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Apresentações Orais

CO.01 a CO.30

20/9/2024

CO.01

UNRAVELING THE NEUROTROPIC POTENTIAL OF OROPOUCHE VIRUS IN THE ADULT HUMAN BRAIN

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Neurotropic viruses can infect the central nervous system (CNS), leading to acute and chronic effects including cognitive impairment. The emergence of neurological symptoms associated with arboviruses such as Zika has raised concerns about the neurotropism of other viruses, including the Oropouche virus (OROV). It has been shown that OROV can reach the CNS and cause meningoencephalitis. However, the extent and mechanisms of OROV damage to the human brain remain debated. Here we have used human brain-derived tissue cultures to investigate the cellular-molecular alterations underlying infection by OROV. Adult human brain slice cultures were prepared from cortical tissue obtained during ressective neurosurgery (Ethics committee approval HCRP #17578/15). Using expansion microscopy, we confirmed that OROV infects human neural cells. OROV infected mainly microglia but also neurons to a lesser extent. In line with this, we have seen that a human microglial cell lineages supports virus production in the absence of cytopathic effects at 48h post-infection. OROV-infected brain slices exhibited increased cytotoxicity (LDH release assay) and up-regulation of pro-inflammatory cytokines. Experimental infection with HSV-1 in brain slices from the same donors revealed that OROV triggered similar levels of toxicity and inflammatory response as HSV-1, a neurotropic virus known to cause encephalitis in adults. The distribution of infected cells differed between OROV- and HSV-1-infected slices, suggesting distinct mechanisms of infection and spreading throughout the cortical layers. Our results highlight the neurotropic potential of OROV to the mature human brain and underscore the importance of further studies on the neurological consequences of OROV infection.

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CO.02

Measles virus mutates during *in vitro* infection of human neurons

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Measles is an infectious disease caused by the Measles virus (MeV), resulting in respiratory and gastrointestinal illnesses, skin rashes, and long-term immunosuppression. The virus can persist in the body, potentially leading to the development of subacute sclerosing panencephalitis (SSPE). The virus produced by neural infection presents mutations in the genes responsible for the H and F proteins, preventing its budding and consequently hindering its isolation. Here we investigated the pathophysiology of viral infection in human neural progenitor cells (NPC) and neurons derived from iPSC. Our findings revealed that both NPC and neurons are susceptible to MeV infection. In NPC, there was a significant increase in viral load in the supernatant, whereas in neurons, the viral load remained stable without significant increase. Using transmission electron microscopy, we demonstrated the formation and budding of virions in NPC and neurons. Viruses generated from neurons undergo mutations in the N gene, which is a possible cause of the low viral budding rate. In conclusion, understanding the effects of MeV infection on the cellular components of the brain is fundamental for advancing our knowledge of CNS infections related to measles. The observed susceptibility of NPC and neurons to MeV infection highlights the need for targeted therapeutic strategies to prevent or mitigate neurological complications caused by this viral pathogen. Additionally, the demonstration of mutations in the in vitro infection of neurons suggests that mutations may occur after infection.

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

CO.03

SARS-COV-2 GENOMIC VARIANTS AND THEIR RELATIONSHIP WITH THE EXPRESSIONAL AND GENOMIC PROFILE OF ANGIOTENSIN CONVERTING ENZYME 2 (ACE2) AND TRANSMEMBRANE SERINE PROTEASE 2 (TMPRSS2)

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SARS- CoV-2 became a global concern in 2020. ACE2 and TMPRSS2 viral receptors were vastly studied, although information on the influence of SARS-CoV-2 variants in the behavior of these receptors remains lacking. So, this study aims to elucidate ACE2/TMPRSS2 nasopharyngeal expression and genomic profiles in response to SARS-CoV-2 variants. For that, 160 SARS-CoV-2-positive nasopharyngeal samples were selected. RNA/DNA were extracted, and ACE2/TMPRSS2 expression was assessed via RT-qPCR. Additionally, SARS-CoV-2 variants were identified using nanopore sequencing and Single Nucleotide Polymorphisms (SNP) in ACE2/TMPRSS2 encoding genes. B.1.1.28, Zeta, Gamma, and Omicron variants were identified in this study. ACE2/ TMPRSS2 levels were higher in response to B.1.1.28 than Omicron (and to Gamma for TMPRSS2). SARS-CoV-2 induces ACE2 expression via interferon signaling, but Omicron´s Spike has a higher ACE2 binding affinity, thus a higher ACE2/Spike affinity potentially reduces ACE2 levels. TMPRSS2 is also induced by SARS-CoV-2, since protease expression is regulated by GATA2, which also regulates cytokine levels such as IL1β, so during infection, IL1β is stimulated, with TMPRSS2 alongside. In Omicron infections IL1β levels are reduced, and the inefficiency of Omicron in using the protease pathway suggests a reduction in TMPRSS2 activity. Only one SNP (rs2285666) showed an increased frequency in response to Omicron compared to B.1.1.28. This SNP increases ACE2/Spike affinity, reinforcing the hypothesis mentioned above. Health professionals expressed higher ACE2/TMPRSS2 levels, possibly due to their higher exposure to SARS-CoV-2, although other factors should be considered.

Financial Support: Fundação de Amparo à Pesquisa e Extensão Universitária (FAPEU - 4/2022 - UFSC).

CO.04

DIFFERENTIAL BST2/THETERIN DOWNMODULATION BY SARS-CoV/2 SPIKE AND ORF7a PROTEINS

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The BST2 (bone marrow stromal cell antigen 2), also known as Theterin, is a cellular membrane protein induced by type I interferon (IFN-I) and restricts viral release. The antiviral function of BST2 was initially demonstrated in infection by human immunodeficiency virus type I (HIV-I) and later in infection by Lassa, Marburg, Ebola, dengue (DENV) viruses, and coronaviruses such as SARS-CoV and HcoV-229E. In this study, we investigated the ability of the spike (S) and ORF7a viral proteins from SARS-CoV and SARS-CoV-2 to modulate BST2. Expression of S and ORF7a proteins followed by flow cytometry and western blot assays revealed that these two viral proteins from both viruses promote the BST2 downmodulation. However, the SARS-CoV-2 S protein demonstrated an enhanced downmodulation of BST2. Furthermore, the SARS-CoV-2 ORF7a protein showed a greater capacity for BST2 deglycosylation, and the ORF7a transmembrane domain was essential for its interaction with BST2. Interestingly, qPCR assay showed that only the SARS-CoV-2 S protein induced the IFN-α gene expression, although this effect did not affect the BST2 gene expression. These preliminary results showed important differences between both β-coronaviruses regarding BST2 downmodulation. Additional studies are being conducted to elucidate the molecular mechanisms involved in the viral proteins-mediated BST2 downmodulation and the possible impact on the pathogenesis of SARS-CoV-2 infection.

