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Apresentações Orais

CO.01 a CO.36

20/9/2023

CO.01

EVALUATION OF SYRIAN HAMSTER (*Mesocricetus auratus*) SUSCEPTIBILITY TO NON-ADAPTED YELLOW FEVER VIRUS STRAINS INFECTION

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Yellow fever is an infectious disease whose best study model is the rhesus monkey. Since the use of non-human primates is limited, other models have been studied, such as the syrian hamster, which requires adapted strains. The aim of this study was to evaluate the hamster susceptibility to yellow fever (YFV) Brazilian strains. Animals were infected with YFV 17DD; PR4408 and RJ155 strains and clinically evaluated for seven days. Samples were analyzed for viral load by RT-qPCR on days 1, 3, 5 and 7 (dpi), presence of viral RNA in the liver by RT-PCR and histopathology of the liver and spleen at 5 and 7 dpi. Animals infected with PR4408 showed progressive weight loss, detection of viral RNA in serum at 3, 5 and 7 dpi and in the liver at 5 and 7 dpi; in addition to periportal inflammatory infiltrates, acidophilic bodies and necroapoptosis areas in the liver and apoptosis and disorganization of the white pulp and enlargement and apoptosis of the red pulp in the spleen. Animals infected with RJ155 showed viral RNA detection in serum at 3 and 5 dpi and in the liver at 5 dpi; in addition to presence of discrete acidophilic bodies and periportal inflammatory infiltrates in the liver and lymphoid hyperplasia and disorganization of the white pulp in the spleen. Animals infected with 17DD didn't show detectable viral RNA in serum and liver; in addition to discreet changes in the histology. These results show that the hamster was more susceptible to PR4408 infection.

Financial Support: FAPERJ, Programa INOVA FIOCRUZ.

CEUA/IOC-019/2021: L-023/2021

CO.02

OROPOUCHE VIRUS INFECTION CAUSES REDOX HOMEOSTASIS IMBALANCE IN TARGET ORGANS OF BALB/c MICE

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Oropouche fever is an acute febrile disease caused by the arbovirus Oropouche (OROV). It is considered as one of the neglecctedemerging disease with more than half a million people infected in the Americas. Currently, there are no antiviral drugs or vaccines available against the infection and little is known about its pathogenicity. In viral pathogenesis, oxidative stress plays a key role in the progression of various viral infections. Therefore this study evaluated the redox homeostasis in the liver, spleen and brain of infected animals. 21-days-old BALB/c were infected subcutaneosly with 10^6 UFP of OROV while the control group received Phosphate Buffer Saline by the same route. On the 4 day after infection the animals were euthanized and the biological samples collected. The infection induced anti-OROV neutralising antibodies, splenomegaly, increased serum levels of the liver transaminases and pro-inflammatory cytokines tumour necrosis factor and interferon-γ. OROV genome were detected in the three organs, with the highest number of RNA copies in the spleen. The levels of Reactive Oxygen Species (ROS) and carbonyl protein were increased in the liver, spleen and brain and the malondialdehyde (MDA) levels was increased in the liver. The activity of the antioxidant enzymes Superoxide Dismutase (SOD) was decreased in the liver and spleen and Catalase (CAT) decreased in the three organs. These results show that OROV infection culminates in an imbalance in redox homeostasis and consequent oxidative stress in target organs of the infection, which provides information to some aspect of Oropouche fever.

FINANCIAL SUPPORT: UFOP, FAPEMIG, CNPq and CAPES.

CO.03

ATTENUATED SABIN POLIOVIRUS INFECTS HYPERTROPHIC HUMAN TONSILS

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Poliovirus (PV) from Picornaviridae, genus Enterovirus, occurs in three wildtype PVs, 1, 2 and 3, and the same three types of attenuated PVs, which compose the trivalent oral poliovirus vaccine (OPV). In Brazil, the current immunization schedule for PV consists in inactivated poliovirus vaccine (IPV) followed by OPV boosters. This study aimed to confirm the existence of natural OPV infection in palatine tonsils and adenoids from children with tonsillar hypertrophy. Samples from 152 patients were tested for OPV by RT-qPCR, and the frequency of detection was 7.23%. Additionally, none of the OPV-positive tissues tested positive for human rhinovirus and 4 of the OPV-positive tissues also tested positive for enterovirus. Attempts to isolate OPV from positive tissues were negative. IHQ revealed the presence of structural proteins with monoclonal antibody for PV, mainly in the epithelium, but also in interfollicular areas of the lymphoid compartment. Serial SIMPLES on the very same tissue sections detected OPV antigen on CD4 T and B lymphocytes. In addition, OPV ex vivo infections of tonsillar explants indicated virus replication over time, with a 20-fold increase in viral RNA copies on the 7^{th} dpi. Neutralization assay did not detect significant differences in neutralizing titers between sera from individuals with and without OPV detection in tonsils. We confirm the presence of asymptomatic carriage of OPV in CD4 T and B lymphocytes in tonsils and adenoids from children. Tonsillar OPV may play a role in maintaining long-lasting immunity and, perhaps, in the shedding of OPV in the community.
Financial support: FAPESP, CNPq and CAPES.

CO.04

ESSENTIAL ROLE OF THE CCL2-CCR2 AXIS IN MAYARO VIRUS-INDUCED DISEASE

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Mayaro virus (MAYV) is an emerging arbovirus member of the *Togaviridae* family and *Alphavirus* genus. MAYV infection causes an acute febrile illness accompanied by persistent polyarthralgia and myalgia. Understanding the mechanisms involved in arthritis caused by alphaviruses is necessary to develop specific therapies. In this work, we investigated the role of the CCL2/CCR2 axis in the pathogenesis of MAYV-induced disease. For this, WT C57BL/6J and CCR2-/- mice were infected with MAYV subcutaneously and evaluated for disease development. MAYV infection induced an acute inflammatory disease in WT mice. The immune response profile was characterized by an increase in the production of inflammatory mediators, such as IL-6, TNF and CCL2. Higher levels of CCL2 at the local and systemic levels, was followed by significant recruitment of CCR2+ macrophages and a cellular response orchestrated by these cells. CCR2-/- mice showed an increase in CXCL-1 levels, followed by a replacement of the macrophage inflammatory infiltrate by neutrophils. Additionally, absence of the CCR2 receptor protected mice from bone loss induced by MAYV. Accordingly, the silencing of CCL2 chemokine expression *in vivo* and the pharmacological blockade of CCR2 promoted a partial improvement in disease. Cell culture data support the mechanism underlying MAYV's bone pathology in which: i) MAYV infection promoted a pro-osteoclastogenic microenvironment mediated by IL-6, TNF and CCL2 and ii) migration of osteoclast precursors was dependent on the CCR2/CCL2 axis. Overall, these data contribute to the understanding of the pathophysiology of MAYV infection and to the identification future of specific therapeutic targets in MAYV-induced disease.

Financial Support: CAPES, CNPq, FAPEMIG, INCT em Dengue

CO.05

DETECTION OF BEAN-ASSOCIATED CYTORHABDOVIRUS AND COWPEA MILD MOTTLE VIRUS IN *Passifora* spp. IN DIFFERENT BRAZILIAN REGIONS.

