genomic identity. Notably, some isolates with over 99.5% genomic identity display markedly different plaque phenotypes, which led us to propose the use of the concept of genomovar among giant algal viruses. Altogether, this work advances our understanding of betachloroviruses and highlights the importance of linking experimental and genomic observations to elucidate viral diversity. Financial support: FAPEMIG, CAPES, CNPq, PRPq, NSF,

CO.05

Type I IFN coordinates resistance and tolerance against Zika virus disease by inducing NK cell recruitment and limiting neutrophil-driven neuropathology in the brain

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Zika virus (ZIKV) neurological disease results from both direct viral replication in the central nervous system (CNS) and host-driven neuroinflammation, with disease severity depending on the balance between antiviral resistance and disease tolerance. Type I interferons (IFN-I) not only restrict viral replication but also regulate inflammatory responses and preserve blood–brain barrier (BBB) integrity. Because the African ZIKV strain MR766 ($ZIKV_{MR766}$) is more resistant to IFN-I than the Asian strain PE243 ($ZIKV_{PE243}$), we investigated how IFN-I shapes CNS disease induced by these distinct strains. Wild-type (WT) and IFNAR-deficient (*IfnR*$^{-/-}$) mice were inoculated intracerebroventricularly (i.c.v.), and viral replication, neurological outcomes, BBB permeability, inflammatory gene expression, immune-cell infiltration, and the contribution of infiltrating leukocytes were evaluated by plaque assay, behavioral testing, RT-qPCR, flow cytometry, and neutralizing antibody-mediated cell depletion. IFN-I conferred antiviral resistance during $ZIKV_{PE243}$ infection, since *IfnR*$^{-/-}$ mice displayed significantly higher viral loads, resulting in mouse death, whereas WT animals controlled virus replication and survived. In contrast, WT and *IfnR*$^{-/-}$ exhibited comparable infectious viral loads during $ZIKV_{MR766}$ infection. Still, IFNAR deficiency accelerated neurological disease and mortality, indicating a predominant role for disease tolerance. These outcomes were associated with BBB disruption and neutrophil infiltration, and neutrophil depletion reduced disease severity without affecting viral replication. Conversely, NK cells only accumulated in the CNS of WT mice, and their depletion increased viral loads without altering disease progression. These findings demonstrate that type I IFN orchestrates infection resistance and disease tolerance during ZIKV CNS infection by promoting NK cell-mediated antiviral control while limiting neutrophil-driven neuroinflammation.

Financial Support: CAPES, CNPq, FAPERJ

CO.06

RISK FACTORS ASSOCIATED WITH POST-CHIKUNGUNYA CHRONIC MANIFESTATIONS IN A COHORT FROM CONTAGEM, MINAS GERAIS, BRAZIL

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Chikungunya virus (CHIKV) infection represents a major public health challenge due to its high rate of progression to the chronic phase, characterized by persistent and debilitating arthralgia. To identify risk factors for chronicity, this study investigated the association between clinical and anthropometric host variables and chronic manifestations. A total of 601 patients diagnosed with Chikungunya in Contagem, Minas Gerais, Brazil, were included in an observational cohort and evaluated 3–7 months after diagnosis. Patients were stratified into three groups: without chronic changes (n=163), with intermittent chronic changes (n=127), and with continuous chronic changes (n=311); mean age was 48.0 ± 18.7 years and 67% were female. During the acute phase, arthralgia (94.3%), myalgia (82.7%), and fever (81.4%) were the most prevalent symptoms, with 69.6% presenting arthritis and only 24.1% reporting complete resolution. Among those with follow-up data, 69% reported ongoing joint symptoms, predominantly pain (87%), with a mean VAS score of 6.2 ± 1.9. Hands (22%) and feet (20%) were the most affected joints. Logistic regression (with vs. without chronic changes) identified female sex (OR=3.42; 95%CI: 2.30–5.12), older age (OR=1.21 per 10 years; 95%CI: 1.09–1.35), and higher BMI (OR=1.04 per kg/m²; 95%CI: 1.01–1.08) as independent predictors of chronicity. These findings demonstrate that demographic and metabolic profiles significantly influence CHIKV disease progression, providing a framework for early risk stratification. Targeted clinical management is essential to mitigate the long-term impact of chronic arthralgia and should be integrated into public health strategies in endemic areas. Financial Support: CNPq, CAPES, and FAPEMIG.

CO.07

Long-term SARS-CoV-2 Persistence in Syrian Hamsters

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SARS-CoV-2 persistence has been proposed as a potential contributor to the pathogenesis of long COVID, with reservoir tissues potentially serving as sites for viral persistence, intra-host evolution, and intermittent viral shedding. Here, we used experimentally infected Syrian hamsters to investigate long-term SARS-CoV-2 persistence across tissues, viral infectivity, and associated immunological and metabolic alterations. Syrian hamsters (Mesocricetus auratus) were intranasally infected with a SARS-CoV-2 parental strain or Gamma and Delta variants and monitored for up to one year, with samples collected at 3, 15, 30, 90, 150, and 365 days post-infection (dpi). During the acute phase, infected animals exhibited significant weight loss, viral shedding, and marked pulmonary inflammation, accompanied by increased expression of pro-inflammatory cytokines at 3 dpi. Infection was confirmed by seroconversion, with sustained IgG responses and low-titer neutralizing antibodies against Omicron. Viral nucleoprotein was detected in multiple tissues up to 365 dpi, while RT-qPCR identified persistent low-level viral RNA in the lungs, brain, spleen, and thymus throughout the observation period, without evidence of productive viral replication. Immune gene expression displayed organ-specific temporal patterns: acute pulmonary inflammation transitioned into broad late-stage suppression, except for sustained TGF-β expression; the brain exhibited a late chemokine signature at 365 dpi; and the thymus showed a delayed immune activation peak at 150 dpi, particularly in Delta-infected animals. Metabolomic profiling revealed a shared acute-phase metabolic signature across variants that largely resolved by 365 dpi, whereas Delta-infected animals retained distinct residual metabolic alterations. Collectively, these findings establish a model of long-term SARS-CoV-2 tissue persistence characterized by organ-specific immune and metabolic signatures.

CO.08

DIFFERENTIAL ASSOCIATIONS OF ZIKA AND DENGUE IgG SEROLOGICAL PROFILES WITH SEROTYPE-SPECIFIC DENGUE RISK: A TEST-NEGATIVE DESIGN STUDY

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Dengue virus (DENV) and Zika virus (ZIKV) are orthoflaviviruses that co-circulate in arbovirus-endemic regions, yet how DENV/ZIKV IgG profiles relate to dengue risk remains unclear. We conducted a test-negative design study of 4,058 patients with dengue-like illness during DENV-2 (2019) and DENV-1 (2021/2022) epidemics to evaluate associations of anti-ZIKV and/or anti-DENV IgG serostatus and antibody levels at health care seeking with DENV-2- and DENV-1-associated outcomes. Cases and controls were DENV RT-qPCR-positive and -negative patients from the same period, with infecting serotypes confirmed by RT-qPCR. DENV/ZIKV IgG profiles were assessed by ELISA, with normalized optical density (OD) ratios used as antibody measures. Adjusted regression models estimated odds of dengue and hospitalization and/or severe dengue (SD) based on serological profile. Anti-ZIKV-only seropositivity was associated with increased odds of DENV-2-associated dengue (OR 11.65, 95% CI 2.88–47.17) and hospitalization and/or SD (OR 11.82, 95% CI 2.74–51.00), but reduced odds of DENV-1-associated dengue (OR 0.34, 95% CI 0.14–0.82). DENV-1/DENV-2-associated dengue odds were low among individuals seropositive to DENV alone (ORs 0.38–0.49) and to DENV/ZIKV (ORs 0.10–0.27). In DENV-1 analyses, higher anti-ZIKV IgG OD ratios were associated with lower odds of both outcomes, whereas anti-DENV IgG OD ratios showed higher odds of DENV-1-associated dengue at intermediate levels and lower odds at high levels. These findings suggest that DENV/ZIKV IgG profiles at health care seeking are associated with dengue outcomes in a serotype-dependent manner but may reflect prior, anamnestic, and/or cross-reactive responses and should not be interpreted as causal evidence of pre-existing immunity.
FAPESP (2013/21719-3, 2022/12576-3, 2022/03645-1 and 2022/09229-0), INCT-One (405786/2022-0).

CO.09

Genomic monitoring and characterization of the hepatitis E virus in the department of Quindío, Colombia.