Financial Suport: Fundação de Apoio à Pesquisa do Distrito Federal (FAPDF); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)

CO.05

DNA METHYLATION ANALYSIS IN BRAINS AFFECTED BY CONGENITAL ZIKA VIRUS INFECTION

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The Zika virus (ZIKV) symptomatic infection in humans generally presents mild symptoms but raises serious public health concerns due to vertical transmissions. Within the fetal environment, the virus shows tropism for the neural progenitor cells, leading to severe congenital neurological malformations. Despite recent advances in understanding ZIKV replication and neuropathogenesis in the developing central nervous system (CNS), little is known about epigenetic mechanisms involved in this process. Therefore, the goal of this study is to identify differentially methylated regions (DMRs) in the DNA of human brains affected by congenital ZIKV infection and validate the findings in an animal model. Through a genome-wide epigenetic association study, we identified 4 DMRs in the genes: *IFI44*, *IQSEC1*, *THBS2-AS1*, and *OR8B8*. Among these genes, *IQSEC1* was selected as a candidate for validation in a murine model due to its biological plausibility. We observed a significant reduction in *Iqsec1* expression in brains of Swiss mice infected with ZIKV during gestation compared to controls at E18 and P0. Global methylation analysis of the *Iqsec1* promoter region did not show significant differences between groups. However, site-specific analyses identified two CHH sites significantly hypomethylated in infected animals (FDR < 0.05), and 12 sites (nine CHH, two CHG, and one CpG) had suggestive values (FDR < 0.06), as evidence for epigenetic modulation by ZIKV in this region. Thus, this study contributes with a pioneering evaluation of DNA methylation patterns in human brain samples and identifies candidate genes for further validation studies.

Financial Support: CAPES, CNPq

CO.06

Antioxidant defense mechanisms modulation in the soybean caterpillar, *Anticarsia gemmatalis* during baculovirus infection

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Defoliator lepidopterans, like the soybean caterpillar A. gemmatalis can develop strategies to counteract stress induced by control methods. For instance, while chemical insecticides are known to cause oxidative stress (OS) through reactive oxygen species (ROS) production, the mechanisms by which insects resist baculovirus-based biopesticides are less clear. OS is typically managed by superoxide dismutase (SOD) and catalase (CAT) activities in lepidopterans by decreasing levels of ROS. Here we aimed to characterize antioxidant-related genes in the soybean caterpillar to understand how its natural baculovirus, AgMNPV, influences the host's oxidative stress response during infection. Thereby, cells were infected in vitro with AgMNPV (MOI 0.1) and analyzed at 0, 24, 48, and 72 h post-infection (hpi). Gene transcription was assessed via RT-qPCR on RNA extracted from infected and non-infected cells, focusing on cat, cytoplasmic and mitochondrial sod, and the transcription factor nrf-2, which regulates antioxidant-related gene expression. ROS levels, SOD and CAT activities, glutathione levels, and lipid peroxidation were measured. As result, we observed during AgMNPV infection a decrease in ROS levels at 72 hpi and CAT activity at 24 hpi compared to the control, with no significant changes in SOD activity, lipid peroxidation or glutathione levels. However, transcription of the two sod isoforms, sod cu/zn and sod mn decreased in 48 and 72 hpi respectively, while nrf-2 transcription decreased at 48 and 72 hpi. These findings suggest that AgMNPV manipulates the host's oxidative stress response by altering transcription and enzyme activity in the caterpillar's antioxidant defense.

Financial Support: CNPq; CAPES

CO.07

Spiroplasma shows a *Wolbachia*-like effect in hampering virus replication in spider mite

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Members of the Acari order, particularly within the Tetranychidae family, are significant agricultural pests due to their extensive diversity, encompassing approximately 1,200 species capable of infesting over 4,000 plant species. These mites not only cause direct crop damage but also serve as vectors for viral pathogens. In this study, we explored the virome of *Tetranychus truncatus* to elucidate the effects of biotic factors (*Spiroplasma* and *Wolbachia* endosymbionts) and abiotic stresses (abamectin and temperature) on viral dynamics. Our metatranscriptomic analyses revealed sequences from prominent viral families infecting arthropods and plants, including the plant-pathogenic *Potato virus Y* and *Cherry virus A*, as well as fourteen newly identified species. These new species were distributed across various viral families: *Nudiviridae* (1, circular-DNA virus); *Kitaviridae* (2), *Dicistroviridae* (2), *Botourmiaviridae* (1), *Virgaviridae* (1), *Betaflexiviridae* (1), and *Nodaviridae* (1) (ssRNA(+) viruses); and *Phenuiviridae* (2, segmented ssRNA(-) viruses). Abamectin treatment was notably associated with the absence of *Potato virus Y* and TtDV-2, suggesting a significant impact of this pesticide on viral diversity. Both *Wolbachia* and *Spiroplasma* single infections led to a decrease in viral diversity and abundance, particularly affecting dicistroviruses, highlighting for the first time the potential of *Spiroplasma* in hampering viral replication. Interestingly, co-infection with *Wolbachia* and *Spiroplasma* anulled this virus restriction effect. Gene expression analysis revealed that responses exclusive to *Wolbachia* and *Spiroplasma* were enriched in similar pathways. Overall, we showed the unexpected interplay within *T. truncatus* microbiome highlighting the putative role of Spiroplasma on virus restriction.