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Bean-associated cytorhabdovirus (BaCV, *Cytorhabdovirus caricae*, *Rhabdoviridae*) and cowpea mild mottle virus (CPMMV, *Carlavirus*, *Betaflexiviridae*) have been identified in beans in Brazil, while other *Cytorhabdovirus caricae* member, the citrus-associated rhabdovirus (CiARV) was identified in passion fruit in China. In this study, we investigated the occurrence of BaCV and CPMMV in passion fruit in Brazil using the following workflow: total RNA extraction, RT-PCR using BaCV and CPMMV-specific primers (BaCV-6491F/BaCV-7178R and CPMMVB22095F/CPMMVB22535R) and Sanger sequencing of amplicons. Passion fruit plants collected in commercial fields (*n*=24 Cuité, Paraíba; *n*=11 Vitória de Santo Antão, Pernambuco; *n*=51 Seropédica, Rio de Janeiro; *n*= 9 Brasília, *n*=19 Planaltina, *n*=20 Brazlândia, Distrito Federal), and *Passiflora* accesses from the Germplasm Bank "Flor da Paixão" -BAG-FP (*n*=55, Planaltina) were evaluated. In the Midwest of Brazil, BaCV was identified in Planaltina in 4/19 of *P. edulis* plants and in 4/55 in *Passiflora* spp. accessions of BAG-FP. CPMMV was identified in 14/55 *Passiflora* spp. from BAG-FP. Using CPMMV-4000F/CPMMV-4500R, an alternative primer pair, CPMMV was identified in 1/19 and 2/9 *P. edulis* from Planaltina and Brasilia, respectively. Additionally, 2/24 and 1 /11 *P. edulis* from Cuité and Vitória de Santo Antão, Northeast region, and 1/51 of *P. edulis* from Seropédica, Southeast region, tested positive for CPMMV, highlighting a variability among passionfruit CPMMV isolates. Mixed infection with other passionfruit-infecting viruses, lettuce chlorosis virus (LCV, *Crinivirus*, *Closteroviridae*), and cowpea aphid-borne mosaic virus (CABMV, *Potyvirus*, *Potyviridae*) was also inspected. Mixed infections of BaCV/CPMMV/CABMV/LCV were confirmed, underscoring a high diversity of viruses infecting passion fruit plants in the country.

Financial Support: Embrapa; FapDF; CAPES; CNPq.

CO.06

LONG-TERM DYNAMICS OF A BEGOMOVIRUS COMMUNITY IN THE NATURAL ENVIRONMENT: THE ODYSSEY CONTINUES

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Studies that address the temporal dynamics of viral communities in the natural environment provide an overview of the complex relationships between different populations, and allow the assessment of factors that govern the emergence of viral pathogens in crops. We have been exploring the dynamics of a begomovirus community infecting *Sida acuta* (*Malvaceae*) for over a decade, collecting samples once a year in an area of Atlantic Forest in Viçosa, MG. A total of 219 DNA-A and 125 DNA-B sequences have been generated to date, corresponding to three begomoviruses: *Oxalis yellow vein virus* (OxYVV), *Sida yellow leaf curl virus* (SiYLCV) and *Sida micrantha mosaic virus* (SimMV). All three populations comprise multiple variants, and we observed fluctuations over time in the proportions of each virus within the community. In the first four years, OxYVV variant I was prevalent. Then, a drastic change in the composition of the community occurred, with SiYLCV becoming prevalent. In the following year the two viruses were present in similar proportions. The passage of the OxYVV population through a narrow bottleneck could explain this drastic change. However, no variation in the effective population size was observed in Bayesian skyline plot analyses. The non-significant negative values of Tajima's D test indicate that populations of OxYVV and SiYLC are evolving mostly through genetic drift, consistent with the absence of a temporal structuring signal. The observed slow evolutionary dynamics is surprising considering the high mutation and recombination rates of begomoviruses, and could be attributed to the stability of the ecosystem.

CO.07

STRUCTURAL CHARACTERIZATION OF THE CAPSID PROTEIN OF VIRUSES BELONGING TO THE NEW GENUS CITLODAVIRUS FROM THE *GEMINIVIRIDAE* FAMILY.

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The *Geminiviridae* family contains circular ssDNA plant viruses that infect significant crops. The family have a distinctive twinned geminated particle that is typical of viruses belonging to the *Begomovirus* and *Mastrevirus* genera. The *Citlodavirus* genus contains viruses with atypically large genomes (> 3.0 kb), suggesting the need for a larger capsid to accommodate their genetic material. However, there is no information about their capsid protein (CP) or particle tridimensional structure. We used the AlphaFold2 tool to generate tridimensional structural models of four citlodavirus CPs, comparing them to the AYVV capsid protein structural derived by Cryo-EM. We analyzed structural features important in determining the capsid conformation, such the N-terminal arginine arm´s length and charge. Citlodavirus CPs shared the same jelly-roll fold of the begomovirus AYVV (DALI Z-score 24.7). The citlodavirus structural models revealed that all 4 CPs have flexible arginine-rich arms that are 60 to 70 amino acids long. These arms are highly positively charged (Net charge Q = +17). However, based on our group´s previous analysis, these charges wouldn't be enough to package the 3.0 kb genome in a typical 110 subunits twined particle. We observed that the citlodavirus capsid proteins presented a distinctive proline-rich motif that demarcates the end of the Arg-arm in AYVV CP but lack the R48 and M59 residues that were found to be critical for assembling a geminated particle of AYVV. This work emphasizes that *Geminiviridae* family may include a diversity of capsid architectures, especially among species with big genomes.

CO.08

A member of N-degradon pathway as a key molecule of a new virus resistance mechanism against polerovirus in plants

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ATE (Arginyl t-RNA transferase) is a component of the N-degron pathway that controls the half-life of proteins by targeting them to degradation via the Ubiquitin Proteasome System. ATE adds an Arg to certain destabilizing residues present at N-termini of target proteins leading them to degradation. Previous results indicate that high levels of ATE mRNA impair cotton leafroll dwarf virus (CLRDV, *Polerovirus*, *Solemoviridae* family) replication and spread in cotton. To deeply understand ATE role in CLRDV resistance, we infected transgenic Arabidopsis plants overexpressing *Ath*-ATE1 with the virus. Arabidopsis plants are susceptible to CLRDV, however in the 35S:ATE1 plants, virus infection was abolished. By the other side, Arabidopsis *ate1.ate2* mutants were highly susceptible to CLRDV. In addition, we silenced ATE of a CLRDV-resistant cotton cultivar by VIGS and observed that ATE silenced plants became susceptible to CLRDV infection. Based on these results, we asked whether ATE can induce the degradation of CLRDV proteins. *Nicotiana benthamiana* leaves were co-infiltrated with an expression vector for ATE (*Gh*-ATE), and CLRDV P0 (silencing suppression protein), P3 (capsid protein), or P4 (short distance movement protein). Protein levels were analyzed by western blot. All the three CLRDV proteins were drastically reduced in the presence of cotton ATE. In contrast, ATE did not degrade GFP and/or GUS in co-infiltration assays. Analysis of co-immunoprecipitation and BiFC are in progress. Our results indicated that degradation of CLRDV P0, P3, and P4 protein induced by *Gh*-ATE might confer the resistance phenotype to CLRDV infection observed in certain cotton varieties.

CO.09

HUMAN ADENOVIRUS QUANTITATIVE RISK ASSESSMENT FOR MUNICIPAL SOLID WASTE WORKERS

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Municipal solid waste (MSW) workers involved in the waste collection are exposed to different risks. The objective of this study was to assess the exposure of MSW workers from inadvertent ingestion of truck leachate to estimate the risk of human adenovirus (HAdV) gastrointestinal (GI) disease. Samples from fresh truck leachate were collected from the basin of solid waste trucks in Rio de Janeiro city, Brazil, ultracentrifuged, and inoculated into A549 cell to determine HAdV infectivity. A quantitative microbial risk assessment (QMRA) was performed to measure the probability of GI illness attributable to inadvertent oral ingestion of truck leachate by two different mechanisms: the direct splashing into the oral cavity or by hand-to-mouth contact (with and without gloves). QMRA was performed by Monte Carlo analysis at Vensim software®. HAdV infectivity ranged from 17 to 667 $TCID_{50}$/ml, and these concentrations applied in the QMRA model. By hand-to-mouth contact, based on a hypothetical model where workers did not wear gloves, a higher probability of developing GI was observed, with a risk of 67%. When compared to the model where workers wear gloves, there was a decrease in the probability to 33%. On the other hand, through the splashing route, the probability of developing GI was 58%. This is the first study to reveal infectious HAdV in fresh truck leachate from solid waste, reinforcing the importance of adopting personal protective equipment to reduce health risks.