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Hepatitis E virus (HEV) genotype 3 (gt3) is the most prevalent zoonotic HEV strain in Latin America. In Colombia, HEV infection is not considered a notifiable disease. This study aimed to determine the presence and molecular characterization of HEV in sows, swine farm wastewater, water effluents, and sweat from patients with indeterminate hepatitis (syn. A, syn. B) in Quindío, Colombia (2019–2023). Fifty-four environmental and swine samples were collected from six municipalities, and five clinical serum samples were obtained from hospitalized patients. HEV RNA was detected by RT-qPCR (MIKROGEN® ampliCube HEV 2.0 Quant). Positive samples were sequenced using Oxford Nanopore Technology (ONT) MinION with k-mer cell digital sequencing (k=25) linked to a local Colombian positive control. Sanger sequencing of a 174 bp ORF2/ORF3 amplicon was performed as independent validation. HEV RNA was detected in 11 sow fecal samples, 3 runoff samples, and 1 effluent sample. Four out of five patients tested positive by RT-qPCR. Nanopore sequencing identified two positive clinical samples. Phylogenetic analysis of the animal linkage (Kimura K2P, 1000 bootstrap replicates) found Colombian isolates within the HEV genotype 3 clade (83% bootstrap). Sanger sequencing confirmed six HEV genotype 3-specific k-mer motifs in the ORF2/ORF3 insert (96–100% identity compared to FJ998008.1). The sequences were deposited in GenBank (accessions PZ262436 and PZ262437), the first Colombian genomic sequences of HEV genotype 3 in international databases. These results confirm the active circulation of HEV gt3 in Quindío, Colombia, and demonstrate that locally calibrated molecular tools are necessary for the surveillance of highly divergent regional strains that are not found in global reference databases.

Financial institution: Doctorado en Ciencias Biomedicas from the University of Quindío

CO.10

MICROBIAL HEALTH RISKS ASSOCIATED WITH RECREATIONAL WATER EXPOSURE AND OYSTER CONSUMPTION IN A SEWAGE-IMPACTED COASTAL AREA OF SOUTHERN BRAZIL

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A Quantitative Microbiological Risk Assessment (QMRA) carried out on Tapera, Ribeirão da Ilha, and Caiacangaçu beaches, in southern Santa Catarina Island, evaluated the health risks associated with ingesting water during swimming and consuming oysters contaminated by *Adenovirus*. The study was divided into two stages, water contact and oyster consumption, and analyzed under two scenarios: current sanitation conditions and a future centralized treatment system. Using historical *E. coli* data (2000–2023) from the local environmental agency (IMA) alongside Monte Carlo simulations in @RISK software, the researchers assessed enteropathogenic *E. coli*, *Rotavirus*, *Cryptosporidium parvum*, and *Adenovirus*. In the water assessment, the highest infection probabilities were linked to *E. coli* due to its high concentration, followed by *Rotavirus* and protozoa. Average annual infection risks in scenario 1 all exceeded the WHO acceptable limit of 0.01%. Even when isolating data within CONAMA Resolution 274/2000 limits (≤800 MPN/100 ml), annual risks still failed WHO criteria, reaching minimum values of 24% for *E. coli* and 0.1% for *Rotavirus*, with Tapera presenting the highest pathogen loads and overall risks. In the oyster assessment, the infection probability reached 100% in both scenarios due to the high ingested dose, the link between water *E. coli* and viral presence in oysters, and the low infective dose of *Adenovirus*. Ultimately, these findings demonstrate that standard bacterial indicators alone are insufficient to safeguard public health in sewage-impacted coastal areas, making viral monitoring an indispensable complement.

CO.11

SCALABLE GENOMIC INTEGRATION INTO SYNDROMIC SURVEILLANCE FOR RESPIRATORY OUTBREAK PREPAREDNESS IN BRAZIL

Moraes L[1,3,2], Viana PAB[4], Santos LA[1,6,5,2], Mendonça-Santos T[1,2], Borelli TC[7], Morais IS[1,6], Aguilar GMO[8], Souza VC[9], Ramos TCA[10], Salvato R[11], Henrique B[12], Roll MM[12], Vieira LL[12], Lemos SRS[13], Cardoso CW[14], Prates APPB[15], Lima H[15], Borba O[16], Gomes M[17,18], Santini-Oliveira M[19], Oliveira PB[20], Resende PC[21,2], Naveca F[22,9,23,2], Thompson F[24], Siqueira MM[21,2], Marcilio I[7,5], Florentino PTV[7], Fonseca J[7,2], Tschoeke D[24,25], Barral-Netto M[1,7,2], Meirelles PM[4,26], Ramos PIP[7,2], Khouri R[1,3,27,6,28,2] - [1]Laboratório de Medicina e Saúde Pública de Precisão, Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, BA, Brazil, [2]Instituto Nacional de Ciência e Tecnologia em Saúde Digital DigiSaude-INCT, Salvador, BA, Brazil, [3]Rede Genômica Fiocruz-Bahia, Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, BA, Brazil, [4]Instituto de Biologia, Universidade Federal da Bahia, Salvador, BA, Brazil, [5]Escola Bahiana de Medicina e Saúde Pública, Salvador, BA, Brazil, [6]Programa de Pós-graduação em Ciências da Saúde, Faculdade de Medicina da Bahia, Universidade Federal da Bahia, Salvador, BA, Brazil, [7]Centro de Integração de Dados e Conhecimentos para Saúde, Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, BA, Brazil, [8]Secretaria Municipal de Saúde do Rio de Janeiro, Rio de Janeiro, RJ, Brazil, [9]Núcleo de Vigilância de Vírus Emergentes, Reemergentes ou Negligenciados, Laboratório de Ecologia de Doenças Transmissíveis, Instituto Leônidas e Maria Deane, Fundação Oswaldo Cruz, Manaus, AM, Brazil, [10]Fundação de Vigilância em Saúde do Amazonas – Dra. Rosemary Costa Pinto, Manaus, AM, Brazil, [11]Centro Estadual de Vigilância em Saúde, Secretaria Estadual de Saúde do Rio Grande do Sul, Porto Alegre, RS, Brazil, [12]Centro Estadual de Vigilância em Saúde, Secretaria de Saúde do Distrito Federal, Brasília, DF, Brazil, [13]Centro Estadual de Vigilância em Saúde, Secretaria Estadual de Saúde de Tocantins, Palmas, TO, Brazil, [14]Centro de Informações Estratégicas em Vigilância em Saúde de Salvador, Secretaria Municipal de Saúde do de Salvador, Salvador, BA, Brazil, [15]Secretaria Municipal de Saúde de Salvador, Salvador, BA, Brazil, [16]Centro Estadual de Vigilância em Saúde, Secretaria Estadual de Saúde da Bahia, Salvador, BA, Brazil, [17]Departamento de Doenças Transmissíveis da Secretaria de Vigilância em Saúde e Ambiente, Brasília, DF, Brazil, [18]Programa de Computação Científica, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brazil, [19]Coordenação Geral de Laboratórios de Saúde Pública, Secretaria de Vigilância em Saúde e Ambiente, Brasília, DF, Brazil, [20]Coordenação-Geral de Inteligência Epidemiológica, Secretaria de Vigilância em Saúde e Ambiente, Brasília, DF, Brazil, [21]Laboratório de Vírus Respiratórios, Exantemáticos, Enterovírus e Emergências Virais, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brazil, [22]Laboratório de Arbovírus e Vírus Hemorrágicos, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brazil, [23]Rede Genômica Fiocruz-Manaus, Instituto Leônidas e Maria Deane, Fundação Oswaldo Cruz, Manaus, AM, Brazil, [24]Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil, [25]Laboratório de Engenharia de Sistema de Saúde, Instituto Alberto Luiz Coimbra de Pós-Graduação e Pesquisa em Engenharia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil, [26]Instituto Nacional de Ciência e Tecnologia em Estudos Interdisciplinares e Transdisciplinares em Ecologia e Evolução, Universidade Federal da Bahia, Salvador, BA, Brazil, [27]Plataforma de Vigilância Molecular, Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, BA, Brazil, [28]Department of Microbiology, Immunology and Transplantation, Rega Institute for Medical Research, Clinical and Epidemiological Virology, KU Leuven, Leuven, Belgium

Timely detection of respiratory outbreaks is a key step for epidemic preparedness. Brazil's Alert-Early System of Outbreaks with Pandemic Potential (ÆSOP) uses anomaly detections in the curves of Primary Health Care encounters data for syndromically detecting increases of influenza-like illnesses. Herein, we present a proof-of-concept study evaluating the integration of a cost-efficient genomic module into ÆSOP, using pooled-sample Next-Generation Sequencing with Hybrid Capture (NGS-HC) to characterize pathogens during both alert and baseline periods across seven cities, representing all Brazilian regions (n=1,137 samples; 114 pools). We performed RT-qPCR for major respiratory viruses in all samples, as a benchmark for NGS-HC results. NGS-HC detected 33 viral species, including Flu A, SARS-CoV-2, RSV, HRV, seasonal HCoVs, together with exploratory profiling of respiratory tract-associated bacteria. Overall concordance with RT-qPCR was moderate (sensitivity 80.81%, specificity 81.27%, weighted kappa = 0.53). Pooling samples led to a tenfold cost-per-sample reduction, with a median turnaround time of 7 days. Syndromic alerts consistently coincided with high impact in public health pathogens, while in non-alert periods there was a predominance of endemic, lower impact viruses. There was a marked regional and seasonal variation in genomic profiles, indicating the need for a national baseline pathogen landscape. We addressed amplicon contamination challenges through laboratory protocols and stringent bioinformatic pipelines. Our findings support the integration of genomic surveillance for early outbreak detection in decentralized health systems, providing a scalable model for low- and middle-income countries to enhance epidemic preparedness.