Financial support: CNPq

CO.08

Mycoviruses inhabiting the fungal cultivar of leafcutter ants elicit defensive RNAi immune responses in their fungal host

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Animal-microbe symbioses are ubiquitous, yet the extent to which reduced immune responses in hosts support these close relationships is often not well understood. Studying host RNA interference (RNAi) pathways, particularly their completeness, functionality, and specificity, provides a promising approach to determine whether and how host immune systems have coevolved with microbial partners. We investigate RNAi pathways in the basidiomycete fungus *Leucoagaricus gongylophorus*, which is cultivated by leafcutter ants, focusing on the fungal immune responses to two newly discovered mycoviruses cohabiting the fungus. Using *in silico* analyses of *L. gongylophorus* DNA and RNA, we found that its genome encodes a full RNAi pathway, which includes the core genes Dicer and Argonaute. Additionally, we also identified RdRp genes that are commonly found in fungi and plants. We then demonstrate that the pathway is active, showing transcriptional activity of these genes and that the resulting small RNAs (sRNAs) show characteristic length of small interference RNAs (siRNAs) generated by RNAi-mediated cleavage. Moreover, we show that each mycovirus induces a different level of immune response in the host, as the abundance and profile of putative virus-derived siRNAs vary significantly. Therefore, we propose that the two mycoviruses have distinct symbiotic roles, with the highly targeted mycoviruses being an opportunistic pathogen and the non-targeted mycoviruses serving as either a commensal or a mutualist. These findings illustrate the broad potential of RNAi pathway analyses to use immune responses to explore hypotheses about the often-hidden costs and benefits that characterize host-microbe symbioses.

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CO.09

Inovirus alters the extracellular vesicle production profile of *Ralstonia pseudosolanacearum,* affecting the plant immune response

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Ralstonia solanacearum species complex comprises Gram-negative phytopathogenic bacteria that cause bacterial wilt disease, which greatly interests the agricultural sector. Bacterial extracellular vesicles (BEVs) are nanoparticles with complex composition and structure. Bacteriophages can be an excellent biocontrol strategy for bacterial wilt, as they alter diverse cell aspects, including the production of BEVs. Our objective is to characterize the BEVs of RSSC in the context of viral infection by an Inovirus and to analyze the plants' response to BEVs. BEVs produced by *Ralstonia pseudosolanacearum* (GMI1000) and the RSBR1 Inovirus-infected (G7) strains were quantified by flow cytometry, and Zeta Sizer measured their size. Gene expression associated with the immune response of tomatoes infected with BEVs and cells was investigated by RT-qPCR. We observed homogeneity in the sizes of the GMI1000 BEVs, unlike the G7 BEVs, which had a more significant variation in size; also, vesicle quantification was higher for GMI1000 than the G7 strain, which produced approximately 10^7 and 10^6 vesicles/mL, respectively. Vesicles purified from infected and non-infected bacterial caused hypersensitivity and tissue necrosis in tomato leaves, and a more aggressive phenotypic response profile was observed in plants infiltrated with BEVs purified from infected bacteria. The relative expression of two genes essential in the immune response was significant for BEVs samples under viral influence. These results suggest that the Inovirus made significant phenotypic changes to the BEVs of *R. pseudosolanacearum*, and further analysis of their effect on plants is needed to use BEVs as a biocontrol.

Financial Support: CNPq, CAPES, Fapemig and Bracell

CO.10

Characterization of a Cellular Communication Mechanism in *Acanthamoeba* Species in Response to Infection by the Giant Tupanvirus

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Amoebae are among the oldest eukaryotes on the planet, having developed unique survival strategies over billions of years. Our knowledge of defense mechanisms in unicellular organisms primarily comes from studies of social amoebae, such as *Dictyostelium* spp. However, studying other groups of primitive eukaryotes, like those in the genus *Acanthamoeba*, can provide valuable insights into the evolution of cellular defense strategies in eukaryotes**.**

Previous studies have shown that *Acanthamoeba* spp., when artificially induced to encyst, become refractory to infection by giant viruses. However, if infected prior to this stimulus, the resistance phenotype is reversed, resulting in a massive production of viral particles. This chronological aspect suggests a cellular communication mechanism among amoebae that could confer an advantage in response to viral attacks**.**

This study aims to identify and characterize cellular communication mechanisms in *Acanthamoeba* in response to infection by tupanvirus (TPV). Cultures of *Acanthamoeba* will be treated with conditioned medium from TPV-infected amoebae and subjected to biological assays, gene expression analysis, electron microscopy, metabolomics, and transcriptomics. Initial results suggest that amoebae treated with the supernatant from infected cultures develop greater protection against TPV infection**.** This protection appears to be associated with increased encystment rates**.** This is the first description of such mechanisms in amoebae outside the group of social amoebae**.**

The expected data can add another piece into the evolutionary origins of the defense mechanisms in eukaryote cells and enhance our understanding of the biology and ecological importance of interactions between *Acanthamoeba* and giant viruses.

Financial Support: FAPESP, MCTI

CO.11

BOTH AGONIST AND ANTAGONIST OF DOPAMINE D2-LIKE RECEPTORS INHIBIT CHIKUNGUNYA VIRUS INFECTION IN NEUROBLASTOMA CELLS

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Chikungunya virus (CHIKV) infection induces oxidative stress, being associated with autophagy activation that benefits viral replication. Dopamine (DA) acts through dopamine receptors (DRs) which reduce oxidative stress. Also, dopamine receptors D2-like (DRD2) inhibit cAMP and activate mTOR, which stimulates cell translation and inhibits autophagy. We hypothesized that DA limits CHIKV replication by DRD2 decreasing oxidative stress and autophagy. Neuroblastoma cells (SH-SY5Y) are highly permissive to CHIKV. Infection with different m.o.i. of Brazilian-ECSA CHIKV isolates showed, by plate assay, a peaking of viral progeny at 36 hpi at a m.o.i. of 0.01 (10^{10} PFU/mL). Also, 100% of cells showed double-strand RNA production by immunofluorescence assay, and translational host cell shutoff by SUnSET at 24 hpi. It was evaluated that CHIKV replication was inhibited in more than 60% by 5 minutes pre-treatment of 10 μM DA (69%), DRD2 agonist Cabergoline (78%) and antagonist Eticlopride (96%) at 24 hpi. Infected cells treated after viral adsorption with 10 μM of these compounds reduced viral replication by 68%, 99% and 97% for DA, DRD2 agonist, and DRD2 antagonist respectively. Curiously the level of ROS, which indicates an imbalance of free radicals leading to oxidative stress, in infected SH-SY5Y cells isn't altered during CHIKV infection and the treatment with DA didn't change either. Therefore, DA and both DRD2 agonist and antagonist inhibit CHIKV replication, but didn't involve modulation of ROS levels and it's possibly related to an alternative pathway of DRD2 which is cAMP-independent.

