
V FAMERP - UTMB
EMERGING INFECTIONS IN THE AMERICAS –
COMMON INTERESTS AND COLLABORATION
BETWEEN NORTH – SOUTH
JULY 25–28TH
2023 – SOCIEDADE DE MEDICINA E CIRURGIA
DE SÃO JOSÉ DO RIO PRETO
SAO JOSE DO RIO PRETO/SP, BRAZIL

EVENTO

V FAMERP-UTMB: Emerginginfections in theAmericas - common interestsandcollaborationbetweennorth - south a ser realizado na cidade de São Jose do Rio Preto, SP, no período de 25 a 28 de Julho de 2023 na Sociedade de Medicina e Cirurgia de São José do Rio Preto. Repetindo o sucesso dos eventos anteriores (FAMERP-UTMB e Escola São Paulo de Arbovirologia), estamos trabalhando para elaborar um evento de alto nível científico, com palestras de renomados pesquisadores nacionais e internacionais, e planejamos oferecer uma programação que abordará temas relevantes e atuais relacionados às principais Doenças Infecciosas Emergentes e seu impactos na esfera científica e social. Certamente, pela excelência dos pesquisadores envolvidos e pela abordagem dos temas de interesse, este evento oferecerá aos participantes um espaço para discussão e difusão de conhecimentos, através de palestras, mesas-redondas, apresentações orais e de pôsteres.

MEETING

V FAMERP-UTMB: Emerginginfections in theAmericas - common interestsandcollaborationbetweennorth - southtobeheld in thecityof São José do Rio Preto, SP, fromthe 25th tothe 28th of July 2023 atthe Sociedade de Medicina e Cirurgia de Sao Jose do Rio Preto. Repeatingthesuccessofpreviousevents (FAMERP-UTMB and Escola São Paulo de Arbovirologia), we are workingtocreate a high-levelscientificevent, withlecturesbyrenownednationalandinternationalresearchers, andweplantooffer a programthatwilladdressrelevantandcurrenttopicsrelatedtotothemainEmergingInfectiousDiseasesandtheirimpacts in thescientificand social sphere. Certainly, duetotheexcellenceoftheresearchersinvolvedandthe approach tothetopicsofinterest, thiseventwillofferparticipants a space for discussionanddissemination ofknowledge, throughlectures, round tables, oral and poster presentations.

INFORMAÇÕES:

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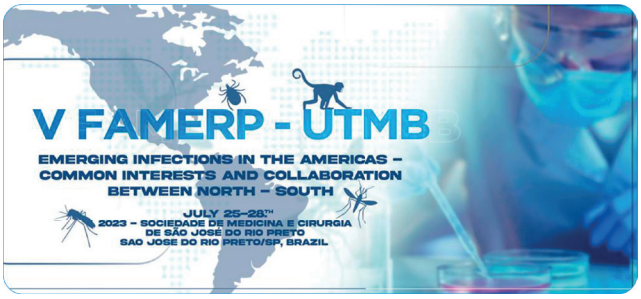

ARCHIVAL FECAL SPECIMENS STORED FROM 1998 TO 2008: EVIDENCE OF HUMAN BOCAVIRUS INFECTIONS IN BRAZIL

Elen Viana[1], Yasmin França[1], Lais Sampaio de Azevedo[1], Roberta SalzoneMedeiros[1], Raquel Guiducci[1], Simone Guadagnucci Morillo[1], **Adriana Luchs[1,*]**

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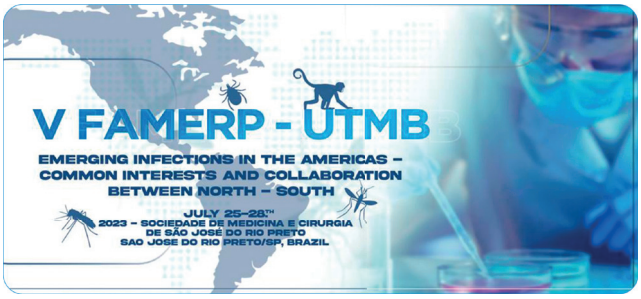
ABSTRACT

Human Bocaviruses (HBoV) have been detected in human respiratory and gastrointestinal infections worldwide. The present study aimed to investigate HBoV in archival fecal specimens stored from 1998 to 2008 in order to understand the natural history of HBoV infection in diarrheal patients in Brazil. A total of 6128 specimens were tested for HBoV using qPCR. Genotypes were identified by conventional PCR and sequencing. HBoV was detected in 6.3% (384/6128). Detection rate significantly varied according to the years 2000/2001 [$\chi2=5.771$, $p<0.05$], 2005/2006 [$\chi2=9.836$, $p<0.05$] and 2006/2007 [$\chi2=12.083$, $p<0.05$ suggesting that HBoV infections show a tendency to occur in natural oscillatory fluctuation. HBoV was frequently observed during the autumn season. The mean and median ages of HBoV positive patients were 7.9 years (ranging from 10 days to 82 years) and 1 year, respectively. Positivity was higher in children ≤5 years [$\chi2=15.311$, $p<0.05$], highlighting that it is an important pathogen in childhood diarrhea. A total of 83 (21.6%,83/384) HBoV specimens were successfully genotyped. HBoV-1 was the most prevalent genotype (80.7%,67/83), followed by HBoV-3 (10.9%,9/83), HBoV-2 (7.2%,6/83) and HBoV-4 (1.2%,1/83). HBoV-1 was the most commonly genotype detected in all months, but more frequently in the coldest and driest months of the year. Since HBoV-1 can display a persistent shedding in feces following prior respiratory infections, its identification in stool samples may not always be linked to symptoms of diarrhea. The present study contributed to the understanding of the natural history of HBoV in diarrheal patients in Brazil, confirmed the long-lasting implication of this pathogen in gastrointestinal disease burden in the country, as well as highlight its genotypic diversity throughout decades. The acquired data are important for studies investigating enteric viruses' prevalence and molecular epidemiology of archival clinical specimens

Keywords: Gastroenteritis, diarrhea, human bocavirus, molecular characterization, surveillance

Financial support: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) #2020/14786-0, #2020/02469-0, #2020/11182-6 and #2021/09064-8. Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). Fundo Especial de Saúde para Imunização em Massa e Controle de Doenças (FESIMA) CAF Nº #001/2021 and #060/2021



MIRNA 511_5P IS A POTENTIAL BIOMARKER FOR OCULAR TOXOPLASMOSIS

Geraldo Magela de Faria Júnior[1], Gláucio Silva Camargos[1], Laurie Sayuri Kumano[1], Isabela Bronchtein[1],-Cristina da Silva Meira Strejevitch[3],Lilian Castiglioni[1],Mariana Previato[2], Vera Lucia Pereira-Chioccola[3],Cinara de Cássia Brandão[1],Luiz Carlos de Mattos[1*].

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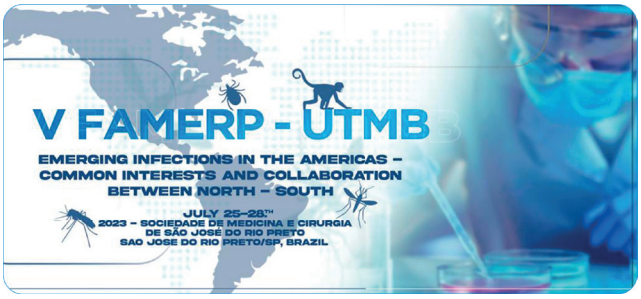
ABSTRACT

Ocular toxoplasmosis (OT) is the most frequent clinical manifestation resulting from infection by Toxoplasma gondii. It is characterized by an intraocular inflammatory process involving the infiltration of immune cells and macrophages, which are activated by proinflammatory cytokines secreted by TH1 cells. There is evidence that the expression of microRNA takes place in the steps of the inflammatory process and among them, the 511 microRNA regulates the production and activation of macrophages. This study evaluated the level of expression of the 511_5p microRNA in the blood plasma of patients with a clinical diagnosis of OT, and in healthy controls. A total of 361 patients from the Retinopathy Outpatient Clinic and the Ophthalmological Surgical Center of the Hospital de Base of Fundação Faculdade de Medicina de São José do Rio Preto (FUNFARME) were enrolled and divided into four groups: G1 - patients with active ocular lesions and reagent serology for T. gondii; G2 - patients with scars and reagent serology for T. gondii; G3 - patients without ocular lesions or scars and other inflammatory diseases of the retina and reagent serology for T. gondii; G4 - patients without ocular lesion or scars and non-reagent serology for T. gondii. All patients underwent clinical and laboratory evaluation to confirm or exclude the diagnosis of OT. Serology tests, RNA extraction, and cDNA synthesis were performed in addition to real-time PCR. The miRNA 511_5p levels were compared among the four groups. The G1 group showed a high blood plasma concentration of the miRNA 511_5p (mean 22,34) compared to the G2 (4.65), G3 (8.91), and G4 (3.52) groups ($p<0.0001$). These data suggest that miRNA 511_5p has significant potential as a biomarker for OT

Key-words: Ocular Toxoplasmosis, *Toxoplasmagondii*,microRNA511_5p

Financial support: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (grants: 2018/09448-8; 2020/14783-0 to CSMS); PIBIC/CNPq CAPES; CNPq (grant: 305976/2022-2)



SIRNA LIPID NANOPARTICLES FOR CXCL12 SILENCING MODULATES BRAIN IMMUNE RESPONSE DURING ZIKA INFECTION

Pedro Augusto Carvalho Costa[1], Walison Nunes da Silva[1], Pedro Henrique Dias Moura Prazeres[1,2], Heloísa Athaydes Seabra Ferreira[1], Natália Jordana Alves da Silva[1], Maria Marta Figueiredo[3], Bruna da Silva Oliveira[4], Sérgio Ricardo Aluotto Scalzo Júnior[1], Felipe Rocha da Silva Santos[5], Aline Silva de Miranda[4], Michael J. Mitchell[6], Mauro Martins Teixeira[5], Vivian Vasconcelos Costa[4], Pedro Pires Goulart Guimarães[1]

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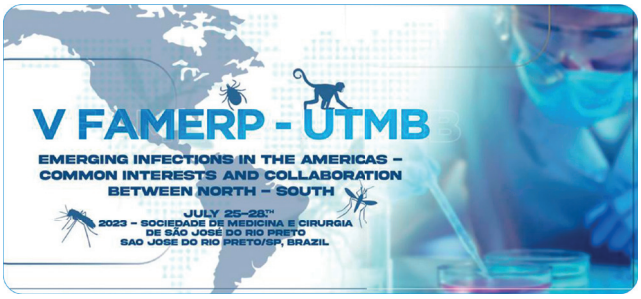
ABSTRACT

Zika virus (ZIKV) neuroimmunomodulation is still poorly understood, and some chemokines are important to maintain tissue homeostasis. CXCL12 is a chemokine that plays a role in neuroinflammation mediating local brain immune response as leukocytes trafficking into the brain to combat virus infection and is upregulated in circulating cells of ZIKV infected patients. Here, we developed a lipid nanoparticle (LNP) to deliver siRNA in vivo to assess the effect of CXCL12 silencing during ZIKV infection. Biodistribution of the LNP was assessed in vivo after intravenous injection. Next, we investigated the ability of LNP to silence CXCL12 in the spleen, liver, and brain and the in vivo effects during ZIKV infection by flow cytometry. LNP encapsulating siRNA significantly inhibited CXCL12 in spleen during ZIKV infection. Systemic silencing of CXCL12 promoted microglial activation in brain as indicated by increased expression of iNOS, TNF-α, and CD206 in these cells. Moreover, lower levels of IFN-□ and IL-17 were secreted by T cell subsets after treatment with LNP. Although CXCL12 silencing after treatment with siRNA-LNP did not change viral load, type-I interferon production was significantly decreased compared to ZIKV and uninfected groups. Furthermore, we found grip strength deficits in the group treated with siRNA-LNP compared to ZIKV and uninfected groups. Our data suggest that upregulation of pro-inflammatory cytokines after CXCL12 silencing could be associated with decreased strength. Systemic silencing shows infiltrated T cells with low cytokine production, however local brain immune response was upregulated due to an increase of iNOS and TNF-α, however these animals still presented a bad prognosis presenting loss of force as no changes in the viral load. Thus, our treatment still needs to improve the outcome but, the identification of the mechanism of systemic silencing modulating local immune response provide novel insights in immunotherapy against this infection.

Financial support: CNPq (442731/2020-5; 305932/2022-5), CAPES (88887.506690/2020-00), FAPEMIG(APQ--00826-21;RED-00202-22 Rede de Pesquisa em Imunobiológicos e Biofármacos paraterapias avançadas e inovadoras).



ULTRASTRUCTURAL ANALYSIS OF MONKEYPOX VIRUS REPLICATION IN VERO CELLS

Amanda Stéphanie Arantes Witt[1], Giliane de Souza Trindade[1], Fernanda Gil de Souza[1], Mateus Sá Magalhães Serafim[1], Alana Vitor Barbosa da Costa[2], Marcos Vinícius Ferreira Silva[2],Felipe Campos de Melolani[2],Thalita Souza Arantes[1],Denilson Eduardo Silva Cunha[1], Rodrigo Araújo Lima Rodrigues[1], Erna Geessien Kroon[1], Jônatas Santos Abrahão[1].

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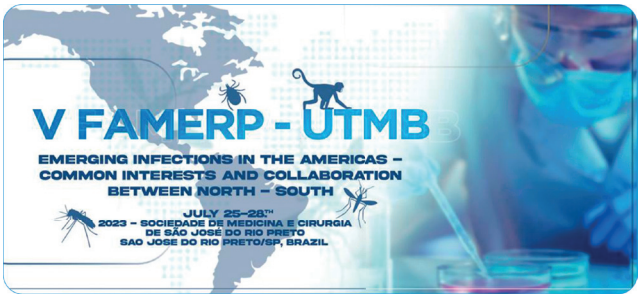
ABSTRACT

The first worldwide monkeypox virus (MPXV) outbreak was reported in early May 2022, with remarkable different clinical aspects from previously described monkeypox infections in humans. Although MPXV is an emerging pathogen with considerable medical importance, yet much of its biological aspects remain to be further investigated. In the present work, we evaluated ultrastructural aspects of MPXV by transmission electron microscopy (TEM). The viral strain characterized in this study was isolated from a male patient infected during the 2022 outbreak. Vero cells were asynchronously infected at MOI of 2, followed by incubation and sample preparation for TEM. In our analysis we were able to successfully identify: (i) adhered intracellular mature virus particles before entry of the host cell; (ii) a reorganization of the rough endoplasmic reticulum cisternae into the so-called "mini-nuclei" structure, which is associated with genome replication; and (iii) noticeably different sites within the viral factory presenting granular or fibrillar aspects, with probable distinct roles on viral multiplication. We also observed viral crescents, different MPXV particle morphotypes, and cellular alterations induced by infection, such as changes in the cytoskeleton structure and multimembrane vesicles abundance. Taken together, to the best of our knowledge, these results revealed for the first-time ultrastructural aspects of different steps of the MPXV cycle.

Key-words: Microscopy, pathogenesis, poxvirus, researchand analysismethods, skin, virusclassification

Financial support: Fundação de Amparo à Pesquisa do Estado de Minas Gerais, Rede Virus- MCTI, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, and Conselho Nacional de Desenvolvimento Científico e Tecnológico.



EPIDEMIOLOGICAL SURVEILLANCE OF THE AICHI VIRUS IN THE DIFFERENT STEPS OF SEWAGE TREATMENT IN THE CITY OF SÃO JOSÉ DO RIO PRETO-SP

Nascimento, Mariah Cristina Antunes; Rosa, Camila Rodrigues; Geraldini, Dayla Bott; Miceli, Rafael Nava; Spilki, Fernando Rosado; Araújo Júnior, João Pessoa; Calmon, Marília de Freitas; Rahal, Paula.

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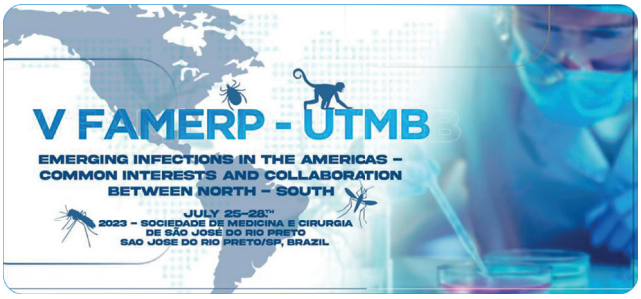
ABSTRACT

Sewage contains several pathogens that can cause diseases, with virusesbeing considered the main source of these, causing various clinical manifestations, such as gastroenteritis. Thus, the objective of this study was widely monitored the Aichi virus (AiV) in three different processing points of sewage treatment carried out in the city of São José do Rio Preto-SP. For that, three samples were collected weekly (before, during and after sewage treatment) by the Autonomous Municipal Water and Sewage Service (SeMAE), during one year, totaling 156 samples. After collection, the samples were sentto the laboratory, and the applied methodology included concentration of viral particles by ultracentrifugation, RNA extraction by TRizol, cDNA synthesis, Nested PCR and electrophoresis for detection, qPCR for quantification and sequencing. Of the 156 samples analysed, the AiV was detected in 124, with frequency in the detection of samples before, during and after treatment of, respectively, 84.6,76.9 and 61.5% (autumn), 100,100and100% (winter), 92.3, 38.4, 34.8% (spring) and 84.6, 92.3 and 84.6% (summer). Likewise, quantification was performed in 104 samples, observing the following medium concentrationof copies/L:$3.7x10^8$;$4.1x10^8$;$1.7x10^7$(autumn),$3.8x10^8$; $1.9x10^8$; $1.6x10^6$(winter),$1.4x10^8$;$5.9x10^5$;$1.4x10^6$(spring)and$4.9x10^8$;$5.4x10^8$; $3.7x10^6$ (summer). So far, 73 samples have been sequenced, all resulting from the Aichi A species, with human Aichi (98.6%) and canine Aichi (1.6%) as its subtypes. The AiV survives in critical conditions, which explains its detectionand quantification in different seasons and even at the end of treatment. The sequencing also concludes that the AiV A specie is predominant in the city, while the literature indicates the predominance of AiV B in Brazil. Therefore, epidemiological surveillance becomes important in many ways, contributing to the identification of possible outbreaks and treatments.

Financial support: Coordination for the Improvement of Higher Education (CAPES) Finance Code: 001



THEMAINTENANCEOFCHIKUNGUNYAVIRUS-INDUCEDDISEASEAND FC--LIKE RECEPTORS (FCyRIIB/FCyRIII)

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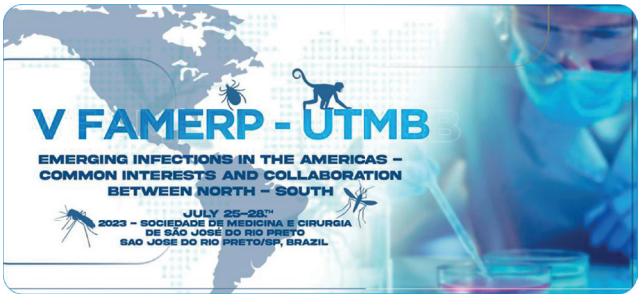
ABSTRACT

Introduction:CHIKV is an arbovirus that causes acute febrile illness and severe chronic arthritis.Within the host,FcyRs recognize the Fc portion of IgG,play a pivotal role in the inflammatory process.This study aims to evaluate the involvement of FcyRIIb and FcyRIII receptors in the pathogenesis of CHIKV infection.Methods:4 week-old WT (C57),FcyRIIb-/- ,and FcyRIII-/- mice were infected intraplantarly with 1x106PFU of CHIKV.Mice were assessed for hypernociception,viral titers in target organs,neutrophil and macrophage infiltration(measured by MPO and NAG enzyme levels,respectively),cytokine profile production and tissue damage.(CEUA:150/2020)Results:CHIKV infection was associated with hypernociception for approximately 21d,viral titer recovery from several organs at early time points,increased MPO and NAG enzymes indicating neutrophil and macrophage infiltration.Tissue damage in hindpaws and joints,correlated with elevated levels of cytokine detection.FcyRIIb-/-mice exhibited exacerbated and prolonged joint hypernociception persisting until the 28th dpi.In contrast,hypernociception in FcyRIII-/-mice was similar to WT mice throughout the evaluated period.Viral titers in hindpaws and plasma were higher in FcyRIIb-/-mice compared to WT animals,while they were lower in FcyRIII-/- .Analysis of neutrophil infiltration on the 1st and 10th dpi showed increased MPO enzyme levels in FcyRIIb-/-animals,accompanied by increased IL1ß,CXCL1,and IL6.Evaluation of macrophage infiltration in FcyRIIb-/-mice revealed increased NAG enzyme levels on the 10th and 14th day,whereas WT littermates peaked on the 7th day.Tissue lesions peaked on the 7th dpi in both groups,although more severe in FcyRIIb-/-animals.Conclusion:Overall,the results demonstrate that the absence of the FcyRIIb receptor plays a crucial role in the persistence of hypernociception and the exacerbation of disease induced by CHIKV. Further investigations are needed to explore FcyRIIb and FcyRIII's role in CHIKV infection.

Key words: Chikungunya, Hypernociception, Virus, Immunoglobulin, Fc-receptor, Inflammation.

Financial Support: CNPq, CAPES, FAPEMIG, FINEP, INCT: Dengue, INCT: interação microrganismo-hospedeiro



UNSUAL CARDIAC MANIFESTATIONS OF DENGUE: A CASE SERIES

Isabela Roberta da Silva[1], Alice Tobal Verro[2]; Ingrid Emily Alencar Bento[2], Bárbara Ferreira dos Santos[2], Bruno Henrique Gonçalves de Aguiar Milhim[2], Mauricio Lacerda Nogueira[2], Cassia Fernanda Estofolete[2].

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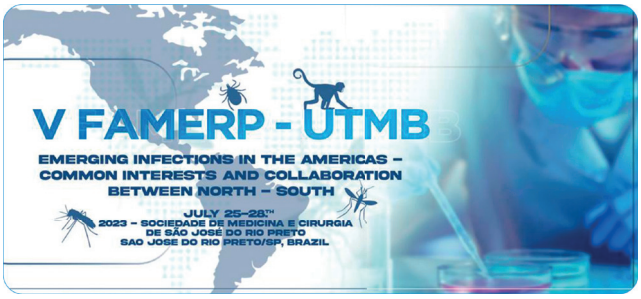
ABSTRACT

Dengue is an significant endemic arbovirus. The disease ranges from asymptomatic cases until severe forms and death. Cardiac involvement by dengue is not uncommon, it ranges from 11.4% to 62.5% of patients, but sometimes it may be under or misdiagnosed, as malignant arrhythmias, myocarditis, heart failure, cardiogenic shock. Case Reports: Here we describre 3 cases of cardiac manifestation of Dengue during an outbreak in an hyperendemic area in 2019. 1. Female, 60, hypertensive, diabetic, with fever, myalgia and headache for 6 days. She started with dry cough and dyspnea in the last 4 days. Physical examination showed 86% saturation with acute hypertensive edema and systolic heart murmur 3+/6+. ECG with atrial fibrillation, unknown by patient. Echocardiogram showed significant increase in the left atrium with preserved contractile function. After 4 days of admission, she was asymptomatic with return of sinus rhythm, being in use of anticoagulant and beta-blocker. 2. Female, 32, healthy, with malaise, arthralgia and retrorbital pain for 7 days. One day before she developed diffuse abdominal pain and vomiting. Physical examination showed 88% saturation with lung congestion. ECG with sinus bradycardia. Echocardiogram showed contractile dysfunction (38%). After 11 days the systolic function was 55% and the patient was asymptomatic using enalapril and carvedilol. 3. Female, 50, healthy, with headache, myalgia, and retrorbital pain for 6 days. She sought care for severe abdominal pain. Physical examination showed painful abdomen without peritonitis and sinus bradycardia. She was discharged after 4 days of hospitalization, totally asymptomatic. Although cardiac manifestations in dengue are common, it is worth elucidating the atypical manifestations in order identificating and managementing of similar clinical conditions

Financial support: This study was supported by the São Paulo Research Foundation (FAPESP) via grant 2013/21719-3 for MLN and 2022/09229-0 for CFE and by INCT Dengue Programgrant 465425/2014-3 (MLN). MLN is partlyfunded bytheCentersfor Research in Emerging Infectious Diseases (CREID), "The Coordinating Research on Emerging Arboviral Threats Encompassing the Neotropics (CREATE-NEO)" grant U01AI151807 by the National Institutes of Health (NIH/USA).



CLINICAL CHARACTERIZATION OF RESPIRATORY SYNCYTIAL VIRUS INFECTION IN ADULTS: A NEGLETED DISEASE?

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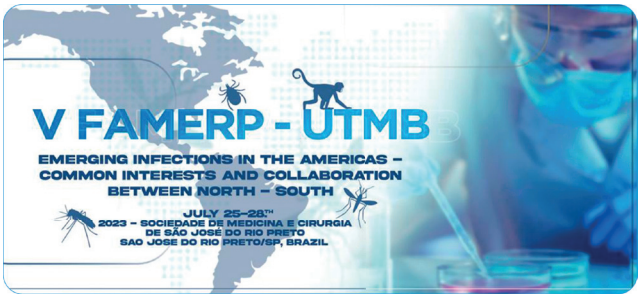
ABSTRACT

Lower respiratory tract infections are a significant cause of disability-adjusted life-year across all age groups, especially in children under 9 years of age and adults over 75. The main causative agents are viruses, such as influenza and respiratory syncytial virus (RSV). This study investigated the incidence of RSV and influenza in adult patients admitted to a referral hospital as well as the clinical profile of these infections. Molecular testing was conducted on nasopharyngeal samples taken from a respiratory surveillance cohort comprising adult (15–59 years) and elderly (60+ years) hospitalized patients who tested negative for SARS-CoV-2 to determine prevalence for influenza and RSV. Influenza was found to be less frequent among the elderly. The main symptoms of RSV infections were cough, fever, dyspnea, malaise, and respiratory distress, while headache, nasal congestion, sore throat, and myalgia were most frequent in influenza. Elderly patients with RSV were not found to have more severe illness than adults under age 60, underscoring the importance of providing the same care to adults with this viral infection.