CO.12

SEROLOGICAL EVIDENCE REVEALS UNDERRECOGNIZED CHIKUNGUNYA VIRUS INFECTION DURING A MAJOR DENGUE OUTBREAK IN MINAS GERAIS, BRAZIL

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The 2024 dengue outbreak in Brazil resulted in an unprecedented number of hospitalizations, posing major diagnostic challenges due to the co-circulation of arboviruses such as *Alphavirus chikungunya* (CHIKV). This study aimed to investigate serological evidence of CHIKV infection among patients hospitalized with suspected dengue. A total of 553 patients admitted to Eduardo de Menezes Hospital, a reference infectious diseases center in Belo Horizonte, were retrospectively evaluated during the outbreak. Molecular testing had previously identified dengue virus (DENV) or CHIKV infection, while 224 cases remained PCR-negative for all tested arboviruses. CHIKV-specific IgM and IgG antibodies were detected using a commercial ELISA. CHIKV IgM was identified in 92 patients (16.6%), including 66 of 216 (30.5%) PCR-negative individuals and 25 of 335 (7.5%) DENV PCR-positive patients, indicating unrecognized CHIKV exposure. Among seropositive individuals, 67 (59.8%) were IgM-positive/IgG-negative, 23 (20.5%) were IgM-positive/IgG-positive, and 22 (19.6%) were IgG-positive only. Hematological analysis revealed thrombocytopenia and leukopenia, with significantly lower leukocyte counts in CHIKV IgM-positive patients compared to IgM-negative individuals within PCR-negative cases, and lower hematocrit levels compared to DENV PCR-positive patients. The median time from symptom onset to hospitalization was 7 days (IQR: 5–8), consistent with the post-viremic phase, limiting molecular detection. These findings highlight the underrecognition of CHIKV infection during dengue outbreaks and reinforce the importance of combined molecular and serological approaches for accurate diagnosis in endemic settings.

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CO.13

FINDING VIRUSES IN THE DARK MATTER: STRUCTURAL EVIDENCE VALIDATES SMALL RNA PROFILING FOR SEQUENCE-INDEPENDENT VIRUS DETECTION

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A significant portion of viral biodiversity remains hidden due to limitations in identifying highly divergent sequences. These novel viruses that evade sequence-based classification are part of the "dark matter" of metagenomics. We previously demonstrated that profiling small interfering RNAs (siRNAs), subproducts of viral infections, can identify putative viral sequences independent of sequence similarity. Here, we analysed 1,362 public mosquito small RNA libraries and identified 47,892 dark matter contigs generating siRNAs. We focused on 731 contigs ≥500 nt with siRNA patterns (+siRNA) and 916 controls (-siRNA). To validate the origin of these divergent sequences, Open Reading Frames (ORFs) were predicted using Prodigal, followed by tertiary structure modeling with AlphaFold3. These models were then compared to available databases using Foldseek to detect structural similarity. Searching against experimentally determined structural databases highlighted a great disparity: the +siRNA group returned 60 unique ORF best hits in CATH50 (23 viral) and 42 in PDB100 (32 viral), while the -siRNA yielded zero viral best hits. Top hits of structural similarity included coat, capsid, and uncharacterized viral proteins. Comparing significant hits against all available deposited structures showed a highly significant enrichment in similarity to viral proteins in siRNA contigs (OR = 177.37, $p < 0.001$). However, only 23% of the unknown contigs with +siRNA were classified via structural similarity, leaving the majority unannotated. Given this robust proof of concept, these structurally unresolved ORFs and their folds may hold vast unexplored potential for discovering entirely novel viral entities in the hidden dark matter of metagenomics.

CNPq|FAPEMIG|CNPq/AWS|CIIA/UFMG

CO.14

Integrative multi-omics uncovers an emerging viral threat in Theobroma cacao plants

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Theobroma cacao seeds are the primary raw material for chocolate and other high-value products. Viral pathogens severely compromise cocoa yield and quality. Using an integrative approach, this study investigated viruses associated with chlorosis and leaf deformation in cocoa plantations in Bahia, Brazil. Macroscopic and microscopic evaluations of symptomatic *T. cacao* leaves revealed severe chlorosis and histological alterations, including cell and nuclear hypertrophy. Deep sequencing of small RNA (sRNA) profiled virus-derived sRNAs (vsiRNAs), confirming the activation of host antiviral RNA interference pathways. Using these vsiRNAs, we assembled two viral sequences: a novel geminivirid, tentatively named *Citlodavirus theobromae*, and a badnavirus resembling the Cacao swollen shoot Ghana S virus (family *Caulimoviridae*), designated as Brazilian cacao swollen shoot Ghana S virus Ilhéus. Mass spectrometry validated the presence of multiple viral peptides from both genomes, confirming viral translation and replication. Interestingly, symptoms were exclusively linked to the geminivirid. While viral DNA from both pathogens was detected across all samples, geminivirid RNA accumulated strictly in symptomatic tissues, establishing a strong correlation between citlodavirus transcription and disease expression. In conclusion, our findings reveal the emergence of a novel geminivirid threat that poses a significant risk to cocoa production in South America.

CO.15

MAPPING CONSERVED NEUTRALIZING ANTIBODY EPITOPES IN HEMAGGLUTININ OF INFLUENZA A AND B VIRUSES CIRCULATING IN BRAZIL AND WORLDWIDE (2024-2025)

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Monitoring the structural conservation of hemagglutinin (HA) neutralizing B-cell epitopes in circulating influenza strains is essential to anticipate vaccine escape. This study analyzed HA sequences from Brazilian and global isolates of H1N1, H3N2, and Influenza B collected between 2024 and 2025 from Global Initiative on Sharing All Influenza Data. Sequences were aligned and mapped against experimentally validated neutralizing antibody epitopes from the Immune Epitope Database and 3D structures from the Protein Data Bank to assess mutational profiles and sequence polymorphisms. Epitope mapping revealed distinct conservation patterns across subtypes. For Influenza A, key neutralizing residues clustered in the highly variable globular head subdomains (H1N1: K147/G275; H3N2: T56/F167) and the more conserved stem region (H1N1: T333/W365; H3N2: T334/D364). Similarly, Influenza B exhibited critical epitope distribution within the vestigial esterase (VE) and receptor-binding site (RBS) of the globular head (K90, D211), alongside stable stem residues, including the fusion peptide (e.g., N348, A381). Across all viruses, high levels of amino acid polymorphism in the globular head—often involving non-conservative substitutions—represent major antigenic drift hotspots, whereas the stem subdomains and fusion peptides remained structurally stable. Identifying these conserved stem residues amidst highly polymorphic head hotspots provides critical insights into viral immune escape. This bioinformatics approach supports the continuous monitoring of circulating variants and highlights structurally conserved targets to guide future seasonal vaccine formulations.
Financial Support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)

CO.16

VIRALQUEST V3: AN UPDATED PIPELINE FOR VIRAL SEQUENCE CHARACTERIZATION AND CURATION

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Viruses are known for their high mutation rates and speciation events — the analysis of new species and variants of this kingdom is a highly demanding task in the current biological data and bioinformatics landscape. Therefore, this work aimed to improve the pipeline that identifies and characterizes viruses and phages in assembled sequences of any NGS samples. We developed the new version of ViralQuest as an attempt to solve this problem. The original workflow starts with the processing of sequences and a pre-selection of viral candidates by using Hidden Markov Models (HMM) and aligning against a viral RefSeq protein database from NCBI. After the selection, the candidates are submitted to an alignment against NCBI's nr and nt databases and Pfam characterization. The new version include features to analyze raw sequencing data and the reconstituted viral sequences, among them: 1) Read coverage (sense/anti-sense) and depth over the viral contigs; 2) Assessment of sequence quality and score for each base; 3) Dotplots with quality indicators — repeats, inversions and low-complexity regions; 4) Sequence quantification for short-read libraries; 5) Cluster analysis of similar viruses in the same sample; and 6) Analyses of overlapping ORFs. These tools and visualizations will support future researchers in analysing data and having publish-ready images for their work. Additionally, the pipeline uses a rule-based score to classify sequences as viral or non-viral and integrates LLMs that leverage ICTV information to help in a contextual analysis. All the details of the new version are available on: https://github.com/gabrielvpina/viralquest.