Financial support: CAPES, FAPERJ, CNPq

CO.12

Impacts of Oropouche virus infection of trophoblast cells on host and viral microRNA regulation

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For many years, the Oropouche virus (OROV) infections were neglected and underreported. However, recent reports have highlighted its re-emergence, with the virus expanding beyond its endemic regions and being implicated in congenital infections and fetal mortality, as well as causing fatalities in otherwise healthy adults. In our study, we confirmed the susceptibility of human trophoblasts derived from healthy placentas to in vitro infection with OROV. Additionally, we investigated the infection of the immortalized trophoblast cell line JEG-3 and analyzed the alterations in the host transcriptome, focusing on microRNAs. Our findings revealed that 10 microRNAs were upregulated while 137 were downregulated. Computational predictions suggested that these changes predominantly affect protein metabolism and post-translational modifications. Furthermore, next-generation sequencing (NGS) identified small RNA sequences aligning with the viral genome, which may be incorporated into the RNA-induced silencing complex (RISC), potentially functioning as viral regulatory RNAs. Using the Vmir software, we predicted potential miRNA precursors from the OROV genome and antigenome segments, resulting in 16 sequences capable of producing 31 secondary structures recognized by the cellular miRNA biogenesis machinery. The prediction of mature miRNAs from these structures was corroborated by sequences detected in our NGS reads, supporting the likelihood of viral miRNA production through non-canonical pathways. The predicted pathways affected by these viral miRNAs suggest significant impacts on developmental processes, cell signaling, and central nervous system homeostasis.

CO.13

KEY MECHANISMS OF DENGUE VIRUS-INDUCED THROMBOCYTOPENIA: MEGAKARYOCYTE CELL DEATH AND LEUKOCYTE-PLATELET INTERACTIONS

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Dengue is the most prevalent arboviral disease globally, manifesting in mild or severe forms. A key feature of dengue is thrombocytopenia, observed in both mild and severe cases, although lower platelet counts are more common in severe cases. Despite extensive research, the mechanisms behind thrombocytopenia are not fully understood. This study characterizes a DENV-2 infection model in type I interferon receptor-deficient mice (A129) and explores the mechanisms of thrombocytopenia during infection. Our results show that A129 mice develop progressive thrombocytopenia starting 24 hours post-infection, accompanied by elevated inflammatory mediators in plasma and spleen, and increased vascular permeability in the liver, spleen, and lungs. Thrombocytopenia is associated with the death of megakaryocytes in the bone marrow, beginning 72 hours post-infection. Additionally, platelets released in the bone marrow undergo activation and progress to apoptosis, resulting in decreased platelet numbers in the marrow. In peripheral blood, platelets become activated after 72 hours, forming aggregates with neutrophils, monocytes, dendritic cells, and NK cells. The lungs are also a target of infection, showing increased viral titers, tissue damage, and elevated fibrinogen levels, which correlate with a decrease in pulmonary megakaryocytes, increased leukocyte-platelet aggregates, and heightened platelet activation. These active platelets aggregate with neutrophils, dendritic cells, and CD4 and cytotoxic T lymphocytes. In conclusion, our results demonstrate that in a systemic severe dengue model in A129 mice, thrombocytopenia arises due to megakaryocyte death, followed by platelet activation and apoptosis in the bone marrow, along with increased platelet-leukocyte aggregates in the lungs and peripheral blood.

Financial Support: CNPq, FAPEMIG, CAPES, INCT,
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CO.14

AUTOPHAGIC PATHWAY IS TRIGGERED BY OROPOUCHE VIRUS INFECTION AND NECESSARY FOR EFFICIENT VIRUS PRODUCTION

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Members of the Orthobunyavirus genus are responsible for several diseases in humans, including Oropouche fever caused by the Oropouche virus (OROV), which has significant epidemiological importance in Brazil and other Central and South American countries. OROV causes a debilitating febrile illness that can progress to meningitis or encephalitis in certain cases. was shown to infect the central nervous system in a animal models. Despite its severity, the molecular processes that drive the progression of OROV disease in humans remain largely undefined. The present study provides evidence that OROV infection in human neuroglioma cells (H4 cells) and HeLa cells induces a complete autophagic flux in host cells, and that elements of autophagic machinery are required for efficient OROV replication. Specifically, OROV infection induces the generation and consumption of LC3-II and p62 during the replication cycle, and depletion of ATG5 and ATG9 leads to reduction of viral protein production and viral particle release. Interestingly, treatment with bafilomycin, an inhibitor of degradative autophagy, does not compromise viral replication or the consumption of autophagic proteins LC3-II and p62. Furthermore, it was observed that cells infected with OROV showed a secretion of proteins related to the secretory autophagy pathway. Therefore, we propose that a form of secretory autophagy may be being induced and contributing to efficient viral release. These results could indicate that the autophagic pathway is subverted by OROV for efficient viral production and propagation.

CO.15

PROSPECTION AND IDENTIFICATION OF NEW ARBOVIRUSES: THE CHARACTERIZATION OF PIRAHY VIRUS (PIRAV), A NEW AND POTENTIALLY EMERGENT ALPHAVIRUS

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The emergence/spillover of a virus is characteristically related both to the evolution and adaptation of viruses and to anthropogenic factors. Unplanned demographic expansion, biodiversity destruction, and global warming directly impact the balance of wild transmission cycles that may impact viral dispersion and new transmission cycles. This situation is especially relevant in Brazil because of the co-circulation and widely dispersed pathogenic arboviruses such as Dengue, Zika, Yellow Fever, Chikungunya and more recently, Oropouche.