Financial support: This study was supported by the São Paulo Research Foundation (FAPESP) via grant 2013/21719-3 for MLN, and 2022/09229-0 for CFE, and by INCT Dengue Program grant 465425/2014-3 (MLN). MLN is Brazilian National Council for Scientific and Technological Development (CNPq) Research Fellows. MLN is partly funded by the Centers for Research in Emerging Infectious Diseases (CREID), "The Coordinating Research on Emerging Arboviral Threats Encompassing the Neotropics (CREATE-NEO)" grant U01AI151807 by the National Institutes of Health (NIH/USA).



EPIDEMIOLOGICAL SURVEILLANCE OF THE INFLUENZA A VIRUS IN SÃO JOSÉ DO RIO PRETO, A ONE HEALTH APPROACH

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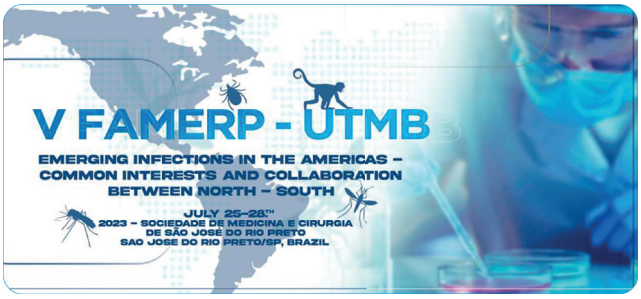
ABSTRACT

The Avian Influenza virus, a member of the Ortomyxoviridae family, is a negative-sense single-stranded RNA virus, whose etiological agent that spreads the disease is Influenza A. Cases of the disease usually involve the transmission of the virus from wild birds, especially aquatic ones, which are natural reservoirs, for domestic birds, other animals and humans. Influenza A virus has been reported in wild birds, especially migratory birds, causing outbreaks in North America and was recently detected in Brazil, in the states of Espírito Santo and Rio de Janeiro. Brazil is part of the migratory routes of wild birds coming from the North American continent, where several outbreaks of Influenza A were recorded, allowing the arrival of the virus in the country and generating reservoirs in the national territory. Thus, the objective of this work is to analyze the presence of Influenza A in avian species in the region of São José do Rio Preto – SP and in raw sewage. The choanopharyngeal/cloacal and blood samples from the birds will come from the Municipal Zoo. In addition, collections will also be carried out at the Municipal Dam and raw sewage at the Sewage Treatment Station. When they are sent to the Genomic Studies Laboratory (UNESP/IBILCE), they are subjected to viral RNA extraction using the TRIzol® Reagent method (Invitrogen®), then RT-PCR is performed to detect the Influenza A virus. Samples were collected from 212 birds, and viral RNA was extracted and analyzed from 135 birds by RT-PCR. The results obtained showed that no positive sample for Influenza A was found. However, with the arrival of bird migration, an increase in the number of cases of infections in Brazil is expected. Birds from the region of São José do Rio Preto, are indicators of the potential for transmission of Avian Influenza to the interior of São Paulo, and it is essential to monitor the circulation, since it is a highly infectious epidemiological disease for animals and humans.

Financial support: Higher Education Personnel Improvement Coordination (CAPES). Financial code 001.



EXTRACELLULAR VESICLE OF SERUM PROTEOMICS DATA REVEALS EXTENSIVE POST-TRANSLATIONAL MODIFICATIONS UPON ZIKA

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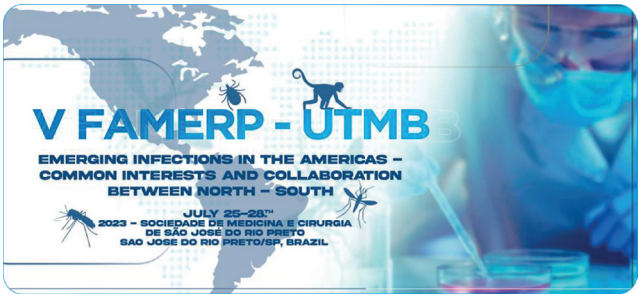
ABSTRACT

Zika virus (ZIKV) is closely related to flaviviruses with similar symptoms; understanding differences in their molecular impact on the host is therefore of high interest. The viruse interact with the host's post-translational modifications, inducing visible changes in serum. The modifications are diverse and of low abundance, they typically require additional sample processing which is not feasible for large cohort studies. Therefore, we tested the potential of isolation and characterization of extravelular vesicles in the blood of zika virus (SCZ) patients and corroborated with data available in the literature (PRIDE). Interestingly, we showed that the activation and regulation of complement is associated with upregulated extravesicular proteins (IGLC7, CA8, RGMB, CSAD, RAB1A, and KRT35) and downregulated (CALML6 and ACSBG1), indicating an important role for the complement cascade in children affected by SCZ. Similar findings were previously described in patients with increased production of alpha interferon (IFNα) by plasmacytoid dendritic cells through interaction with extracellular vesicles from a cell line of hepatocarcinoma infected by the hepatitis C virus - HCV, that is, this result demonstrates a defense strategy of the organism, activating the innate immune response of the host. The NFkB pathway stands out in EVs for its wide range of actions. We remined all published mass spectra from 125 unenriched serum samples from ZIKV patients for the presence of phosphorylated, methylated, oxidized, glycosylated/glycated, sulfated, and carboxylated peptides. We identified 384 modified peptides with significantly differential abundance in ZIKV patients. Amongst these, methionineoxidized peptides from apolipoproteins and glycosylated peptides from immunoglobulin proteins were more abundant in ZIKV patient serum and generate hypotheses on the potential roles of the modification in the infection. The results demonstrate how dataindependent acquisition techniques can help prioritize future analyzes of peptide modifications.

Financial support: FAPESP - Research Support Foundation of the State of Sao Paulo, 2019/27294-0 Postdoctoral fellowship, 2016/22158-3 Thematic project



AIRWAYIMMUNE MEDIATORSTORMFORCOMPREHENSIVE ANALYSIS OF DISEASE OUTCOME IN CRITICALLY ILL COVID-19 PATIENTS

Leticia Gomes de Pontes[1], Juan Jonathan Gonçalves[2], Camila Pacheco Silveira Martins da Mata[1,3], Alice Aparecida Lourenço[3], Ágata Lopes Ribeiro[3], Geovane Marques Ferreira[3], Thais Fernanda de Campos Fraga-Silva[4], Fernanda Mesquita de Souza[4], Vanessa Egídio Silveira Almeida[3], Iara Antunes Batista[3], Carolina D`Avila-Mesquita[5], Ariel E. S. Couto[5], Ligia C. B. Campos[5], Adriana Alves Oliveira Paim[1], Linziane Lopes Ferreira[1], Patrícia de Melo Oliveira[1], Lorena de Almeida Teixeira[1], Daisymara Priscila de Almeida Marques[1], Henrique Retes de Moraes[1], Samille Henriques Pereira[1], Joaquim Pedro Brito-de-Sousa[2], Ana Carolina Campi-Azevedo[2], Vanessa Peruhype-Magalhães[2], Márcio Sobreira Silva Araújo[2], Andréa Teixeira-Carvalho[2], Flávio Guimarães da Fonseca[1,6], Vânia Luiza Deperon Bonato[4], Christiane Becari[4,5], Denise Ferro[7], Mayra Gonçalves Menegueti[7], Amanda Alves Silva Mazzoni[8], Maria Auxiliadora-Martins[8], Jordana Grazziela Coelho-dos-Reis[1] and Olindo Assis Martins-Filho[2]

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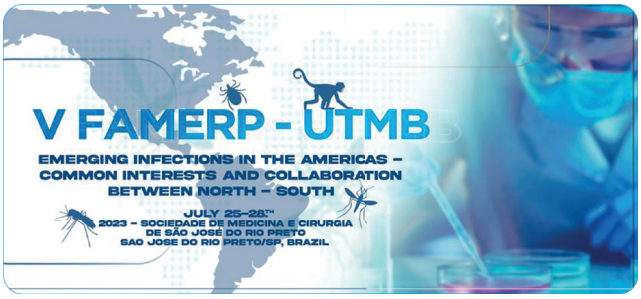
ABSTRACT

This observational cross-sectional investigation was comprised of a convenience sample including 675 biological specimens (serum and tracheal aspirates) from critically ill COVID-19 patients admitted to intensive care unit (ICU) and controls at the peak of SARS-CoV-2 B1 lineage epidemiological status of hospital areas. Patients with COVID19 were admitted to the ICU of Hospital Risoleta Tolentino Neves (Belo Horizonte, MG) and Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto, University of São Paulo (Ribeirão Preto, SP). In the present study, the levels of serum and airway soluble chemokines, pro-inflammatory/regulatory cytokines, and growth factors were quantified in critically ill COVID-19 patients (total n=286) at distinct time points (D0, D2-6, D7, D8-13 and D>14-36) upon Intensive Care Unit (ICU) admission. Increased levels of soluble mediators were observed in serum from COVID-19 patients who progress to death. An opposite profile was observed in tracheal aspirate samples, indicating that systemic and airway microenvironment diverge in their inflammatory milieu. While a bimodal distribution was observed in the serum samples, a unimodal peak around D7 was found for most soluble mediators in tracheal aspirate samples. Systems biology tools further demonstrated that COVID-19 display distinct eccentric soluble mediator networks as compared to controls, with opposite profiles in serum and tracheal aspirates. Regardless of the systemic-compartmentalized microenvironment, networks from patients progressing to death were linked to a pro-inflammatory/growth factor-rich, highly integrated center. Conversely, patients evolving to discharge exhibited networks of weak central architecture, with lower number of neighborhood connections and clusters of pro-inflammatory and regulatory cytokines. All in all, this investigation with robust sample size landed a comprehensive snapshot of the systemic and local divergences composed of distinct immune responses driven by SARS-CoV-2 early on severe COVID-19.



HIGHTROUGHPUT SCREENING OF COMPOUND LIBRARY AGAINST OROPOUCHE, MAYARO AND SAINT LOUIS ENCEPHALITIS VIRUS USING A CELL-BASED CULTURE ASSAY

[1]Jacqueline Farinha Shimizu, [1]Ana Amélia Sanchez Iacia, [1]Jéssica do Nascimento Faria de Souza, [1]Mariana Piccoli Goncalves, [1,2]Alexandre Borin, [1]Artur Torres Cordeiro, [1]Rafael Elias Marques.

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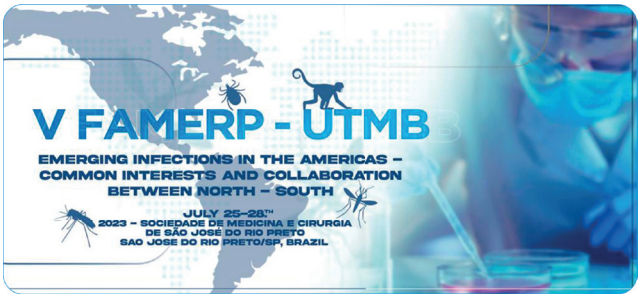
ABSTRACT

The Oropouche (OROV), Mayaro (MAYV) and Saint Louis Encephalitis (SLEV) virus belong to Bunyaviridae, Togaviridae and Flaviviridae family, respectively. These viruses are arboviruses and can be transmitted by the bite of infected mosquitoes. The clinical signs of arboviruses are usually mild. However, serious complications can also occur. Currently, there is no vaccine or treatment available. The use of high throughput screening (HTS) for drug discovery and repurposing of human approved drugs has shown as an alternative for neglected diseases treatments. The discovery of antiviral compounds may help in understanding virus biology and development of future treatments. This projected aimed to stablish an HTS assay in Huh-7 cells and identify antiviral compounds by screening a customized TargetMol library against Oropouche, Mayaro and Saint Louis Encephalitis viruses. To stablish a phenotypic assay in Huh-7 cells, they were seeded in 384-well plates at different concentrations and incubation time. We also tested different Multiplicity of infection (MOI) for each virus. After standardization of the assay, the different viruses were screening against 7,784 compounds at 10 µM to determine whether the compounds protect cell cultures from virus-induced cytopathic effect. The Z-factor was calculated by comparing infected wells (positive control) with the non-infected wells (negative control) and a Z-factor above 0.5 was considered a reliable assay. Compounds that exhibited an inhibition of cytopathic effect greater than 50% compared to negative control were considered as HITs candidates. Twenty-eight compounds were selected as HIT candidates against SLEV, 13 and 50 compounds against OROV and MAYV, respectively. To confirm these HITs candidates, dose-response curves will be performed to obtain the EC50 and CC50 values. Compounds that have an EC50 value less than 10 µM and a selectivity index (SI) greater than 4 will be considered as candidate compounds.

Financial support: MCTI–Redevíruse Finep.



BUSCA DAATIVIDADE ANTIVIRAL DE PRODUTOS NATURAIS CONTRA OS VÍRUS MAYARO E CHIKUNGUNYA

Tamiris Vanessa Miguel de Souza; Eliza Flores de Souza; Anna Beatriz Oliveira, Mendes; Daniela Nabak Bueno Maia; Mariana Costa Ferreira; Alisson Samuel Portes, Caldeira; Thaís Magalhães Acácio; Carlos Leomar Zani; Tânia Maria de Almeida Alves, **Jaquelline Germano de Oliveira.**

Instituto Rene Rachou–FIOCRUZ MINAS
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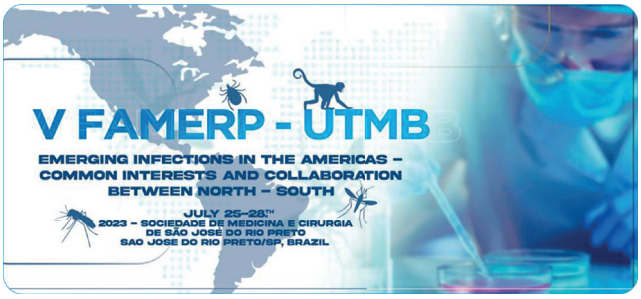
ABSTRACT

Mayaro virus (MAYV) and Chikungunya virus (CHIKV), genus Alphavirus of the Togaviridae family, are emerging viruses that cause febrile illnesses characterized by headache, rash, nausea, vomiting, and especially pain in muscles and joints. Since there are no antiviral drugs available to treat patients, our aim is to screen fungi and plant extracts to identify natural products (NP) with antiviral properties against MAYV e CHIKV. Ninety-five extracts were screened in vitro for their antiviral properties against both viruses by their viral cytopathic effect reduction followed by the 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method, previously validated for this purpose. Selected extracts were submitted to the bioguided fractionation using high- and ultrahigh-performance liquid chromatography coupled to high-resolution mass spectrometry aiming the molecular formula determination of the NP into the active fractions. To date, four extracts from Orchidaceae (01) and Amaryllidaceae (01) plants and two extracts from unidentified fungi from Antarctica showed protection against CHIKV ranging from 40 to 70%. We detected antiviral activity against MAYV in one extract obtained from a fungus, Pseudogymnoascus sp, with approximately 70% of protection. By bioguided fractionation of a Hippeastrum puniceum bulb, we detected six NP, including an alkaloid with antiviral activity described for DENV-2, ZIKV and SARS-CoV-2. None of these six compounds has been described as active against CHIKV and MAYV. Indeed, the antiviral activity of these substances against both viruses are under investigation. Therefore, we expect to find at least one NP that could be used as a prototype for the development of antivirals to treat patients suffering from diseases caused by those arboviruses.

Financial support: Fiocruz Minas, Projeto INOVA Fiocruz, Fapemig, CNPq, CAPES; Plataforma de Bioprospecção, Programa P.G. Ciências da Saúde do IRR;



ANALYSIS OF THE ACTION OF MOLECULES AS ANTIVIRAL AGENTS AGAINST THE MAYARO VIRUS (MAYV).

Pâmela Jóyce Previdelli da Conceição[1],Gabriela Miranda Ayusso[1], Maria LetíciaDuarte Lima[1], Tamara de Carvalho[1], Cintia Bittar Oliva[2], Ana Carolina Gomes Jardim[3], Bo Zhang4, Bruna Fleck Godoi[5], Murilo Helder de Paula[5], Flavio da Silva Emery[5], Paula Rahal[1], Marília de Freitas Calmon[1]

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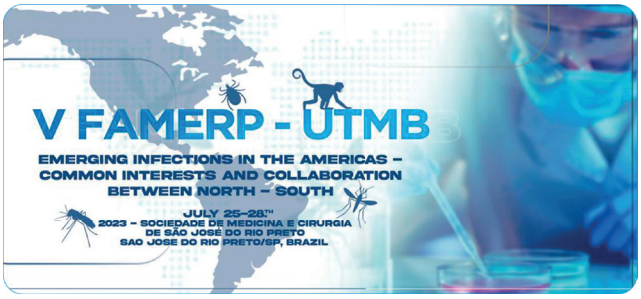
ABSTRACT

Arboviruses represent an important problem in public health worldwide, with high economic and social impact. The Mayaro virus (MAYV), Togaviridae family, genus Alphavirus, was isolated for the first time in Trinidad, 1954. Since then, MAYV has caused several sporadic outbreaks, affecting mainly rural areas and forest regions of the Americas. Currently, there is no effective treatment with a curative effect on the disease. Therefore, the search for new antiviral substances has intensified in the last decade due to the limited number of drugs and the increase in the number of cases of infections caused by MAYV. (-)-Epigallocatechin gallate (EGCG) is the most abundant catechin in green tea. Studies have shown that EGCG has extensive antiviral activity. The QHM series of compounds are synthetic (QHM-0011, QHM-0020, QHM-0110, QHM-0230) and natural (QHM-001 – lapachol) hydroxynaphthoquinones with promising antiviral and antiparasitic activities. This work aims to evaluate the action of these compounds as an antiviral against the MAYV in BHK-21 and VERO E6 cells. Initially we performed cell citotoxicity with different concentrations of these compounds incubated in BHK-21 and VERO E6 cells for 24 hours. After we also performed dose-dependence assays in BHK21 and VERO E6 cells incubated with different concentrations of the compounds and CMV-MAYV-NLuc at MOI 0.05. The compounds presented maximum non-toxic concentration at: EGCG (BHK-21:50µg/mL; VERO E6:25µg/mL), QHM0001 (10µM for both strains), QHM0011 (BHK-21:75 µM; VERO E6:37.5µM), QHM0020 (BHK-21: 45µM; VERO E6: 5.625µM), QHM0110 (25 µM, both strains) and QHM0230 (50 µM, both strains). After analyzing the dose-dependence assays, the compounds with the highest selective index (SI) were: EGCG (13.26 in BHK-21 and 5.85 in VERO E6) and QHM0011 (2.49 in BHK-21 and 26.70 in VERO E6). With these previous results, we suggested that these compounds may be efficient in the inhibition of the replication cycle of MAYV.

Financial support: CAPES/CNPq
"This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001" and "Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)".



SURVEILLANCE OF INFLUENZA A VIRUS AND NEWCASTLE DISEASE VIRUS IN MIGRATORY WILD BIRDS CAPTURED ON THE COAST OF PARANÁ STATE, BRAZIL.

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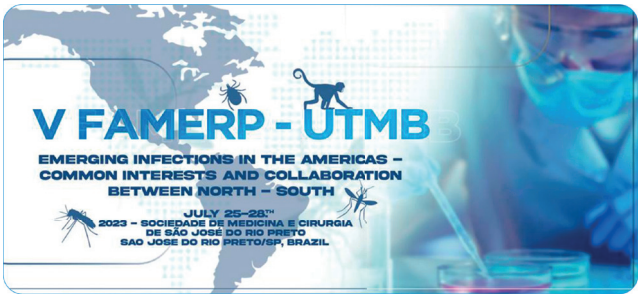
ABSTRACT

Influenza A virus (IAV) and Newcastle disease virus (NDV) are easily transmitted between animals and humans, causing epidemics and pandemics seen worldwide. Migratorybirdsare naturalreservoirsofthesevirusesand dueto their zoonoticpotential they infect chickens, shorebirds and mammals, as well as humans. The epidemiological outbreaks of the Newcastle virus are related to its virulence factor, classified as lentegenic, mesogenic and velogenic.Influenza virusesareclassified intwo groups:low pathogenic avian influenza virus (LPAI) and highly pathogenic avian influenza virus (HPAI). In recent years, the most devastating pandemic was caused byH1N1 subtype oftheInfluenza Avirus in2009, whichwas named Swine Flu. Currently, cases ofavian influenza, caused by the H5 and H3 subtypes, considered highly pathogenic avian influenza (HPAI), have been notified around the world. So, the aim of this study is to detect Influenza and Newcastle viruses in migratory birds from the coast of the state of Paraná, Brazil. A total of 114 oral and cloacal swabs were collected from 57 migratory birds. The total RNA were extracted by Trizol according to manufacture's intrusctions. After, the RNA was used to detect the viruses by real time PCR using TaqMan technology, virus-specific primers, and probes designed to detect Influenza A vírus and Newcastle Disease virus.To date, all samples analyzedwere negative for Influenza A virus and Newcastle Disease virus. With climate change, thousands of bird species perform annual migration in search of better conditions. Brazil is considered one of the most popular countries for migratory wild birds due to its tropical climate. Thus, the monitoring of these birds enables a rapid detection of these circulating avian viruses, which contributes to the prevention and reduction of viral circulation, important for the national and international trade of chicken meat, in addition to helping animal and human health.

Financial support: CAPES/CNPq
"This studywas financed inpart bythe Coordenação de Aperfeiçoamento dePessoalde Nível Superior – Brasil (CAPES) – Finance Code 001" and "Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)".



MANIFESTATIONS AND CLINICAL EVOLUTION OF PATIENTS CHRONICLYINFECTEDBYHIV,HBVANDHCVVIRUSESCOINFECTED BY DENGUE

Tolentino-BinhardiFM[1]; Polotto de Santi M[1];Buzutti PC[2];Soares MMCN[1]; Fernandes JG[2]; Bocchi M[3]; Montanha JOM[4]

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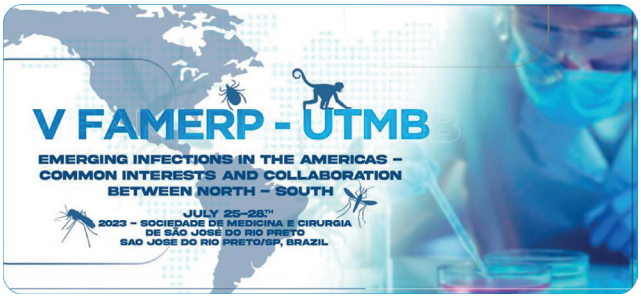
ABSTRACT

Introduction: Dengue is an acute febrile disease that attacks the organism in a systemic and dynamic way, being considered a neglected tropical disease of extreme importance. Another problem of great public health impact, in Brazil and in the world, are chronic viral diseases such as viral hepatitis, which can manifest acutely or chronically and infect millions of people, and HIV, considered a dynamic and unstable disease. The manifestations of dengue in a population with a known prevalence of HIV, HBV and HCV have never been studied in our region. Objective: to evaluate the manifestations and clinical evolutions in patients chronically infected with the HIV, HBV and HCV viruses, co-infected with DENV in the region of São José do Rio Preto-SP, from 2009 to 2016, to know if there are potential risks for these patients. Material and method: data from 4,845 patients chronically infected with HIV, 502 patients infected with HBV and 1,388 infected with HCV were evaluated. The results obtained were compared with SINAN spreadsheets containing data from patients with DENV, belonging to the 66 municipalities of the Epidemiological Surveillance Group-29 (GVE-29). After identifying the co-infected patients, the results of the viral load of HIV, HBV and HCV before and after dengue infection and the evolution of the clinical picture of these patients were collected. Results and conclusion: the co-infection rates found were 2.4%, 2.2% and 3.2% for HIV/DENV, HBV/DENV and HCV/DENV respectively. It was observed that 71% of patients with HIV showed a decrease or no change in viral load and also a small increase in CD4+ T cell count. Chronic HCV carriers had a higher rate of co-infection and diversity of symptoms when compared to HBV carriers. The increase in viral load in Hepatitis occurred more in HBV carriers. DENV-1 was the only serotype detected and no evolution to severe dengue and/or death was found.

Financial support: OwnFinancing



GENETIC CHARACTERIZATION OF G12P[9] AND G12P[6] ROTAVIRUS STRAINS IDENTIFIED IN BRAZIL BETWEEN 2011 AND 2020

Yasmin França1, **Raquel Guiducci[1,*]**, Roberta Salzone Medeiros[1], Ellen Viana[1], Lais Sampaio de Azevedo[1], Simone Guadagnucci Morillo[1], Antonio Charlys da Costa[2], Adriana Luchs[1]

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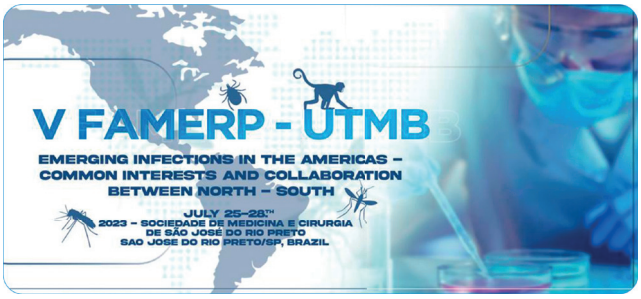
ABSTRACT

The species A rotaviruses (RVA) are important gastroenteric pathogens that infect humans and animals. G12 RVA is currently recognized as a globally emerging genotype and have been described in combination with several P-types. In Brazil, G12 in combination with P[9] and P[6] has been continuously detected following their sporadic and confined pattern of detection. In addition, G12P[9] genotype has been described in human-animal reassortment events. To date, few complete genomes of G12P[6] and G12P[9] RVA strains have been described in Brazil. This study aimed to determine the genomic constellation of G12P[9] and G12P[6] RVA strains detected in Brazil between 2011 and 2020. The eleven gene segments of three Brazilian G12P[9] and four G12P[6] RVA strains were amplified using RT-PCR and Sanger sequencing. The genotype of each gene segment was assigned using phylogenetic analysis. All G12P[9] strains displayed an AU-like genetic backbone constellation (G12-P[9]-I3-R3-C3-M3-A3-N3- T3-E3-H6), exhibiting genetic relationships to animal RVA strains such as bovine, canine, feline and chiropter, suggesting the occurrence of reassortment events. The four G12P[6] strains showed the typical DS-1-like genetic backbone (G12-P[6]-I2-R2-C2- M2-A2-N2-T2-E2-H2). Genetic analysis indicated that the G12P[6] strains circulating in Brazil were closely related to RVA sequences originating from distinct continents, and there is no evidence for the introduction of a particular G12P[6] variant in the country. In addition, no evidence of animal ancestry was observed in Brazilian G12P[6] strains. Rotarix® vaccine has been included in the National Immunization Program in March/2006, with an excellent uptake in subsequent years and high vaccination coverage. Tracking virus evolution and gaining an understanding of the role that animal RVA strains play is important for continued development of vaccine strategies and genotyping surveillance.