Financial Support: Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB)

CO.17

CAP-SNATCHING OF RIBOSOMAL PROTEIN TRANSCRIPTS AND STRESS GRANULE DISASSEMBLY IN OROPOUCHE VIRUS INFECTION

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Oropouche virus (OROV; species *Orthobunyavirus oropoucheense*) is an arbovirus that has recently caused widespread outbreaks in Brazil. OROV uses a cap-snatching mechanism, in which 5'-capped fragments of host RNAs are incorporated into viral transcripts, ensuring efficient engagement of the host translation machinery. In other bunyaviruses, this strategy has been associated with decay of translation-related 5'TOP mRNAs and modulation of cytoplasmic RNA granules, a host mechanism of viral restriction independent of PKR or IFN pathways. We aimed to determine whether prototypic and epidemic OROV infections deplete translation-related mRNAs and impair RNA granule assembly. Following a 12h kinetic course, we infected Huh7 and HEK293 cells with OROV BeAn or SC-2024, then treated them with sodium arsenite ($NaAsO_2$) to induce stress granule (SGs) formation. RNA granules were visualized by immunofluorescence using different markers (P-bodies: DCP1a/DCP2; SGs: G3BP1/TIA-1). Infected cells showed disrupted SG assembly at 6 hpi and peaked at 12 hpi, with no major differences between BeAn and SC-2024. At 6hpi, 12hpi, and 18hpi, we quantified host mRNAs encoding ribosomal proteins (RPL10, RPL37A, RPS8, RPS23, RPS3A) and translation factor EEF2 by RT-qPCR. We also used a reverse OROV genome primer to detect capped viral RNA. RPS23, RPS8, EEF2, RPL37A, and RPS3A mRNAs had a 2.5-fold decrease over the course of infection. In parallel, the corresponding capped OROV RNAs increased at least 5-fold for all targets except RPS23. Those results support that OROV inhibits host translation, disrupting RNA granules by progressive depletion of translated-related mRNAs and the accusation of capped viral RNAs.

Key words: Oropouche virus; cap-snatching; RNA granules
Financial Support: IDOR, Ciência Pioneira, FAPEMIG

CO.18

LIPID DROPLET TARGETING STABILIZES SARS-CoV-2 ORF6 AND ENHANCES ITS INTERFERON ANTAGONIST ACTIVITY

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Lipid droplets (LDs) are increasingly recognized as organelles exploited by viruses, yet how viral protein association with LDs affects their function remains poorly understood. Here, we investigated whether LD targeting stabilizes the SARS-CoV-2 accessory protein ORF6 and modulates its ability to suppress interferon (IFN) signaling. Huh7 cells expressing C-terminally tagged ORF6-mCherry were treated with oleic acid (OA) to stimulate LD biogenesis or with DGAT1/2 inhibitors to block it. ORF6 localization was assessed by confocal microscopy and LD purification, protein stability by cycloheximide chase and proteasome/lysosome inhibition assays, and IFN antagonism by GAS-luciferase reporter assays and pSTAT1 immunofluorescence. We found that OA-induced LD formation promoted ORF6 redistribution from the endoplasmic reticulum to LDs and increased ORF6 protein levels in a dose-dependent manner. An LD-targeting-deficient mutant (ORF6 4Q) showed reduced protein abundance, increased aggregation and ubiquitination, and impaired ability to inhibit STAT1 nuclear translocation and GAS transcriptional activity. Proteasome inhibition rescued the mutant's anti-IFN function, indicating that LD localization itself is not strictly required for ORF6 activity, but that stabilization of the protein is critical. LD induction delayed ORF6 proteasomal degradation, whereas ER stress reduced ORF6 levels. These findings demonstrate that LD association protects ORF6 from proteasomal degradation, limits its aggregation, and enhances its IFN-antagonist activity, supporting the view that LDs are exploited during SARS-CoV-2 infection to promote viral immune evasion.
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CO.19

Integrated single-cell, spatial transcriptomic and metatranscriptomic analyses reveal cellular and spatial organization of HSV-1 transcriptional states in human tonsils

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Herpes simplex virus type 1 (HSV-1) establishes lifelong infection, yet its transcriptional programs in lymphoid cells remain poorly understood. We integrated single-cell RNA sequencing, spatial transcriptomics, and a metatranscriptomic pipeline to determine HSV-1-infected cell types, viral transcriptional states, investigate host cell differentiation status and the spatial organization of antiviral responses based on publicly available sequences from a human palatine tonsil atlas. HSV-1 transcripts were detected in 81 cells (0.19%) and 52 spatial spots (1.88%), revealing marked cellular heterogeneity. Plasma cells exhibited the highest viral transcript burden and the greatest enrichment of replicating viral states, whereas $CD4^{+}$T cells were predominantly associated with intermediate viral states. Trajectory analysis showed that HSV-1 late transcripts were concentrated in terminally differentiated plasma cells enriched for regulatory genes and endoplasmic reticulum homeostasis. Conversely HSV-1-infected plasma cells had reduced transcripts related to antigen presentation, immune communication, and secretory programs. HSV-1-positive epithelial regions were more active for interferon-mediated antiviral pathways and remodeling of mitochondrial homeostasis, autophagy, and lipid metabolism. Spatial analyses indicated that antiviral activity was highest around HSV-1-positive regions, decreasing as distance from viral foci increased, supporting the existence of spatially organized antiviral niches. Ligand-receptor analyses identified enrichment of cytokine, chemokine, innate immune, and antigen presentation pathways surrounding infected regions. These findings provide the first integrated single-cell, spatial transcriptomic, and metatranscriptomic characterization of HSV-1 infection in human palatine tonsils, revealing associations between host cell differentiation, viral transcriptional programs, and spatial immune organization.

Financial Support: CAPES, CNPq, FAPESP

CO.20

MPXV Infection Modulates Phospholipid Composition and Activates AIM2 and NLRP3 Inflammasomes in an Ex Vivo Human Lymphoid Tissue Model

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MPXV amplification in lymphoid tissues drives systemic dissemination and inflammatory pathology, but mechanisms underlying inflammasome activation remain incompletely understood. This study investigates MPXV-induced immune responses in human tonsillar explants, focusing on inflammasome pathways and host metabolic remodeling. Human tonsillar explants were infected with MPOX (MOI 0.01; 5×10^5 $TCID_{50}$/mL) or mock-inoculated. At 3 and 7 days post-infection, tissues were analyzed by: (1) RT-qPCR for pro-inflammatory cytokines (IL-6, IL-1β, TNF-α, IL-17), interferons (IFN-α, IFN-β, IFN-γ), pattern-recognition receptors (cGAS, STING), and inflammasome components (AIM2, NLRP3); (2) immunofluorescence and Western blot for AIM2, NLRP3, N-terminal gasdermin-D (GSDM-N); and (3) liquid chromatography-tandem mass spectrometry (LC-MS/MS) for lipidomic analysis. MPOX replication reached robust levels by day 7, quantified by RT-qPCR. IL-6 and IL-1β mRNA were significantly upregulated compared to mock controls ($p<0.05$), whereas cGAS/STING markers showed minimal elevation. Immunofluorescence revealed robust AIM2 punctate labeling co-localizing with viral antigen. Western blot and immunofluorescence confirmed N-terminal GSDM, indicating AIM2 inflammasome activation and pyroptotic cell death. NLRP3 protein was detected but showed weaker caspase-1 cleavage, suggesting AIM2 as the dominant activator. Lipidomic analysis revealed a significant reduction in the phosphatidylcholine/phosphatidylethanolamine (PC/PE) ratio in infected tissues, indicating membrane remodeling potentially linked to viral egress. This study provides the first evidence that MPXV infection in human lymphoid tissue synergistically activates the AIM2 and NLRP3 inflammasomes and triggers pyroptosis in response to cytosolic viral DNA, accompanied by host metabolic remodeling. These findings elucidate key mechanisms of MPOX pathogenesis and identify inflammasome-based pathways as therapeutic targets.