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CO.10

THE TUBULAVIRUS RSIBR1 ALTERS THE FORMATION AND STRUCTURE OF *Ralstonia pseudosolanacearum* BIOFILM

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Tubulaviruses are filamentous bacteriophages that chronically infect prokaryotes and may induce reduced or increased host aggressiveness. The *Ralstonia solanacearum* species complex (RSSC) is the etiologic agent of bacterial wilt that affects several crops and is a known host of tubulaviruses. Further, this complex is a notorious biofilm producer in plants' xylem, disrupting water flow, resulting in wilting and killing the plant. The tubulavirus Ralstonia solanacearum inovirus Brazil 1 (RSIBR1) can reduce the aggressiveness of RSSC, eliminating their ability to kill plants. In this sense, we aimed to evaluate whether *R. pseudosolanacearum* GMI1000 (RpsGMI), an aggressive strain, shows changes in RNA expression and biofilm profile when infected by RSIBR1. We found 160 genes deferentially expressed (DEGs) related to nitrogen and sulfur metabolism, transmembrane transport, biofilm, secretion systems, chemotaxis, and expression regulation. In the following steps, we will explore and describe these DEGs' relationship with pathways related to aggressiveness. We also showed that RSIBR1 induces motility reduction of RpsGMI. The motility alterations may involve fimbriae and flagella, which are also involved with biofilm formation. RpsGMI infected by RSIBR1 had increased biofilm formation than virus-free RpsGMI and shows a distinct biofilm formation profile at different conditions, such as varying the glucose concentration and nutrient replacements over time. At last, it was observed that tubulaviruses infection altered the biofilm structure *in vitro* and in xylem vessels. In both situations, RpsGMI biofilm is a dense and homogeneous structure; however, in RpsGMI infected, it became a heterogeneous and non-dense structure.
Financial Support: CNPq, FAPEMIG, Suzano, Bracell.

CO.11

ISOLATION AND CHARACTERIZATION OF A "JUMBO" PHAGE COLLECTED IN WATER SAMPLES FROM MINAS GERAIS

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Bacteriophages are viruses that control and modulate bacterial communities, being found in association with their host bacteria. A study was conducted to assess the diversity and biotechnological potential of coliphages (viruses that infect *Escherichia coli*) from a stream in Mário Campos-MG, Brazil. Three collection points along the stream were selected, and 13 coliphages were isolated using *E. coli* 30 as bait. Among them, the isolate EC3, showed a broad host range and was selected for characterization. Transmission Electronic Microscopy showed particles with a myovirus morphology, with a head of about 85nm of diameter and a tail 112nm long. EC3 complete genome was sequenced using the MiSeq platform, assembled using SPAdes software and annotated using Prokka database. The virus has a dsDNA genome with 348.694 nucleotides, making EC3 a "jumbo" phage. It has 597 predicted ORFs, most of them codifying hypothetical conserved proteins with unknown function. Also, proteins involved in DNA metabolism, particle structure, lyse and 7 tRNAs were annotated. Sequence comparison to GenBank database by BLASTN algorithm, revealed EC3 is close to phages belonging to *Asteriusvirus* genus. The comparison with all *Asteriusvirus* deposited sequences by VIRIDIC showed it shares 95,3% of similarity with Escherichia phage vB_Eco_slurp01, a sequence unassigned to the two *Asteriusvirus* species currently recognized by ICTV. Further studies will be conducted to investigate EC3 potential as a biotechnological tool for bacterial control. These findings contribute to understanding the diversity of coliphages in the stream and highlight the potential of these viruses for biotechnological applications.

Financial Support: FAPEMIG, PAPq/UEMG.

CO.12

THE GENOMIC LANDSCAPE OF *Chlorella variabilis* -INFECTING GIANT VIRUSES

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Chloroviruses are a clade of chlorovirus that infects the Chlorella variabilis algae. These viruses have large genomes of about 350 kbp in size, encoding hundreds of CDS and up to 14 tRNAs, present in an islet at the genome center. Interestingly, one of these viruses is capable of infecting a specific lineage of the algae, named Syngen2-3, but not others. This work aimed to describe the genomic characteristics of chloroviruses isolates of lake samples from different parts of the world. The data were obtained as already assembled genomes and raw sequencing data. Canu software was used to assemble PacBio long-reads and gene predictions were performed using GeneMarkS. The annotation pipeline was performed with Diamond, HHpred, and InterPro servers. Statistical tests of normality and homoscedasticity were performed, followed by analysis of variance. Genome collinearity was accessed using BRIG and mauve. Analysis of the newly discovered viruses revealed chloroviruses with the largest genomes to date, with over 400 kbp. Our results indicated that four genomic features (genome size, count of CDSs, tRNAs and %GC) are statistically different in the viruses that infect only the Syngen strain of the algae. Furthermore, we found large regions of dissimilarity in the genomes of PBCV1 and OSy-NE5 when compared to the other genomes. These regions contain genes related to the interaction with the host cell machinery and viral capsid proteins. In this sense, we speculate that host specificity in these viruses arises from modifications in their replicative module, with subsequent changes in the structural module.

Financial Support: NSF, UNCISGP, PRPq-UFMG, CAPES, FAPEMIG

CO.13

The longevity of IgM and IgG anti-SARS-CoV-2 antibodies after COVID-19 and the impacts of reinfection and booster dose on humoral responses.

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The COVID-19 pandemic had a huge impact on health systems over the world. Healthcare works were highly exposed to the virus, and thus represents an interesting population for the study of dynamic of the immune response and its relation to reinfection cases. Here, we followed 52 healthcare workers from Brazil during 450 days to track using a rapid test their IgM and IgG anti-SARS-CoV-2 response after the infection, vaccination and booster doses. Additionally, reinfection suspension cases were monitored and confirmed by RT-qPCR. Forty-six (88%) participants had suspected reinfections; of these, 19 (37%) cases were confirmed by RT-qPCR, all presenting mild symptoms. Reinfection was more prevalent in women than that in men ($p=0.011$). After infection, the IgM levels dropped sharply in all participants, with over 47% of them becoming seronegative by the 60th day. For IgG, 90% of the participants became seropositive within the first 30 days after infection, but this antibody level also dropped after this period reaching the lowest level on day 270 (68.5 ± 72.3, $p<0.0001$). Booster dose and reinfection increased the levels of both antibodies, with the interaction between them resulting in an increase in IgG levels of 130.3 arbitrary units. No association between antibody levels and the occurrence of reinfection was observed. Overall, our data indicate that acquired humoral immunity declines over time and suggests that IgM and IgG antibody levels are not associated with the prevention of reinfection.