Key words: Gastroenteritis, interspecies transmission, genotying, sequencing,molecular epidemiology

Financial support: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) #2020/14786-0, #2020/02469-0, #2020/11182-6 and #2021/09064-8. Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). Fundo Especial de Saúde para Imunização em Massa e Controle de Doenças (FESIMA) CAF Nº #001/2021 and #060/2021. Laboratórios de Investigação Médica, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo #HC-COMVIDA



DEPLETION OF INTESTINAL MICROBIOTA AND SUSCEPTIBILITY DEVELOPMENT TO SAINT LOUIS ENCEPHALITIS VIRUS

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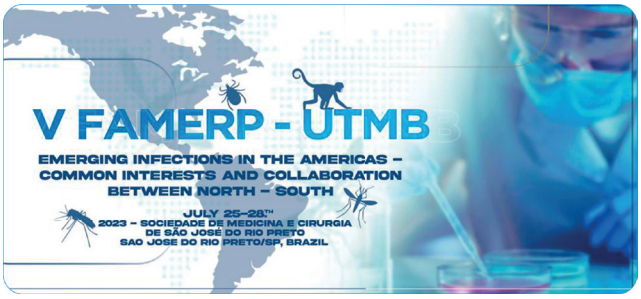
ABSTRACT

St. Louis encephalitis virus (SLEV), a member of the Flavivirus genus, is transmitted by Culex mosquitoes and can lead to severe neurological disease with high mortality rates. Studies on other flaviviruses have highlighted the potential impact of gut microbiota on viral infections. However, the specific role of the intestinal microbiota in SLEV susceptibility remains understudied. In this study, we hypothesized that depletion of the intestinal microbiota would render mice more susceptible to SLEV infection due to impaired immune response development. We used C57BL/6 mice treated with a cocktail of antibiotics to deplete their microbiota, while an untreated control group was infected with SLEV. We observed a reduction in the expression of genes and bacterial density of the microbiota in the infected animals, confirming successful microbiota depletion. Our results demonstrated that mice with depleted microbiota and infected with SLEV exhibited increased susceptibility to disease development, with higher mortality rates (83%) compared to mice with intact microbiota (50%). Notably, microbiota depletion did not influence the viral load in infected animals (both 107 PFU), suggesting that susceptibility may be related to factors other than viral replication. Furthermore, analysis using ELISA assays revealed alterations in the cytokines and chemokines important for the immune response, such as decreased INF-γ, in mice with depleted microbiota. These findings shed light on previously unknown aspects of viral biology in the context of St. Louis encephalitis. Overall, our study provides evidence for the role of microbiota depletion in the susceptibility to SLEV, which resulted in increased disease severity and altered immune responses, highlighting the importance of further investigation of the intricate interplay between the microbiota and viral infections affecting the central nervous system

Financial support: We thank FAPESP and UNICAMP for financial support and CNPEM for infrastructure support



EXPERIMENTAL MODELS TO STUDY MAYARO AND CHIKUNGUNYA VIRUSES CO-INFECTIONS IN C57BL/6 MICE.

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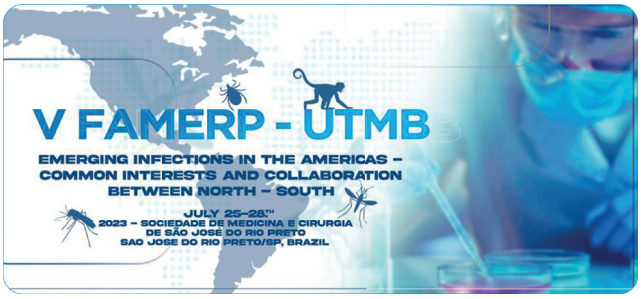
ABSTRACT

Ecological changes have contributed to an upsurge in epidemic outbreaks of arboviruses in Brazil, including Chikungunya (CHIKV) and Mayaro (MAYV) viruses. However, unlike CHIKV, MAYV is significantly neglected due to the resemblance of clinical symptoms to those of Chikungunya and Dengue. Recent studies have demonstrated that prior exposure to an alphavirus elicits a humoral response in exposed individuals, offering protection against reinfection and partial cross-protection against heterologous infection. Therefore, our aim was to establish models of co-infection with CHIKV and MAYV in immunologically competent adult C57BL/6 mice to investigate how the co-infections modulate the progression of infection for both viruses. To do so, mice were infected with 5x10 6 PFU of both MAYV and CHIKV on the right footpad, under the following conditions: 1. Concurrent coinfection; 2. pre MAYV and 7 days later CHIKV; 3. pre CHIKV and 7 days later MAYV; 4. pre CHIKV and 14 days later MAYV; 5. pre MAYV and 14 days later CHIKV. Viral load in the blood (viraemia), joint inflammation, footpad viral load (in vivo bioluminescence imaging) and histology were conducted. Our data demonstrated that concurrent co-infection didn't alter footpad inflammation, blood viraemia, footpad viral load and footpad tissue damage. Interestingly, previous exposure 7 or 14 days with MAYV, prevented CHIKV viraemia, footpad viral load and peak of inflammation, even though tissue damage and inflammatory cells infiltration in the footpad was observed. Mice that were previously exposed to CHIKV, either 7 or 14 days, prevented MAYV-induced inflammation despite MAYV viral load was detectable in the blood and footpad, and inflammatory cells were present in the subcutaneous area and tendon. Taking together, our data suggests that previous exposure with an alphavirus is able to prevent the peak of inflammation induced by the second virus.

Financial support: FAPESP2022/13236-1,2018/24470-0



MONITORING OF ROTAVIRUS INFECTION IN DOMESTIC DOGS AND CATS IN BRAZIL DURING A 10-YEAR FOLLOW-UP (2012-2021): FULL GENOTYPE HARACTERIZATION OF BRAZILIAN CANINE G3P[3] STRAINS

Lais Sampaio de Azevedo[1,*], Fernanda Faria Costa[2], Monique Beerens Abdul Ghani[3], Ellen Viana[1], Yasmin França[1], Roberta Salzone Medeiros[1], Raquel Guiducci[1], Simone Guadagnucci Morillo[1], Dieli Primo[1], Ricardo Duarte Lopes[2], Michele Soares Gomes- Gouvêa[4], Gislaine Celestino Dutra da Silva[5], Antonio Charlys da Costa[6], AdrianaLuchs[1]

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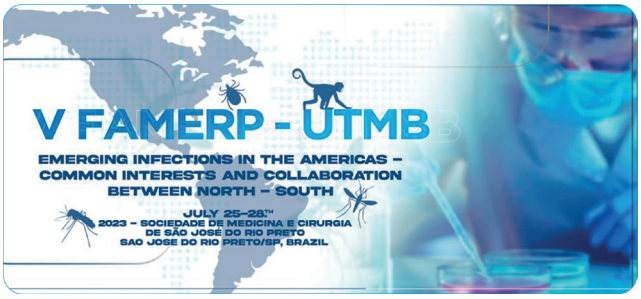
ABSTRACT

There is a dearth of information on the molecular epidemiology of rotavirus in pets in Brazil. This study aimed to monitor rotavirus infections in household dogs and cats, characterize full-genotype constellations, and obtain data on evolutionary relationships. Between 2012 and 2021, 600 fecal samples from dogs and cats (516 and 84, respectively) were collected at pet clinics in 6 municipalities of São Paulo state, Brazil. Rotavirus screening was conducted using ELISA, PAGE, RT-PCR, sequencing and phylogenetic analysis. Rotavirus species A (RVA) was detected in 0.5% (3/600) of the animals. No non-RVA species were recognized. All positive RVA samples were collected from dogs (0.6%, 3/516) attended in the city of São Paulo in 2017. The three positive canine RVA strains possess G3-P[3]-I2-R3-C2-M3-A9-N2-T3-E3-H6 constellation, a potential novel genetic constellation never formerly identified in dogs. All genome segments except the NP2 and VP7 genes were closely related to the genes from feline, canine and feline/canine-like human RVA strains, as expected. The NSP2 gene clustered with bovine, rat and human strains into a new N2 lineage, here named Lineage XXIII, suggesting reassorting events. The VP7 genes were phylogenetically closer to sewage Uruguayan G3 strains resembling animal strains, suggesting a wider distribution of these strains in the pet's population of South American countries. Phylogenetic analysis revealed potential new lineages for NSP2 (I2), NSP3 (T3), NSP4 (E3), NSP5 (H6), VP1 (R3), VP3 (M3) and VP6 (I2) segments. This is the first report describing the complete constellation sequence of canine G3P[3] RVA strains detected in Brazil. Epidemiological and genetic information obtained here is expected to provide an updated understanding of RVA circulating in Brazilian canine population and highlights the need for collaborative efforts to apply One Health approach in RVA research field.

Keywords: Rotavirus, Pets, Reassortment, Interspeciestransmission, Lineages
Financial support: This study was supported by the Sao Paulo Research Foundation (FAPESP) through the grant numbers #2015/12944-9 and #2020/14786-0 to AL, #2020/02469-0 to YF and #2020/11182-6 to RSM. AL is also supported by Fundo Especial de Saúde para Imunização em Massa e Controle de Doenças (FESIMA) CAF Nº #001/2021 and #060/2021. ACC is supported by a scholarship from Hospital das Clínicas, Faculty of Medicine, University of São Paulo (HCFMUSP) with funds donated by NUBANK under the #HCCOMVIDA scheme.



ANALYSIS OF SARS-COV-2 VARIANTS OF CONCERN CIRCULATING IN THE CITY OF UBERLÂNDIA, MINAS GERAIS: ONE-YEAR FOLLOW-UP.

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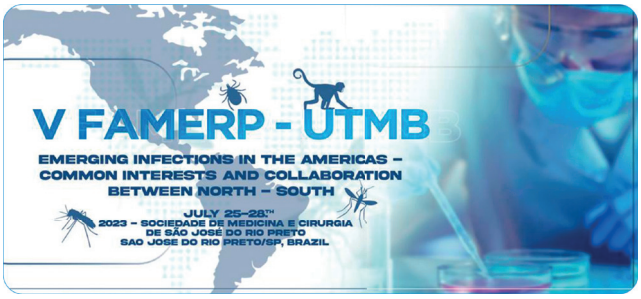
ABSTRACT

Variants of SARS-CoV-2, the causative agent of the Coronavirus Disease 2019 (COVID-19), have been rising during the ongoing pandemic, and have been associated with viral virulence, generating the named Variants of Concern (VOCs). Brazil has become an important epicenter of COVID-19, with the 5 reported VOCs found to circulate in the country. Here we performed two RT-qPCR protocols to investigate the circulation of all described VOCs, using 850 clinical samples of COVID-19 confirmed cases collected in Uberlândia, from December 2020 to March 2022. In order to evaluate the molecular properties of circulating virus, we performed sequencing of the spike genomic region of SARS-CoV-2. Our analyses by RT--qPCR demonstrated that circulation of the VOC Alpha occurred early in December 2020, Gamma introduction was late in January 2021, Delta circulated in the region from August 2021, and Omicron from December 2021. The scenario of VOCs circulation also changed over time. In December 2020, the majority of samples were identified as VOC Alpha, however, with the insertion of the Gamma variant late in January, 90% of the samples in March were classified as Gamma. From August to December 2021 all sequences were identified as Delta, however, from the first detection of Omicron by the end of December 2021, all ssamples analyzed belonged to this variant. The sequences of the spike region obtained were analyzed in Pangolin platform and the results of sorting into variants confirmed the findings obtained by RT-qPCR. Our results suggests that over time, with flexibility of social isolations measures, and due to advantageous mutations, the viral spread of VOCs end up becoming predominant in the region.

Financial support: CAPES,INCT,CNPq,Fapemig,Fapesp\MRC.



INVESTIGATION OF IGM ANTIBODIES AGAINST CKIKUNGUNYA VIRUS IN SMALL MUNICIPALITIES IN SÃO JOSÉ DO RIO PRETO REGION

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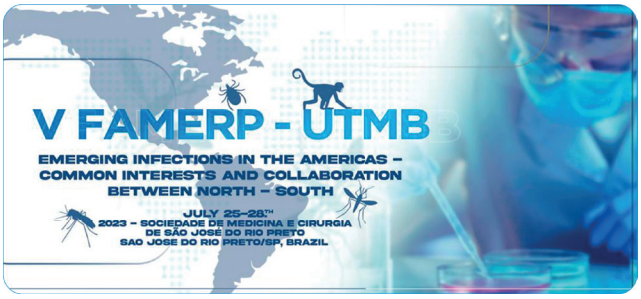
RESUMO

Chikungunya virus (CHIKV) is an alphavirus of Togaviridade family first isolated in 1952 in Tanzania. This virus causes a febrile acute disease and is transmitted by the bite of infected Aedes species mosquitoes, vectors that also transmit dengue and yellow fever. When infected, patients have symptoms that include severe joint and muscle pain, rashes, and fever, as well as prolonged periods of disability. CHIKV has recently reemerged causing millions of infections in many countries, including Brazil. One of the diagnostic method widely used is the enzyme immunoassay (ELISA) that can detect IgM antibodies presençe in serum. So, the objective of this studie was to investigate the presence of IgM antibodies against Chikungunya virus in patients with seronegative IgM antibodies against dengue in an endemic dengue region. From the period of january to may 2023, 229 serum samples were evaluated for chikungunya virus-specific IgM antibodies using the ELISA technique. Our findings showed that 32,7% (75/229) of the samples were positive for IgM antibodies. The municipality with the highest number of cases was Estrela D'Óeste presenting 18 positive cases, followed by Sales (9 cases), Santa Fé do Sul (7 cases), Jales and Oridiúva (5 cases each), the other municipalities studied has less than four cases each. Regarding the age group, 12% were aged between 4 and 9 years old, 20% between 10 and 19 years old, 56% between 20 and 59 years old and 12% between 60 and 80 years old. Regarding gender, 33% were male and 42% were female. Our results suggest that outbreaks are occurring in small municipalities in the region, with emphasis on Estrela D'Óeste that has approximately 9000 inhabitants and 24% of all positive cases of the study. Therefore, it is extremely urgent to adopt measures against the spread of mosquitoes in these municipalities in an attempt to stop a chikungunya large epidemic in the next 2024 summer in São José do Rio Preto region.

Financial support: OwnFinancing



EFFECTS OF USUV INFECTION DURING MOUSE PREGNANCY

Marina Alves Fontoura[1,2]; Laís Durço Coimbra[1,2]; Alice Nagai[2]; Giuliana Eboli Sotorilli[1,2]; Alexandre Borin Pereira[1,2]; Rebeca de Paiva Froes Rocha[1,2];Aline Freitas de Paula Melo[1,2];Rafael Elias Marques[2]; Murilo Carvalho[2]

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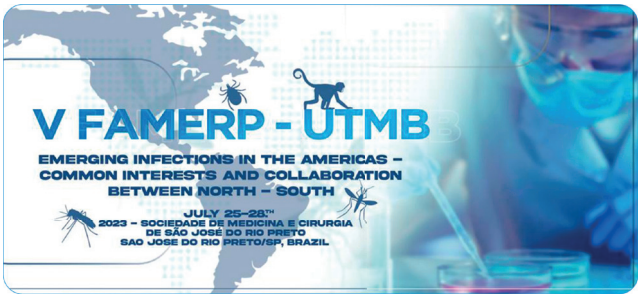
RESUMO

Usutu virus (USUV) is an emerging flavivirus which normally cause mild illness in adults. Albeit rarely in humans, USUV can infect the central nervous system of vertebrates leading to encephalitis and death. However, some flaviviruses such as ZIKV and SLEV exhibits an additional competence to cross the placental barrier and disturb embryonic development. This ability is widespread among flavivirus, likely including USUV. Here, we developed a mouse model to investigate USUV infection effects during pregnancy, in both wild-type and susceptible Type I IFN receptor knockout (IFNAR-/-) strains. For developmental studies, dams were infected at different timepoints, and maternal and embryonic tissue were collected for morphological analysis and USUV quantification. Our results for the wild-type mouse model showed a phenotypical penetrance of about 2% of malformed embryos from intravenously infected dams. In the susceptible IFNAR-/- model, the subcutaneous infection with 101 , 102 , and 103 PFU of USUV is lethal within 9 days post infection (dpi), showing weight loss and signs of disease starting at 3 dpi. The phenotypical penetrance raised to 80% for the higher inoculum and USUV was detected in maternal sera, brain, spleen, liver, kidneys, placentas, and embryos. Morphological analysis suggests that USUV infection impairs the conceptus' vasculature and microcirculation, impacting the pregnancy's outcome. We found a profile of acute inflammation with a considerable rise in cytokines and chemokines, particularly in mediators of the granulocyte response. Infection in the placenta reduced populations of immune cells required for vascular remodeling during the studied gestational period. Affected embryos exhibited vascular rarefaction, cardiac malformations, and alterations in neural tube closure. Altogether, USUV infection proved to be capable to comprom

Keywords: Flavivirus,USUV,mouse model, pregnancy

Financial support: CAPES,CNPq(141253/2019-3),FAPESP (2018/16453-8)



ANTIVIRAL FINDINGS OF BENZOTIAZOL DERIVATIVES AGAINST ZIKA VIRUS *IN VITRO*

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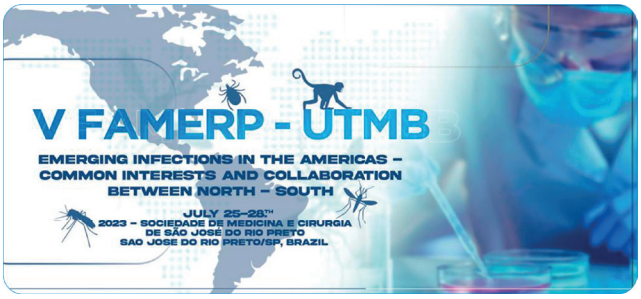
ABSTRACT

Introduction: Zika virus (ZIKV) is the etiologic agent of Zika fever, and was previously associated with cases of microcephaly, drawing the attention of health authorities worldwide. However, no vaccine or antiviral drugs are currently available. In this context, Benzotiazol derivatives have demonstrated antiviral activity against flaviviruses, as a potent inhibitor of the NS2B-NS3pro enzyme. Here we demonstrated the anti-ZIKV activity of Benzotiazol compounds in vitro. Materials and methods: Vero E6 cells were infected with ZIKVPE243 at a multiplicity of infection (MOI) of 0.01 in the presence or absence of compounds for 72 hours. For a dose-response assay, ZIKVPE243 and compounds at concentrations ranging from 1.6 µM to 200 µM were simultaneously added to cells, and virus titers were quantified by immunofluorescence assay. For replicon assays, BHK21-RepZIKV_IRES-Neo cells were treated with different concentrations of each compound, and virus replication levels were quantified by measuring Renillaluciferase activity 72h post treatment. Results: Benzotiazol compounds (1), (2), (3), and (4) inhibited up to 72.1%, 72.9%, 69.1%, and 74.4% of viral infection, with a Selectivity Index of 4.03, 25.08, 9.16, and 1.19, respectively. By using BHK21-RepZIKV_IRESNeo cells, Benzotiazol compounds 1-4 presented an antiviral activity against ZIKV replication inhibiting 23.4%, 63.4%, 60.4%, and 40.3%, respectively. Conclusions: Our findings show that Benzotiazol compounds present antiviral activity against ZIKV, suggesting the action of these derivatives potentially by interfering with functioning of viral nonstructural proteins, such as NS2B-NS3pro, being useful templates for the development of future antiviral drug candidates against Zika fever.

Financial support: FAPEMIG,CNPq,CAPES.



RUTHENIUM-NO COMPLEXES AS A BROAD-SPECTRUM ALTERNATIVE AGAINST ZIKA AND CHIKUNGUNYA VIRUSES

Natasha Marques Cassani[1] , Uriel Enrique Aquino Ruiz[1] , Igor Andrade Santos[1] , Daniel Oliveira Silva Martins[1,2] , Giovanna André Antoniucci[1], Ana Laura Oliveira Costa[1] , Evelyn Christine de Sousa Arantes[3] , Renata Galvão de Lima[3] , Ana Carolina Gomes Jardim[1,2]

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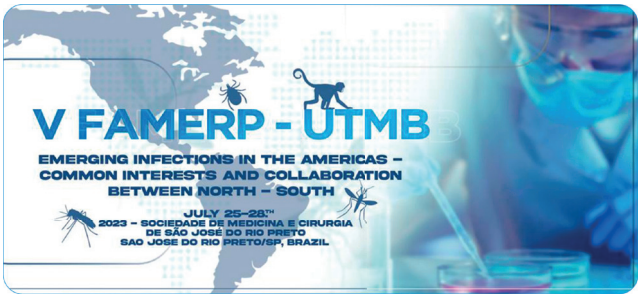
ABSTRACT

Introduction: Since its discovery and association with human infection, Chikungunya virus (CHIKV) and Zika virus (ZIKV) have shown potential health concern, unleashing serious outbreaks throughout the world. The need to find treatment that can help abrogate these viral infections of extreme clinical concern instigate the search for potential molecules that can act as potent antiviral drugs. In this sense, Ruthenium (Ru) complexes have shown interesting activity against RNA viruses, based on their chemical and biological properties. Here, the antiviral activity of four Ruthenium and nitric oxide (NO) coordinated molecules were evaluated against CHIKV and ZIKV. Materials and methods: Vero E6 were infected with ZIKVPE243 at a multiplicity of infection (MOI) of 0.01, and BHK-21 with CHIKV-nanoluc at a MOI of 0.1, in the presence or absence of compounds for 72 and 16 hours, respectively. For replicon assays, BHK21- RepZIKV_IRES-Neo cells and BHK-CHIKV-NCT were treated non-cytotoxic concentrations of each compound, previously determined by MTT viability assays, and Renilla-luciferase activity levels were measured 72h later. Results: The compounds Ru1, Ru-2, Ru-3, and Ru-4 were able to inhibit CHIKV and ZIKV in vitro, with a dosedependent activity, resulting in the selective index (SI) of 24.8, 1.7, 6.9, and 1.8, respectively for CHIKV, while 12.2, 3.3, 14.5, and 3.2 for ZIKV, respectively. Additionally, the treatment of stable cell lines harboring replicons with Ru-1 and Ru-2 inhibited 81.4% and 39% of CHIKV replication, respectively, and 72% and 84.2% for ZIKV, respectively. Conclusions: These data demonstrate the potential of these molecules as potential inhibitors of CHIKV and ZIKV infections.

Financial support: FAPEMIG,CNPq,CAPES.



GENE DUPLICATION AS ONE OF THE MAIN DRIVE FORCES FOR GENOMIC GIGANTISM AMONG NUCLEOCYTOVIRUS

Talita Bastos Machado,[1] Agnello César Rios Picorelli,[2] Bruna Luiza de Azevedo,[1] Isabella Luiza Martins de Aquino,[1] Victória Fulgêncio Queiroz,[1] Rodrigo Araújo Lima Rodrigues,[1] João Pessoa Araújo Junior,[3] Leila Sabrina Ullmann,[3] Luiz Eduardo Vieira Del Bem,[4] Thiago Mendonça dos Santos,[4] Rafael Elias Marques,[5] Samuel Leite Guimarães[5] and Jônatas Santos Abrahão[1]

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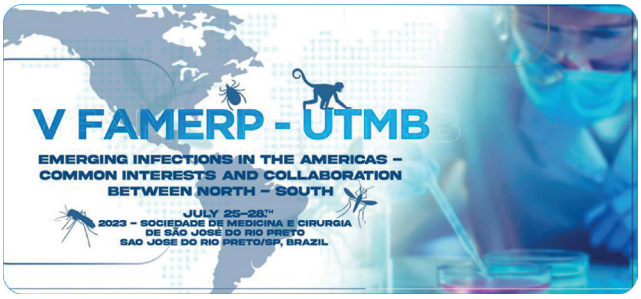
ABSTRACT

Giant viruses' (GV) gigantic genomes is one of the most intriguing features of the virosphere. Lateral gene transfer and even de novo gene creation have been proposed to explain GV genomic gigantism, however, those mechanisms seem to be restricted to specific GV families. A more universal phenomenon has not been identified. Here we describe the discovery of cedratvirus pambiensis, an amoebal GV isolated in Brazil (SISGEN: A02C870). Although c. pambiensis particles morphology and replication cycle are very similar to that described to other cedratviruses, the whole genomic sequencing revealed the largest cedratvirus genome ever described, with 623,564 base pairs and 842 predicted proteins (among them, 76 ORFans). The reasons of such largest genome were investigated and revealed an unprecedent number of paralogous genes. About 73% of c. pambiensis genome is composed by genes with two or more copies. Large clusters/families of paralogous genes were identified, coding for several functions of predicted proteins, with until >70 copies, and widely geographically spread in the genome. The in-depth investigation on the mechanisms and origins of gene duplication reveals that both tandem-like copy and distal transfer of sinthenic blocks of genes contribute to c. pambiensis genomic expansion. Finally, a comprehensive analysis of the genomes of all known GV families suggest that gene duplication is the most important driven force related to genomic gigantism among nucleocytovirus. The expansion of viral genomes by successive duplications followed by genes-copies independent evolution may be related to the rise of new gene functions and better viral adaptation to a variety of niches

Keywords: Giant viruses, amoebas, cedratviruses, genomics, paralogy.