We recently characterized a new alphavirus, named Pirahy (PIRAV) from mosquitoes collected in the state of Paraná. Although no epidemiological link between PIRAV with infections in humans or animals has been established so far, *in vitro* assays revealed that PIRAV replicates in several vertebrate cell lines and in primary cultures of human PBMC. Furthermore, genomic signature analysis supports these results showing a dinucleotide and codon usage balance compatible with several vertebrate hosts and mosquitoes. Phylogenetic analyses placed PIRAV basal to the Venezuelan equine encephalitis complex. Genome analyses, electron microscopy, and biological characterization show the potential of this virus to infect new hosts. Furthermore, PIRAV was able to infect mosquitoes of the genus *Aedes* orally and disseminate in the vector's tissues. In murine model, infection with PIRAV lead to weight loss and death in 100% of IFNAR-KO mice inoculated with 10^4 to 10^7 pfu by subcutaneous infection route.

These findings emphasize the importance of investigating the epidemiological role and the potential of PIRAV to infect new hosts alerting for the potential emergence of a new arbovirus in South America.

CO.16

SURVEILLANCE OF VIRAL PATHOGENS IN CATS WITH RESPIRATORY INFECTIONS DURING THE COVID-19 PANDEMIC IN SOUTHERN BRAZIL

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Feline infectious upper respiratory tract disease (URTD) is a highly contagious illness, caused by various pathogens. The COVID-19 pandemic has highlighted the risk of zoonotic spillover events, especially with animals in close contact with humans, like cats. This study investigated common respiratory viral agents and SARS-CoV-2, in domestic cats with URTD clinical signs during the pandemic in Southern Brazil. Nasal, ocular and oropharyngeal swabs were collected from 120 symptomatic cats in contact with COVID-19-positive humans within 15 days, and samples were tested using PCR. Feline herpesvirus (FHV-1) was detected in 38.3% of the animals, statistically associated with sneezing and predisposing factors like junior cats and multi-cat environments. Feline calicivirus (FCV) was present in 34.1% of the cats and associated with sneezing, stomatitis and predisposing factors such as kittens, senior cats, outdoor access and feline leukemia virus (FeLV) positive cats. One female cat had detectable SARS-CoV-2 RNA in nasal and oral samples, showing clinical signs of conjunctivitis, stomatitis and nasal discharge. This cat was coinfected with FHV-1 and *Mycoplasma felis*. Close contact with infected owners likely facilitated the transmission. Confirmatory testing through additional nasal swabs and bronchoalveolar lavage consistently detected SARS-CoV-2 RNA. These findings align with recent studies indicating that cats can be asymptomatic or exhibit mild clinical signs when infected with SARS-CoV-2, especially with coinfections complicating the prognosis. Although SARS-CoV-2 infection in cats appears rare, monitoring feline populations is crucial to safeguard animal and human health, preventing potential interspecies viral transmission.

Financial Support: FAPERGS.

CO.17

OUTBREAK OF *Morbillivirus canis* IN NEOTROPICAL PRIMATES: A HEALTH ALERT

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Morbilliviruses are significant pathogens affecting global health in both humans and animals. Canine distemper, caused by Canine morbillivirus (CDV), is one of the deadliest infectious diseases in dogs and other carnivores, alongside rabies. Natural and experimental infections demonstrate that morbilliviruses can easily cross species barriers. In 2022, a 39-day surveillance study at a zoo revealed seven black-tufted marmosets (*Callithrix penicillata*) found dead or captured, some showing neurological signs such as myoclonus. Necropsies revealed skin thickening and brain lesions, including gliosis and neuronal necrosis, with samples collected for further analysis. Genetic material was extracted from oral and rectal swabs, as well as from the brain, skin, lungs, spleen, liver, and kidneys, followed by PCR detection of herpesvirus, measles virus, and CDV. All but one sample tested positive for CDV, and three were also positive for Callitrichine gammaherpesvirus 3. Immunohistochemistry identified CDV and herpesvirus antigens in various tissues. Evolutionary analyses using sequencing were conducted to investigate phylogenetic relationships, providing insights into the genetic connections between the viruses found in the marmosets and those in other species. This marks the first report of a lethal CDV outbreak in free-ranging neotropical primates. Recent adaptations of CDV to non-human primates increase the risk of zoonotic transmission to humans. The detection of CDV-related disease causing mortality in neotropical primates raises concerns about future spread and underscores the need for a One Health approach, emphasizing the importance of including CDV in primate surveillance efforts.

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CO.18

REEMERGENCE OF THE OROPOUCHE VIRUS OUTBREAK IN MUNICIPALITIES IN RONDÔNIA AND BORDER CITIES IN AMAZONAS IN 2024: CLINICAL FINDINGS.

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The Oropouche virus (OROV) is a re-emerging arbovirus of public health concern. This disease has caused several outbreaks of acute fever in Central and South American countries, with more than half a million reported cases. Before the Zika and chikungunya epidemics, it was the second most reported arbovirus, behind dengue. This study sought to report the clinical signs and symptoms presented by patients who tested positive in real-time PCR for the Oropouche virus in municipalities in Rondônia and border towns in Amazonas. Using RT-qPCR molecular techniques, OROV-positive cases were identified among a cohort of individuals previously negative for Malaria spp. who had reported symptom data filled in via an internal notification form. In a cohort of 1.128 individuals, we identified 372 OROV-positive cases circulating in the state of Rondônia and state border locations in the Western Amazon, the period from January to May 2024, with 100% of the cohort characterized as infected individuals with reported symptoms, ranging mainly from fever 96.77%, headache 95.16%, back pain 85.48%, 85.22% myalgia, retro-orbital pain 72.31% and 28.76% among other reported symptoms (diarrhea, abdominal pain and bitter mouth). The diagnosis of arboviruses, such as the OROV virus, is ineffective when only the symptoms are assessed, since all arboviruses and other pathologies such as malaria, which is endemic in the region, share similar symptoms.