Financial Support: FAPEMIG, CNPq, Fiocruz (programa Inova Fiocruz)

CO.14

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

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Monkeypox virus (Mpox) is an Orthopoxvirus genetically distinct from other members of the Poxviridae family. In the year 2022, several cases of Mpox were reported in countries where the disease is not endemic. Some studies suggest that a vaccine based on the Modified Vaccinia virus Ankara (MVA) is effective Against Mpox.The objective of this work is to optimize and produce a Brazilian vaccine based on the MVA virus in compliance with GLP and to evaluate it's in vivo immunogenicity. CEFs cells were infected with MVA virus in 0.1 M.O.I for 48 hours, followed by purification. Balb/C mice were immunized by subcutaneous (S.C) and intramuscular (I.M) routes for comparison purposes with 1x108 MVA virus using homologous prime and boost protocol. After 14 days of prime and boost, the animals' blood was collected to evaluate the production of neutralizing antibodies by PRNT and IgG by ELISA. Total IgG production was observed in both groups evaluated after prime, reaching a titer of 5x103 in group I.M and 1x103 for group S.C. After the boost there was a significant increase in total IgG titers, 1x106 for group I.M and 2x105 for S.C. The Production of neutralizing Antibodies, postprime/boost, capable of neutralizing 50% of the viral particles, occurred in the 1/320 Dilution, in Group I.M and 1/160 in Group S.C. Our results show that a vaccine against Mpox based on the MVA virus produces high levels of total IgG and neutralizing antibodies, in vivo, after a single boost, showing protective potential after challenge with Mpox.
Financial Support: CAPES, FAPEMIG, CNPq.

CO.15

Neutralizing antibodies against SARS-CoV-2 induced by SpiN-Tec MCTI-UFMG during Fase 1 clinical trial

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In March 2020, the WHO declared the SARS-CoV-2 pandemic, leading to a world-class race to develop vaccines that are effective, safe, and produced in record time to combat the pandemic. Brazil, within its public institutions, began the development of several vaccines, each with a different strategy/approach. In this work, we present the Fase 1 neutralizing antibodies data from people vaccinated with the SpiN-TecMCTI-UFMG, which uses a chimeric protein subunit as an approach, and compared it with the commercial vaccine Covishield®. For this, 36 people aged between 18 and 55 years that were previously vaccinated with CoronaVac® with a booster with Pfizer were divided into three arms: B1 (20ug), B2 (60ug), and B3 (100ug). Each arm had twelve individuals receiving active comparator Covishield® (n=3) or SpiN-Tec MCTI-UFMG (n=9). Safety clinical data was evaluated, and serum samples were collected before vaccination D0 and post-vaccination D14 and D28 to detect neutralizing antibodies by $PRNT_{50}$ for SARS-CoV-2 Wuhan and Omicron BA.1, and VNT_{50} for subvariants XBB1.5 and XBB1.16. The vaccine showed to be safe, tolerable and immunogenic. Neutralizing antibodies against SARS-CoV-2 were detected in 100% of vaccinated participants. Antibody detection was higher on D28 when compared to D0 in all groups in the follow-up. Neutralizing antibodies showed higher titers for Wuhan, Omicron BA.1, and lower titers for XBB 1.5 and XBB 1.16 subvariants. No significant difference between Covishield® and SpiN-Tec MCTI-UFMG was found. We conclude that vaccination increased the titer of neutralizing antibodies both for participants vaccinated with SpiN-Tec MCTI-UFMG or the active comparator. Financial Support: Prefeitura de Belo Horizonte, Rede Virus/MCTI, INCT-Vacinas/CNPq, Fiocruz, Emenda Parlamentar (Bancada de MG na Câmara Federal), CAPES /MEC

CO.16

PREVENTION OF LETHAL SARS-COV-2 REPLICATION IN HUMAN ACE2-TRANSGENIC MICE THROUGH ERROR PRONE SUGGESTS THAT DACLATASVIR DOSE CAN BE ADJUSTED FOR EARLY COVID-19 THERAPY

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SARS-CoV-2 became a pandemic threat, with the number of confirmed infections ramping up globally. Due to the continuous worldwide circulation of 2019 coronavirus disease (COVID-19) variants with improved transmissibility and ability to escape the humoral immune response elicited by vaccines or prior infection. Repurposing of clinically approved drugs is the fastest pathway towards an effective response to a pandemic outbreak, therefore antiviral drugs are still necessary to treat. The main class of antiviral drugs nucleoside/nucleotide analogs, have demonstrated limited clinical benefit against COVID-19. This class inhibits the SARS-CoV-2 RNA polymerase when incorporated into the newly synthesized virus genome, they may be excised by this virus's proofreading exonuclease. We examined the hepatitis C virus (HCV) nonstructural protein 5A (NS5A) inhibitor daclatasvir for potential repurposing against SARS-CoV-2. Based on molecular docking, enzymatic assays and cellular-based studies, evidence was provided in favor of these compounds possessing anti-exonuclease activity. To further test the hypothesis that SARS-CoV-2 exonuclease is susceptible to HCV NS5A inhibitors, we evaluated if inhibition of exonuclease by these NS5A inhibitors would result in more mutations in the virus genome. The NS5A inhibitor daclatasvir increased the number of mutations in the SARS-CoV-2 genome, in especial A to U and C to U changes were enhanced by almost 60 times. Daclatasvir was also tested *in vivo*. By also inducing error-prone *in vivo* replication, daclatasvir prevented lethal SARS-CoV-2 replication in transgenic mice expressing human ACE2 (K18-hACE-2), reduced cell death and inflammatory responses that could allow further clinical development against COVID-19.

Funding: CAPES, CNPq, FAPERj

CO.17

THE ZIKA VIRUS INFECTION INDUCES ULTRASTRUCTURAL CHANGES, MITOCHONDRIAL DAMAGE ALONG WITH OXIDATIVE STRESS IN HUMAN TROPHOBLASTIC CELL LINE HTR-8/SVNEO

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Zika fever is an important disease that gained importance in Brazil in 2015, due to the correlation between Zika virus (ZIKV) infection and congenital zika syndrome. Based on this, this project aims to understand the consequences that ZIKV infection can cause on placental homeostasis, studying its effect on a trophoblastic cell lineage, HTR-8/SVneo. Initially, we infected the cells using 1 MOI of virus/Mock for 24h. Then, infected and control cells were processed for analysis by transmission electron microscopy and evaluated the mitochondrial membrane potential of infected cells by flow cytometry. Finally, we analyzed the activity of enzymes and reactive species related to cellular oxidative stress, such as Superoxide Dismutase (SOD), Catalase (CAT) as Malondialdehyde (MDA) and Nitric Oxide (NO) through colorimetric reactions. The ultrastructural analysis of infected cells showed mitochondrial alterations, such as rupture of membranes and loss of mitochondrial cristae, abundant endoplasmic reticulum with dilated cisternae and the presence of agglomerates of viral particles, in relation to the control that presented normal morphological aspects. Cells infected showed loss of mitochondrial membrane potential, indicating that the infection induces mitochondrial damage. In addition, infected cells showed a significant increase in the activity of SOD antioxidant enzymes such as MDA and NO-reactive species compared to the control. CAT enzyme activity was lower in infected cells compared to control, due to the rapid depletion of the enzyme. These results allow a better understanding of the disease, elucidating the effects of infection on the placental cell line. **Financial support**: IOC-FIOCRUZ, UERJ, CNPq and FAPERJ.