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



YELLOW FEVER VIRUS INFECTION OF LIVER CELLS RESULTS IN OXIDATIVE DAMAGE

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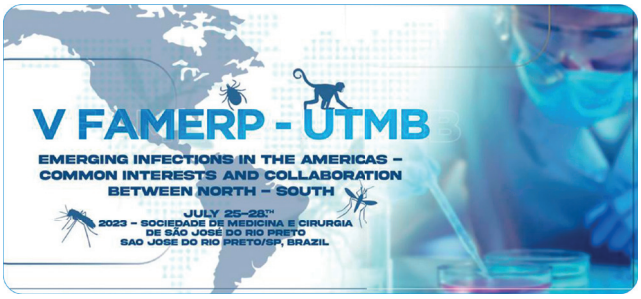
ABSTRACT

Yellow fever virus (YFV) is an arbovirus endemic in tropical and subtropical regions of Africa and the Americas, whose maintenance cycle is wild. However, changes in the epidemiological pattern of sylvatic Yellow Fever (YF) have been observed since the 2000s and the unprecedented magnitude of the last YF outbreak in Brazil (2016-2018), since the eradication of urban YF in 1942, are an alert. YF has a broad spectrum of severity, with clinical manifestations in humans ranging from febrile and self-limiting to fatal cases. Although YF is an old disease, for which there is an effective and safe vaccine, little is known about the pathogenesis, clinical aspects of the disease and patient management. Thus, studies that aim to better understand the factors associated with YFV infection are important as they can help to better understand the disease. In this case, more and more studies have shown that oxidative stress triggered by viral infections seems to contribute to the pathogenesis. So, the aim of this study was to evaluate whether YFV would cause oxidative damage to biomolecules when infecting human liver cells. For this, in infected and uninfected HepG2 cells were evaluated: the production of Reactive Oxygen Species (ROS) and the dosage of biomarkers of oxidative stress, such as lipid peroxidation products, protein oxidation and DNA damage. When measuring these oxidative parameters, it was found that YFV infection generated a significant increase in ROS at 2-4 days post-infection (dpi). In addition, YFV infection (4dpi) generated increased levels of lipid peroxidation, protein carbonyl and 8-hydroxy-2'-deoxyguanosine (a DNA damage marker). This way, the results obtained so far suggest that YFV infection can generate an imbalance in redox homeostasis, inducing oxidative damage in cellular constituents, which can contribute to the hepatic pathogenesis of YFV.

Keywords:Yellowfever, Oxidative stress, Pathogenesis

Financial support: CNPq, NIH, Capes, Fapemig.



ANALYSIS OF THE INFLAMMATORY AND HISTOPATHOLOGICAL PROFILE OF HUMAN FATAL CASES INFECTED BY DENGUE VIRUS SEROTYPE 4

Arthur da Costa Rasinhas[1], Gabriela Cardoso Caldas[2,3], Ana Luísa Teixeira de Almeida[2], Fernanda Cunha Jácome[1], Jorge José de Carvalho[4], Kíssila Rabelo[4], Felipe de Andrade Vieira Alves[4], Ronaldo Mohana-Borges[5],Debora Ferreira Barreto Vieira[2],Milla Bezerra Paiva[3], Priscila Conrado Guerra Nunes[1], Flavia Barreto dos Santos[1].

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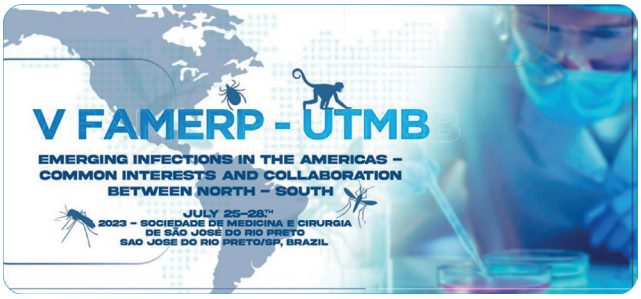
ABSTRACT

Dengue, the tropical febrile disease caused by dengue virus (DENV), is responsible for well over 300 million cases worldwide. Although cases of DENV-4 usually present milder symptoms, factors such as secondary infections by heterologous serotypes can often result in fatal cases. The present study aims to characterize the immunohistopathological profile of the hepatic, pulmonary, cardiac and renal involvement in fatal cases infected with DENV-4 that occurred between 2011 and 2013, in the state of Ceará, Brazil. The inflammatory profile of the tissue was evaluated through immunohistochemistry. For histopathological analysis, the samples were stained with hematoxylin & eosin and analyzed in a bright field microscope. Production of TNFα and IFN-γ by inflammatory cells was detected in all analyzed organs. Extensive presence of CD68+ cells was also detected in the hepatic and pulmonary interstice, and, to a lesser extent, in the heart. In the liver, the most common find was periportal necrosis, with steatosis and mononuclear cell infiltration, but without signs of hemorrhage. In the lung, pneumocyte necrosis was only observed in areas of alveolar edema. Septal and alveolar hemorrhage was present, with thickening of the alveolar walls, due to inflammatory cell migration. In the kidney, glomerular capillary congestion was seen in most of the analyzed cases. Hemorrhage and inflammatory cell infiltration was also observed in the cortical region of the kidney, with proximal and distal tubule necrosis, supported by the detection of low levels of VEGF in necrotic structures. Finally, in the heart, while dengue associated cytokines were detected, and inflammatory cell infiltration was observed, no signs of tissue damage were present. Although the findings reported in this study are consistent with what is described in dengue fatal cases, the absence of heart alterations indicates that DENV-4 could cause a milder infection in this tissue, unlike other serotypes.

Financial support: CAPES, IOC.



REPURPOSING POTENTIAL OF FDA APPROVED SULFONAMIDE METALLOCONJUGATED DERIVATIVES AGAINST CHIKUNGUNYA VIRUS

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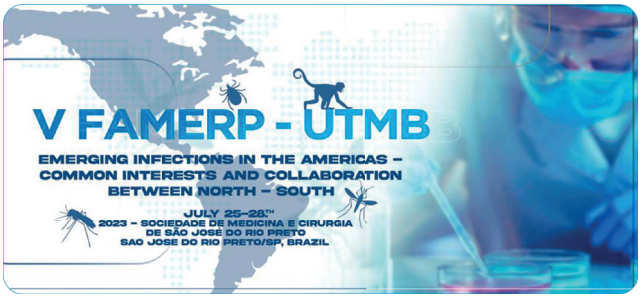
ABSTRACT

Chikungunya virus (CHIKV) is an arbovirus transmitted by mosquitoes of the genus Aedes. It causes Chikungunya fever, a disease characterized by body and joint pain, high fever, arthralgia, and presents the potential to progress to a chronic condition. There is no specific antiviral treatment against CHIKV, demonstrating the urgent demand for antiviral candidates. Here we investigated the antiviral activity of the FDA approved antiparasitic sulfonamide and its silver metallo-conjugated Schiff-base derivatives against CHIKV in vitro. For cell viability assay, BHK 21 cells were treated with each compound (SFX, SFX-SL, AgSFX and AgSFXSL) at 50, 10, 2 and 0.4 μM. After 24h treatment, an MTT assay was performed. For antiviral assay, the higher non-cytotoxic concentration of each compounds was selected to treat BHK21 cells in the presence of CHIKV-nanoluc at an M.O.I of 0.1. for 16 h, when replication levels were measured through luminescence using Promega Renilla Luciferase Assay Kit (Promega, USA). EC50 and CC50 assays were performed and used to calculate the selective index (SI – CC50/EC50). To time-of-drug addition assay, compounds were added to the cell at different times of virus infection (M.O.I of 0.1), at 50 μM for SFX-SL, and 2 μM for AgSFX and AgSFXSL. The compounds SFX-SL, AgSFX and AgSFX-SL inhibited CHIKV replication by 88%, 84.4 % and 94.2%, respectively. The SI results were: SFX-SL = 10.59; AgSFX, = 3.90 and AgSFX-SL = 7.86. Compound SFX-SL inhibited 60% of viral replication in the entry assay and 96% in the post entry assay, whereas compounds AgSFX and AgSFX-SL showed potent activity in the entry and post entry steps (98 and 99%, respectively). Our results suggest that the presence of silver atom contributed to an increase in antiviral activity in the early stages of CHIKV infection

Financial support: Fapemig, CNPq, CAPES, Fapesp.



BUILDING A GENOMIC AND EPIDEMIOLOGICAL SURVEILLANCE NETWORK SEQV BR

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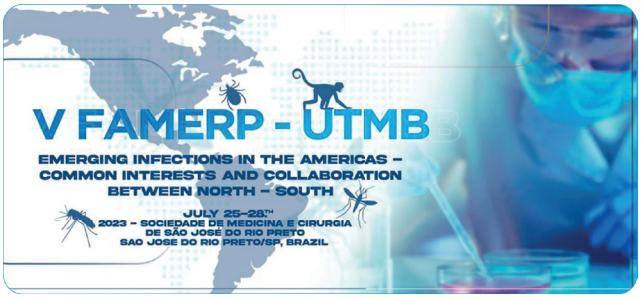
ABSTRACT

The advancement of the SARS-CoV-2 pandemic in Brazil and the emergence of the gamma variant (P.1) in the state of Manaus have necessitated efforts to enhance the sequencing capacity of circulating strains throughout the country. Our objective is to establish a sequencing network connecting all 27 federative units of Brazil, with the aim of equipping at least one laboratory in each state with the necessary infrastructure for genetic sequencing of SARS-CoV-2 in various locations across the country. The project commenced in March 2021 with the support from of the Mulheres do Brasil group. A total of 23 laboratories from 27 states were included in the network, named SEQV Br. In the initial phase, 20 laboratories were provided with an Mk1C sequencer, along with training on sequencing SARS-CoV-2 using the Oxford Nanopore Technologies methodology and reagents. The goal was to achieve sequencing of up to 100 samples per month over a six-month period. A total of 2,226 sequences achieved coverage above 80% in comparison to the reference SARS-CoV-2 genome and were submitted to GISAID. The second phase will focus on training the 23 collaborating laboratories of the SEQV network in the metagenomics approach (MinIon), a technique validated by our group and with lower cost. The samples used will be obtained from febrile cases without a definitive diagnosis. The participating centers will receive reagents to process up to 50 samples per month for a duration of six months. Establishing the SEQV network is a fundamental strategy to prepare for a potential new epidemic in the country

Keywords: Sequencing network, Metagenomics, MinIon, SARS-CoV-2.

Financial support: Mulheres do Brasil Institute



DETECTION OF INSECT-SPECIFIC VIRUSES IN THE URBAN AND SYLVATIC VECTORS OF YELLOW FEVER VIRUS COLLECTED IN THE BRAZILIAN AMAZON.

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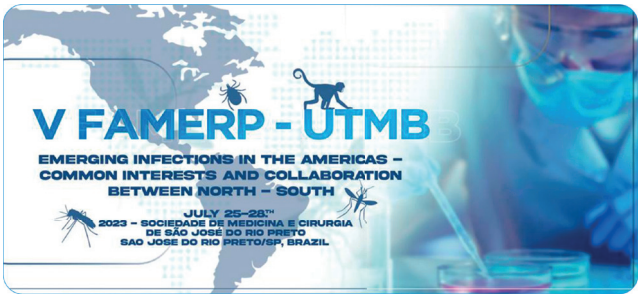
ABSTRACT

Background: Culicidae are important vectors and viral reservoirs of arboviruses and insectspecific viruses (ISVs). Mosquitoes of the genera Aedes, Sabethes, and Haemagogus comprise species of great epidemiological relevance since they act as vectors in transmission cycles of arboviruses. Methods: From May 2021 to June 2022, mosquito collections were carried out in the Adolfo Ducke Forest reserve, bordering Manaus, Amazonas state, Brazil. Mosquitoes identified morphologically as species of Aedes, Sabethes, and Haemagogus genera are being investigated in this work. So far, 286 pools belonging to the genus Haemagogus, 122 pools belonging to the genus Aedes and 32 pools belonging to the genus Sabethes have been macerated, and the supernatant obtained has been used for viral isolation and molecular investigation for medically important flaviviruses and alphaviruses using RT-qPCR with specific and generic primers and for ISVs using PCR and specific primers. Results: All pools tested negative for arboviruses such as YFV, DENV, ZIKV, CHIKV, and MAYV. HumaitáTubiacanga virus (HTV) was detected in 25 pools (8.8%) of Haemagogus spp. and one pool (3%) of Sabethes spp. Phasi Charoen-like virus (PCLV) was detected in 13 pools (4.5%) of Haemagogus spp., 22 pools (18%) of Aedes spp., and one pool (3%) of Sabethes spp. We sequenced the PCR products by the dideoxy method, confirming the presence of HTV and PCLV in Haemagogus spp. and Aedes spp. In addition, we had 20 pools (7%) of Haemagogus, nine pools (7.3%) of Aedes, and one pool (3%) of Sabethes positive for flavivirus. Isolation of HTV and PCLV was confirmed in C6/36 cells by end-point PCR. Our next steps are genomic/biological characterization and electron microscopy. Conclusions: These findings reinforce the little we know about the circulation of ISVs and the importance of entomological and viral surveillance in Brazilian mosquitoes, especially in the Amazon rainforest, a hotspot of circulation/maintenance of arboviruses.

Financial support: CAPES (88887.676127/2022-00); CREATE-NEO (NIH grant 1U01Al151807).



EVALUATION OF HUMORAL IMMUNE RESPONSE AFTER YELLOW FEVER INFECTION: AN OBSERVATIONAL STUDY ON PATIENTS FROM THE 2018 OUTBREAK IN BRAZIL

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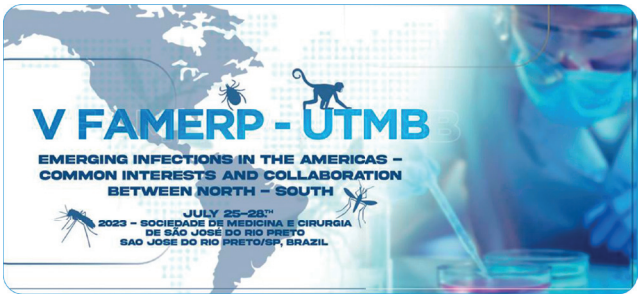
ABSTRACT

Between 2016-2018 three major yellow fever (YF) outbreaks occurred in southeastern Brazil, in which 2155 cases and 745 deaths were reported, most of these in Minas Gerais (MG) state. Patients from these outbreaks represent a unique opportunity to assess the immune response triggered by wild-type strains of the YFV in humans. The plaque reduction neutralization test (PRNT) is considered the gold standard for quantifying anti-YFV nAb and this test is routinely performed using the 17DD vaccine strain, which presents several mutations in its genome, resulting in an immune response different from that generated against the wild-type YFV. The present study aimed to evaluate the immune response of patients from the 2018 outbreak in MG, with different disease outcomes compared to healthy vaccinees, through the quantification of nAb, using PRNT protocols with wild-type (isolated from the outbreak) and vaccine (isolated from the Fiocruz vaccine) YFV strains, to better understand the response elicited against these viruses. Soluble factors (CXCL8, CXCL9, CXCL10, CCL2, CCL3, CCL4, CCL5, IFN-α, TNF-α, IL-1b, IL-6, IFN-γ, IL-1ra, IL-4, IL-5, IL-10, IL-17A, G-CSF, CM-CSF and M-CSF) were also measured and determined for the outbreak patients. Results suggest that the humoral immune response after a natural infection of YFV can be more powerful than that induced by vaccination (geometric mean nAb levels against wild-type YFV of 3,899 among outbreak patients, compared to a geometric mean nAb levels of 343, among vaccinees). Both outbreak patients and vaccinees presented different levels of nAb against the wild-type and 17DD strains, and overall, the intensity of the neutralization activity against different strains of YFV varied according to the viral challenge. This study provided evidence that the immune response triggered by the vaccine and wild-type strains of YFV is different, which could be explained by the presence of genetic and biochemical differences between these viruses.

Financial support: CAPES,NIH, FAPEMIG,CNPQ and Fiocruz Minas.



MOLECULAR AND TEMPORAL INVESTIGATION OF SARS-COV-2 CIRCULATION IN SÃO JOSÉ DO RIO PRETO (SP)

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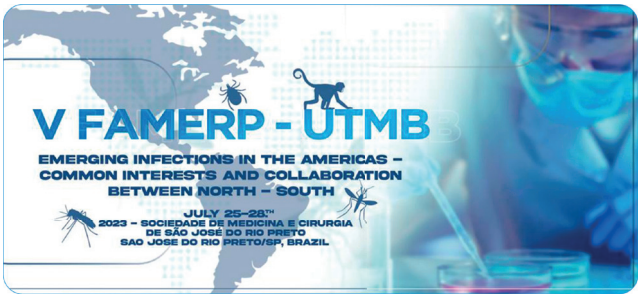
ABSTRACT

SARS-CoV-2 is the causative agent of COVID-19. São José do Rio Preto is a reference public health center in São Paulo State, making it an important municipality within the Brazilian COVID-19 pandemic scenario. We investigated the dynamics circulation of SARS-CoV-2 in the city from March to November 2020. A total of 963 nasopharyngeal swab samples were selected. Samples were previously tested for COVID-19 and collected from the local population. Viral RNA was extracted using Quick-RNA™ Viral Kit (Zymo Research). SARSCoV-2 RNA presence was investigated by RTqPCR using Allplex™ 2019-nCoV Assay (Seegene Brazil). A total of 865 samples were selected for whole-genome sequencing. The library preparation was performed using the Illumina CovidSeq Test (Illumina Inc, USA) and the QIAseq SARS-CoV-2 Primer Panel (Qiagen, USA). Sequencing was implemented on the Illumina MiSeq System (Illumina Inc, USA), using 300-cycle MiSeq Reagent Kit v3 (Illumina Inc, USA). Genomes were assembled using the ARTIC Nextflow pipeline (github.com/connorlab/ncov2019-artic-nf/tree/illumina). Maximum Likelihood Phylogenetic reconstruction was performed by IQ-TREE v. 2.2.0 (www.iqtree.org/). Generated genomes were separated according to coverage. A total of 628 genomes with more than 90% coverage were analyzed. The first wave of the COVID-19 pandemic in Brazil was characterized by the circulation of multiple lineages. Our phylogenetic analysis shows that São José do Rio Preto follows this same pattern. We were able to detect the circulation of 11 lineages in the city during the study period. The lineage B.1.1.28 was the most prevalent. VOI Zeta evolved from the B.1.1.28 lineage and was one of the variants that emerged during the second wave of the pandemic in 2021. We were able to detect Zeta circulation in São José do Rio Preto in March 2020. Our data shows that São José do Rio Preto was probably an importer and an exporter of SARSCoV-2 variants, contributing to the spread of the virus in Brazil.

Financial support: CAPES, CREATE-NEO and RedeVírus MCTI – Corona-ômica.



THE RETURN OF DENGUE VIRUS SEROTYPE 3: HISTOPATHOLOGICAL ALTERATIONS IN LIVER AND LUNGS OF IMMUNOCOMPETENT MICE

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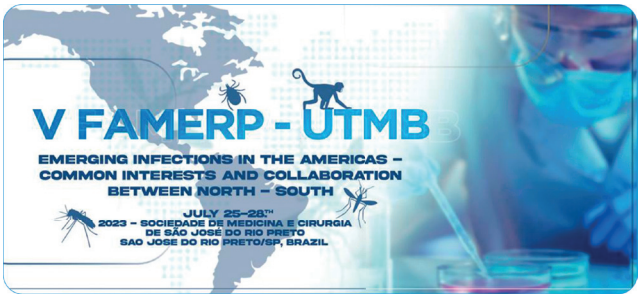
ABSTRACT

In 2023, dengue virus serotype 3 (DENV-3) reemerged after more than 15 years without being responsible for a dengue epidemic in Brazil. The greater probability of epidemics, due to the lower number of immune people, and of severer cases attributed to this serotype reinforce the importance of its study. If, on the one hand, the liver is recognized as a primary target organ in dengue infection, on the other, lung alterations are rarely described despite their relevance. For this study, two-month-old immunocompetent BALB/cJ mice were infected with 104 PFU of DENV-3 (non-neuroadapted clinical isolated strain) by intravenous route. Five animals were evaluated by time of infection and the negative control group was MOCK-inoculated. After 3, 7, 10, 14 or 21 days after infection (DAI), mice were euthanized, liver and lungs were collected and processed for analysis by bright field microscopy. Histomorphometric parameters were evaluated in 30 fields for each animal and included: counting uni- and binucleated hepatocytes and measuring the luminal area of liver sinusoid capillaries and the thickness of alveolar septa. The liver showed vascular congestion, hydropic degeneration and hypoxic necrosis. Relevant hemorrhagic focuses only occurred at 3 DAI. The tendency was dilation of the sinusoidal lumen up to 10 DAI. Despite necrosis, the number of total hepatocytes was higher among infected mice than in the negative control group except at 7 DAI, time in which the highest percentage of binucleated hepatocytes also occurred. In the lungs, vascular congestion, bronchiolar hemorrhage and small foci of inflammatory infiltrate and alveolar hemorrhage were observed. The alveolar septa thickening reached highest values at 14 DAI. Although most mice showed significant regression of morphological changes at 21 DAI in both the liver and lungs, the level of involvement seen in these organs highlights the importance of studying this reemerging serotype.

Financial support: CAPES, IOC/Fiocruz



SECONDARY DENGUE VIRUS INFECTION LEADS TO SEVERAL DAMAGE IN LIVER AND LUNGS OF BALB/CJ MICE

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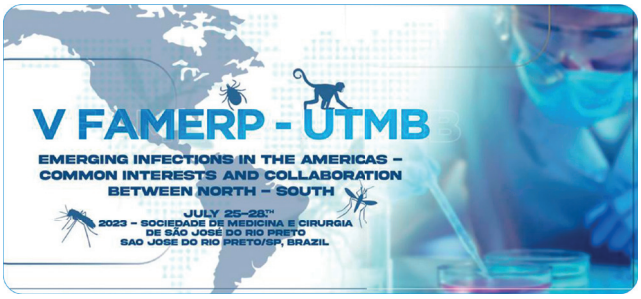
ABSTRACT

Dengue is a systemic viral infection and has a wide clinical spectrum. Although most cases are asymptomatic, severe forms of the disease might include multi-organ involvement, hemorrhage and hypovolemic shock and lead to death. Epidemiological studies suggest that severe dengue occurs mainly during a secondary infection with a heterologous serotype and, among the four serotypes of the dengue virus (DENV), DENV-2 and DENV-3 are related to severer cases. This study aimed to evaluate the impact of secondary infection with a non-neuroadapted clinical DENV strain on the morphological hepatopulmonary profile of immunocompetent mice. Twomonth-old BALB/cJ mice were infected with 104 PFU of DENV-3 and, two months later, infected with 104 PFU of DENV-2, both by intravenous route. Five animals were analyzed by time of infection and the negative control group was MOCK-inoculated. After 3, 7, 10, 14 or 21 days after infection (DAI), mice were euthanized, liver and lungs were collected and processed for analysis by bright field microscopy. Histomorphometric parameters included: counting uni- and binucleated hepatocytes and measuring the luminal area of liver sinusoid capillaries and the thickness of alveolar septa (30 fields per animal). In the liver, hepatocellular necrosis, hydropic degeneration, vascular congestion, foci of inflammatory infiltrate and reactive hepatocytes were observed. The mice at 10 DAI showed most alterations and the highest values of hepatocytes per field, binucleation and dilation of the sinusoidal lumen, alteration that had broad individual variation. In the lung, diffuse hemorrhage occurred at 7 and 10 DAI, in which the thickness of the alveolar septa was three times greater than in the negative control group. Intense endothelial activation and recruitment of inflammatory cells were also observed. Despite the severity of certain alterations, a degree of recovery from the injuries was observed in mice from the last times of infection.

Financial support: CAPES, IOC/Fiocruz



SEMISYNTHETIC COMPOUNDS DERIVED FROM LAPACHOL WITH ANTIVIRAL ACTIVITY AGAINST ALPHA AND FLAVIVIRUSES

Stephanie Paola Borjas Reyes[1], Nancy Aracely Juarez Contreras[1], Daniel Oliveira Silva Martins[1,2], Natasha Marques Cassani[1], Bruno Amaral Meireles[3], Diego Pandeló José[3], Ana Carolina Gomes Jardim[1,2]

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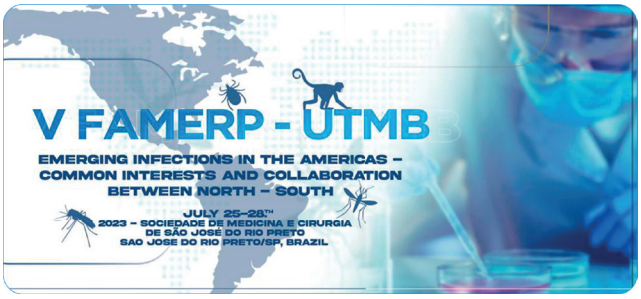
ABSTRACT

Introduction: Arboviruses from the family of Alphavirus, including Chikungunya virus (CHIKV) and Mayaro virus (MAYV), as well as Flavivirus, notably Zika virus (ZIKV), have been causes of growing concern due to their propensity to cause widespread epidemics, with a significant impact in vulnerable communities. Despite their ability to cause severe disease, there are still no effective treatments against them, making imperative the need to develop antiviral therapies. Derivatives of Lapachol, a naphthoquinone compound, seem to be promising candidates as antiviral agents since their activity against other pathogens has already been reported. Therefore, in this study we evaluated the antiviral activity of six semisynthetic compounds derived from Lapachol against the CHIKV, MAYV, and ZIKV. Materials and methods: Vero E6 were infected with ZIKVPE243 at a multiplicity of infection (MOI) of 0.01, and BHK-21 with MAYVnanoluc or CHIKV-nanoluc at a MOI of 0.1, in the presence or absence of compounds for 72, 24 and 16 hours, respectively. Results: Compounds Lap-1, Lap-2, Lap-4, Lap-5 and Lap-6 presented antiviral activity in vitro. Lap-1 was able to reduce 85% of ZIKV replication. Lap-2 and Lap-6 inhibited CHIKV replication in 73% and 64% respectively, while Lap-4 was able to inhibit 66% and 93.1% of CHIKV and MAYV replication, respectively. Lap-5 also had an antiviral activity against MAYV, inhibiting 83.8% of its replication. Conclusions: These findings shed light in the potential of semisynthetic derivatives of Lapachol for the development of antiviral therapies against Alpha and Flaviviruses in vitro, representing relevant candidates to the development of future broadspectrum antiviral agents.

Financial support: FAPEMIG, CAPES, CNPq.