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CO.19

Virome analyses from stools and water samples reveal the potential of CrAssphage as a human fecal contamination indicator and characterize six novel whole genomes with gene deletions

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CrAssphage belongs to *Crassvirales* order, which comprises four families: *Intestiviridae*, *Crevaviridae*, *Suoliviridae*, and *Steigvirida*. This group of phages is found in various environments and is particularly prevalent in the mammalian gut. No studies have described the *Crassvirales* in the Rio dos Sinos watershed. Our study is the first to describe six new genomes and highlight their potential as indicators of human fecal contamination in the region. To achieve this, we conducted shotgun metagenomic sequencing on samples collected from human stool, surface water, and groundwater within a watershed in Southern Brazil. The contigs of *Crassvirales* were assembled using *de novo* assembly and reference mapping. To classify CrAssphage genomes, phylogenetic analyses of the TerL, MCP, and portal proteins were conducted using the CrassUS pipeline. Six new complete genomes of CrAssphage from the *Intestiviridae* family were identified, with lengths ranging from 95,313 to 97,065 bp and exhibited mean coverage depths ranging from 8.5 to 501.8. Among the six analyzed genomes, five contained a deletion in a gene encoding a putative endonuclease, and two exhibited deletions in a gene coding for a hypothetical protein. Only one genome showed no deletions. CrAssphage contigs were detected in three groundwater samples, one from a highly urbanized area, and two from rural areas. All positive samples contained contigs from the *Intestiviridae* family, while only one groundwater sample included contigs from the *Steigvirida* family. Although CrAssphages were only recently discovered, they have emerged as a robust indicator for tracking human fecal contamination.

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CO.20

Marsupials (Marsupialia: Didelphidae) from the State of São Paulo: Potential Reservoirs of Zoonotic Viruses

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The pathogenic microbiota of species coexisting with humans are important subjects of a One Health approach. After the SARS-CoV-2 pandemic, bats became important objects of studies related to viral infection tolerance especially due to their long history of coevolution of around 50 million years with these pathogens. Marsupials of the *Didelphidae* family evolved approximately 100 million years earlier and some species have adapted to urban environments, becoming synanthropic animals. Zoonotic viruses such as rabies have already been reported in these animals, but they are still very neglected. In marsupials, this and other viruses may be present in greater abundance and be of further relevance in epidemiological vigilance efforts. In this research we used molecular biology techniques to investigate the presence of viruses in the *Arenaviridae, Coronaviridae* and *Paramyxoviridae* families in didelphids from the state of São Paulo. Oral and rectal swabs were collected from wild marsupials and viral RNA extraction was performed with subsequent synthesis of complementary DNA. For screening, the PCR technique was applied with degenerate primers specific for each virus family. Samples from 52 didelphids were analyzed, of which 26.9% indicated to be positive for a potentially zoonotic virus: 19.2% were positive for *Arenaviridae*, 11.5% for *Coronaviridae* with a co-infection rate of 3.85%. Positive samples will be sequenced and phylogenetically analyzed to better understand the relevance of the findings. This study indicates that didelphids from the state of São Paulo may be infected with potentially zoonotic viruses and should be included in One Health approach studies.

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CO.21

Mucus-adherent bacteriophages as a preventive strategy for a lethal *Pseudomonas aeruginosa* challenge

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Pseudomonas aeruginosa is an opportunistic pathogen associated with infections in mechanically ventilated patients, cystic fibrosis, cancer, severe burns, and immunocompromised individuals. Bacteriophages (phages) are promising agents for inhibiting the growth of *P. aeruginosa* strains resistant to antibiotics. The bacteriophage mucosal adhesion model proposes that metazoans harbor phages on their mucosal surfaces. The goal of this study was to evaluate the potential of phage-mucus interaction to prevent infections caused by *P. aeuginosa*. We isolate two phages (named VAC1 and VAC3) that can infect *P. aeruginosa* PA14 strain. They have very similar morphologies and a conserved synteny with a high degree of identity, except for a specific region of 1.062 bp present in the 5' region of VAC3 phage. *In vitro* studies demonstrated only an increase in viral production of VAC3 phage in the presence of mucin. C57Bl/6 mice were treated with both phages via the nasal route and after 24 hours of treatment, phage quantification was performed in the trachea, lung and nasal lavage. The results indicated that phage VAC3 was found in the trachea of all animals while phage VAC1 was not detected. Animals pretreated with phage VAC3 and infected 24 hours post-treatment with *P. aeruginosa* PA14 had the highest survival rate, fewer clinical signs, the lowest bacterial lung load and few histopathological changes in their lungs. Therefore, the use of mucus-adherent bacteriophages could be useful as a new alternative to prevent *P. aeruginosa* infections in patients at high risk of acquiring this type of infection.

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CO.22

MONITORING NEUTRALIZING ANTIBODIES AGAINST ORTHOPOXVIRUS VACCINIA AFTER IMMUNIZATION WITH JYNNEOS

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Mpox is an infectious disease caused by Orthopoxvirus monkeypox (MPXV), which became the most significant threat of the genus *Orthopoxvirus* (OPVs) after smallpox was eradicated. Due to high genetic similarity, OPVs are capable of inducing cross-immune protection. Therefore, considering the health aggravation caused by MPXV, the JYNNEOS vaccine, which uses a Modified Vaccinia Ankara, was approved to prevent MPXV. The monitoring the neutralizing antibody response is essential for evaluating vaccine efficacy and analyzing cross-immunological protection caused by OPVs. A total of 26 volunteers who received JYNNEOS were recruited to participate in this study. Serum collections were carried out before the first dose of the vaccine and on days 7, 14, 30, 60, and 120 after the vaccination. Plaque reduction neutralization assays (PRNT) in 24- and 6-well plates were performed using Vaccinia virus and BSC-40 cells to detect and titrate anti-OPVs neutralizing antibodies. We observed that from 30 days after the first dose of the vaccine, a higher number of individuals had antibodies. After receiving the second dose of JYNNEOS, everyone had anti-orthopoxvirus antibodies. Through $PRNT_{50}$, we identified that there was 50% viral neutralization at dilutions of up to 1:640 within 60 days after the vaccine. A greater production of neutralizing antibodies against VACV was observed after application of the second dose of JYNNEOS.