CO.18

CRISPR/Cas systems as a viral disruption strategy: A reactivation of HIV-1 latency study

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CRISPR/Cas9 methodologies were used to cut the HIV-1 from the genome of cells or cellular proteins important for viral infection and replication. Despite antiretroviral treatment efficacy, it does not lead to the complete eradication of HIV infection. Consequently, reactivation of the virus from latently infected cell reservoirs is a major challenge toward cure efforts. The "Block and Lock" methodology aims to control HIV-1 latency reactivation, promoting a functional cure. We utilized the CRISPR/dCas9-KRAB platform to block drug-induced HIV-1 reactivation in latently infected T cells and myeloid cells. We identified a set of five sgRNAs targeting the HIV-1 proviral genome (LTR1-LTR5), having the lowest nominated off-target activity, and transduced them into the latently infected lymphoid (J-Lat 10.6) and myeloid (U1) cell lines. One of the sgRNAs (LTR5), which binds specifically in the HIV-1 LTR NFκB binding site, was able to promote robust repression of HIV-1 reactivation in latently infected T cells stimulated with PMA and IngB, both potent PKC stimulators. Reactivation with HDAC inhibitors, such as SAHA and Panobinostat, showed the same strong inhibition of reactivation. Additionally, we observed a 100 times reduction of HIV-1 RNA expression levels in the latently infected myeloid cell line, U1 induced with IngB. Taken together, our results show that the KRAB fused CRISPR/dCas9 system can robustly prevent the HIV-1 latency reactivation process, mediated by all ltency reactivators, both in myeloid and lymphoid HIV-1 latently infected cells. In addition, we demonstrated that KRAB repressor protein is crucial to reactivation.
Financial support: Cnpq

CO.19

B cells require MyD88 signaling for antibody responses that prevents Oropouche virus-induced mouse neurological disease

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Oropouche oropoucheense (OROV) is a bunyavirus transmitted by insects that induces neurological disease in humans. There is a growing concern about the potential expansion of OROV beyond its established borders in Brazil. However, the host factors responsible for OROV pathogenesis have remained unclear. In this study, we present evidence demonstrating that B cells, rather than T cells, play a critical role in controlling viral replication and its dissemination to the central nervous system in mice. Notably, wild-type (WT) mice exhibited robust antibody production within six days of infection, indicating a protective function of B cells. Moreover, the transfer of serum antibodies containing neutralizing IgM to mice lacking both T and B cells (*Rag1*$^{-/-}$ mice) effectively prevented neurological disease. Conversely, mice with a specific deficiency in *MyD88* signaling in B cells (*CD19*-Cre^{+} *MyD88*^{fl} mice) were susceptible to OROV with neurological disease and showed reduced levels of OROV-specific IgM and IgG antibodies, which displayed suboptimal avidity, neutralization, and potency compared to WT mice and mice with intact MyD88 signaling in B cells (Cre^{-} *MyD88*^{fl} mice). Additionally, *MyD88*-deficient mice exhibited decreased spleen cellularity, reduced numbers of marginal zone B cells and plasmablasts, and increased infection and lethality. These findings highlight the importance of early MyD88 signaling in B cells for an effective antibody response that limits OROV infection and neurological disease in mice during the primary infection with OROV.

Financial Support: FAPESP, CNPq, CAPES, FAEPEX, PIPAE.

CO.20

THE ANTIVIRAL LECTIN CYANOVIRIN-N INHIBITS SARS-COV-2 VARIANTS IN VITRO AND IN VIVO MODELS.

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SARS-CoV-2 is an enveloped virus responsible for the recent deadly COVID-19 pandemic. The SARS-CoV-2 virion surface is coated with the heavily glycosylated Spike protein, crucial for viral attachment and entry into target cells. Throughout the pandemic, several SARS-CoV-2 variants of concern (VOCs) emerged harboring important mutations in Spike coding gene, that drastically impacted the effectiveness of current prophylaxis for COVID-19, such as vaccines and monoclonal antibody-based therapy. However, the Spike N-glycosilated sites shown to be conserved among those VOCs, being an opportune target to inhibit SARS-CoV-2 infection. In this context, the present study evaluated the activity of the antiviral lectin Cyanovirin-N (CV-N) against SARS-CoV-2, demonstrating that CV-N binds with high affinity to Spike N-glycosylated sites, specifically in the S1 region. Despite not neutralizing the Spike receptor binding domain (RBD), CV-N was able to inhibit the entry of SARS-CoV-2 in several in vitro assays, being notably more efficient against newer VOCs such as Delta and Omicron, as observed in plaque neutralization test with replicating SARS-CoV-2 strains. In hamsters challenged with SARS-CoV-2, intranasal CV-N administration reduced viral load and lung damage, besides weight loss recovery in CV-N treated animals. In conclusion, our data indicates promising therapeutic potential of CV-N against emerging SARS-CoV-2 variants. Financial support: CNPq, FAPERJ, EMBRAPA, NCI.

CO.21

Molecular and evolutionary analysis of canine circovirus in dogs from animal shelters in Belem, Para, Northern Brazil

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Introduction: Canine circovirus (CanineCV) is an important emerging virus associated with gastroenteritis in dogs worldwide. In this study, we investigated the presence of CanineCV in dogs with diarrhea in Northern Brazil. Material and Methods: The study involved the collection of fecal samples and rectal swabs from asymptomatic (n=56) and symptomatic (n=91) dogs. PCR and Sanger sequencing was performed with Rev 533/For genomic primers for the *rep* gene region. Genomic libraries were made by enzymatic fragmentation and sequenced on an Ion S5 Platform. Phylogenetic inferences were performed by maximum likelihood. The evolutionary rates, the most recent common ancestor and geographic origin were estimated using the Bayesian Markov Chain Monte Carlo. Results: CanineCV was detected by PCR in 15% (22/147) of dogs from animal shelters in Belem, between 2019-2020. We observed an association between infection and the presence of diarrhea in animals younger than one year of age ($p>0.01$). The Brazilian strains grouped in the China genotype, with 99.54-100% nucleotide homology. The most recent common ancestor was estimated in 2017, with evolution rate of $1.6x10^{-3}$s/s/y. Viral family diversity was also investigated, with emphasis on the families of enteric pathogenic viruses *Parvoviridae*, *Picornaviridae* and *Astroviridae*. Discussion: This study highlights the importance of CanineCV as emergent virus causing diarrhea. Our results contribute to the understanding of the role of CanineCV in enteric diseases and evolutionary molecular characterization of circulating genotypes. Furthermore, we increased the understanding of the fecal virome in diarrheal dogs provided data for the monitoring viral gastroenteric diseases. Financial support: Instituto Evandro Chagas

CO.22

UNRAVELING THE PHYLODYNAMICS OF 2009 H1N1 PANDEMIC INFLUENZA VIRUSES: HUMAN-TO-SWINE INTRODUCTIONS IN BRAZIL

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Following its introduction into the human population, the 2009 H1N1 pandemic virus (H1N1pdm09) was repeatedly introduced into swine populations globally with subsequent onward transmission among pigs. To explore human-to-swine H1N1pdm09 introductions in Brazil, an extensive phylogenetic analysis was conducted and included 4,141 H1pdm09 hemagglutinin (HA) and 3,227 N1pdm09 neuraminidase (NA) gene sequences from humans and swine isolated between 2009 and 2022. Phylodynamic analysis revealed that during the period between 2009 and 2011, there was a rapid transmission of the H1N1pdm09 virus from humans to swine in Brazil. Multiple introductions of the virus were observed, but most of them resulted in self-limited infections in swine, with limited onward transmission. Only a few sustained transmission clusters were observed during this period. However, after 2012, the number of human-to-swine H1N1pdm09 transmissions in Brazil decreased significantly. Throughout this time, the virus underwent continuous antigenic drift, achieving a balance between swine-to-swine transmission and extinction, with minimal sustained onward transmission from humans to swine. This study reveals the crucial dynamics that govern the transmission and evolution of H1N1pdm09, emphasizing the need for continuous surveillance and understanding of the viral evolution to mitigate potential risks to both human and swine populations.