MOLECULAR INVESTIGATION OF FLAVIVIRUS IN NON-HUMAN PRIMATES IN THE STATE OF MINAS GERAIS, 2017-2019

Gabriel Dias Moreira[1a],Matheus Soares Arruda[1a],Gabriela Fernanda Garcia Oliveira[1a], Thais Alkifeles Costa[1a],Nikos Vasilakis[2a],Kathryn Hanley[3a],Betânia Paiva Drumond[1a]

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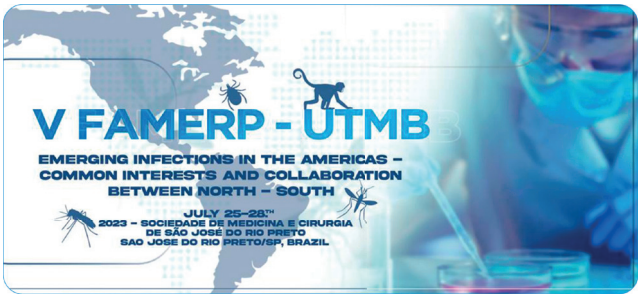
ABSTRACT

The genus Orthoflavivirus, Flaviviridae, includes several viruses of significant medical importance, such as yellow fever virus (YFV), Zika virus (ZIKV) and dengue virus (DENV). They are transmitted by the bite of arthropod vectors to vertebrate hosts, including humans and non-human primates (NHP). DENV has circulated among humans in Brazil for centuries; ZIKV was first detected in Brazil as early as 2013 and launched a massive epidemic resulting in hundreds of cases of Zika congenital syndrome, and an outbreak of YFV spread across Brazil in 2016, moving from its typical range in Amazonia into the densely populated eastern portion of the country, including Minas Gerais (MG) and Sao Paulo. Since these viruses share vectors and hosts, the aim of this study was to investigate the presence of orthoflavivirus RNA in liver samples from NHP carcasses collected in MG, during the yellow fever outbreak of 2017 to 2019. We investigated 189 liver samples collected from Callithrix sp. carcasses that were previously tested negative for the presence of YFV RNA using RTqPCR targeting 5'UTR region of YFV genome. Samples were submitted to total RNA extraction and subsequent sybr RTqPCR using pan flavi primers targeting NS5 gene. Tests were run in duplicate and in the presence and absence of reverse transcritase. Assayed samples included 74 collected in 2017, 65 in 2018 and 50 in 2019. Out of all the samples tested, 14 were positive (amplicons detected just when tests were run in presence of reverse tanscriptase). After melting curve analysis the samples presented melting temperatures ranging from 82-85°C which are expected for different orthoflaviviruses. However, the majority of samples presented melting temperatures of 84°C, which is expected for YFV amplicons. All positive samples will be resubmitted to RT-PCR with primers for different orthoflaviviruses and to nucleotide sequencing to better understand the role of NHP as a host for orthoflaviviruses in MG.

Financial support: CREID/NIAID/NIH -U01 AI151807; FAPEMIG



INVESTIGATION OF VIRAL PERSISTENCE OF MPXV IN DIFFERENT BIOLOGICAL FLUIDS

Raissa Heloisa de Araujo Eliodoro[1,2], Fábio de Rose Ghilardi[1,2], Erika Manuli[1,3], Alessandra Luna-Muschi[1,2], Geovana Maria Pereira[1,2], Ester Cerdeira Sabino[1,2]

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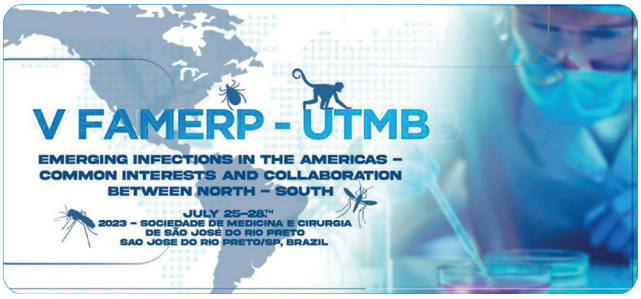
ABSTRACT

Background: Monkeypox was declared an International Public Health Emergency in June 2022. Little is known about the duration of the period of transmissibility and viral persistence in body fluids, data is needed to implement control measures. Objective: To evaluate the viral persistence of MPXV in different biological fluids. Methods: This is a cohort study, in which patients with a confirmed diagnosis of Monkeypox treated at the Hospital das Clínicas of the School of Medicine of the University of São Paulo were included. Samples of skin lesions and genital area, blood, urine, saliva and semen were serially collected until the lesions resolved. Viral DNA was extracted and subjected to MPXV detection by RT-PCR, to measure Cts and verify viral persistence in different fluids. Viral shedding was inferred as a Ct value < 34. Results: Between July 27 and November 29, 2022, 8 individuals were included, all male, with a mean age of 35 years (IQR 29-41, range 23-54). At enrollment, all subjects had skin and genital lesions, with a mean Ct of 22.1 (IQR 19-36, range 14-36), and all 4 body fluid samples were RT-PCR positive. The average Ct values observed in saliva, blood, urine, semen were 29.6(IQR 22-35, range 19-39), 35.2(IQR 33-37, range 33-39), 33(IQR 31-34, range 30-34) and 35(IQR 35-36, range 34-36), respectively. Ct values were progressively increasing until the lesions were resolved. In the last collection, on average, two samples remained positive. Our study suggests that individuals can be potentially transmissible up to 14 days in saliva, 8 in blood, 6 in urine and 5 in semen. Conclusion: We demonstrated that MPXV viral DNA can be detected in body fluids in an average of 46 days, but Ct values

Keywords: Monkeypox, viralpersistence, bodysites.



MOLECULAR AND SEROLOGICAL DIAGNOSIS OF CHIKUNGUNYA VIRUS IN SERUM SAMPLES FROM PATIENTS WITH DENGUE-LIKE SYMPTOMS IN SÃO JOSÉ DO RIO PRETO, SÃO PAULO, BRAZIL, 2021-2023

Victor Miranda Hernandes[1] , Beatriz de Carvalho Marques[1] , Victoria Bernardi[1] , Igor da Silva Teixeira[1] , Leonardo Cecílio da Rocha[1] , Andreia Negri[2] , Nikos Vasilakis[3] , Maurício Lacerda Nogueira[1,3], Lívia Sacchetto[1]

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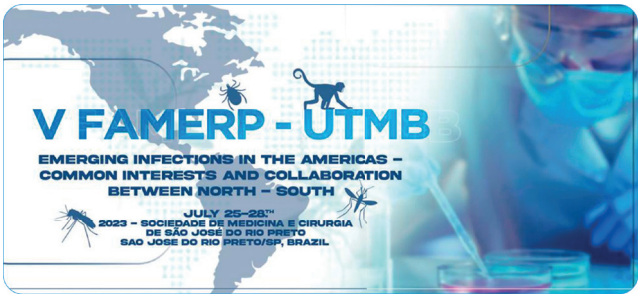
ABSTRACT

The Alphavirus genera include arboviruses of medical importance reported worldwide in subtropical and tropical areas. In the Americas, potential emergent viruses of these genera are the Mayaro virus (MAYV), equine encephalitis viruses (VEEV, EEEV, WEEV), and chikungunya virus (CHIKV). These arboviruses are at risk of emerging/reemerging in Brazil and have often neglected by epidemiological and molecular surveillance. Thus, arboviruses investigation in endemic areas with favorable factors for their emergence is crucial to identify the circulation of new viruses and prevent outbreaks/epidemics. This work aims to characterize molecularly and serologically for alphavirus samples from patients with dengue-like symptoms in São José do Rio Preto, São Paulo, Brazil. So far, from January 2021 to May 2023, we molecular screened 3,368 samples using RT-qPCR for MAYV and CHIKV. All samples were negative for MAYV. However, five patients were positive for the CHIKV RNA. Regarding CHIKV positive samples, one was from July 2022 from a 69-year-old woman. The other four CHIKV positive samples were from February to April 2023 from two women aged 73 and 39 and two men aged 64 and 68. For the same period, we also serologically screened 3,057 serum samples using CHIKV IgM ELISA (EUROIMMUN). A total of 790 samples were positive for CHIKV IgM (25.8%), 39% from males, and 60.1% from female patients. Demographic and clinical data will be further analyzed to correlate CHIKV exposure with possibly associated factors. Additionally, the positive samples in RT-qPCR will be sequenced to determine the CHIKV genotype. These findings provide a better understanding of the circulation of these arboviruses, which have a great risk of emerging in SJRP like we are observing with the detection of CHIKV this year. Additionally, the diseases caused by these viruses have a similar clinical picture to dengue, endemic in the municipality, making clinical diagnosis between them a challenge and reaffirming the need for differential diagnosis.

Financial support: CAPES (88887.676127/2022-00), CREATE-NEO (NIH grant 1U01AI151807).



VIRAL METAGENOMIC PROFILE OF BRAZILIAN BATS OF THE SPECIE MOLOSSUS MOLOSSUS - PRELIMINARY RESULTS

Vivaldo Gomes da Costa[1]; Ana Júlia Chaves Gomes[1]; Dayla Bott Geraldini[1]; Cíntia Bittar[1]; Rafael Rahal Guaragna Machado[2]; Matheus Rodrigues Beguelini[3]; Guilherme Rodrigues Fernandes Campos[4]; João Pessoa Araujo Jr[5]; Fábio Sossai Possebon[5]; Maurício Lacerda Nigueira[4]; Marília Freitas Calmon[1]; Paula Rahal[1]

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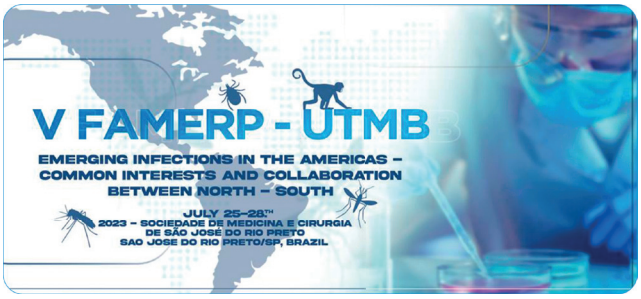
ABSTRACT

Studies on viral diversity in animal reservoirs are crucial for comprehensive surveillance aimed at identifying and tracking emerging zoonotic diseases. In recent decades, bats have been recognized as significant reservoir hosts for emerging viruses. This study aimed to conduct a metagenomic investigation of bat lung viruses in Molossus molossus. RNA was extracted from lung tissues of 14 bats using Trizol, and a RNA pool with 5000 ng of input was used to generate RNA-seq libraries following the instructions of the Zymo-Seq Ribo-free Total RNA Kit (Zymo Research), including dual indexing. The pooled RNA-seq library was subjected the high-throughput sequencing using Illumina Miseq platform (nano-flow cell, 300-cycle). The quality of the reads was assessed using FastQC, and adapter removal and cleaning (trimming) were performed using BBDuk. Subsequently, the trimmed reads were taxonomically classified using Kaiju. Metagenomics analysis revealed sequences of Retroviridae (~84%), Poxviridae (1%), Herpesviridae (1%), and Flaviviridae (genus Pestivirus) viral families. The group of unclassified viruses accounted for a significant proportion of the hits (5%) and will be further explored in future studies. Among the non-Retroviridae species, the presence of belonging to the genus Pestivirus (Bovine Viral Diarrhea Virus 2 [4%] and Pestivirus A [2%]) was notable. Collectively, viral species within the aforementioned viral families will be investigated for their potential as re-emerging zoonotic pathogens. The metagenomic data analysis provided additional insights into the viral biodiversity of the Brazilian bat population. Currently, we are analyzing additional libraries to determine viral frequency and diversity. Therefore, future work will involve more detailed shotgun metagenomic analyses.

Keywords: Virome; Next generation sequencing; Metagenomics; Bat

Financial support: UNESP (Edital PROPe 13/2022).



IDENTIFICATION OF ENTEROVIRUSES AND ABSENCE OF DETECTION OF FLAVIVIRUS AND CHIKUNGUNYA VIRUS IN PEDIATRIC PATIENTS ITH SUSPECTED CENTRAL NERVOUS SYSTEM INFECTION IN THE METROPOLITAN REGION OF BELO HORIZONTE (MG) DURING 2022

Victor Valadares Summers Albuquerque, Aline Almeida Bentes, Aléxia Stenner Rodrigues Radicchi Campos, Ana Beatriz Alvim Avelar, Ana Luiza França Vieira, Erna Geessien Kroon, Betânia Paiva Drumond.

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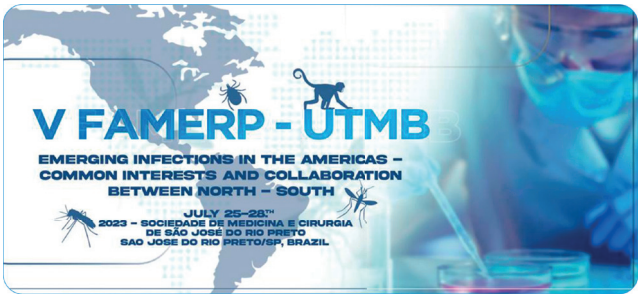
ABSTRACT

Meningitis is a compulsorily notifiable disease in which viruses are responsible for most infectious cases. Children are the group most affected by central nervous system (CNS) infections. Although cases of viral origin generally, have a benign outcome, it is possible to develop cognitive and motor sequelae. Enteroviruses (ENTV) are commonly related to these cases. Brazil is considered endemic for arboviruses such as dengue virus, yellow fever virus, Zika virus (ZIKV), and chikungunya virus (CHIKV), which are associated with CNS infections. This study aimed to investigate the presence of arboviruses and ENTV in cerebrospinal fluid (CSF) of pediatric patients with meningitis, encephalitis, myelitis, or meningoencephalitis. Therefore, CSF samples were collected from 110 patients (between 0 and 16 years old) treated at a reference hospital in the metropolitan region of Belo Horizonte, Minas Gerais, in 2022. The samples were subjected to total RNA extraction and RTq-PCR using specific primers for host beta-actin gene, CHIKV, ZIKV and pan-flavivirus primers. All samples showed amplification for the beta-actin gene, 9 showed amplification for ENTV, and there were no positive samples for the tested arboviruses. The absence of detection of arboviruses in the samples suggests that the presented cases were not caused by these viruses. It is expected that ENTV are responsible, in greater frequency, for these infections. Since arboviruses have already been identified as pathogens of CNS infections in children, the continuity of these investigations provides correct diagnosis and data for epidemiological surveillance.

Keywords: viral meningoencephalitis; children; cerebrospinal fluid; diagnosis; epidemiological surveillance.
Financial support: FAPEMIG, CAPES, CNPq



ESTA SEM TITULO???????????/

A meningite é uma doença de notificação compulsória, que tem os vírus como responsáveis pela maioria dos quadros infecciosos. Crianças são o grupo mais afetado por infecções no Sistema Nervoso Central (SNC) e apesar dos casos de origem viral, em geral, terem desfecho benigno, é possível que haja danos cognitivos e motores. Os enterovírus (ENTV) são comumenterelacionadosaestescasos.OBrasiléconsideradoendêmicoparaarboviroses como ascausadaspelosvírusdadengue,febreamarela,zikavírus(ZIKV)echikungunya(CHIKV), que são associados a infecções no SNC. O objetivo deste estudo foi investigar a presença de arbovírus e de ENTV em líquido cefalorraquidiano (LCR), de pacientes pediátricos com meningite, encefalite, mielite ou meningoencefalite. Assim, foram incluídos 110 pacientes (entre 0 e 16 anos de idade) atendidos em um hospital de referência, na região metropolitana deBeloHorizonte,MinasGerais,em2022.Os110pacientestiveramo LCRcoletado,eestes foram encaminhados para extração do RNA total e submetidos a RTq-PCR usando primers específicos para gene de beta-actina do hospedeiro, CHIKV, ZIKV e primers pan-flavivírus. Todas as amostras apresentaram amplificação para o gene da beta-actina, 9 amostras apresentaram amplificação para ENTV. e não foram identificadas amostras positivas para o genoma dos arbovírus testados. A ausência de detecção de arbovírus nas amostras sugere que os casos apresentados não foram causados por estes vírus. É esperado que os ENTV sejam responsáveis, em maior frequência, por estas infecções. Uma vez que já foram identificados arbovírus como patógenos de infecções no SNC em crianças, a continuidade destas análises é importante para o correto diagnóstico e para o fornecimento de dados para vigilância epidemiológica.

Palavras-chave: meningoencefalite viral; crianças; líquido cefalorraquidiano; diagnóstico; vigilância epidemiológica.
Agênciasdefomento:FAPEMIG, CAPES,CNPq

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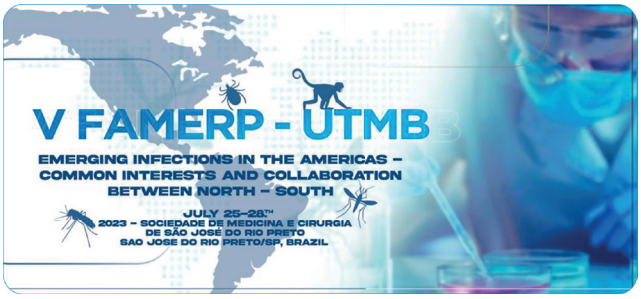

ANGIOTENSIN-(1-7) POTENTIATES EARLY HOST IMMUNE RESPONSES, AND EXERTS PRO-RESOLVING EFFECTS IN A MODEL OF SEVERE DENGUE INFECTIO

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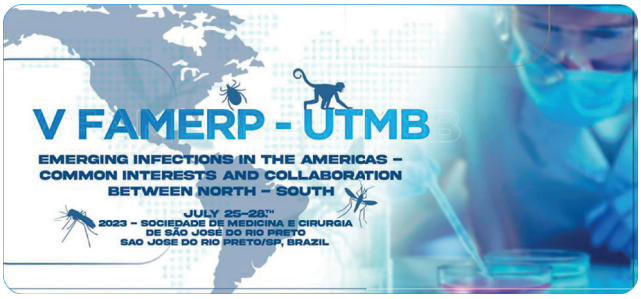
ABSTRACT

Host immune responses play a significant role in the pathogenesis and severity of dengue. Severe dengue is not only the result of an exacerbated inflammatory response but also a consequence of dysregulated or 'failed' pro-resolving mechanisms. Therapeutic strategies that utilize pro-resolving molecules have great potential for treating acute inflammatory infectious diseases. Angiotensin-(1-7) [Ang-(1-7)], a biologically active peptide of the renin-angiotensin system (RAS), acts on its receptor Mas (MasR) to promote inflammation resolution. We investigated the potential pro-resolving role of Ang-(1-7) during dengue. A129 mice were infected with DENV-2 by treating groups with a solution containing [Ang-(1-7)] at a dose of 100 ug/Kg administered subcutaneously (s.c.), B.I.D, starting 36 hours post-infection. Euthanasia was performed 3- and 5-days. DENV infection resulted in increased clinical scores, including body weight loss, thrombocytopenia, and leukocytosis. Increased inflammatory mediators in plasma and spleen were detected, characterizing the cytokine storm phenomenon. Therapeutic treatment with Ang-(1-7) prevented the increase in clinical scores as well as the thrombocytopenia induced by infection. Treatment potentiated the numbers of circulating leukocytes in whole blood, with a predominance of lymphocytes. In contrast, on the 5th day, Ang-(1-7)-treated mice showed a decrease in the numbers of circulating leukocytes. Interestingly, Ang-(1-7) potentiated the levels of inflammatory markers in plasma (CXCL1) and spleen (CXCL1, INFγ, TGF-β, IL12, and IL6) at 3dpi, which was associated with reduced viremia and similar viral titers recovered in target organs. Overall, these findings suggest that Ang-(1-7) accelerates resolution of inflammation by improving lymphocyte numbers and activation, as well as potentiating early proinflammatory responses that culminate in a systemic reduction of viral titers and amelioration of clinical symptoms

Keywords: Dengue; Angiotensin-(1-7); Inflammation; Resolution; Infection

Financial support: CNPq, CAPES, FAPEMIG, FINEP, INCTdengue and Host Microorganism Interaction.



ACTIVATION OF THE PI3Kγ PATHWAY DEMONSTRATES PRO-VIRAL EFFECTS AND CONTRIBUTES TO DENGUE PATHOGENESIS IN A MURINE MODEL.

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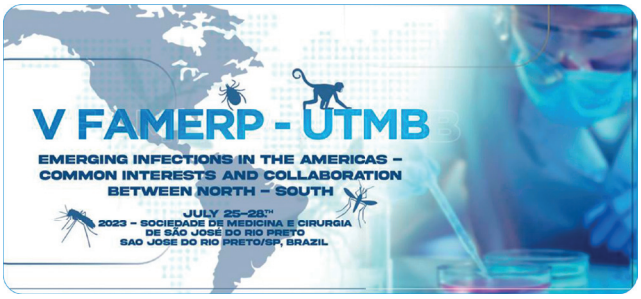
ABSTRACT

Dengue fever is a significant arboviral disease that affects millions of people worldwide. Currently, there are no specific approved drugs for treating dengue. Activation of the phosphatidylinositol 3-kinase γ (PI3Kγ) pathway has been associated with various physiological and infection-driven events, including pro-viral effects. Here we investigated the role of PI3Kγ in dengue pathogenesis. For a severe murine model of dengue disease, wild-type (WT) and PI3Kγ knockout (PI3Kγ -/-) mice were infected i.v. with an adapted isolate of DENV-3 (102 PFU). Mice were monitored for survival (16 days), or euthanized at 7 dpi to evaluate dengue disease parameters. In a mild dengue disease model, WT and PI3Kγ -/- mice were infected i.p. with a clinical isolate of DENV3 (106 PFU). In addition, WT mice were treated with a selective inhibitor of PI3Kγ (AS605240; 50mg/Kg) via subcutaneous route 1h before infection. After 24 h postinfection, the mice were euthanized for evaluation of markers of disease. Furthermore, interferon α/β receptor knockout mice (A129) were inoculated i.v. with DENV-3 (104 PFU) and treated with AS650240 between the 2nd and 4th days post-infection. Mice were euthanized at 4 dpi to evaluate several aspects of disease. Blocking PI3Kγ reduced lethality, thrombocytopenia, hemoconcentration, and liver damage induced by DENV infection compared to WT mice in both severe and mild murine models. PI3Kγ blockade impaired viral replication in blood, spleen, and liver, while reducing vascular permeability and cytokine production. Pharmacological inhibition of PI3Kγ ameliorated thrombocytopenia and liver damage and it reduced viral titers and IL-6 levels in the spleen. PI3Kγ pathway appears to exert potent pro-viral effects during DENV infection and plays a significant role in the pathogenesis of dengue. Thus, inhibition of PI3Kγ shows benefits to the host and represents a promising host-directed target to treat dengue disease.

Keywords: Dengue virus,inflammation,PI3K, virus replication

Financial support: CNPq, CAPES, FAPEMIG, FINEP, INCTdengue and ISN CAEN grant.



IN-DEPTH STUDY OF THE MAYARO VIRUS REPLICATION CYCLE

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ABSTRACT

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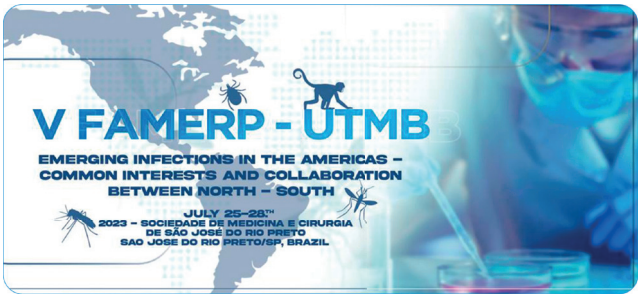
One of the most concerning epidemiological scenarios for the near future is the potential emergence of newly circulating viral lineages of highly epidemic relevance. Although this concern was very recently brought to light with the emergence of another sample of a pandemic coronavirus, some historically neglected arboviruses were already the reason of great attention for the scientific community due to serious outbreaks and diseases caused in the past. Among them, we can mention the so-called mayaro fever, caused by an Alphavirus named Mayaro virus (MAYV). The MAYV fever is characterized by an acute febrile illness that may cause a severe and debilitating process of arthralgia, remarkably similar to what occurs for other arboviral diseases (e.g.,CHIKV fever). Notwithstanding, studies on the biology of these viruses are extremely scarce, and our knowledge of them is based on the information we have for other described alphaviruses. With the absence of a licensed vaccine or clinically effective drugs against MAYV, understanding its multiplication cycle becomes a key point to easily identify and block important steps of viral replication. In this work, we begin an in-depth characterization of the replication cycle of MAYV in VERO and C6/36 cells, representing both of its main hosts. One-step-growth curve assays were performed, along with cell counting during different time-points of infection. Even after infection, VERO cells seem to grow until 24h.p.i., starting to suffer from lysis at later time-points. For these cells, MAYV particles start to be produced as early as 6.h.p.i. For C6/36, a slight increase of cells is observed until 12h.p.i, but at later time-points, their numbers look similar to the observed at the beginning of the cycle. For these cells we also detected MAYV particles being released into the supernatant portion at intervals between 10h.p.i and 12h.p.i. For a complete viral cycle description, other data are still to be gathered concerning the MAYV genome replication during these time periods and the observation of MAYV-infected cells by electron microscopy assays.

Financialsupport: FAPESP, processo2021/13615-0



INFLUENCE OF COVID-19 VACCINE ON INTRA-HOST GENETIC VARIABILITY OF SARS-COV-2

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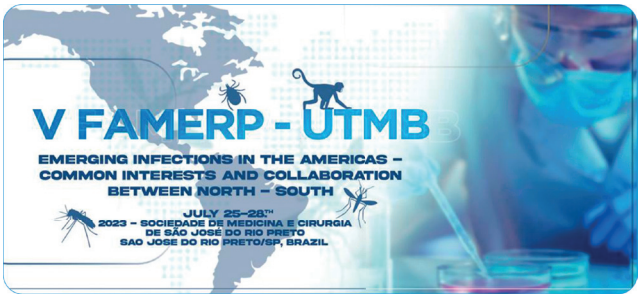
ABSTRACT

Virus Intra-Host Genetic Diversity (IHGD) can influence transmission and virulence by evading host immune response and disease severity, especially for SARS-CoV-2 variants. First, to evaluate the IHGD in unvaccinated (Uv, individuals without any dose of vaccine) and vaccinated (V, individuals vaccinated with two doses of CoronaVac) patients, we analyzed 120 COVID-19 samples from São José do Rio Preto region, obtained from April to July 2021. Total RNA was extracted, and the whole-genome sequencing was performed with Illumina CovidSeq. Using Pangolin COVID-19 Lineage Assigner Tool, these genomes were classified into Gamma lineage. The intrahost single nucleotide diversity analysis was carried out using LoFreq, and annotation and prediction of genetic effects was annotated using the SnpEff. The inference of selective pressures was performed using HyPhy to detect codons evolving on diversifying (DS) or purifying selection (PS). Our results evidenced that vaccination with CoronaVac favors negative selection at the intra-host level, in different genome regions, especially in nonstructural protein-coding genes, preventing further SARSCoV-2 genetic diversity and reinforcing the importance of vaccination to reduce virus transmission. After this, we aim to analyze the influence of booster doses on IHGD of SARS-CoV-2 in COVID-19 patients infected with other variants. So far, total RNA and whole-genome sequencing have been carried out on 904 samples from patients with two or three doses of vaccine, obtained from October to December 2022. Genomes were classified in the VOCs Delta and Omicron, of which 561 correspond to patients who received two doses of vaccine, and 343 correspond to patients who received three doses. Our next steps are subdividing the samples based on vaccination status, vaccine technologies and SARS-CoV-2 variant to compare intra-host diversity in different conditions and investigate possible immune-escape mutations among vaccinated individuals.