CO.21

CRYO-EM-GUIDED IDENTIFICATION OF AN UNKNOWN VIRUS REVEALS THE FIRST STRUCTURAL REPRESENTATIVE OF THE FAMILY *INSEVIRIDAE*

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Mosquito-derived C6/36 cells are widely used for arbovirus propagation, yet they frequently harbor persistent insect-specific viruses that remain poorly characterized. During cryo-electron microscopy studies of an orthoflavivirus propagated in C6/36 cells, we unexpectedly identified a population of non-enveloped icosahedral particles. Single-particle analysis yielded a 3.49 Å reconstruction of the complete viral particle, while localized reconstruction improved the asymmetric unit to 3.14 Å resolution, enabling *de novo* atomic model building. Structural comparison suggested a toti-like virus; however, no available atomic model adequately fit the density. Manual sequence inference failed to identify the virus by BLASTp searches. To overcome this limitation initially through an entirely *in silico* approach, the cryo-EM map was analyzed with ModelAngelo, and the longest predicted fragment was subsequently queried using BLASTp, identifying *Aedes aegypti* totivirus 2 (AaTV2) as the closest sequence. Using this prediction for molecular confirmation, newly designed primers, two-step RT-PCR, and Sanger sequencing confirmed the viral identity. Independent metagenomic analysis provided a near-complete genome sequence and definitive species identification. The complete capsid sequence enabled refinement of the first atomic model representing the recently established family *Inseviridae*. Phylogenetic analysis demonstrated that the virus previously designated AaTV2 clusters within AaTV, supporting their classification as a single species. Molecular screening further revealed persistent infection in independently maintained C6/36 cell cultures from multiple sources. Beyond providing the first structural representative of the family *Inseviridae*, these findings reveal a previously unrecognized viral contaminant that may influence arbovirus propagation *in vitro* and, potentially, vector competence in mosquitoes.
Financial Support: MCTI

CO.22

GENOMIC CHARACTERIZATION AND INTRAPOPULATION DIVERSITY OF CO-INFECTING ALPHA- AND BETABACULOVIRUSES FROM SPODOPTERA FRUGIPERDA IN BRAZIL

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The fall armyworm, Spodoptera frugiperda, is a major agricultural pest worldwide primarily controlled with chemical insecticides, which may cause environmental impacts and select resistant populations. Baculoviruses are promising biological control agents, yet their genomic diversity and occurrence as mixed infections remain poorly understood. Here, we characterized two co-infecting baculoviruses recovered from S. frugiperda larvae collected in Sete Lagoas, Minas Gerais, Brazil: a novel granulovirus (SpfrGV-SL) and a nucleopolyhedrovirus (SfMNPV-SL). Occlusion bodies were purified and examined by scanning electron microscopy, revealing granular and polyhedral morphotypes. Viral DNA was sequenced using Illumina technology, followed by genome assembly, annotation, comparative genomics, repeat analysis, phylogenomics, and intrapopulation variation analyses. SpfrGV-SL comprised a 142,001-bp genome with 45.7% GC content and 154 predicted ORFs, whereas SfMNPV-SL had a 132,773-bp genome with 50.9% GC content and 144 ORFs. No homologous repeat (hr) regions were identified in SpfrGV-SL, whereas SfMNPV-SL contained eight hrs. SpfrGV-SL encoded two dUTPases, including one sharing 63% amino acid identity with an Achroia grisella dUTPase while retaining conserved catalytic motifs; SfMNPV-SL contained only the orthologous baculoviral dUTPase. Phylogenomic analyses recovered SpfrGV-SL as the most divergent lineage within the SpfrGV clade, whereas SfMNPV-SL showed 99.8% nucleotide identity to the Brazilian isolate i19. Comparison with i19 identified 22 SNPs, eight nonsynonymous. SfMNPV-SL contained 671 intrapopulation SNPs (2.4–36.5%), whereas SpfrGV-SL harbored only 16 SNPs (3.5–9.4%). These findings reveal contrasting genomic diversity between naturally co-infecting alpha- and betabaculoviruses and expand current knowledge of baculovirus evolution in S. frugiperda. Financial Support: CNPq, FAP-DF, Instituto Serrapilheira, CAPES.

CO.23

MAPPING THE DIVERSITY OF INSECT-SPECIFIC VIRUSES IN SOUTH AMERICA

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Arthropods are the largest animal group and harbor a remarkable diversity of viruses, including insect-specific viruses (ISVs). Although ISVs replicate exclusively in insects, they have attracted growing interest because of their potential use in arbovirus biocontrol and their value for understanding the evolutionary transition from single-host to dual-host viruses. A scoping review was conducted to compile all published reports of ISV detection in South America, following the PRISMA 2020 guidelines. The literature on ISVs was reviewed using Web of Science, Scopus, CAPES Periodicals Portal, and PubMed. Search terms combined “mosquito-specific” OR “insect-specific” AND “virus*”, with South American country names included in searches. From the first reported ISV isolation in 1977 through 2026, 572 virus detections representing 58 taxa were identified in 89 insect species across eight South American countries. Most studies were conducted in Brazil (66.4%) and Colombia (28.8%), followed by Argentina (1.8%), Trinidad and Tobago (1.6%), Paraguay (0.5%), Peru (0.5%), Chile (0.2%), and Bolivia (0.2%). ISVs were detected primarily in insects from the families Culicidae, Ceratopogonidae, Crambidae, Psychodidae, and Reduviidae. These viruses were classified into several viral families that also include medically important arboviruses, such as Flaviviridae, Togaviridae, Reoviridae, and Bunyaviridae. Phylogenetic analyses suggest that ISVs, particularly those within Flaviviridae and Bunyaviridae, are ancient viruses that have likely coevolved and diversified alongside their insect hosts. Expanding knowledge of insect-specific viruses advances our understanding of viral evolution and virus–vector interactions, with potential applications in arbovirus surveillance and biological control. This is particularly relevant in South America, a global biodiversity hotspot that experiences recurrent large-scale arboviral epidemics.
FINANCIAL SUPPORT: FAPESP

CO.24

INVESTIGATION OF CHLOROVIRUS OCCURRENCE AND DISTRIBUTION IN WATER AND SEDIMENT SAMPLES AT WESTERN NEBRASKA SPRINGS

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Chloroviruses are large dsDNA viruses known to infect unicellular algae from the *Chlorellacea* family. Although they are traditionally considered aquatic viruses, their ecological roles may be more complex. This study aims to evaluate the extent and type of chloroviruses in Gusher Springs, Western Nebraska, and compare their frequency and diversity in the water column to surface sediments. In October 2025, sampling was conducted at Gusher Springs, a total of 90 water samples and 31 sediment samples were gathered, the physicochemical parameters of those samples (pH, oxygen saturation, temperature, and conductivity) were also analysed. For viral isolation, plaque assays were conducted in lawns with three host algae, *Chlorella heliozoae, Micractinium conductrix,* and *Chlorella variabilis* Syngen 2-3. Following the physicochemical analysis of the samples, oxygen levels were the only parameter to show significant changes when the collection points were compared, ranging from 50% to 94%. A total of 135 viral isolates were obtained infecting *C. variabilis* (73 isolates from water and 49 from sediment) and *C. heliozoae* (2 isolates from water and 11 from sediment samples). No *M. conductrix* isolates were found. Viral plaques exhibited varying sizes and phenotypes, indicating a diverse viral population. This study revealed that chlorovirus particles are significantly more abundant in sediment samples. This finding challenges the existing paradigm that chloroviruses are strictly aquatic viruses. Microscopic evidence suggests that these viruses are replicating in non-*zoochlorella* algae living within biofilms on the sediment. These results emphasize the importance of sediment as a site of replication for chloroviruses in freshwater. Financial support: FAPEMIG, CNPq, SF-EPSCoR, University of Nebraska—Lincoln.

CO.25

ARBOVIRUS CIRCULATION ALONG THE BR-319 HIGHWAY IN AMAZON REGION, BRAZIL, 2024-2026

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The BR-319 highway, connecting the Brazilian states of Amazonas and Rondônia, traverses the Amazon rainforest. *Since its construction in the 1970s, ongoing reconstruction,* increasing land-use pressure*, and environmental degradation have* intensified *human-wildlife interactions,* potentially increasing the risk of viral *emergence/reemergence.* Between 2024 and 2026, we investigated arboviral infection and exposure among symptomatic and asymptomatic humans, as well as domestic animals, from three communities along the highway: Realidade (58.9%), Igapó (36.4%), and Jacaretinga (4.7%). Human serum samples (n = 465; mean age, 40 years; range, 1 month–85 years; 53.4% female) and samples from domestic and farm animals (n = 80; 67.2% dogs, 19.0% cats, 8.6% cattle, and 5.2% pigs) were screened by RT-qPCR for alphaviruses, flaviviruses, and bunyaviruses, and by ELISA, FRNT, and PRNT for antibodies against Mayaro virus (MAYV), Oropouche virus (OROV), Zika virus (ZIKV), chikungunya virus (CHIKV), and dengue virus (DENV). Although all human and animal samples tested negative by RT-qPCR, serological analyses revealed widespread human exposure to multiple arboviruses, with seroprevalence rates of 43.8% for OROV, 42.5% for DENV, 19.0% for CHIKV, 19.0% for ZIKV, and 10.7% for MAYV. Among the animal samples analyzed to date, five dogs had neutralizing antibodies against OROV; notably, all of their owners were also seropositive for OROV. These findings demonstrate widespread exposure to multiple emerging and re-emerging arboviruses along the BR-319 corridor. Moreover, the detection of OROV-neutralizing antibodies in domestic dogs highlights the importance of continuous One Health surveillance in Amazonian hotspots, where environmental disturbance may facilitate viral spillover and spillback between wildlife, domestic animals, and humans.

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CO.26

Serological surveillance of Madariaga virus in equids and domestic birds in Viçosa, state of Minas Gerais, southeast Brazil.