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CO.23

H5N1 highly pathogenic avian influenza virus detected in terns belongs to the clade 2.3.4.4B

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Brazil reported the first H5 highly pathogenic avian influenza virus (HPAIV) detection in wild birds in May 2023. Up to August, 72 outbreaks caused by H5N1 were reported in seven states. Espirito Santo is the most affected state, with 29 outbreaks. The PREVIR team sampled wild birds in the Rio Doce estuary, ES, from June 17 to 27, 2023. Molecular detection was performed using a specific AIV RT-qPCR targeting the M gene, and positive samples were selected for virus isolation and next-generation sequencing. Maximum-likelihood phylogenies for each gene segment based on General Time Reversible (GTR) model with a gamma distribution and the clock-likeness estimated by TempEst v1.5.3 software were performed. Samples from 81 wild birds, including 13 Charadriiformes, were collected. Five samples from Cabot's terns and royal terns were detected by RT-qPCR with Ct values ranging from 20 to 35. Viruses were successfully isolated and sequenced. Our study provides evidence for the presence of H5N1 HPAIV from clade 2.3.4.4B in Cabot's tern and royal tern in Espirito Santo. HA sequences had amino acid signatures of the clade 2.3.4.4B, including 123P, 133A, and 156A. The cleavage site had five insertions of R and K. All genes clustered and formed a well-supported monophyletic clade, suggesting their close ancestry with South American wild bird sequences. Brazil seemed to be the last country reached by this virus in the South American region. Therefore, local surveillance of bird species with a high risk of infection, such as terns, should be intensified. Financial support: CAPES, RedeVírus, CNPq-MCTI (400172/2022-4).

CO.24

Study of Bluetongue Virus (BTV) and Epizootic Hemorrhagic Disease Virus (EHDV) circulation in the Municipal Parks and Zoobotanical Foundation of Belo Horizonte (FPMZB-BH)

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BTV and EHDV are insect-borne Orbivirus transmitted by Culicoides spp. They have a worldwide distribution, causing hemorrhagic diseases in domestic and wild ruminants. BTV has 36 serotypes and EHDV 7. Ten serotypes of BTV and two of EHDV have been identified in Brazil causing outbreaks in sheep and deer. In 2020 a female gray brocket (Subulo gouazoubira) were found dead in the enclosure of Foundation of Municipal Parks and Zoobotanical Garden of Belo Horizonte (FPMZB-BH) and a EHDV infection was confirmed. In the light of this situation, studies related to BTV, EHDV circulation were conducted in FPMZB-BH. Sera samples collected from 72 animals (14 species), from 2012 to 2022, were tested for BTV and EHDV. Results showed that 75% (54/72) and 19% (11/59) of samples were seropositive for BTV and EHDV, respectively. It was identified that 100% (9/9) of the tested Oryx were positive for BTV and 50% (3/6) for EHDV. Other African ruminants were also positive for BTV as well as an elephant and a tapir. Evaluating the data, it was possible to conclude that many of positive animals were infected in the zoo. This study show that Orbivirus are circulating in the FPMZB-BH, been a risk for ruminants that live there and for new introduced animals, that may be seronegative for BTV and EHDV serotypes that are circulating in the Zoo.
Financial Support: CNPq, CAPES and FAPEMIG.

CO.25

BRAZILIAN HUMAN ROTAVIRUS G8 STRAINS DETECTED OVER A 13-YEAR PERIOD: GENOMIC CONSTELLATION OF THE NOVEL BOVINE-LIKE G8P[8] STRAINS WITH THE DS-1-LIKE BACKBONE

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Rotavirus G8 is mostly identified in animals and occasionally in humans. Recently, an elevation in G8 detection was noticed outside Africa. The main goals of the investigation were to track G8 infections in Brazilian population between 2007-2020, characterize the full genome and perform phylogenetic analysis to understand its genetic diversity and evolution. A total of 12,978 samples were screened for RVA using ELISA, PAGE, RT-PCR and Sanger sequencing. G8 genotype represented 0.6% (15/2434) of all RVA-positive samples. G8P[4] 33.3% (5/15), G8P[6] 46.7% (7/15) and G8P[8] 20% (3/15). All the twelve G8 strains displayed a DS-1-like genetic backbone with four different genotype-linage constellations. Brazilian G8P[8] strains were derived from cattle and clustered with newly DS-1-like G1/G2/G3/G9/G8P[8] strains. IAL-R193/2017/G8P[8] belonged to a VP1/R2.XI lineage and were grouped with bovine-like strains identified in Asia. IAL-R558/2017/G8P[8] had a “distinct” VP1/R2 lineage never previously described. Our findings suggest the Brazilian bovine-like G8P[8] are continuously evolving and reassorting with local RVA strains. Brazilian G8P[6] strains have been reassorted with co-circulating American strains and phylogenetic analyzes revealed that these strains have genetic origin from Africa. Rather than being African-born, Brazilian G8P[4] were imported from Europe. None of the Brazilian strains analyzed exhibited signs of zoonotic reassortment and continued in Brazil according to their localized pattern, that not suggest a potential emergence taking place in the country. Our study demonstrates the diversity of G8 RVA strains in Brazil and add up to the understanding of G8P[4]/P[6]/P[8] RVA genetic diversity and evolution on a global scale.

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CO.26

EVALUATION OF THE IMPACT OF AMINO ACID SUBSTITUTIONS IN HCV CORE PROTEIN ON THE DEVELOPMENT OF NONACOHOLIC STEATOHEPATITIS

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Nonalcoholic steatohepatitis (NASH) in patients with hepatitis C virus (HCV) infection has an prevalence of 55.0% and can lead to cirrhosis. Studies show that core protein is capable of inhibiting the activity of microsomal triglyceride transfer protein, leading to accumulation of triglycerides. The aim of this study was to evaluate the impact of amino acid substitutions in the core protein on development of NASH. Serum from 61 patients with chronic HCV were analyzed, 39 had NASH and 22 didn't. The complete HCV core (996 bp) was amplified (RT-PCR) and sequenced (Sanger). In our group, 67.2% (41/61) were female and the mean age was 65 ± 10.2 years, 52.4%(32/61) had subgenotype 1b. All substitutions found were more frequent on the NASH group when compared to no-NASH. In the NASH group, R70Q and L91M were present in 43.6% (17/39) and 35.9% (14/39), respectively. The T189A was the most prevalent substitution with 69.2% (27/39). The Y164F occurred in 12.8% (5/39), L182F in 10.2% (4/39) and T186I in 10.2% (4/39), found only in subgenotypes 3a. The substitutions Y164F, L182F, T186I and T189A have not been linked to NASH in studies yet. This study contribute to a better understanding of the mechanisms that lead to the accumulation of fat in the liver during HCV infection and help as a tool to control NASH rates in the population.
Financial support: Fiocruz

CO.27

Respiratory Virus Surveillance during the COVID-19 pandemic in Rio de Janeiro State, Brazil

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Various viruses' infections may be associated with acute respiratory syndromes, which have overlapping signs and symptoms, making it impossible to identify the etiological agent by clinical examination. Some viruses are more related to severe cases, so they are often included in surveillance programs, such as *Alphainfluenzavirus influenzae* (FLUVA) and *Betainfluenzavirus influenzae* (FLUVB) and *Orthopneumovirus hominis* (HRSV) in children. However, many other viruses circulate in the general population and can emerge as aggressive phenotypes, as seen with SARS-CoV-2. During the COVID-19 pandemic, all diagnostic efforts were devoted to confirming the infection of SARS-CoV-2, so information regarding the circulation of other respiratory viruses was scarce. Therefore, this study aimed to describe which respiratory viruses circulated in the population of Rio de Janeiro State during the pandemic. Between August 2021 and December 2022, 21,797 samples from suspected COVID-19 cases were also tested for HRSV, FLUVA, and FLUVB. A set of samples was also enrolled for evaluating the presence of Rhinovirus, Bocavírus, *Metapneumovirus hominis*, Parainfluenza types 1, 2, 3 e 4 Virus, the coronaviruses 229E, OC43, HKU1, NL63, enterovírus, and Adenovirus. During the evaluated period, the percentage of detection was 14.55% for Rhinovirus, 12.52% for Adenovirus, 11.93% for SARS-CoV-2, 11.23% for Influenza A, 10.60% for RSV, and 8.87% for Metapneumovirus. Our result reinforces the need to overcome the labor and economic challenges to implement broad and efficient surveillance for respiratory viruses.