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CO.23

PRE-CLINICAL AND CLINICAL DEVELOPMENT OF A POTENT COMBINATION OF BROMELAIN AND N-ACETYLCYSTEIN TO TREAT VIRAL RESPIRATORY DISEASES

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Respiratory viral diseases are lethal syndromes caused by pandemic virus such as SARS-CoV-2 and Influenza, which continue as public health threats to society. COVID-19 persists as a continuous respiratory disease of concern and is often associated with sputum retention and cytokine storm, for which there are limited therapeutic options at late stages of disease. In this regard, we evaluated the use of BromAc®, a combination of Bromelain and Acetylcysteine (NAC) in pre-clinical and clinical settings. For the pre-clinical studies, we aimed at examining the mucolytic and anti-inflammatory effect of BromAc® in tracheal aspirate samples from critically ill COVID-19 patients requiring mechanical ventilation. BromAc® showed a robust mucolytic effect in a dose dependent fashion on COVID-19 sputum ex vivo. In addition, BromAc® showed anti-inflammatory activity, reducing the cytokine storm at concentrations of 125 and 250 µg. In addition to showing safety in phase 1 clinical studies, BromAc is currently being investigated in a phase 1/2 clinical study in a nebulized form to treat respiratory viral diseases and is in the final stages of approval by ANVISA. In summary, pre-clinical and clinical investigations demonstrate BromAc® as a potential therapeutic intervention for respiratory diseases with strong inflammatory outcomes, setting the stage for the imminent clinical development of BromAc® in Brazil.

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CO.24

IN SILICO DESIGNING OF MULTI-EPITOPE VACCINE AGAINST CANINE PARVOVIRUS USING REVERSE VACCINOLOGY

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Canine parvovirus (CPV-2) is a highly contagious virus that affects dogs worldwide, with the VP2 protein as its main immunogenic structural component. Currently, it presents variants CPV-2a, 2b, and 2c, distinguished by changes in amino acid residue 426 of VP2. Other amino acid changes are also identified in VP2 and are responsible for the diversity of disease outbreaks in Brazil. Although vaccination is the main preventive measure against the virus, conventional vaccine development methods are challenged by the diversity of viral strains. Therefore, the objective of the study was to investigate CPV-2 variants in Brazil and design an updated in silico vaccine based on reverse vaccinology. Firstly, field and vaccine strains were collected from GenBank, analyzed and compared at the amino acid level using Mega-X software. After that, B and T cell epitopes were predicted using BepiPred-2.0 web server and NetMHCpan-4.1, and evaluated for antigenicity, allergenicity and toxicity, using VaxiJen v2.0, AllerTOP v2.0 and ToxinPred softwares, respectively. The vaccine was constructed with selected epitopes linked to an adjuvant and optimized for expression in *Escherichia coli.* Structural and molecular dynamics predictions confirmed the antigenicity and stability of the vaccine, and molecular docking demonstrated interaction with the canine Toll-like receptor 4. Immunological simulations demonstrated a progressive immune response post-vaccination, including increased antibody production and T-helper cell activity. The multiepitope vaccine targeted prevalent CPV-2 variants in Brazil and potentially other regions of the world.

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CO.25

EXPERIMENTAL PRODUCTION OF INFECTIOUS PCV2b VIRAL PARTICLES FROM SYNTHETIC GENOMES

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Circovirus porcine 2 (PCV2) is a prevalent virus in the global swine population, causing significant economic losses. PCV2 possesses a single structural protein, the Cap protein, which is associated with protective immunity. Cultivation of PCV2 from biological samples is challenging and rarely successful. Therefore, the aim of this study was to induce viral replication by transfecting synthetic DNA into porcine testis (ST) cell lines. The DNA containing a complete PCV2 sequence was commercially obtained and inserted into a plasmid. The PCV2 DNA was excised from the plasmid using restriction enzymes and circularized. This DNA was then used to transfect the cultured cells. After 5 days, the supernatant was collected, and an infection assay was performed. Viral DNA from the cell cultures was quantified by qPCR at 24-hour intervals in both the transfection and infection assays, showing a directly proportional relationship to cell confluence. Capsid protein production was confirmed by ELISA, and particle formation was demonstrated by transmission electron microscopy. The process was thus considered successful. This study is the first description of this process in porcine testis cells, which is crucial for studies on viral biology, antiviral drugs, and vaccines.

Financial Support: FAPESP.

CO.26

AEDES AEGYPTI POPULATIONS CAN BE CHARACTERIZED USING GENOMIC FEATURES OF PHASI CHAROEN-LIKE VIRUS

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Aedes aegypti is an important mosquito and is implicated in the transmission of some arboviruses, such as Dengue, Chikungunya, and Zika viruses. The metavirome studies of *A. aegypti* have identified the prevalence of Phasi Charoen-like virus (PCLV), an insect-specific virus that belongs to the taxonomic order *Bunyavirales*. The study retrieved and filtered PCLV genome sequences from NCBI GenBank, focusing on those associated with *A. aegypti*. Codon usage bias was analyzed using Relative Synonymous Codon Usage (RSCU) values, followed by clustering and PCA, conducted in Python. For *A. aegypti* population genetics, genomic diversity was assessed using seven markers (18S, 28S, Arginine Kinase, COI, COII, Enolase, and ITS2), using the raw sequencing data, which were retrieved from SRA and used to markers genomic assembly. Phylogenetic trees for both PCLV and *A. aegypti* were constructed using the Maximum Likelihood, and tree topologies were compared using RF and SPR metrics in R. Here, we included 37 complete genomes (segment L, M, and S) available for analysis from six countries across three continents. Phylogenetic analysis revealed two main lineages: Lineage I included sequences from Kenya, China, Brazil, and Guadeloupe, while Lineage II consisted of sequences from India and Thailand. The analysis of the diversity of *A. aegypti* revealed a tree topology similar to the viral genealogy, suggesting coevolution between the mosquito and PCLV. This approach demonstrates the grouping of the virus sequences as a function of mosquito distribution, suggesting potential ecological and evolutionary implications to understand the virus-mosquito interactions and arbovirus transmission.

Financial Support: FAPESP.