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Madariaga virus (*Alphavirus madariaga*, MADV) is a zoonotic arbovirus that causes neurological disorders in equids, and febrile disease in humans from South and Central America. MADV is believed to circulate in Brazil between *Culex* spp. mosquitoes and wild birds, where equids and humans act as dead-end hosts. To contribute with the active surveillance of MADV in Brazil, we conducted a serosurvey in equids and domestic birds from the rural area of Viçosa, located in the Zona da Mata, state of Minas Gerais, southeast Brazil. From November 2023 to June 2024, blood samples were collected from 148 equids and 89 domestic birds. Of these, 92 equids and 27 domestic birds of the local university had full descriptions of their clinical status, history of traveling and vaccinations. So far, 141 equids and 24 cage-free laying hens were screened by plaque reduction neutralization test ($PRNT_{90}$) for MADV, isolated from equids in 2019 (GenBank: MZ389693.1). Preliminary results indicate 24% (n=12) of the equids from private properties, 15.38% (n=14) of equids of the university herd, and two (8.33%) chickens were reactive to MADV. Among the 14 suspected equids from the university, 13 had no history of traveling or vaccination for equine encephalitis suggesting potential natural and local exposure. Despite confirmatory $PRNT_{90}$ for MADV are still pending, these preliminary results may undiscover potential MADV circulation in rural areas and reinforce the need for continuous monitoring of zoonotic arboviruses in domestic animals, humans and vectors in Brazil. Financial Support: FAPEMIG, CAPES, CNPq e Funarbe.

CO.27

Molecular surveillance of enteric viruses in clinically healthy dogs and cats from Brazil, 2012-2021: detection and genetic characterization of coronaviruses, astroviruses and bocaparvoviruses

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Despite growing awareness of enteric viruses affecting companion animals, molecular epidemiological data from South America remain limited. This study investigated adenoviruses (AdV), astroviruses (AstV), coronaviruses (CoV), bocaparvoviruses (BoV), and picobirnaviruses (PBV) in asymptomatic domestic dogs and cats from Brazil. A total of 600 fecal samples obtained from dogs (n = 516) and cats (n = 84) in São Paulo State between 2012 and 2021 were analyzed using PCR-based assays for AdV, AstV, CoV and BoV, whereas PBV was screened by PAGE. Positive amplicons were sequenced and analyzed phylogenetically. Overall, 49 samples (8.2%) tested positive for at least one virus. AstV and BoV were the predominant viruses (3.7% each; 22/600), followed by CoV (0.8%; 5/600), while AdV and PBV were not detected. AstV and BoV were detected in 3.5% (18/516) and 2.1% (11/516) of dogs, respectively, and in 4.8% (4/84) and 13.1% (11/84) of cats. No viral co-infections were identified. Phylogenetic analyses assigned CoV strains to FCoV-I and CCoV-II and AstV strains to MAstV-5, revealing considerable genetic diversity and the absence of host-associated clustering. BoV comprises FBoV-1 (10/11), FBoV-2 (1/11) and CBoV-2 (2/11) genotypes, all closely related to strains circulating worldwide. Notably, the genetic proximity between CBoV-2 strains infecting domestic and wild carnivores reinforces the relevance of a One Health framework for investigating viral evolution and host adaptation. This study represents the first comprehensive molecular survey of feline and canine bocaparvoviruses in Brazil and demonstrates that clinically healthy companion animals may contribute to the unnoticed circulation of enteric viruses. FAPESP #2020/14786-0; FAPESP #2025/25687-6; CNPq #310056/2023-3; CAPES #2022-0/88881.689557; PROAP-AUXPE #2022/115; FESIMA CAF #049/2025.

CO.28

Genomic Surveillance Uncovers Silent HPAIV H5N1 2.3.4.4b Circulation in Brazilian Wildlife

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The global expansion of highly pathogenic avian influenza virus (HPAIV) H5N1 clade 2.3.4.4b across multiple host species has emphasized the need for integrated genomic surveillance strategies to improve wildlife monitoring and early detection of emerging viral threats. In this study, swab samples collected from wild birds and mammals along the coast of Paraná and in the northwestern region of São Paulo, Brazil, were investigated for avian influenza virus (AIV) detection. A total of 661 animals were analyzed, including individuals that showed no clinical signs or histopathological findings suggestive of avian influenza during monitoring activities. Molecular screening identified three AIV-positive animals (0.45%; 95% CI: 0.16–1.33), including two avian species (*Sula leucogaster* and *Thalasseus acuflavidus*) and one marine mammal (*Otaria flavescens*). Whole-genome sequencing identified HPAIV H5N1 clade 2.3.4.4b in a Brown Booby (*Sula leucogaster*) sampled on the Paraná coast (GISAID: EPI_ISL_1897537). Phylogenetic and phylogeographic analyses revealed the relationship of this genome with HPAIV H5N1 sequences previously detected in southern and southeastern Brazil, providing insights into viral diversity and spatial dissemination patterns. The detection of HPAIV H5N1 genomic sequences in samples from non-suspect wildlife cases highlights the importance of molecular and genomic approaches as complementary tools for virus monitoring. These findings reinforce the relevance of genomic surveillance within a One Health framework to characterize viral circulation, evolution, and potential dissemination pathways in wildlife populations.

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CO.29

Systemic movement of a cilevirus upon mechanical inoculation of *Nicotiana benthamina* plants by an infectious clone

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The genus *Cilevirus* (family *Kitaviridae*) comprises plant-infecting viruses with bipartite (+)ssRNA genomes. In nature, cileviruses cause non-systemic diseases with infection remaining restricted to cells at the inoculation sites established during *Brevipalpus* mite feeding. Solanum violifolium ringspot virus (SvRSV; *Cilevirus solanum*), a member of subgroup 2 of the genus, naturally infects *Solanum violifolium* plants and is transmitted by *B. obovatus* and *B. yothersi*. To investigate SvRSV biology, we generated an infectious clone and agroinoculated transgenic *Nicotiana benthamiana* plants expressing the TuMV HC-Pro RNA silencing suppressor. Sap extracts from agroinfiltrated leaves were then mechanically inoculated onto WT and HC-Pro-expressing *N. benthamiana* plants. Fourteen days after inoculation (dai), local symptoms appeared on inoculated leaves of both genotypes. Unexpectedly for cilevirus infections, chlorotic ringspots and atypical lesions developed on non-inoculated apical leaves of WT plants at 20 dai. RT-PCR and transmission electron microscopy detected viral RNA, abundant virions and viroplasms in both inoculated and upper leaves, confirming systemic infection. Symptoms subsequently appeared on additional non-inoculated leaves, and the virus was detected in roots between 28 and 40 dai. In contrast, lesions on inoculated leaves of HC-Pro plants progressed to severe necrosis, whereas systemic leaves and roots remained virus-free. Although these observations were obtained under laboratory conditions in an experimental host for SvRSV, they provide the first evidence of systemic movement by a cilevirus, suggesting the involvement of a complex plant-virus interplay hampering viral systemic infection in nature. Ongoing studies are investigating the roles of RNA silencing and hormone-mediated defense pathways in this process.

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CO.30

IN-CELL CRYO-ELECTRON TOMOGRAPHY OF ZIKA VIRUS REPLICATION ORGANELLES: FROM A SUBGENOMIC REPLICON TO AUTHENTIC INFECTION

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Zika virus (ZIKV) is an Aedes-transmitted flavivirus associated with neurological complications including congenital Zika syndrome in newborns of infected pregnant women and Guillain-Barré syndrome in adults. Despite its re-emerging potential, no antivirals or vaccines are available. ZIKV remodels the host's endoplasmic reticulum (ER) into viral replication organelles (VROs), essential for genome replication and viral propagation. Here, we combined antiviral characterization with in-cell cryo-electron tomography (cryo-ET) to investigate whether replication inhibition alters VRO architecture. We first analyzed a mammalian cell line harboring a subgenomic replicon, in which structural genes are replaced by a luciferase reporter. Cells were treated with three different compounds: Remdesivir (RDV), ASAP-0036543 and (+)-JNJ-A07, targeting, respectively, the viral polymerase, the viral protease and the NS3–NS4B interaction. Remdesivir and ASAP-0036543 inhibited replication (EC50 1.23 and 1.60 µM at 24 h; 0.17 and 0.11 µM at 48 h), while (+)-JNJ-A07 was inactive. Next, non-treated replicon cells grown on grids were thinned by cryo-focused-ion-beam milling and imaged by cryo-ET. No VROs were identified, only sparse ER-associated vesicles of compatible size, indicating that replication alone does not reconstitute ER architecture. We therefore moved to the analysis of infected Huh7 cells, using anti-double-stranded-RNA immunofluorescence and observed that RDV reduced infection dose-dependently (MOI 10) and remained effective when added 18 h post-infection (MOI 30), whereas ASAP-0036543 showed minimal effect. Tomograms of untreated cells revealed abundant VROs, consistent with reported dimensions. Our findings establish an in-cell cryo-ET approach to investigate antiviral action on ZIKV replication, with cryo-ET of RDV-treated cells ongoing. Financial support: FAPESP (process nº 2025/21936-1)