Financial support: FAPESP (2019-21711-9)



EVALUATION OF NEW MOLECULES WITH POTENTIAL ANTIVIRAL ACTIVITY AGAINST SARS-COV-2

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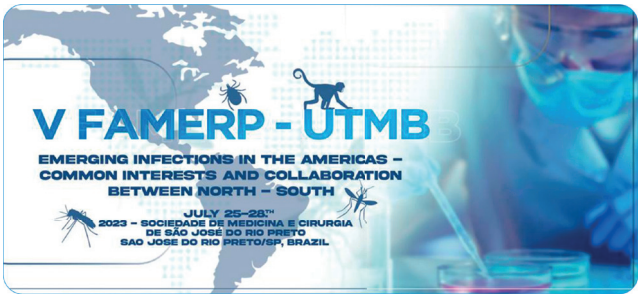
ABSTRACT

COVID-19, a disease caused by SARS-CoV-2, has become a global pandemic and represents a serious threat to the public health system. The current treatment strategy for patients with severe COVID-19 is usually performed using anti-inflammatory drugs, such as dexamethasone, and drugs that inhibit viral multiplication such as molnupiravir or paxlovid, which are expensive antivirals. In order to contribute to the therapy of COVID-19 in future waves and pandemics of SARS-CoV-2, five compounds, named here LVBA2, LVBA3, LVBA4, LVBA5 and LVBA10, as well as a Reference Drug (RD) from which they are derived were tested in vitro in SARS-CoV-2 infection assays in Vero CCL-81 cells. Cytotoxicity of the compounds and cell viability were evaluated by Alamar BlueTM assay while quantification of viral genomic copies, was performed by qRT-PCR for E and RdRp genes. It was observed that the tested compounds did not show high cytotoxicity up to the concentration of 100 uM. Furthermore, there was a reduction in the genomic copy number of SARS-CoV-2 in the in vitro treatments with compounds LVBA4, LVBA5 and LVBA10, showing significant reduction in IC50 (uM) values when compared to the reference compound. Complementary antiviral and transmission electron microscopy assays are being performed to support the applicability of the compounds in in vivo trials. The study is expected to contribute by providing new alternatives for broad spectrum antiviral therapies, which will bring benefits in combating COVID-19 and SARS-CoV-2 new waves

Financial support: CNPQ, CAPES, UFMG, Mucpharm.



METHOTREXATE AS A POTENTIAL ADJUVANT THERAPY FOR CHIKUNGUNYA VIRUS-INDUCED ARTHRITIS

Matheus R.Gonçalves[1]; Victor R.M. Costa[1]; Thaiane P. Moreira[2]; Simone de Araújo[1]; Celso M. Queiroz-Junior[1]; Flávio A. Amaral[3]; Daniele G. Souza[2]; Mauro Teixeira[1,3]; Vivian V. Costa[1]

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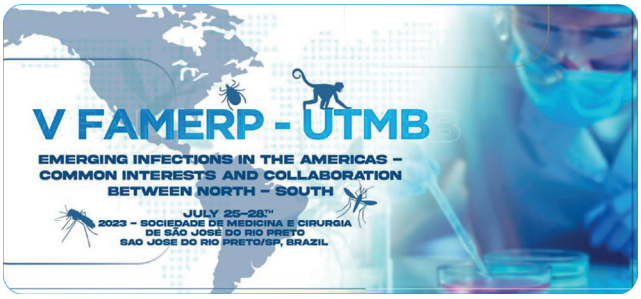
ABSTRACT

CHIKV infection leads to Chikungunya Fever, a disease characterized by chronic inflammatory polyarthralgia. Methotrexate (MTX), a folate antagonist, is widely used in the treatment of various inflammatory diseases due to its anti-inflammatory and immunomodulatory properties. MTX plays a crucial role in reducing inflammation by decreasing the production of pro-inflammatory molecules and increasing the production of anti-inflammatory cytokines. Therefore, our study aimed to evaluate the impact of MTX on the pathogenesis of CHIKV infection, assessing its potential in mitigating inflammation and improving CHIKV-induced arthritis and hypernociception. We divided 3-4-week-old C57BL/6 mice into five groups: uninfected, infected and treated with placebo, and infected and treated with MTX, starting one day before, three days after, and five days after CHIKV infection. MTX treatment (0,38 mg/kg, i.p.) was administered every other day for 21 days. Hypernociception was assessed using the electronic Von Frey test for 21 days. Viral titers determined by viral titration, while cell infiltration analyzed by indirect quantification of neutrophils and macrophages in the paw, measured by myeloperoxidase (MPO) and N-acetyl-β-D-glucosaminidase (NAG) activities, respectively. Inflammatory mediators measured by ELISA, and histopathological lesions examined by H&E staining. Results revealed that all regimen of MTX treatment reduced joint hypernociception and increased levels of the anti-inflammatory cytokine IL-10. Interestingly, increased levels of the pro-inflammatory cytokine TNF and augmented neutrophil infiltration (but not macrophage infiltration) were observed in the plantar footpad of mice. MTX did not affect viral titers. Based on these findings, methotrexate shows promise in the treatment of hypernociception induced by CHIKV. Furthermore, combining MTX with an antiviral drug may represent a more effective strategy for treating CHIKV disease.

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



BROMAC® ATTENUATES CYTOKINE STORM AND MODULATES IMMUNE CELL SUBPOPULATIONS IN AN IN VITRO STIMULATION MODEL WITH SARS-COV-2

Ferreira, Geovane Marques[1]; Reis, Erik Vinícius De Sousa[1,2]; Lopes-Ribeiro, Ágata[1] ; Gomes-De-Pontes, Letícia[1] ; Araújo, Franklin Pereira[1,5]; Lourenço, Alice Aparecida[1] ; Ferreira, Linziane Lopes[1] ; Teixeira, Caio Wilker[1] ; Dias, Laura Correa[1]; Clarindo, Felipe Alves[1]; Retes, Henrique Morais[1]; Santos, Thaiza Aline Pereira[1]; Valle, Sarah J.[2,4] ; Coelho-Dos-Reis, Jordana Grazziela Alves[1,3]

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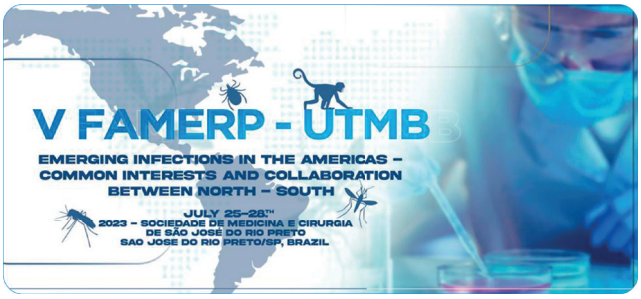
ABSTRACT

COVID-19 is a deadly disease caused by the SARS-CoV-2 pandemic, which has remained a public health threat for over three years. In contrast to the severity of COVID-19, so far, there are few drugs capable of efficiently preventing or delaying the development of severe COVID19. In this regard, BromAc® is a combination of bromelain and acetylcysteine (NAC), currently used for the treatment of pseudomyxoma (Phase 3) and has been studied for repositioning in the treatment of COVID-19. BromAc® has already demonstrated ex-vivo mucolytic and antiinflammatory activity in tracheal aspirate samples from patients with severe COVID-19. To examine the anti-inflammatory effect of BromAc®, an assay was standardized and performed using the inactivated SARS-CoV-2 virus in an in vitro system with peripheral blood cells in the presence or absence of the compound. Luminex® assays and flow cytometry were performed. BromAc® demonstrated anti-inflammatory activity, reducing the action of the cytokine storm, chemokines, growth factors and regulatory cytokines in the treated samples, in comparison with the samples stimulated with the virus. After stimulation, the cell suspension was incubated with antibodies for labeling subpopulations of lymphocytes, neutrophils, and monocytes. The results show that BromAc® was able to modulate the populations of CD16+ neutrophils and CD14+ monocytes observed after stimulation with iSARS-CoV-2, with a lower percentage being observed in the treated samples. BromAc® treatment has also been shown to increase the HLADR activation marker in CD14+ monocyte populations. It is also possible to observe that the treatment with BromAc® decreases the production of TNF by the CD19+ B cells, in comparison with the group stimulated with the inactivated virus. These results indicate a robust antiinflammatory effect of BromAc® in an in vitro stimulation system with SARS-CoV-2, indicating its potential as a therapeutic strategy for COVID-19.

Financial support: :CAPES, CNPQ, FAPEMIG, Mucpharm.



GENOMIC SURVEILLANCE AND EVOLUTIONARY DYNAMICS OF DENGUE VIRUS SEROTYPE 1 AND 2, SÃO PAULO, BRAZIL

Lívia Sacchetto[1], Beatriz de C.Marques[1], Victoria Bernardi[1], Igor da S.Teixeira[1], Cecília A. Banho[1], Victor M. Hernandes[1], Andreia F. Negri[2], Nikos Vasilakis[3], Maurício L. Nogueira[1,3]

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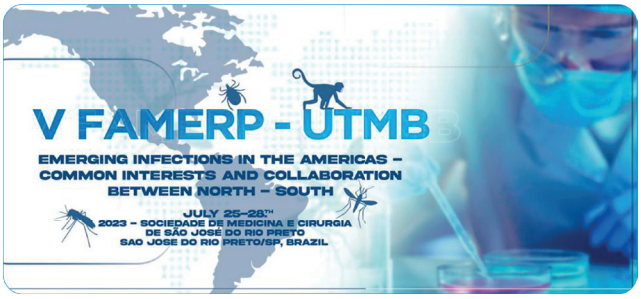
ABSTRACT

Background: Brazil is considered a hyperendemic country for dengue with the cocirculation of all dengue virus serotypes (DENV1- 4) with great genetic diversity. In the last decades, several outbreaks have been caused by the introduction/reintroduction of different serotypes. In São José do Rio Preto, the circulation of DENVs is characterized by frequent serotypes/lineages replacement, which make the city a great site to study the virus dynamics, diversity, and impact. Methods: We molecular screened for DENVs a total of 5,654 serum samples collected from patients with dengue-like symptoms from 2020 to May 2023. Results: In this period, DENV1 and DENV2 were detected. The overall positivity rate for dengue in 2020 was 21%, with the prevalence of DENV2 circulation (90%). In 2021, DENV2 was detected in 36% of DENV-positive samples and DENV1 in 64%, showing an overall positivity rate of 13%. We observed a serotype replacement in the municipality over 2021, with DENV2 replaced by DENV1. Emphasizing, from 2022 up to May 2023, DENV1 was detected in 90% of DENVpositive samples (n=486). Genomic sequencing of DENV1-2 is being conducted (n=780) to better understand the evolution of the virus. Preliminary phylogenetic analyses evidenced that DENV2 genomes are grouped within the American/Asian genotype (III) (BR3 and BR4 lineages), and DENV1 genomes are grouped within genotype V (predominance of L1). We are analyzing the genetic diversity of dengue circulating lineages in SJdRP during these past years and correlating it with epidemiological and clinical data. Most reported and positive cases occurred during the epidemic period of arbovirus in Southeast Brazil. However, we showed DENV detection in all months. Major dengue cases were classified as dengue without warning signs. Conclusions: Our findings demonstrate a DENV serotype replacement event and reinforce the critical role of molecular/genomic surveillance in dengue hyperendemic municipalities

Financial support: CREATE-NEO (NIHgrant 1U01AI151807).



PREDICTORS OF SEVERITY BASED ON CLINICAL, DEMOGRAPHIC AND SEROLOGICAL ASPECTS IN PEDIATRIC PATIENTS WITH SUSPECTED DENGUE DURING THE 2019 DENGUE EPIDEMIC IN BRAZIL

Flora de Andrade Gandolfi[1]; Bárbara Ferreira dos Santos[1]; Bruno Henrique Gonçalves de Aguiar Milhim[1]; Gislaine Celestino Dutra da Silva[2]Mauricio Lacerda Nogueira[3,4]; Cássia Fernanda Estofolete[3].

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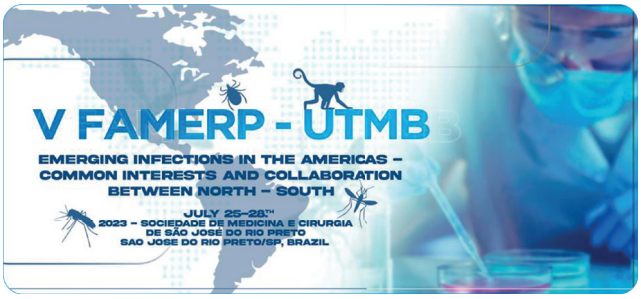
ABSTRACT

Dengue is the main arbovirus in terms of morbidity-mortality. Clinical presentation ranges from mild/asymptomatic to severe disease. The difficulty of diagnostic in children leads to late appropriate clinical treatment. Thus, the interaction between clinical, epidemiological, and virological characteristics in dengue-confirmed patients, during the 2019 epidemic, was analyzed based on the risk of severe forms of the disease. For that, data from 341 patients up to 15 years old, were analyzed retrospectively, based on medical records and serum samples. The patients samples were confirmed for DENV by NS1, RTPCR and serological test (anti-dengue IgM detection). Out of the total, 54.84% were included in the study, 30.48% were hospitalized due to dengue with warning signs (DWS) or severe dengue (SD). The most frequent dengue signs and symptoms were fever 88.24%, leukopenia 51.91% and exanthema 42.25%. The predominant warning signs were abdominal pain 81.67%, fluid accumulation 46.67% and bleeding 38.33%. For SD cases the most frequent were shock 57.14%, respiratory distress and neurological involvement 42.86%. No death was identified on confirmed patients. Age ranges or sex did not associate with risk of DWS/SD. The risk of DWS/SD were associated with the presence of comorbidities OR 4.2 (CI95% 1.65-10.694; $p<0.003$), immunosuppression OR 11.167 (CI 95% 1.218-102.40; $p<0.33$) or anti-Zika IgG presence OR 8.75 (CI 95% 3.115-24.578; $p<0.001$). The detection of anti-dengue IgG did not influence the development of DWS/SD (OR 1.226, CI 95% 0.369-0.887, $p=0.622$). The study was developed in an endemic region, with a high seroprevalence of IgG antibodies for dengue in adult population, raising the question of whether or not the object of this study might also been exposed to multiple dengue infections. Thus, highlighting the need for further studies to better evaluate the virological status of this population to understand and mitigate the evolution to severe forms of the disease.

Keywords: Arbovirus; Dengue; Pediatric. Financial support: -



LACK OF EVIDENCE OF MAYARO VIRUS EXPOSURE IN FREE-RANGING MARMOSETS OF AN ANTHROPIZED AREA OF ZONA DA MATA, SOUTHEAST BRAZIL

Larissa Berdine G.Jesus[1], Bruno B.Morente[1], Rafaela A.Lima[1], Clara Maria Ferraz[1], Ana Alice P.Pereira[1], Gabriel A.S.Oliveira[1], Mariana F.Moreira[1], Giulia Yumi Kaku[1], Ingrid F.Souza[1], Gabriela S.Bem[1], Ana Catarina V. Veloso[1], Isabela N.Mascarenhas[1], Ana Maria Marques[2],Fabiano R.Melo[2],Fabiana A.Voorwald[2],Alex Pauvolid-Corrêa[1]

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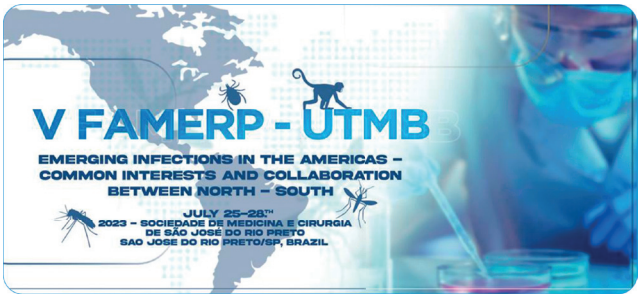
ABSTRACT

Background: Mayaro virus (MAYV) is an enzootic arbovirus maintained among nonhuman primates and sylvatic mosquitoes in Central and South America, including Brazil. MAYV has been involved in outbreaks of febrile illness and arthralgia in Northern and West-Central regions of the country. Despite genetic similarities with other alphaviruses, such as chikungunya virus, transmission cycles are more related to the sylvatic yellow fever. MAYV has been detected in wild-caught mosquitoes of several genera including Aedes, Culex, Psorophora, and Coquillettidia, but the main vectors are acrodendrophilic species of Haemagogus and Sabethes genera. The expansion of these vectors into urban areas increases the risk of introducing MAYV into previously non-enzootic regions and potential urbanization. Methods: Aiming to evaluate the exposure of non-human primates to MAYV in Southeast Brazil, serum samples from 38 hybrid marmosets (Callithrix sp.) collected in an anthropized area of Zona da Mata were tested for specific neutralizing antibodies by plaque reduction neutralization testing (PRNT). Results: All serum samples presented PRNT titers for MAYV <10 and considered seronegative. Conclusions: Findings presented here suggest no exposure to MAYV of hybrid marmosets sampled in the Zona da Mata, southeast Brazil. Active surveillance is an instrumental approach for the detection of cryptic and subclinical activity of enzootic arboviruses and may serve as a warning system to implement appropriate actions to prevent outbreaks.

Keywords: Mayaro virus, marmosets, PRNT, Zona da Mata, Brazil

FinancialSupport: Universidade Federal de Viçosa e Wildlife Conservation Network



UNVEILING THE ROLE OF ANNEXIN A1 IN CONTROLLING EXCESSIVE INFLAMMATORY RESPONSE UNLEASHED BY BETACORONAVIRUS INFECTION

Filipe Resende Oliveira de Souza*, Celso Martins Queiroz-Junior, Larisse de Souza Barbosa Lacerda, Ian de Meira Chaves, Isabella Zaidan, Laís Grossi, Lirlândia Pires de Sousa, Fernanda Marim, Renato Santana de Aguiar, Jordane Pimenta, Mauro Martins Teixeira, Vanessa Pinho da Silva, Vivian Vasconcelos Costa

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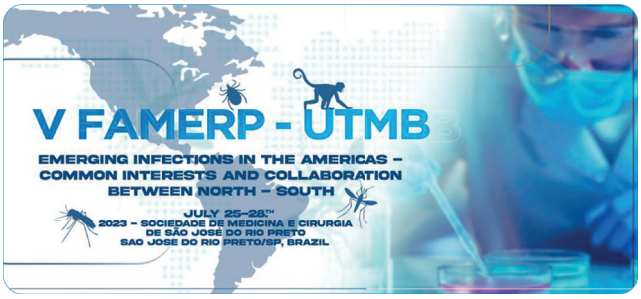
ABSTRACT

Resolution of inflammation is a critical process for restoring tissue homeostasis after injury. Annexin A1 (AnxA1), a pro-resolutive molecule produced by various cell types, plays a fundamental role in the resolution of acute inflammation by modulating intracellular signaling pathways and binding to formyl peptide receptor 2 (FPR2). Misplaced inflammation contributes significantly to tissue damage and mortality associated with infectious diseases like COVID-19. We hypothesized that inadequate engagement of AnxA1 underlies the cytokine storm and pulmonary pathology observed in betacoronavirus infections. To investigate this, we examined AnxA1 levels in the plasma of a cohort comprising low and high risk of deterioration according to National Early Warning Score 2 (NEWS2) in COVID-19 patients. Additionally, we utilized a murine model of severe coronavirus infection, characterized by different disease parameters, such as lung lesion and mortality. Wild-type (WT) and AnxA1 knock-out mice (AnxA1KO) were intranasally inoculated with 102 PFU/mouse of a murine betacoronavirus (MHV-3) (CEUA UFMG 249/2020), and MHV-3-induced disease was evaluated. Our findings revealed increased circulating levels of AnxA1 in severe COVID-19 patients. In mice, MHV-3 infection elevated AnxA1 expression in the lungs of WT mice. Conversely, AnxA1KO mice exhibited significantly increased lung injury and exacerbated production of CXCL1 and CXCL2 chemokines in the lungs compared to WT-infected littermates. Interestingly, AnxA1KO mice demonstrated diminished expression of IFN-β despite similar viral titers recovered from lung tissue compared to the infected WT group. Overall, our results demonstrate that Annexin A1 plays a crucial role in controlling excessive inflammatory responses triggered by betacoronaviruses without compromising the host's ability to deal with infection. Collectively, these findings suggest that AnxA1 could serve as a promising therapeutic target for betacoronavirus infections.

Financial support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Instituto Nacional de Ciência e Tecnologia em Dengue e Interação Microorganismo-Hospedeiro (INCT Dengue e Interação Microorganismo-Hospedeiro), Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG).



TRAMETINIB PROTECTS A129 MICE AGAINST LETHAL ZIKA VIRUS INFECTION IN A SEX-DEPENDENT MANNER

Colquehuanca NEA[1] , Cruz ALC1 , Mendonça DC[1] , Souza JPC [2] , Costa TA[1] , Arruda MS[1] , Oliveira GFG[1] , Albuquerque VVS[1] , Moreira GD[1] , Guimarães ACDS[1] , Viegas SSFM[1] , Bonjardim CA[1] and Drumond BP[1]

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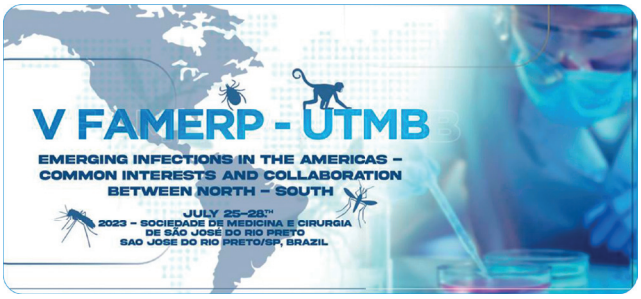
ABSTRACT

Zika virus (ZIKV), a member of the orthoflavivirus genus, is an emerging virus that has demonstrated its epidemic capacity on a global scale. Recent outbreaks of ZIKV have revealed that it can cause Congenital Zika Syndrome in neonates and Guillain-Barré syndrome in adults. Despite the lack of specific vaccines or antiviral treatments, repurposing existing drugs offers a promising strategy. Trametinib, an MEK/ERK kinase inhibitor, has shown antiviral activity against RNA viruses, including dengue and yellow fever. Its mode of action potentially involves interfering with viral particle morphogenesis and release during the replication cycle. Therefore, the objective of this study was to evaluate the efficacy of oral trametinib treatment against lethal ZIKV infection using 5- week-old A129 mice. No signs of toxicity were observed with oral doses of trametinib (2 and 3 mg/kg) administered daily for 8 days. Assessment of renal and hepatic function showed a similar outcome profile to the control group that received only the vehicle formulation. Trametinib treatment, initiated 24 hours after lethal ZIKV infection, improved survival by reducing weight loss in a dose-dependent manner and mitigated or eliminated severe signs of the disease (facial edema/conjunctivitis and limb paralysis). Interestingly, the effectiveness of trametinib treatment was found to be dependent on the sex of the mice, with 66.6% of male and only 33.3% of female mice surviving lethal ZIKV infection. Trametinib also significantly reduced ZIKV titers in the mice's brains and testes, indicating its effectiveness against lethal infections caused by an African strain of ZIKV. These results support trametinib as a safe and effective candidate for lethal ZIKV infections, while emphasizing the importance of considering sex as a crucial variable in preclinical studies for reliable and applicable outcomes. Further investigations should explore underlying mechanisms and its efficacy against other orthoflavivirus.

Financial support: Fundação de Apoio a Pesquisa do Estado de Minas Gerais (FAPEMIG)-CBB–APQ-01670-11;CBB–AUC-00071-15;FAPEMIG/PPSUS (PesquisaParaoServiçoÚnicodeSaúde)– CBB –APQ-04178-17.



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

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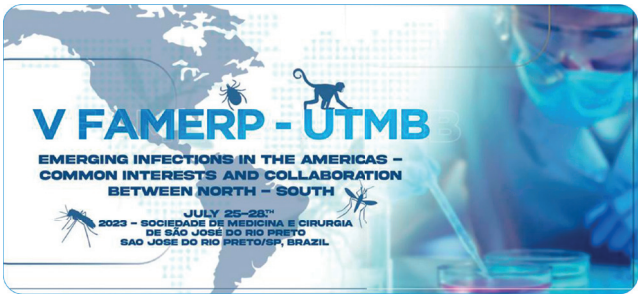
ABSTRACT

The Saint Louis encephalitis virus (SLEV) is a single-stranded positive-sense RNA arbovirus belonging to the Flaviviridae family, Flavivirus genus. Typically, its cycle is limited to infecting mosquitoes and birds, but it is known that SLEV can infect humans, causing mild to moderate symptoms, such as fever and headache, in most cases. However, the infection can progress to more severe conditions, with the presence of neurological disorders such as meningitis and encephalitis, which can increase the chances of fatality to 30%. Due to the scarcity of studies on this virus, this study aims to assess the genetic variability and adaptive dynamics of SLEV, in cell culture, mimicking the transmission cycles between the invertebrate host (mosquito) and the vertebrate host (human). To evaluate the intra-host adaptive dynamics in mosquitoes, the Aedes aegypti embryonic cell line (AAG2) was consecutively infected for 5 passages with an MOI of 0.0001, and after 120 hours, the supernatant was collected for viral titration and subsequent consecutive infection in the same cell line. So far, the consecutive passages in AAG2 have shown an increase of one log per passage, with a drop of one log in the fifth passage, without alterations in the plaque morphology. The same process is in progress with a mammalian cell line (LLC-MK2) to evaluate variability in the mammal host, following the same protocol used for AAG2. In the future, this approach will be used to assess the adaptive dynamics of SLEV at the inter-host level, conducting alternating infections between the two cell lines, with 5 passages for each lineage, thus totaling 10 alternating passages. After the infection cycles, the inter- and intra-host viral genetic variability will be determined by next-generation sequencing (NGS) through the sequencing of all consecutive and alternate passages, where bioinformatics tools will be applied to evaluate the frequency dynamics of viral variants.