CO.27

GENETIC VARIABILITY OF BLAINVILLEA YELLOW SPOT VIRUS (*Begomovirus blainvilleae*) REVEALS RECOMBINANT VARIANTS AND A D ISTINCT NONANUCLEOTIDE MOTIF

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Viruses from wild plants often lead to the emergence of new economically important agricultural pathogens through frequent spillover events. However, knowledge of Blainvillea yellow spot virus (*Begomovirus blainvilleae*, BlYSV), a geminivirus that exhibits a sealed container relationship with the plant *Blainvillea rhomboidea*, remains limited. To better understand the ecology and evolution of this virus, leaf samples were collected in the states of Minas Gerais, Rio Grande do Norte, and Alagoas between 2022 and 2023. A total of 23 DNA-A clones of BlYSV were obtained, and using 31 additional sequences retrieved from NCBI, the population structure was assessed through DAPC and phylogenetic analysis. Four variants (named A to D) were identified, one of which (A) was classified as a different strain (<94% nucleotide sequence identity for the complete DNA-A segment). A group of isolates, although not forming a monophyletic group or variant, was structured into an independent subpopulation. Five recombination events were identified. A shift in the occurrence of variants was observed in the municipalities of Minas Gerais, with a new recombinant variant B replacing variant C. Additionally, the *AC4* gene of variant B isolates is evolving under positive selection. Moreover, four sequences in strain A (n=6) exhibited a nucleotide substitution (A2667G) within the conserved geminivirus nonanucleotide motif (from TAATATTAC to TAATGTTAC). This mutation does not alter stem-loop formation. However, interestingly, this subpopulation exhibits reduced genetic variability compared to others. Thus, further studies should evaluate the biological significance of this new mutation.

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CO.28

PERSISTENCE AND DIVERSITY OF WESTERN AFRICA ZIKA VIRUS IN FOREST AREAS OF AFRICA WITHOUT URBAN ADAPTATION

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Zika virus (ZIKV) is an emerging mosquito-borne virus of concern for public health, but very little is known about its transmission patterns and ecology, especially in African countries. Here, we aimed to characterize new ZIKV sequences collected in Senegal from 2011 to 2015. This study used virus strains from the Institute Pasteur de Dakar collection, obtained from mosquitoes collected in the Kédougou region of southeastern Senegal (four from 2011 and seven sequences from 2015). The ZIKV were cultured in AP61 and Vero cells. The ZIKV sequencing was conducted using next-generation sequencing. The genomic sequences were analyzed using phylogenetic, phylogeographic, and codon adaptation analysis. The new genomes are part of the African genotype, within the ZIKV Western Lineage. Sequences were strongly clustered by sampling location and date of the collection. The phylogeographic reconstructions indicated that the African genotype emerged in Western Africa between 1957 and 1974 mostly following an East-to-West migration pattern. Since the emergence of the ZIKV in Western Africa, we found a decrease in the effective population size, but stagnating in recent years. Over time, no significant increase was recorded in the Codon Adaptation Index values for *Aedes aegypti* and *Homo sapiens*. In summary, the results indicate that the African genotype ZIKV continues to circulate in forest areas, using sylvatic mosquitoes as vectors in Western Africa, with a long period of persistence at a local scale, with an accumulation of diversity over time, and without any evidence of adaptation based in CAI values to the urban environment.

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CO.29

A playground for capsidless viruses: mixed infection in *Sclerotinia sclerotiorum* Brazil isolates

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The fungus *Sclerotinia sclerotiorum* is a phytopathogenic agent responsible for white mold disease in diverse crops. Mycoviruses have been pointed out as a promissory strategy for biocontrol because they can induce hypovirulence in pathogenic fungi. Thus, it is essential to discover and characterize novel viruses to develop and improve new technologies. Therefore, our objective was to prospect RNA viruses infecting *S. sclerotiorum*. For that, sclerotia from 44 different isolates were grown on potato dextrose agar (PDA) medium with antibiotics. After mycelium growth, discs were transferred to a PDA medium covered by cellophane for seven days in the dark at 28°C. The mycelium was powdered, and total nucleic acids (TNA) were extracted using the silica protocol. After that, the TNAs were treated with S1 nuclease and DNAse, according to the manufacturer's manual. From that, 14 samples harbor double-stranded RNA (dsRNA), a characteristic present only in RNA viruses. The fragment sizes ranged from 500bp to 10000bp, and at least four dsRNA patterns were observed, indicating that the isolates are infected with different viruses. Some isolates have more than one fragment, suggesting they may have segmented viruses or mixed infections. We performed Illumina High Throughput Sequencing of four isolates to characterize those dsRNA. A high diversity of viruses from the *Mitoviridae* family was detected, and viruses from *the Botourmiaviridae* and *Endornaviridae* families were also found, suggesting that *S. sclerotiorum* is a promiscuous host for capsidless mycoviruses. At least three circular sequences having a hammerhead motif were assembled, suggesting the presence of viroid-like.

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PORCINE PARVOVIRUS TYPE 1: A PHYLOGENETIC, EVOLUTIONARY AND STRUCTURAL ANALYSIS

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Porcine parvovirus type 1 (PPV1) is globally recognized as the etiological agent responsible for one of the primary diseases causing reproductive failures in swine herds, known by the acronym SMEDI. Although it has been identified since the mid-1960s, PPV1 continues to be isolated worldwide, leading to significant financial losses. Vaccination remains the primary preventive measure against the virus. However, the emergence of new variants globally raises concerns regarding the efficacy of currently commercialized vaccines. This study aimed to understand the evolutionary dynamics of PPV1 through a detailed analysis of 200 complete sequences dated from 1963 to 2021 of the gene which encodes the VP2 protein, main structural protein of the viral capsid, using bioinformatics software such as BEAST2, RDP5, BepiPred, NetMHCpan, and others. The methodology involved data collection, genetic sequence analysis, followed by phylogenetic tree construction, simulation of different VP2 proteins, and prediction of positive selection sites and B and T cell epitope regions. Phylogenetic analysis revealed a total of five groups. Two of those groups did not contain vaccine reference variants. The first group is predominantly composed of strains isolated in Europe, and the second is mainly divided between Asian and European strains. Predictions and simulations confirmed structural differences at various protein positions among the five groups identified in the phylogenetic tree and their three reference vaccine variants (NADL-2, 27a, and PPV014), which were classified as epitope regions and positive selection sites, suggesting that the strains used as vaccine references are possibly not providing protection against the new variants.