CO.31

HUMAN METAPNEUMOVIRUS INDUCES A GLOBAL H3K27ME3 REDUCTION TO DRIVE THE TRANSITION FROM COLD TO HOT PHENOTYPES IN A LUNG ADENOCARCINOMA MODEL

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Lung adenocarcinoma (LUAD) frequently presents as immunosuppressive "cold" tumors, driven by PRC2-mediated H3K27me3 epigenetic silencing. Transitioning to inflamed "hot" tumors is critical for effective antitumor responses. Viral infections offer a powerful mechanism to drive this shift by directly infecting tumor epithelial cells and establishing an acute inflammatory microenvironment. Here, we identify human metapneumovirus (hMPV), a respiratory virus triggering pulmonary inflammation, as a potent epigenetic reprogrammer in a cancer model. We hypothesize hMPV infection transitions cold to hot LUAD phenotypes via global H3K27me3 reduction, derepressing inflammatory networks. We established an infection model using an hMPV-GFP reporter (MOI 0.1) in 3D-A549 LUAD and normal bronchial epithelial cultures. We applied a multi-omics approach integrating H3K27me3-ChIP-seq, RNA-seq, RT-qPCR, confocal microscopy, and OLINK proteomics with GSEA and EnrichR to correlate transcriptomes with ChIP-seq databases. Patient-derived PBMC transcriptomics evaluated systemic chemotactic responses. Tumor cells exhibited high viral replication coupled with a global H3K27me3 decrease. Transcriptomics revealed a transition toward a "hot" phenotype, driven by EZH2 downregulation and KDM6A upregulation. Analyses confirmed that derepressed genes with baseline EZH2 occupancy drove chemotaxis, antigen presentation (MHC-I/II), apoptosis, and G2/M arrest. Matched RNA-seq/OLINK profiling revealed robust secretion of antitumor chemoattractants *CXCL10* and *CXCL11*. Furthermore, patient transcriptomics showed peripheral CXCR3 repression, suggesting infiltration originates from lymphoid organs within the pulmonary niche. In conclusion, hMPV actively dismantles H3K27me3-mediated repression to reprogram LUAD cells. Our findings highlight a novel viro-epigenetic mechanism, providing a promising framework to utilize respiratory viruses as innovative immunotherapies and epigenetic priming strategies against refractory solid tumors.

CO.32

Mayaro virus remodels stress granules and depends on PKR for efficient replication

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Viruses have co-evolved with host cells and exploit cellular machinery to ensure efficient replication while evading antiviral responses. Stress granules(SGs) are cytoplasmic structures that form upon stress and have been traditionally categorized as playing antiviral roles. However, alphaviruses reorganize SGs to favor replication. During alphavirus infections, canonical SGs are suppressed while virus-modified granules(vmGs) are induced, a process mediated by the viral protein nsP3, which remodels these structures to support infection. The host PKR and RNase L pathways cooperate in the assembly of SGs and RNase L-dependent bodies (RLBs) with antiviral activity. In this study, we investigated the role of PKR and RNase L in *Mayaro virus* (MAYV) infection, their relationship with vmG formation, and the impact of prior cellular stress on viral replication. A549 parental, $PKR^{-/-}$, and RNase $L^{-/-}$ cells were infected with MAYV-eGFP and MAYV-HA to assess viral replication and vmG formation. RNase L deficiency resulted in only a modest increase in viral titers, whereas PKR deficiency modestly reduced viral replication and the percentage of infected cells. MAYV induced vmGs in both conditions, with clear signal overlap between the stress-granule marker G3BP1 and viral nsP3. However, vmGs in PKR knockout cells appeared smaller than those in parental cells. Additionally, inducing cellular stress by blocking calcium pumping into the endoplasmic reticulum with 1 µM thapsigargin prior to infection drastically reduced viral replication, indicating that this form of cellular stress can affect MAYV replication. These findings indicate that PKR supports efficient MAYV replication, whereas RNase L exerts a modest antiviral effect.

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CO.33

SMARTPHONE-ASSISTED PLATAFORM FOR RAPID MPXV DETECTION USING COLORIMETRIC LOOP-MEDIATED ISOTHERMAL AMPLIFICATION

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Monkeypox virus (MPXV) re-emerged as a global public health concern in 2022, highlighting the urgent need for rapid, affordable, and decentralized diagnostic tools capable of overcoming the limitations of laboratory-based methods such as polymerase chain reaction (PCR). Herein, we developed a molecular point-of-care (mPOC) platform for mpox detection based on a colorimetric loop-mediated isothermal amplification (LAMP) assay. Six primers targeting highly conserved regions of the viral genome were designed, and the amplification conditions were optimized using a design of experiments (DoE) combined with chemometric analysis to maximize assay performance. To provide an objective and quantitative readout while eliminating the subjectivity associated with visual interpretation, a smartphone-assisted digital analysis was integrated using images acquired in a custom-designed 3D-printed imaging device. Colorimetric responses were quantified using the Value (V) parameter of the HSV (Hue, Saturation, Value) color space, enabling reliable discrimination between positive (n=12) and negative (n =16) samples. The proposed platform demonstrated excellent diagnostic performance, achieving an area under the receiver operating characteristic (ROC) curve of 0.91, supporting its suitability for decentralized testing. Furthermore, analytical specificity was confirmed by the absence of cross-reactivity with Vaccinia virus and others. Overall, the developed platform combines isothermal amplification with smartphone-based image analysis to provide a low-cost, portable, and user-friendly diagnostic strategy for MPXV detection. This approach transforms a widely available mobile device into an accurate molecular diagnostic tool, representing a promising alternative for mPOC applications, particularly in resource-limited and decentralized healthcare settings.

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CO.34

OPTIMIZING AN ARYLFURAN DERIVATIVE INHIBITOR TARGETING ZIKA AND DENGUE VIRUS PROTEASES: IMPROVING *IN VITRO* POTENCY AND MULTI-VIRAL INHIBITION DRIVEN BY COMPUTATIONAL SIMULATIONS

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Global outbreaks of dengue and Zika fever have occurred in recent years, the largest to date happening in Brazil in 2024, with approximately 6.5 million cases of dengue fever and over 6,000 deaths, as well as over 40,000 cases of Zika fever. Currently, there are no antiviral drugs to treat these infections, for which the NS2B-NS3^{pro} enzyme complex is a promising target to design optimized inhibitors having the potential for a multi-flavivirus activity against ZIKV and DENV. We identified an arylfuran derivative that inhibited the ZIKV protease (IC_{50} of 17 μM) but showed evidence of acting through aggregation. Thus, we designed and assessed the structure-activity relationship of eight new structural analogs, using a combination of molecular docking and molecular dynamics simulations, as well as aggregation predictions. Two out of eight compounds were predicted as potential multi-inhibitors. We synthesized all eight compounds and tested against ZIKV and DENV proteases. One compound showed inhibitory activity against both enzymes, supporting computational predictions, despite showing fluorescence interference. Three other compounds were active against the DENV protease in low micromolar range (*e.g.*, IC_{50} of 19 μM) and showing no promiscuous inhibition behavior, also supporting the computational predictions. The confirmed inhibitors are to experimentally validated in antiviral assays *in vitro*. Notably, designing new structural analogs using computational simulations is still a promising approach to identify new therapeutic starting points targeting viral proteases, ultimately aiming for broad-spectrum antiviral drug candidates to tackle the ongoing threat of current and future flavivirus infections.

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CO.35

TARGETING THE HOST TO HALT THE VIRUS: A MAPK INHIBITOR AS A POTENT REPURPOSING CANDIDATE AGAINST CHIKUNGUNYA VIRUS

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Chikungunya virus (CHIKV) is characterized by explosive replication kinetics and rapid dissemination, leaving the host immune system struggling to mount a timely defense. The virus achieves this speed by hijacking host signaling pathways, specifically the MAPK (MEK/ERK) cascade, to fuel its life cycle. But, despite the clear role of MAPK in viral replication, there are currently no approved antivirals that exploit this vulnerability. Therefore, we investigated the repositioning of trametinib, a clinically approved MEK1/2 inhibitor, as a strategy to "blindside" CHIKV replication. We evaluated trametinib's efficacy through asynchronous and synchronous growth kinetics, virucidal, dose-response, antiviral kinetics, antigen detection, and ultrastructural microscopy assays. Our results reveal that trametinib acts as a potent antiviral, slashing infectious particle production by a massive 1,000-fold. Furthermore, we observed a 70-fold decrease in total viral particles. Ultrastructural analysis pinpointed the mechanism: trametinib disrupts early-stage membrane remodeling and the assembly of viral replication complexes. To our knowledge, this is the first evidence that a selective, FDA-approved MEK inhibitor can neutralize CHIKV in vitro. By repurposing an existing drug, we significantly reduce development timelines and costs. These findings suggest that inhibiting host MEK can "decelerate" viral amplification enough to allow immune clearance, offering a high-impact therapeutic strategy for managing CHIKV outbreaks.