Financial support: CAPES



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

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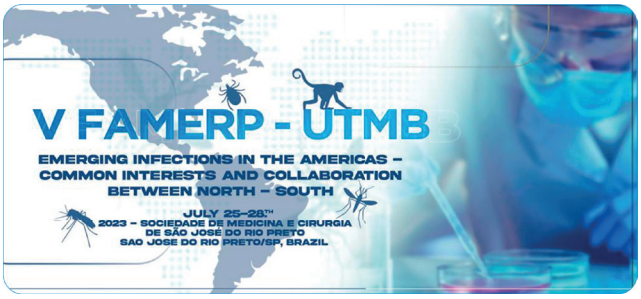
ABSTRACT

Mayaro virus (MAYV) is an arbovirus which has emerging potential and may become a significant problem in tropical countries. As an arbovirus, it circulates in vertebrate and invertebrate hosts. Therefore, this study evaluates the genetic variability and adaptive dynamics of MAYV during transmission cycles between mosquitoes and humans in vitro. To measure inter-host variability, infection cycles were performed using mosquito embryonic cell line (AAG2) and human cell line (HFF1). To mimic the transmission cycle of MAYV between hosts, HFF-1 cells were infected with an MOI of 1. After 72 hours, supernatant was collected for viral titration and subsequent infection of AAG2 cells by the same protocol. Subsequently, the virus was titrated and inoculated back into HFF1 for a new cycle. To evaluate inter-host variability, both cell lines were sequentially infected with the same MOI and incubation time, without host alternation. A total of 8 alternated passages were performed, with 4 passages in HFF1 and 4 passages in AAG2. During the first 5 passages, the viral titer fluctuated according to the cell line, decreasing after infections in AAG2 cells and recovering after infections in HFF1 cells. However, after passage 6, in AAG2, the viral titers decreased for both cell lines and were not recovered. For the intra-host evaluation, a significant increase in viral titers was observed, for both cell lines after consecutive passages, until the second passage. However, after third passage, a slight decrease in viral titer was observed in HFF1 cells, while a sharp decline occurred in AAG2 cells, inhibiting the fourth passage in this cell line. Our results suggest an increase in MAYV fitness during human cells infection, while the opposite can be observed in mosquito cells, both in alternated and consecutive cycles. These findings will be confirmed by NGS, allowing the evaluation of MAYV genetic diversity at inter and intra-host levels, detecting mutations emergence and maintenance.

Financial support: CAPES.



WILDTYPE YELLOW FEVER VIRUS BR-2018 VIRULENCE IN C57BL/6 IFNAR-/- MICE BY FOOTPAD ROUTE

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ABSTRACT

Yellow fever virus (YFV) is an arbovirus responsible for an acute viral hemorrhagic disease, with clinical manifestations varying from very mild infection to severe and lifethreatening illness, with a mortality rate ranging from 20 to 50%. YFV is endemic in tropical areas of Africa, Central and South America, with massive outbreaks causing thousands of deaths in recent years, showing that YFV is still a serious public health concern despite the vaccination. Recent YF outbreaks occurred from 2017-2018, in the southeast region of Brazil, with several cases and deaths, caused by a new lineage of YFV belonging to South American genotype. In the present study we aimed to study and characterize wildtype YFV_BR_2018 in experimentally infected C57BL/6 IFNAR-/- mice. For this, four weeks old C57BL/6 IFNAR-/- mice were infected with YFV-BR-2018 (103 p.f.u.) or 17DD (105 p.f.u.), used as control, by footpad route. Mice were evaluated for clinical signs and weighed daily. The results indicate higher survival rate in mice infected with YFV 17DD, with 80% survival in both females and males, whereas those infected with YFV_BR_2018 had a survival rate of 60% and 40% in females and males, respectively. Signals were also different, with 100% of males and females infected with YFV_BR_2018 presenting various signs such as several ruffling fur and arching back, conjunctivitis, edema, paralysis, tremors, or penis inflammation, for males. Whereas males and females infected with YFV 17DD presenting milder signs, like mild or moderate ruffling fur and arching back, with just a single male and female presents conjunctivitis, and just one male presents paralysis and penis inflammation. In general, for both strains, males showed more severe signs than females. The results indicate that YFV_BR_2018 is more virulent than YFV 17DD and the sex is a variable that might interfere in the outcome of YFV infection

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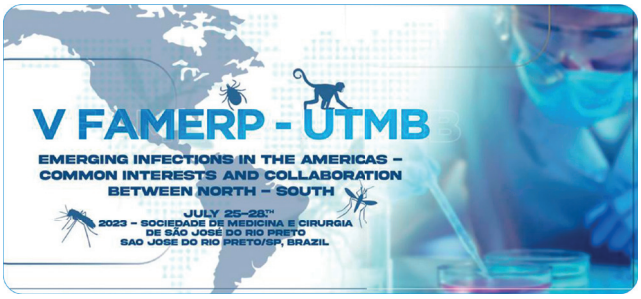

PREVIOUS ZIKA INFECTION IN ACUTE DENGUE

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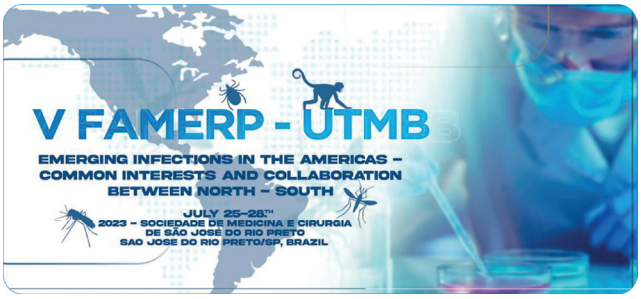
ABSTRACT

Although dengue is a disease known for years in the world and has been affecting several continents, some 28 aspects remain unclear. One of them is about the possible factors that may influence the development of 29 severe forms of the disease. Much has been discussed about the influence of a previous dengue episode, but 30 the global spreading of other flaviviruses to areas where dengue was already circulating has aroused 31 interest regarding a role like the other dengue serotypes. This study analyzed the influence of antibodies 32 produced in response to Zika infection on the clinical course of a subsequent infection with dengue 33 serotype 2 in patient samples collected during the epidemics in 2019 and 2022 in the São José do Rio 34 Preto-SP. We enrolled 1,043 laboratory-confirmed dengue patients through the Polymerase Chain Reaction 35 (PCR) method and investigated their prior infection to Zika or dengue using an enzyme-linked 36 immunosorbent assays (ELISA). Furthermore, we assessed the cytokine expression profile in the patients' 37 samples and conducted statistical analysis on our data. This study was conducted in a tropical area where 38 flaviviruses cocirculate, suggesting that previous Zika infection may be a risk factor for development of 39 more severe forms of dengue disease and hospitalization. Contrary to the currently accepted mechanism of 40 SDD in secondary dengue infection, the mechanism here appears not be associated with ADE, and neither 41 DENV load, vascular leakage or cytokine storm were increased. In fact, individuals with previous Zika 42 infection had increased levels of IL-10 and IL-17A and no difference in IFN-y, IL-6, and IL-1β:IL-1ra 43 ratios. These findings highlight the complex interactions between viruses and hosts and warn to fact of the 44 knowledge acquired about the interaction among different dengue serotypes can not necessarily be applied 45 to different flaviviruses.

Financialsupport: São Paulo Research Foundation (FAPESP 2022/12576-3), INCT Dengue Program grant, 48 Brazilian National Council for Scientific and Technological Development (CNPq), The Coordinating Research on Emerging Arboviral Threats Encompassing the Neotropics (CREATE-NEO)



CLINICAL IMPACT OF DENV AND SARS-COV-2 CO-INFECTION IN HOSPITALIZED PATIENTS

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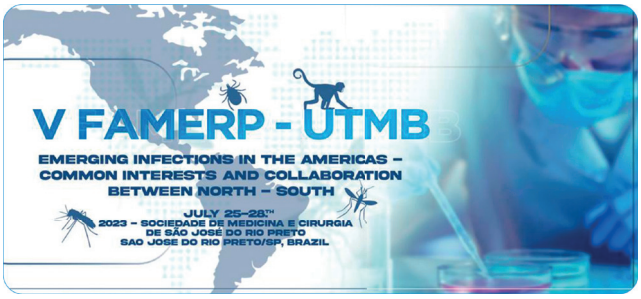
ABSTRACT

Since its emergence in 2019, the coronavirus disease (COVID-19) has spread worldwide, consuming public health resources. However, the world still needs to deal with the burden of other infectious diseases that continue to thrive. Tropical countries, such as Brazil, are annually affected by cyclic dengue epidemics, with some regions being hotspots for other flavivirus transmissions. Until now, little is known about the impact of a co-infection between DENV and SARS-CoV-2. Thereon, we investigated sera samples from COVID-19 positive patients collected from February to June 2021, months historically related to DENV outbreaks. 400 samples were tested for DENV by serology and molecular assays. No DENV PCR positive was observed, but 78% were DENV IgG positive, 6% DENV IgM positive, and 0.25% DENV NS1 positive in ELISA. DENV IgM and IgG antibodies were isolated by chromatography from co-positive samples, and 62.5% of the samples were positive for neutralizing antibodies (FRNT80) for DENV IgM, indicating a recent infection. Also, IL-1β was increased in co-infected patients. Regarding the clinical aspects, diabetes was the only relevant comorbidity ($p=0.046$), though at a lower frequency than expected. A high rate of global hospitalization (94.9%) and mortality (50%) was found, with a significant increase in invasive mechanical ventilatory support (86.96%) in co-infected cases, suggesting an impact on patients' evolution. Nonetheless, previous dengue exposure may seem a protective factor against several illnesses as primary dengue patients and dengue naïve tended to evolve to worse outcomes than the secondary infected. Our data shows that the differentiation between both diseases is a great concern for tropical countries and should be explored more to improve patient management and effectiveness in the surveillance of these pathogens.

Keywords: dengue virus, SARS-CoV-2, co-infection, arboviruses.

Financialsupport: FAPESP, NIH, CAPES.



EVALUATION OF ANTIBODY RESPONSE AGAINST SARS-COV-2 OMICRON SUBLINEAGES IN INDIVIDUALS VACCINATED WITH CORONAVAC FOLLOWED BY A HETEROLOGOUS TWO BOOSTER DOSES PROTOCOL

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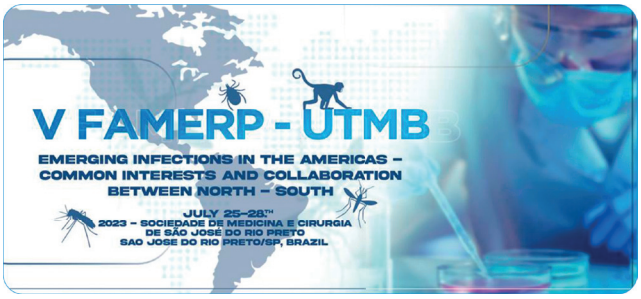
ABSTRACT

The vaccines against SARS-CoV-2 have enabled a gradual return to normalcy across the world. However, their production and distribution was not fast enough and allowed the emergence of variants capable to evade immune response induced by prior infections and vaccination. Thus, our study evaluated antibody response of a Brazilian cohort vaccinated with two doses of CoronaVac followed by two booster doses with BNT162b2 or Janssen against Omicron sublineages BA.1, BA.5 and BQ.1.1. A total of 160 individuals were included and divided into 3 time points: 9, 12 and 18 months after CoronaVac doses. At each time point, individuals were divided into 3 subgroups: No booster, 1 booster and 2 boosters (except for the 9 months group, where second booster was not available yet). Samples were subjected to a viral microneutralization assay, against Omicron sublineages, to evaluate neutralization titers and seroconvertion rate. For BA.1, in the 9 months group, the first booster significantly increased VNT50 mean (133.1 to 575.8) and seroconvertion rate (33.3% to 76.6%) when compared to no booster subgroup. In contrast, in the 12 months group, a reduction in VNT50 mean was observed as the first and second booster doses were administered (1292.3, 1048.1 and 949.1 for no booster, 1 booster 2 boosters groups, respectively). However, seroconvertion rate increased as the booster doses were distributed (85%, 90% and 100%, respectively). For the 18 months group, VNT50 mean decreased between no booster and 1 booster groups, but the neutralization mean after second booster significantly increased (1881.4, 1402.5 and 2361.5, respectively). Seroconvertion rate, in this point, was maintained at 100% for all the subgroups. Our data shows a positive impact, over time, of booster doses in the serological response against BA.1. The same process will be applied for BA.5 and BQ.1.1 to evaluate the antibody neutralization against sublineages that already showed capacity to evade immune response.

Financial support: Oswaldo Cruz Foundation (FIOCRUZ) and FAPESP (process number 2020/07419-0).



EXPLORING THE INTERACTION BETWEEN ALPHA-1 ADRENERGIC RECEPTOR AND DENGUE VIRUS REPLICATION

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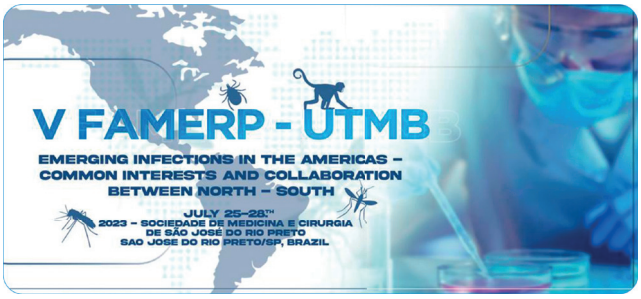
ABSTRACT

Dengue virus (DENV) replication involves the activation of lipophagy pathways, resulting in the degradation of triglycerides and the release of fatty acids stored in lipid droplets. This metabolic process is crucial for ATP generation, which is essential for viral multiplication. Hence, targeting these pathways holds promise as a specific antiviral strategy. Transcriptomic analysis of DENV-2-infected hepatocytes revealed differential expression of genes involved in lipid metabolism, including the alpha-1 adrenergic receptor (ADRA1A). ADRA1A has been implicated in mediating sympathetic nervous system responses related to lipophagy regulation and fatty acid β-oxidation. This study aims to assess the interplay between ADRA1A and DENV replication, using ADRA1A antagonists to validate it as a potential therapeutic target against dengue fever. Among commercially available ADRA1A antagonists in Brazil, we selected Tamsulosin Hydrochloride (TH) based on its low cost, minimal hepatotoxicity, and non-psychotropic effects. Initial assays focused on determining the EC50 of TH formulation. Initial assays were conducted to determine the effective concentration (EC50) of the TH pharmacological formulation. Vero and AML12 cells were infected with DENV-2 at an m.o.i of 5 and exposed to 12 concentrations of TH. After five days of culture, the viability was analyzed. AML12 infected with DENV-2 and exposed to 0.3 nM and 0.5 nM of TH demonstrated 57% and 65% higher viability, respectively, compared to the control. Moreover, the EC50 of TH was determined as 9.5 nM for AML12 cells and 31 nM for Vero cells. These preliminary results suggest that TH may confer protection against DENV-2 infection in AML12 cells. By targeting ADRA1A, this study provides insights into the mechanisms underlying DENV replication and highlights the potential therapeutic implications.

Financial support: FAPEMIG and Fiocruz



ARBOVIRUSES IN FREE-LIVING MAMMALS IN SOUTHERN BRAZIL

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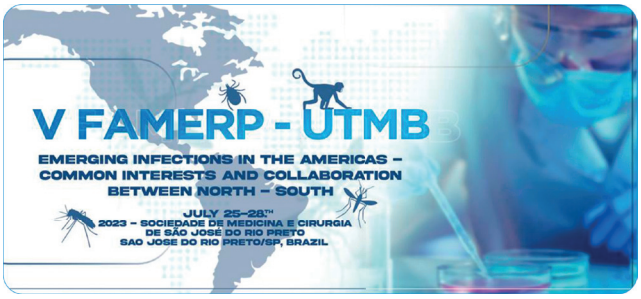
ABSTRACT

Arthropod-borne viruses (arboviruses) are a major threat to human health worldwide. About four billion people live in areas at risk of arbovirus transmission. Dengue virus (DENV), the most widespread arbovirus, is estimated to cause 390 million infections every year. While Zika (ZIKV) and Chikungunya (CHIKV) viruses started spreading globally in the early 2000s and have since caused great outbreaks in Asia and the Americas. Humans are known to be the main reservoir host maintaining the epidemic cycles of dengue but it's unclear if dengue virus is also maintained in a similar enzootic cycle, as well as other arboviruses. It's known that arboviruses transmitions likely originated from sylvatic cycles maintained between susceptible non-human primates and Aedes mosquitos in Asia. Sylvatic cycles have also been described in African forests, and little is known about the circulation of these viruses in wild mammals in the Americas. In this study we have assessed the viral diversity present in oral and fecal swabs collected from 88 free-living animals, totalling 13 different species through metagnomics assays. Samples were collected between March 2022 and March 2023 in the Vale dos Sinos Region, Southern Brazil. DENV and CHIKV were identified in 14 pools, a total of 19 animals, from the species Didelphis albiventris, Akodon montensis, Coendou spinosus and Myocastor coypus. The genome coverage varied from 1.04% to 99.3%. The highest DENV genome coverage was found in Didelphis albiventris (83.56%) and the highest CHIKV genome coverages were found in Akodon montensis (99.3%) and Coendou spinosus (98,9%). Arboviruses are capable of infecting a number of animal species. The role of these animals as amplifying reservoirs is still uncertain and more studies are being conducted to evaluate the importance of these findings.

Financialsupport:Capes/CNPq/Finep.



METAGENOMICAPPLICATIONFORDETECTIONOFVIROMESINTICKS COLLECTED FROM WILD ANIMALS

Geraldini, Dayla Bott; Beltrão, Rafael Cesário; Bittar, Cíntia; Gismene, Carolina; Possebon, Fábio Sossai; Mariutti, Ricardo Barros; Campos, Guilherme Rodrigues Fernandes; Neto, Guilherme Guerra; da Costa, Vivaldo Gomes; Lofego, Antônio Carlos; Calmon, Marília de Freitas; Nogueira, Mauricio Lacerda; Araújo Junior, Joao Pessoa; Rahal, Paula

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ABSTRACT

Ticks are capable of transmitting a wide variety of pathogens, affecting both wild and domestic animals and humans, posing a serious risk to public health. Most tick-borne viruses have RNA genomes prone to accumulating mutations. This characteristic results in high variability, that can lead to the emergence of new viruses and spillover to new host, which may pose a threat to public health. Here we performed a metagenomic analysis of the virome of ticks collected from wild animals received in the Municipal Zoo of São José do Rio Preto - SP. A total of 273 ticks were collected and separated into 71 pools. RNA was extracted and species identification was confirmed by molecular analysis through the mitochondrial gene. Samples were pooled for next-generation sequencing, resulting in four genomic libraries that were sequenced in the MiSeq platform. The analyzes showed important molecular evidence of a potentially new segmented virus, belonging to the Jingmenvirus group, most closely related to the Jingmen tick virus (JMTV). Specific primers and probe were designed, and RT-qPCR was performed in the 71 pools. Four pools were positive for the viral genome of JMTV_Like_Brazil described in this work. The viral genome was detected in the tick species Amblyomma sculptum and Amblyomma nodosum, these ticks were collected from vertebrate hosts Myrmecophaga tridactyla, Callithrix penicillata and Cerdocyon thous. An analysis of the structures of the viral proteins of the four segments revealed a unique loop in segment 1, this region blocks access to a helix that belongs to a part of NS5 in flaviviruses. This study contributes to epidemiological surveillance in the region and helps to understand the risks of the emergence of zoonoses, which can affect the fauna of the region, in addition to possible impacts on human health. In short, further analyzes are needed to evaluate the epidemiological characteristics of this potentially new JMTV_Like_Brazil in the wild

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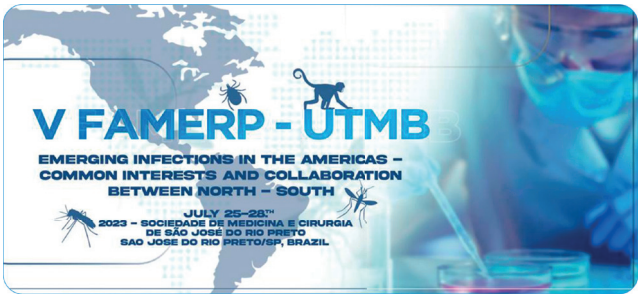

PREDICTING POTENTIAL TRANSMISSION RISK OF EVERGLADES VIRUS IN FLORIDA USING MOSQUITO BLOOD MEAL IDENTIFICATIONS

Kristin E.Sloyer[1], Narayani Barve[2], Dongmin Kim[1], Tanise Stenn[1], Lindsay P.Campbell[1], and Nathan D. Burkett-Cadena[1]

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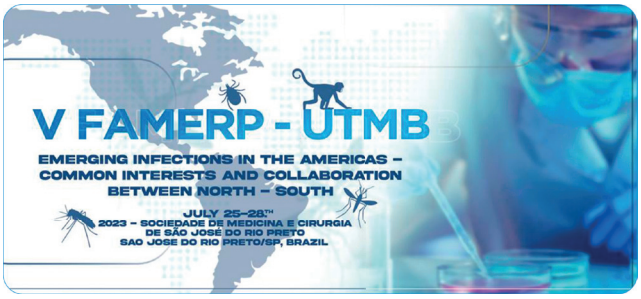
ABSTRACT

The overlap between arbovirus host, arthropod vectors, and pathogen distributions in environmentally suitable habitats represents a nidus where risk for pathogen transmission may occur. Everglades virus (EVEV), subtype II Venezuelan equine encephalitis virus, is endemic to southern Florida and is transmitted by the endemic vector Culex cedecei between muroid rodent hosts. We developed an ecological niche model to predict areas in Florida suitable for EVEV transmission from georeferenced vector-host interactions determined by blood meal analysis from blood-engorged Cx. cedecei. Thirteen environmental variables were used for model calibration, including bioclimatic variables derived from daily temperature and precipitation values, and land use/land cover data representing percent land cover. Maximum temperature of the warmest month, minimum temperature of the coldest month, and precipitation of the driest month contributed 31.6%, 28.5% and 19.9% to model performance. The land cover types contributing the greatest to model performance were percent landcover of emergent herbaceous and woody wetlands which contributed 5.2% and 4.3% respectively. Results showed high suitability for Cx. cedecei feeding on rodents throughout southwestern Florida, and pockets of high suitability along the northern east coast, while areas with low suitability included the Miami-Dade metropolitan area and most of northern Florida and the Panhandle. Comparing predicted distributions of Cx. cedecei feeding upon rodent hosts in the present study to historical human cases of EVEV disease, as well as antibodies in wildlife show substantial overlap with areas predicted moderate to highly suitable for these vector/host associations. The findings of this study likely predict the most accurate distribution of the nidus of EVEV to date, indicating that this method enhances inference of transmission areas better than models which only consider the vector or vertebrate host species individually.

Financial support: This work was funded by Florida Department of Agriculture and Consumer Services contract #27475 and USDA National Institute of Food and Agriculture, Hatch project 1021482



ROLE OF IRF5 IN DENDRITIC CELLS FOR MAYARO VIRUS INFECTION IN A MURINE MODEL

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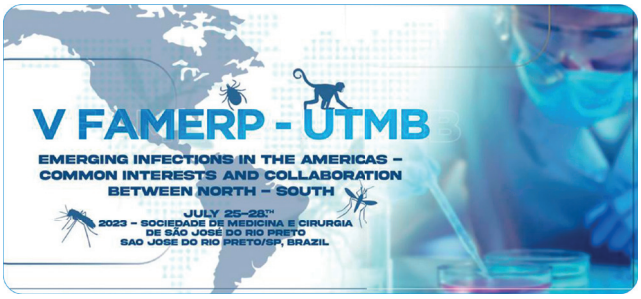
ABSTRACT

The intensification of climate change and environmental changes caused by increased deforestation, both for agribusiness and urban expansion, has intensified, in recent decades, the contact of human populations with new pathogens. Currently, in Brazil, one of the most worrying infections caused by viruses that are transmitted by arthropods (arboviruses) is the Mayaro virus (MAYV) infection, which causes an arthritogenic febrile illness similar to Chikungunya. The control of infection by this virus is usually related to innate immunity with intense participation of macrophages and dendritic cells. The transcription factor IRF5 (Interferon Regulatory Factor 5), in turn, is associated with the immune response of other cell types during infection by arboviruses from other viral families. Therefore, the objective of this work was to investigate the MAYV replication profile in an animal model with IRF5 depletion only in CD11c+ dendritic cells, using CD11c-Cre+ Irf5fl mutant animals and Cre– Irf5fl controls. After the inoculation of 10^6 PFU of MAYV in the paw of these animals, it was observed that about 30% of the mutants for Irf5 had swelling of the paw and were susceptible to infection. In these animals, about 10^9 PFU/g of MAYV was detected in the paw, 10^7 PFU/g in the heart, and 10^6 PFU/g in other tissues such as the brain, spinal cord, kidneys, lungs, spleen and liver. In addition, 10^5 PFU/g of MAYV was found in the paw, inguinal lymph nodes and kidney of mutants that had no signs of disease on day 42 postinfection. Based on these results, it was verified that IRF5 in dendritic cells is important for the control of the initial infection and development of the disease caused by MAYV in a murine model, as well as suggesting its participation in the control of persistence and late disease. Therefore, further investigation of the mechanisms involved is necessary

Financial support: SAE-Unicamp, FAPESP, CNPqand CAPES.