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CO.28

GENOMIC SURVEILLANCE OF ARBOVIRUSES (DENGUE AND CHIKUNGUNYA) DURING THE MOST RECENTE EPIDEMY OF 2023.

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The rapid spread and increase of Dengue-DENV and Chikungunya-CHIKV cases in Brazil raised concerns of the impact of arboviruses on public health services. We performed a genomic surveillance and epidemiological analysis to estimate the introduction and spread of DENV and CHIKV during the most recent epidemics in 2023. We investigated 61,353 and 16,838 positive diagnoses for DENV and CHIKV, respectively in Brazil during 2022-2023 period. 391 samples collected in 2023 from MG were genotyped for DENV1-4 and co-infection events were investigated. 239 samples were positive for DENV-1 and four classified as DENV-2. 38 samples positive for dengue were positive for CHIKV, and three samples positive for CHIKV were positive for dengue (co-infection rate 10.49). We generated 80 novel CHIKV, 151 DENV-1 and four DENV-2 new high-quality genomes. Phylogeographic analyzes showed that all CHIKV genomes were classified as ECSA lineage, and all genomes are closely related to genomes isolated from the northeast of the country, suggesting importation events from this region. In contrast, all DENV-1 genomes generated were classified as genotype 5 with polyphyletic distribution associated to other DENV viruses from all Brazilians regions. Most of the samples grouped in one cluster, in which MG genomes are the main source of export to other Brazilian regions. The higher rates of reemergence and co-infection of arboviruses reinforce the importance of increasing number of vectors during the most recent epidemics and the clinical outcome of individual carrying both viruses should be followed by the health authorities.

FINANCIAL SUPPORT: ITPs, MCTI, FINEP, CNPq, CAPES

CO.29

STUDY OF OROPOUCHE VIRUS TRAFFICKING AND ITS RELATIONSHIP WITH THE MODULATION OF THE AUTOPHAGIC PATHWAY DURING HOST CELL INFECTION

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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). However, our understanding of the cell biology of Orthobunyaviruses and their strategies to evade the host's antiviral response remains limited. OROV has been reported as a neurotropic virus, highlighting the importance of studying the infection process. This study provides evidence that OROV infection in human neuroglioma cells (H4 cells) induces a complete autophagic flux and generates viral factories that colocalize with autophagosome markers. In addition, the requirement of autophagic proteins in OROV replication was observed, specifically of ATG5 protein, described by OROV infection of H4 shRNA ATG5 cells, where both viral protein production and viral particle release were significantly reduced compared to H4 shRNA luciferase cells (Control). Additionally, the presence of autophagic and lysosomal proteins in the extracellular vesicle fraction together with the viral particles was evaluated. Notably, the secretion of p62, Lamp1, pro-cathepsin-D, and cathepsin-D was detected in the extracellular vesicle fractions of cells infected with OROV. These results suggests the participation of the autophagic pathway in OROV infection.

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CO.30

HOW HIF-1α DELETION IN DENDRITIC CELLS IMPACTS CHIKV INFECTION IN MICE

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Chikungunya virus (CHIKV) is an emerging arbovirus associated with arthralgia or arthritis in humans. The chronic disease has similar characteristics of rheumatoid arthritis (RA) in which dendritic cells (DCs) and a hypoxic environment are implicated in pathogenesis. Hypoxia-inducible factor (HIF-1α) is a transcription factor that is activated during hypoxia and plays a central role in immune responses activating the transcription of hypoxia-inducible genes. Thus, we believe that the metabolic imbalance of DCs may play an essential role in the pathogenesis of CHIKV and in the development of chronic inflammation. To analyze the effect of HIF-1α in DCs for CHIKV replication and pathogenesis, we analyzed the viral load and genetic expression after infection when HIF-1α was inhibited in DCs in vivo, using CD11ccre+HIF-1$\alpha^{fl/fl}$ animals, or in vitro, using BAY 87-treated bone marrow-derived dendritic cells (BMDCs). The inhibition of HIF1-α in BMDCs does not affect CHIKV replication but increases the expression of glycolytic enzymes involved in inflammation. Interestingly, CD11c^{cre+}HIF-1$\alpha^{fl/fl}$ CHIKV-infected mice had a dramatic increase in footpad swelling during acute infection and a statistically significant increase in viral load 3 days after infection in comparison with controls. In concordance, these animals had alteration in local and systemic production of cytokines and chemokines at 3 and 7dpi, favoring monocytes infiltration. In summary, the deletion of HIF-1α in dendritic cells indirectly promotes an increase in infectivity, inflammation, cytokine production and monocyte migration during the acute phase of CHIKV infection in mice.

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CO.31

Alterations in Adipocyte Lipid Droplet Dynamics: The Role of SARS-CoV-2 in Viral Pathogenesis

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Overweight and obese patients showed a high prevalence of severe acute respiratory syndrome during the COVID-19 outbreak caused by the SARS-CoV-2 virus. Thus, this study aims to elucidate the interactions between adipose tissue and SARS-CoV-2 and its implications for viral pathogenesis.
We demonstrate that SARS-CoV-2 infects murine and human adipocyte cell lines (3T3-L1 and A41, respectively) at different stages of differentiation (0, 3, 5, 9, 13, and 17 days) and produces infective viral particles as detected by qPCR and plaque assay. We also found viral double-stranded RNA close to the lipid droplets (LD) by immunofluorescence, and a reduction of LD size in human adipocytes 12 hours post-infection, which suggests that LDs are being consumed and may serve as an energy source for viral replication in adipocytes. But it was followed by an increase in LDs, which may also indicate that the infection alters the lipid droplet dynamics in these cells. We also detected viral genome in adipose tissue depots of hamsters infected with SARS-CoV-2 after 2 months of a high-fat diet, but not in the chow-fed group.
Using the lipidomic approach, we found that the infection in human adipocytes increases phosphatidylcholine (PC) and phosphatidylethanolamine (PE), which suggests an increase in the fluidity of the cell membrane. In murine adipocytes, we noticed a significant increase in diacylglycerol (DG), whose abundance directly reflects on the LD size. Taken together, we demonstrate that SARS-CoV-2 alters lipid droplet dynamics and lipid metabolism in adipocytes, influencing the permissiveness and pathogenesis of SARS-CoV-2.
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Key words: SARS-CoV-2, adipocytes, Lipid droplets

CO.32

The Impact of Monkeypox Virus on Lymphoid Tissue: Assessing Inflammatory Responses in an Ex Vivo MPOX infection

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Human monkeypox virus (MPOX) is a neglected zoonotic orthopoxvirus closely related to the smallpox virus. Following the increase in human-to-human transmission in May 2022, MPOX has become a global concern. We investigated the ex vivo infection of human tonsillar explants by MPOX to evaluate activation of inflammatory pathways, considering that swellings lymph node it is a hallmarker symptom. Human tonsil explants were cultured in Transwell inserts with an air-liquid interface, and MPOX was carefully inoculated on the epithelial side. After 2 hours, the tissue was washed, and fresh medium was added. Explants were harvested at 72 hours and 7 days post-infection for molecular assays, in situ labeling, and lipidomics analyses. MPOX infection resulted in a significant increase in viral loads, with strong viral protein signals observed in the lymphoid parenchyma. Tissue destruction, necrosis, were evident at 7 days post-infection, as shown by intense architectural loss, eosinofilia. Necroptosis and apoptosis were not relevant, as indicated by staining with MLKL and Caspase 3, respectively. The upregulation of IL-6 and IL-1B mRNA, along with positive labeling of AIM2, NLRP3, and N-term GSDM, by in situ fluorescence and Western blot assay, indicated the activation of inflammasome pathways and pyroptosis in MPOX-infected explants. Furthermore, lipidomics analysis revealed significant alterations in phosphatidylethanolamine and phosphatidylcholine species, enhencing cellular membrane fluidity. In conclusion, these findings highlight the susceptibility of tonsillar explants to MPOX infection and the involvement of inflammasomes AIM-2 and NLRP-3 in MPOX lymphoid pathogenesis and inflammation. Financial support: Fapesp 2019/26119-0