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CO.36

Nanoencapsulation of *Spodoptera frugiperda multiple nucleopolyhedrovirus* in NaDES-stabilized lipid nanocarriers for enhanced biological control

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The increasing demand for sustainable pest management has intensified interest in enhancing the field performance of baculovirus-based bioinsecticides against the fall armyworm, *Spodoptera frugiperda*. To overcome environmental instability, this study evaluates the encapsulation of *Spodoptera frugiperda multiple nucleopolyhedrovirus* (SfMNPV) within lipid nanocarriers stabilized by natural deep eutectic solvents (NaDES), composed of murumuru butter, soybean oil, and tomato hydrolate. The methodology integrated nanocarrier synthesis, ultrastructural analysis via transmission electron microscopy (TEM), in vitro cytotoxicity assays (MTT and Alamar Blue) on insect (Sf9) and mammalian (L132) cell lines, and in vivo mortality bioassays on 2nd and 3rd instar caterpillars. TEM confirmed the successful encapsulation of viral DNA and budded virions. In vitro assays revealed a dose-dependent cytotoxicity of the nanocarriers, demonstrating that formulations below 0.1% preserve cell viability, whereas higher concentrations induce acute cellular toxicity. Initial in vivo results showed that formulations lacking NaDES caused mortality due to direct carrier intoxication rather than viral infection, while NaDES-stabilized formulations exhibited better biocompatibility but sub-optimal viral mortality. Consequently, updated bioassays applying a higher viral load specifically to 3rd instar larvae are currently underway to overcome midgut release barriers and maximize infectivity. In conclusion, integrating green nanotechnology with viral biocontrol offers a robust framework for eco-friendly pest management, provided the viral load-to-carrier ratio is optimized to balance efficacy and nanocarrier biocompatibility.

Financial Support: CNPq, FAPDF, Embrapa, Bionte.

CO.37

Ecology and Evolution of avian influenza viruses in South America: A continental synthesis before the HPAI H5N1

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Understanding how avian influenza viruses (AIVs) circulated in South America before the introduction of H5N1 is essential for interpreting the current epidemiological scenario. For the first time, an integrative eco-evolutionary framework was produced by combining a systematic synthesis of surveillance studies (2000–2024), available genomic data, ecological metadata and phylogenetic analyses to reconstruct the continental history of LPAI AIV circulation. A total of 73 surveillance studies comprising more than 120,000 samples were integrated into a standardized database together with curated genomic records retrieved from public repositories. Epidemiological, ecological, maximum likelihood, and Bayesian analyses were subsequently combined to investigate surveillance patterns, subtype diversity, host associations, and evolutionary relationships. Despite extensive surveillance efforts, AIV detection remained consistently low across South America, while subtype diversity was concentrated in waterfowl and Charadriiformes, with characteristic host-specific subtype distributions. Comparative analyses of internal genomic segments consistently recovered two major South American phylogenetic, one associated to southern cone, and other associated to northern and central regions, with each groups showing contrasting ecological composition. Bayesian phylogeographic analyses further supported distinct transitions among these phylogroups, revealing each major central host and pattern of dissemination. Together, these independent lines of evidence reveal that LPAI circulation in South America is organized as structured eco evolutionary system maintained by distinct host communities and regional evolutionary lineages. This study establishes the first continental baseline integrating surveillance, ecology and viral evolution before the H5N1 panzootic and provides a comprehensive framework for interpreting the emergence and future evolution of avian influenza viruses in South America.

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CO.38

Evolutionary Dynamics of SARS-CoV-2 XBB-FLip Lineages in Brazil, 2023

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In Brazil, SARS-CoV-2 XBB lineages expanded rapidly in early 2023 following the sequential acquisition of key Spike substitutions (S:F486P, S:F456L, and S:L455F), with the latter two defining the XBB-FLip lineages. This study investigated whether their spread during the second half of 2023 was driven mainly by convergent evolution or by the heterogeneous spatial connectivity across Brazil. We generated 4,856 complete SARS-CoV-2 genomes through the Fiocruz and Lacen Genomic Networks and integrated genomic, epidemiological, and human mobility data using phylogenomic and statistical approaches. A total of 78 XBB-FLip lineages were identified between August and November 2023, of which 20 independently acquired the Spike substitution S:A475V through convergent evolution and became dominant by October-November. Phylogeographic reconstruction indicated that 19 major XBB-FLip lineages originated within Brazil, emerging in the Southeast (7), North (8), Northeast (2), and Central-West (2) regions. The Southeast acted as the principal source of viral dissemination, accounting for approximately 65% of all primary migration events, whereas the remaining regions each contributed about 10%. Negative binomial regression demonstrated that both air and road passenger connectivity were strongly associated with the number of primary viral migrations. Furthermore, S:A475V-positive lineages displayed approximately 65% more viral migrations beyond their epicenter and an estimated 10% relative fitness advantage compared with S:A475V-negative lineages. These findings indicate that the spread of XBB-FLip lineages in Brazil resulted from the combined effects of convergent viral evolution and heterogeneous human mobility, emphasizing the importance of integrated genomic surveillance for the early detection and monitoring of highly transmissible SARS-CoV-2 variants.
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From viral genotypes to phenotypes: resurrecting paleoviruses to unravel HPV biology

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Human papillomaviruses (HPVs) have co-evolved with humans for hundreds of thousands of years, yet the evolutionary origins of HPV16 oncogenicity remain inferred almost exclusively from modern genomes. Ancient viral genomes provide a unique opportunity to directly investigate how oncogenic traits emerged. We aim to reconstruct ancient HPV genomes from archaic and prehistoric humans, identify ancestral variation in the E6 and E7 oncogenes, and experimentally test the phenotypes of reconstructed ancient HPVs generated by synthetic genomics. Ancient sequencing datasets from Neanderthals (~50 kya), Ust'-Ishim (~45 kya), and the Tyrolean Iceman Ötzi (~5.3 kya) were screened for HPV DNA. Viral sequences were authenticated using ancient DNA damage profiles, genome coverage, randomized controls, phylogenetic analyses, and consensus genome reconstruction. HPV sequences were detected in Neanderthal datasets, whereas high-confidence HPV16 genomes were reconstructed from Ust'-Ishim and Ötzi. These findings demonstrate that oncogenic HPV16 lineage A infected anatomically modern humans at least 45,000 years ago. Phylogenetic analyses placed the Ötzi genome closest to lineage A1 and the Ust'-Ishim genome closest to lineage A4. Both ancient genomes carried nonsynonymous substitutions in E6 and E7 relative to contemporary HPV16 reference sequences, revealing previously unknown ancestral protein variants that may have influenced viral fitness and oncogenic potential. We are synthesizing reconstructed ancestral HPV16 genomes carrying ancient E6/E7 variants to determine whether ancestral viral genotypes produce distinct molecular and cellular phenotypes. This paleovirological strategy establishes a direct genotype-to-phenotype framework for investigating the evolution of HPV oncogenicity across human prehistory. Financial Support: FAPESP, CNPq.

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A DIVERGENT POXVIRUS ASSOCIATED WITH CUTANEOUS LESIONS IN VULNERABLE GIANT ANTEATERS (*Myrmecophaga tridactyla*) FROM BRAZIL

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Poxviruses infect diverse hosts, yet their diversity and impact on wildlife remain poorly understood. This study characterized cutaneous lesions associated with a divergent poxvirus in free-ranging giant anteaters monitored in a conservation program from Brazil, using macroscopic, histopathological, ultrastructural, and molecular approaches. Among 17 skin lesions examined, two adult female giant anteaters presented ~0.5-cm nodular lesions on the limbs. Histopathology revealed epithelial hyperplasia and poxvirus-associated cytopathic changes, including intracytoplasmic inclusion bodies. Transmission electron microscopy identified elliptical poxvirus-like particles (336–555 nm × 196–302 nm). Molecular screening detected poxvirus DNA in both lesions through amplification of a high-GC region of the RNA polymerase gene. The positive samples were sequenced and phylogenetic analyses were conducted. A 400-bp fragment showed 88% nucleotide identity to molluscum contagiosum virus. According to ICTV criteria (>98% nucleotide identity for species demarcation), the detected virus represents a divergent poxvirus lineage. Previous reports of poxvirus infections in giant anteaters include severe multispecies outbreaks and self-limiting cutaneous disease; however, the lesions observed here were clinically benign and not associated with systemic disease. Given the vulnerability of giant anteaters and their slow population recovery, infectious diseases may represent an additional conservation challenge. Whole-genome characterization is underway to further define the evolutionary position and genomic features of this virus. These findings expand knowledge of poxvirus diversity in wildlife and emphasize the importance of molecular surveillance for detecting viruses in threatened species.

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