CO.33

THE VIROME OF THE SPIDER MITE *TETRANYCHUS TRUNCATUS*

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Tetranychus truncatus is a spider mite species belonging to the cosmopolitan family *Tetranychidae* that infests over 1100 plant species from 250 different families posing as a treat to crop productivity. The damage caused by *Tetranychidae* is approximately US$ 470 billion annually, 18-20% of the total global crop production. Mites are phytopathogens vectors, but there's little knowledge about the associated virome to *Tetranychidae*. Here, we investigated 10 publicly available transcriptomes that represented a wide range of sample conditions, from temperature stress to symbiont infection status looking for virus presence. Using an integrative assembly strategy, we identified twenty-one assembled transcripts associated with viral sequences, thirteen of then were unique and likely represent complete viral genomes according to the closest species. Analyses of conserved domains and phylogeny indicated these viral sequences are related to diverse species distributed among several families, such as the circular-DNA *Nudiviridae*(1); ssRNA(+) viral families such as *Kitaviridae*(2)*, Dicistroviridae(2)*, *Ourmiaviridae*(1), *Virgaviridae*(1), *Betaflexiviridae*(1) and *Nodaviridae(1)*; and segmented ssRNA(-): *Phenuiviridae(2)*. Two other viral-sequences representing known viruses were also observed: polyproteins of *Cherry virus A* (*Betaflexiviridae)* and *Potato virus Y* (ssRNA(+), *Potyviridae*). Overall, these results extend our understanding on *T. truncatus* virome diversity and taxonomy. The key findings of our study are the identification of the first DNA virus on *Tetranychidae* and six viruses belonging to families known to infect plants as their natural hosts. Therefore, further investigation is required to determine the potential role of *Tetranychus truncatus* as a vector of phytopathogenic viruses.

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CO.34

DETECTION AND QUANTIFICATION OF WOLBACHIA AND ITS INFLUENCE ON VIRAL LOAD IN MOSQUITOES USING SMALL RNA LIBRARIES

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Mosquito-borne diseases cause millions of infections worldwide annually. Mosquito co-infections shape the dynamics of arbovirus transmission. *Wolbachia pipientis*, an endosymbiotic bacterium, controls *Aedes aegypti* virus transmission, while insect-specific viruses induce the contrary effect on arbovirus transmission in the same mosquito. Utilizing a small RNA-based metagenomics approach optimized for virus identification, we have redirected its application to detect and quantify Wolbachia, within small RNA libraries. A total of 102 libraries were analyzed, including 18 from laboratory *Ae. aegypti* as a control group (all artificially infected with Wolbachia and half treated with antibiotic to remove the infection), and 84 field-collected libraries (17 of *Ae. albopictus*, 56 of *Ae. aegypti*, 4 *Ae. japonicus*, 5 *Haemagogus sp.* and 2 *Sabethes sp.*). In the control experiment libraries, only the libraries not treated with the antibiotic showed significant amounts of Wolbachia reads. Wolbachia was only detected in wild-caught mosquito libraries of *Ae. albopictus*, in which 11 out of 17 displayed substantial Wolbachia small RNA reads aligned specifically to the wAlb strain, which commonly infects this mosquito species in the field. We found *Cell-Fusing Agent Virus* (CFAV) in *Ae. aegypti* control libraries, and two viruses in wild-caught *Ae. albopictus* libraries. Statistical analysis indicated a negative correlation between CFAV viral load and Wolbachia load, suggesting Wolbachia may inhibit insect-specific virus replication. The same trend was observed for the *Ae. albopictus* viruses. Our findings highlight the potential impact of Wolbachia on the mosquito viromes, offering valuable insights for future surveillance projects.

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CO.35

UNRAVELLING THE BACULOVIRUS-ORTHOMYXOVIRUS CONNECTION: INSIGHTS FROM THE GP64 GENE TRANSFER AND HOST SPECIFICITY ANALYSES

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The Baculoviridae family includes insect-infecting viruses with large circular double-stranded DNA genomes. The Alphabaculovirus genus specifically infects lepidoptera and is divided into Group I and Group II. Each group has a major envelope glycoprotein, GP64 and F protein, respectively, crucial for viral budding and cell entry. GP64 shares structural and sequence similarity with the envelope glycoprotein (GP) of Thogotovirus, a distant virus belonging to Family Orthomyxoviridae (ssRNA-), suggesting the horizontal gene transfer (HGT) from an orthomyxovirus ancestor. However, more evidence is needed to support this hypothesis. To explore this connection, we replaced the *gp64* gene of AcMNPV baculovirus with the GP of a bee orthomyxovirus sequenced in our lab. In vitro experiments involving viral passage, virus titration, western blot, and qPCR showed that this modified GP rescued baculovirus infectivity in lepidopteran cell lines. Additionally, it functioned as a fusogen, as confirmed by pH-sensitive syncitia formation assay. Furthermore, fluorimetry and cell cytometry assays revealed that this GP significantly enhanced baculovirus entry and gene transduction in mosquito cell lines. We conducted datamining in the SRA database, characterizing the genome and phylogeny of a novel thogotovirus. This thogotovirus was discovered in an RNAseq project of a lepidopteran insect, clustering with baculovirus gp64 in the GP-based phylogeny. Molecular dynamic analyses based on protein homology models explored the flexibility of GP across viral species and its implications for host specificity.

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CO.36

ARBOVIRUS AND INSECT-SPECIFIC VIRUS SURVEILLANCE IN MOSQUITOES COLLECTED IN A DENGUE HYPERENDEMIC AREA IN NORTHWEST OF SÃO PAULO STATE, BRAZIL

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Insect-specific viruses (ISVs) are a group of RNA viruses that have a host tropism to insects and are incapable to replicate in mammalian cells. ISVs may influence arbovirus vector competence and can potentially be used as biological control agents or vaccine delivery platforms. From March 2022 to April 2023 in São José do Rio Preto (SJRP), São Paulo, Brazil, a dengue hyperendemic area, we collected 642 pools of mosquitoes, consisting of *Culex*, *Aedes*, *Psorophora*, *Sabethes*, *Anopheles*, *Haemagogus*, and *Limathus* species, in urban (443 pools) and forest fragments (199 pools). 306 pools were screened by viral isolation, sequencing, RT-qPCR for medically important flaviviruses and alphaviruses and PCR for ISVs. Humaitá-Tubiacanga virus (HTV) was detected in 15% of *Aedes*, *Culex*, and *Sabethes*. Phasi Charoen-like virus (PCLV) was detected in 19% of *Aedes* and *Culex*. Culex flavivirus (CxFV) was detected in 9% of *Culex*. Guapiaçu virus (GUAPV) in 1% of *Limathus*, *Culex*, and *Psorophora*. 35% of *Aedes* and *Culex* tested positive in a pan-Flavivirus PCR assay. All PCR products were confirmed by Sanger sequencing. Pools with only one mosquito have shown co-infections between ISVs: HTV and PCLV; flavivirus and PCLV; HTV and flavivirus; CxFV and flavivirus; and HTV, PCLV and flavivirus. All pools tested negative for medically important arboviruses, including dengue, Zika, Yellow Fever, Mayaro, Oropouche and chikungunya viruses. Our results demonstrate the diversity of the virome of mosquitoes sampled in various ecotypes in SJRP, warrant further studies assessing their contribution to vector competence and their interactions with circulating arboviruses in the region.

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