FPR2: A NOVEL PROMISING TARGET FOR THE TREATMENT OF CORONAVIRUS INFECTION

Filipe Resende Oliveira de Souza*,Celso Martins Queiroz-Junior,Larisse de Souza Barbosa Lacerda, Ian de Meira Chaves, Jordane Pimenta, Lirlândia Pires de Souza, Mauro Martins Teixeira, Vanessa Pinho da Silva, Vivian Vasconcelos Costa

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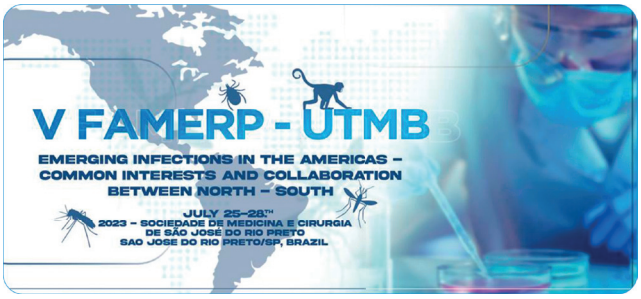
ABSTRACT

Resolution of inflammation is a crucial process for restoring tissue homeostasis following an injury. Formyl peptide receptor 2 (FPR2) is a G-protein coupled receptor (GPCR) primarily expressed by macrophages and neutrophils. It plays a fundamental role in the resolution of inflammation by binding to various pro-resolving molecules, including Annexin A1, Lipoxin A4, and Resolvin D1. Inappropriately regulated inflammation is a major contributor to tissue damage and mortality associated with various infections, such as COVID-19. Here, our aim was to assess the role of the FPR2 receptor during Betacoronavirus infection. Wild-type (WT) mice and FPR2/3 knockout mice (FPR2/3KO) were intranasally infected with a strain of murine betacoronavirus (MHV-3) (CEUA 249/2020), which mimics severe COVID by causing pneumonia and mortality in mice. Mice lacking FPR2/3 receptors showed approximately 50% protection from lethality when exposed to higher MHV-3 inoculums, compared to WT mice, in which 100% succumbed to infection by day 5-7. Interestingly, a logarithmic reduction in MHV-3 inoculum resulted in 100% protection against lethality in FPR2/3KO mice, while all WT mice were deceased by day 6-10 post-infection. MHV-3 inoculation led to altered hematological parameters in infected WT mice, such as leukopenia and lymphopenia, which were completely reversed in the absence of FPR2 receptors. FPR2/3KO mice also exhibited reduced viral titers in the lungs and spleen, decreased lung damage, diminished production of inflammatory mediators (such as CXCL1 and CCL2), and reduced expression of ISG-20 in the lungs. Pharmacological blockade of FPR2 using a selective inhibitor WRW4 (8mg/kg, intraperitoneal route, daily) resulted in reduced systemic viral titers in the spleen without exerting any additional protective effects on the lungs. In conclusion, these findings suggest that inhibition of FPR2/3 receptors could represent a promising therapeutic target against Betacoronavirus infections.

Financial support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Instituto Nacional de Ciência e Tecnologia em Dengue e Interação Microorganismo-Hospedeiro (INCT Dengue e Interação Microorganismo-Hospedeiro),Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG).



SURFACE FIBRILS ORGANIZATION AS SPECIFIC TRADEMARKS OF DIFFERENT MIMIVIRUS LINEAGES

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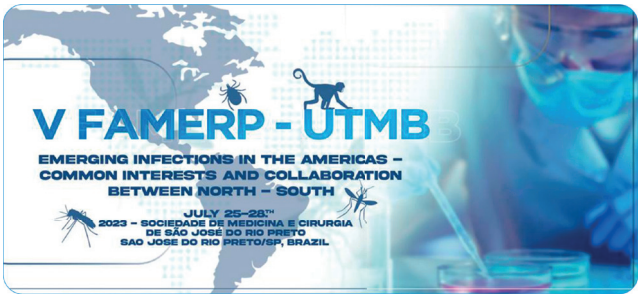
ABSTRACT

Among the most intriguing structural features in the known virosphere, mimivirus surface fibrils fuel debates on evolutionary traits of viral gigantism. Fibrils are approximately 150 nmlong highly glycosylated proteinaceous filaments, covering the mimivirus capsid surface, which expand the viral particle up to 750 nm diameter and promote particle adhesion to host cell. Although mimiviruses are abundant in a plethora of worldwide biomes, there is no comparative analysis on fibrils organization and abundance among distinct mimiviruses lineages. Here, by combining a set of methods and analyses, including electron microscopies, image processing and genomic sequencing, we describe, for the first time to our knowledge, lineage-dependent profiles of fibrils organization among mimiviruses. Microscopical analyses of 15 mimiviruses revealed at least three profiles of isolates based on fibrils organization: i) those with abundant fibrils, distributed homogeneously on the capsid surface, as previously described; ii) isolates with particles almost fiber-less; and iii) isolates with particles containing fibrils in abundance, but organized as clumps. Hallmark genes sequencing revealed that those well-defined fibrils organization profiles correspond to evolutionary-related viruses, belonging to lineages A, B and C, respectively. To demonstrate that fibrils appearance is not due microscopy artifacts, isolates were analyzed concomitantly in pairs or trios, reinforcing the aforementioned diversity on fibrils organization. The analyses of the most abundant protein described in fibrils, the GMCoxidoreductase, revealed lineage B (almost fiber-less) as the most divergent among mimiviruses. At last, virological assays demonstrated that lineage A mimiviruses are more efficient to trigger phagocytosis in amoebas then lineage B and C mimiviruses. Taken together, our data suggest a notable diversification on mimiviruses fibrils organization after lineages radiation.

Financial support: CNPq, CAPES, FAPEMIG, CM-UFMG, PRPqandPRPg UFMG.



EVALUATION OF THE ANTIVIRAL ACTIVITY OF QUERCETIN HYDRATE AGAINST OROPOUCHE VIRUS

Marielena Vogel Saivish[1,2], Gabriela de Lima Menezes[3,4], Roosevelt Alves da Silva[3], Umberto Laino Fulco[4], Gislaine Celestino Dutra da Silva[1], Igor da Silva Teixeira[1], Natalia Franco Bueno Mistrão[1], Lívia Sacchetto[1], and Maurício Lacerda Nogueira [1,2,5]

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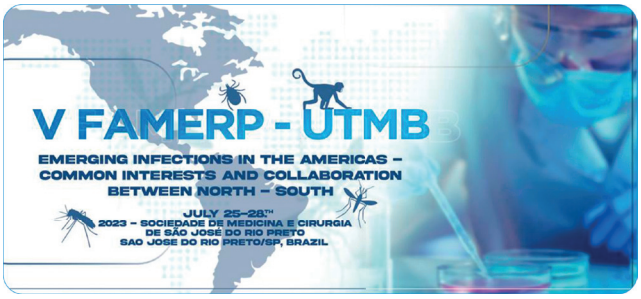
ABSTRACT

Most of the orthobunyavirus members are arthropod-borne viruses (arboviruses) transmitted by ticks or mosquitoes, some of which could lead to human disease (such as fever and encephalitis). They are endemic in many countries, including Brazil, highlighting the need for preparedness, control, and prevention. Currently, there are no antiviral drugs available to treat orthobunyavirus infections. Our purpose was to investigate the inhibitory activity of quercetin hydrate against the Oropouche virus (OROV), an orthobunyavirus already detected in Brazil. Quercetin hydrate inhibited OROV with an EC50 value of 53.52 ± 26.54 μM under post-infection treatment conditions in Vero cells. Furthermore, OROV plaque formation was strongly inhibited. In addition, we used molecular docking to analyze the Gn viral protein-compound interaction. Favorable binding energies were observed between quercetin hydrate and the Gn viral protein, suggesting that this molecule may be a promising inhibitor. In conclusion, quercetin hydrate was an inhibitor of OROV infection. However, we encourage further studies to be conducted to investigate the existence of these interactions. Future in vitro evaluations will help further elucidate these hypotheses. Finally, using computation tools to postulate potential mechanisms of action in antiviral compounds can help develop and rationally evaluate molecules with drug potential.

Financial support: This research was funded by the São Paulo Research Foundation (FAPESP), grant 2013/21719-3. M.L.N. is partially supported by the Centers for Research in Emerging Infectious Disease via The Coordinating Research on Emerging Arboviral Threats Encompassing the Neotropics (CRE-ATE-NEO) grant 1U01AI151807-01 awarded by the National Institutes of Health (NIH/USA). M.V.S. was supported by an FAPESP PhD Scholarship, process 2020/12875-5. G.L.M. was supported by a CAPES PhD Scholarship, process 88887.659079/2021-00.



COMPARATIVE ANALYSIS OF THE GLOBAL EXPRESSION GENES INVOLVED IN THE EVOLUTION OF PATIENTS TO POST-CHIKUNGUNYA CHRONIC INFLAMMATORY JOINT DISEASE

Milena Gomes Cabral [1,2] , Mariana Severo Ramundo [1,2], Felipe Ten-Caten [1,2], Guilherme Cordenonsi da Fonseca[3], Otavio Brustolini[3], Alexandra L. Gerber[3], Ana Paula Guimarães[3], Erika Regina Manuli [1,2], Marina Farrel Côrtes [1,2,4], Geovana Maria Pereira [1,2], Carolina Dos Santos Lázari [5,6], Patrícia Brasil 5,Clarisse S Bressan[7], Isabella de Moraes[7], Helder I Nakaya [8,9,10],Gláucia Paranhos-Baccalà[4], Ana Tereza R Vasconcelos[3],Ester Cerdeira Sabino[1,2*]

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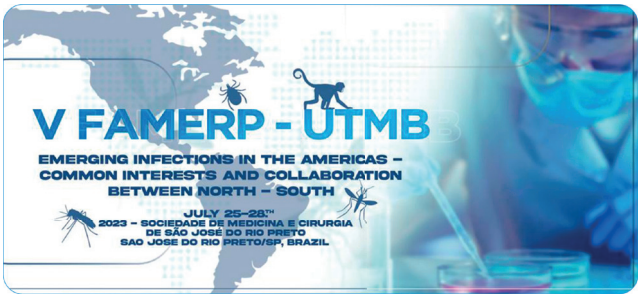
ABSTRACT

Chikungunya virus was first detected in Brazil in 2014, and has rapidly spread, becoming a public health emergency in the country. Chikungunya fever is characterized by fever, myalgia, and polyarthralgia. After three months, this setting can evolve to Post-Chikungunya Chronic Inflammatory Joint Disease (pCHIKV-CIJD), leading to increased morbidity levels and economic losses. We aimed to clarify the mechanisms involved in the chronification process by comparing the transcriptome of non-pCHIKV-CIJD and pCHIKV-CIJD patients. Patients were included in a prospective cohort with clinical evaluation and collection of biological materials in acute phase, sub-acute phase, and 90-day follow-up. Patients that remained with arthritis signs and altered imaging exams in the follow-up were considered as pCHIKV-CIJD. RNA was isolated from whole blood, and total and small RNA libraries were built and sequenced using Illumina. Differentially expressed genes (DEGs) and miRs (DEMs) were obtained, and pathway enrichment analysis was conducted, using edgeR and fGSEA, respectively. Validated miR target genes were found using miRTarBase. Interactome was constructed using the NPinter database. Here, pCHIKV-CIJD patients had an increase of 83 DEGs in acute phase to 458 DEGs in the subacute phase compared to non-pCHIKV-CIJD. In the subacute phase, 65,1% of DEGs were composed of long non-coding RNAs. Neutrophil Degranulation and MHCI Mediating Antigen Processing and Presentation were altered in both phases. Signaling by Interleukin was altered only in the acute phase. Chronic patients had altered miRs in both phases. In the acute phase, two up-regulated miRs possibly led to the down-regulation of the DDIT4 gene, already described as important in development of Osteoarthritis. In conclusion, this study sheds light on the mechanisms involved in the chronification process of chikungunya fever focusing on the immune pathways that appear to be significantly depleted in chronic patients.

Key-words:Chikungunyavirus, arthralgia, transcriptome.
Financial support: FAPESP; Biomerieux SA



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

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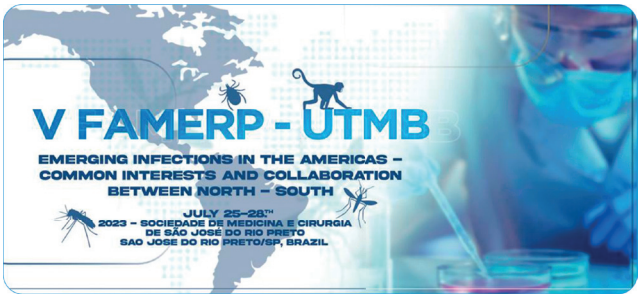
ABSTRACT

Monkeypox virus (Mpox) is an Orthopoxvirus genetically distinct from other members of the Poxviridae family, including the Variola virus, Vaccinia virus, and Cowpox virus. In 2022, several cases of Mpox were reported in countries where the had never been reported before, causing more than 87000cases and 141 deaths to date. Modified Vaccinia virus Ankara (MVA) has a solid history as a vaccine agent in the prevention of smallpox, and some studies suggest that an MVA-based vaccine 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 the Principles of Good Laboratory Practice (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 with an interval of 21 days between doses. After 14 days past prime and boost, animals' blood was collected to evaluate the production of neutralizing antibodies by PRNT and IgG by ELISA. The production of IgG in the S.C group was observed after vaccine priming and had a significant increase post boost, reaching a titer of 104 and neutralizing antibodies titer of 1x102 . As for the I.M group, we observed a significant difference in IgG production when compared to the S.C group, with titers of 103 in the I.M group. The production of neutralizing antibodies, post-prime, capable of neutralizing 50% of the viral particles, occurred in the 1/40 dilution, in the groups immunized by both routes. 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: MCTI(RedeVirus), CAPES, FAPEMIG, CNPq.



MOLECULARCHARACTERIZATIONANDGROWTHKINETICSOFAWILD-TYPE YELLOW FEVER VIRUS PLAQUE SIZE VARIANTS IN MAMMALIAN CELL LINES

Letícia Trindade Almeida, Andreza Parreiras Gonçalves, Ketyllen Reis Andrade de Carvalho, Paula Eillanny Silva Marinho, Thaís Alkifeles Costa, Betânia Paiva Drumond, Andréa Teixeira de Carvalho, Pedro Augusto Alves.

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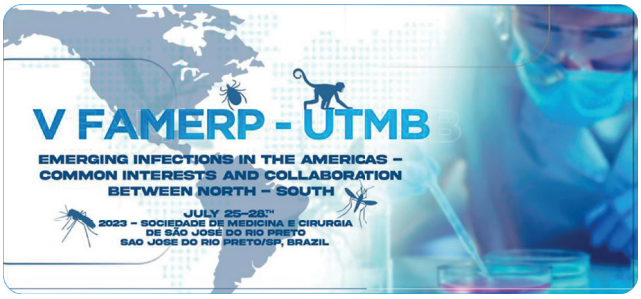
ABSTRACT

Recently (2016-2018), a major Yellow Fever Virus (YFV) outbreak occurred in Brazil causing almost 750 deaths, with 997 cases. This study aimed to perform a molecular and biological characterization of the silvatic YFV circulating during the 2018 outbreak. For that, a serum sample collected from a Yellow Fever acute phase patient was used for viral isolation. The isolated sample presented plaque-size variants and required purification, that was performed through limiting dilution and plaque purification. Small (B2) and large (P3) plaque-size variants were obtained, and then characterized through sequencing of the envelope (E) gene, by Sanger method. An A-G mutation was detected at the 451nt position of the E gene of the B2 variant, which resulted in an isoleucine-valine substitution. A nextgeneration sequencing was then performed, using a pool of primers that cover the entire YFV genome, to confirm the mutation previously detected and to check the variants in the whole sequence. After alignment it was possible to confirm the same mutation at the E gene and to detect two other mutations in the NS4b gene of the B2 variant (an A-C mutation that resulted in an asparagine- threonine substitution and an A-G mutation that resulted in a lysineglutamate substitution). Growth kinetics of both B2 and P3 variants were performed in Vero, HepG2 and BHK-21 mammalian cells, using the 17DD YFV strain as a control. We performed a multiplication curve (MC) (MOI 0.01), that demonstrated a similar behavior for all variants and strains evaluated. The peak of multiplication occurred 3 days p.i. (in HepG2 and BHK cells) or 4 days p.i. (in Vero cells). In HepG2 cells, no viable viral particles from the B2 were obtained in any of the time-points analyzed. We have done other experiments like biological characterization of the viruses in C6/36 cells and further analyze the genome of the variants, to better understand these wild-type YFV variants.

Financial support: FAPEMIG, CAPES, CNPQ, Instituto René Rachou- Fiocruz Minas,NIH.



LOSS OF IMMUNODOMINANT HLA-A2-RESTRICTED EPITOPES FROM SARS-COV-2

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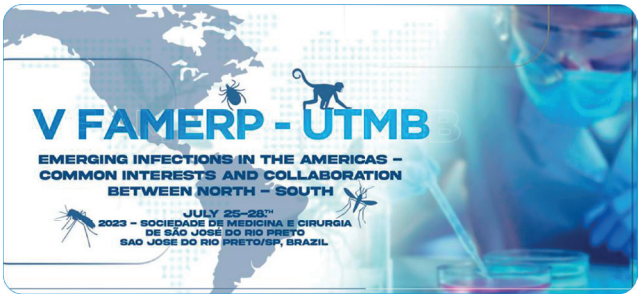
ABSTRACT

The SARS-CoV-2 pandemic represents one of the biggest challenges of modern society. The virus has evolved rapidly generating several variants of concern (VOCs), highlighting the importance of tracking new variants and their immunogenicity. In the present work, coronavirus subfamilies and the main SARS-CoV-2 VOCs comprising alpha, beta, gamma, delta, and omicron were evaluated by the presence of immunodominant epitopes that can bind to human MHC-I using in silico and in vitro epitope mapping tools. Our results show that abundant MHC binders for all coronavirus families were found with significant predilection to HLA-A2, the human MHC-I of higher frequency in the population. However, regardless of the higher number of peptides identified with HLA-A2- restriction, these epitopes displayed the lowest predicted combined scores. HLA-A2-restricted epitopes from the SARS-CoV-2 Wuhan strain have demonstrated the highest score amongst subfamilies of coronavirus, with a progressive decrease in combined score for HLA-A2-restricted epitopes when the original strain was compared to the new variants. This pattern can be interpreted as indicative of viral escape from the immune system. In fact, the epitope signature analysis has revealed major epitope loss for structural (S) and non-structural (ORF1ab) proteins of SARS-CoV-2 variants in comparison to the original Wuhan strain, bringing new insights regarding the antiviral CD8+T cell response against SARS-CoV-2. On the other hand, N epitopes remain as the most conserved and reactive immunodominant viral peptides across SARS-CoV-2 variants of concern. The Omicron subvariants also continue evolving in the same pattern observed for previous variants, with a major loss of reactive epitopes in hotspots of both structural and non-structural viral proteins. The data presented herein can contribute to the development of new vaccinal platforms to induce broad cellular antiviral response, aiming at controlling viral transmission.

Financial support: CAPES, CNPq, FAPEMIG.



THE USE OF COMBINED IN SILICO AND IN VITRO TOOLS FOR THE ASSESSMENT OF ARBOVIRAL MHC CLASS I-RESTRICTED-EPITOPE SIGNATURES REVEAL SITES OF IMMUNODOMINANCE AND POOR OVERLAPPING PATTERNS AMONG MEDICALLY IMPORTANT ARBOVIRUS IN BRAZIL

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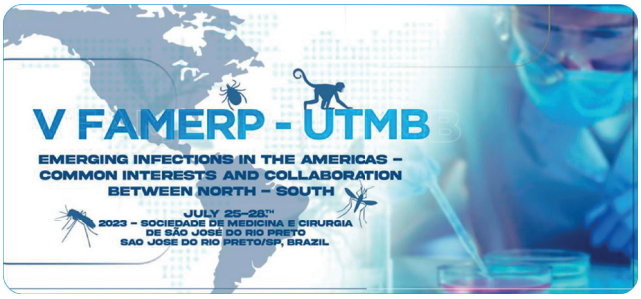
ABSTRACT

The repetitive annual burden of arboviruses in Brazil has placed them as one of the most pressing healthcare problems in the country for the past decades. Considering that acute viral infections triggered by arboviruses induce a rapid activation and expansion of CD8+T cells, the present work sought to identify MHC-I-restricted peptide signatures for arbovirus using in silico and in vitro peptide microarray tools. For that, peptide sequences from 5 arbovirus and 8 different viral serotypes were analyzed, including YFV17DD vaccinal strain. Haplotype HLA-A*02.01 was the dominant human MHC for all arboviruses, with the identification of only three overlapping peptides between two or more flavivirus sequences, suggesting poor overlapping of virus-specific immunogenic peptides amongst arboviruses. Aiming at the assessment of MHC-I-peptide reactivity, a peptide microarray was designed, using concentrations of 1ug/mL, 10ug/mL, and 30ug/mL of a dimeric protein containing HLA-A*02:01 molecules. Global analysis of in vitro reactivity indicated a dose-response effect in the microarray with an elevated number of DENVreactive peptides. Furthermore, a lower number of arbovirus/YFV-17DD overlapping peptides presented reactivity when compared to non-overlapping peptides. In addition, the assessment of HLA-A*02:-01-reactive peptides across virus polyproteins highlighted nonstructural proteins as "hot-spots" for CD8+ T cell epitopes. Data analysis supported these findings, indicating major hydrophobic sites in the final segment of non-structural protein 1 throughout 2a (Ns2a) and in non-structural proteins 2b (Ns2b), 4a (Ns4a) and 4b (Ns4b). To our knowledge, these results provide the most comprehensive and detailed snapshot of the immunodominant peptide signature for arbovirus with MHC-class I restriction, which may bring insight into the design of future virus-specific vaccines to arboviruses and for vaccination protocols in highly endemic areas.

Financial support: CAPES, CNPq, FAPEMIG.



MORPHOLOGIC, GENETIC, AND PHYLOGENETIC CHARACTERIZATION OF BUSSUQUARA VIRUS, AN UNDERSTUDIED MOSQUITO-BORNE FLAVIVIRUS

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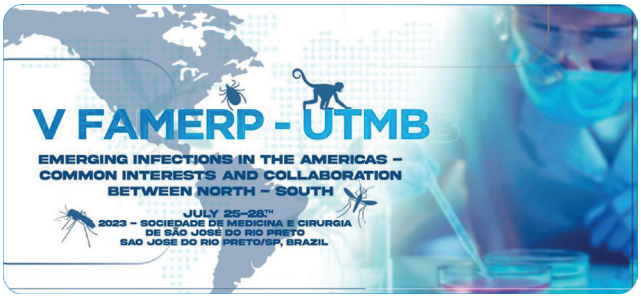
ABSTRACT

Bussuquara virus (BSQV) is amosquito-borne flavivirus that is circulating throughout Central and South America and the Caribbean. It is a relatively unknown member of the understudied Aroaserocomplexwithin the familyFlaviviridae.BSQV has beendetected in a wide range of species including mosquitoes (primarily Culexspp), small and large mammals, and birds. There is yet only one study that reported successful viral isolation and detection of BSQV-specific antibodies in humans. This study aims to describe the initial morphologic, genomic, phylogenetic and infectivity relationships of the four known BSQV strains available for use. Viral stocks were expanded before viral RNA extraction with chloroform was performed for next generation sequencing. Phylogenetic analysis was inferred based on a dataset ofopen reading framesthat included all representative species in the Flaviviridae family. Transmission electron microscopy (TEM) images were produced in both Vero CCL81 and C6/36 cell lines. Replication kinetics studies were performed with inoculation (MOI=0.01) of 3 BSQV strains on cell lines of mosquito and mammalian origins (C6/36, Vero CLL81, BHK, OK, Huh7).Phylogeny revealed that all four BSQV strains clustered within the Aroaserocomplex. However, one of four strains was discovered to be more genetically related to Naranjal virus, and itwas removed from further analysis. The virion size was roughly 40nm in diameter and in line with flaviviruses.Replication kinetics showed a similar peak of viral load between the various cell lines on days 4-5 post-infection, with minimal cytopathic effect in C6/36. but complete destruction of the monolayer in the four mammalian cell lines. Ourdata suggest that BSQV poses a threat to humans as a generalist arbovirus with multiple mammalian hosts and mosquito vectors. Additional replication kinetics need to be performed in cell lines of other mosquito genera (Culex spp) and birds to better understand the potential transmission cycle in endemic regions.

Key words: Bussuquara virus, arbovirus, TEM, phylogeny, in vitro characterization

Financial support: The study is partly funded by the Centers for Research in Emerging Infectious Diseases [CREID], "The Coordinating Research on Emerging Arboviral Threats Encompassing the Neotropics [CREATE-NEO]" grant U01 AI151807 by the National Institutes of Health [NIH]. The funders had no role in the design of the study, collection, analyses, or interpretation of data, writing of the manuscript, or in the decision to publish the results



EVALUATION OF SARS-COV-2-SPECIFIC T LYMPHOCYTES IN DENGUE--INFECTED CHILDREN

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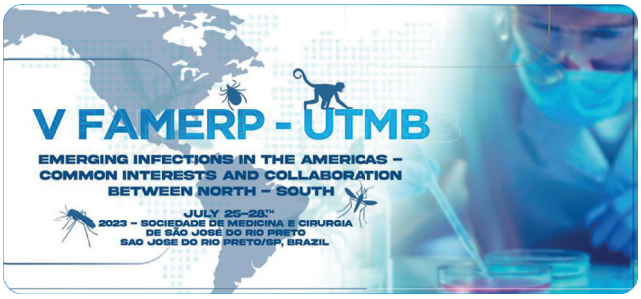
ABSTRACT

The dengue virus is a flavivirus that causes a disease that ranges from mild to severe conditions that can lead to death. The disease has three stages of development, febrile, defervescence and convalescence. During each of them different cytokines are produced both for the effective combat of the disease and for the control of the inflammatory response. The balance between the pro-inflammatory response and the anti-inflammatory response results in asymptomatic/mild diseases, on the other hand, the exacerbation of the pro-inflammatory response can result in immunopathologies. Previous infections can promote protection or worsening of the disease through cross-reactivity, as observed in patients infected with SARS-CoV-2 who had already had contact with other coronaviruses. Therefore, T lymphocytes may play an important role in eliminating infection and controlling inflammation through cross-reactivity. This work aims to verify the presence of T cells with cross-reactivity for SARS-COV-2 in children infected by dengue. PBMC's of children (1-13 years) collected of the years 2013-2014 were used. PBMC's were thawed and cultivated in the presence of the DENV1 Envelope peptide library (ENV D1) or SARS-CoV-2 Nucleocapsid for 14 hours. After the incubation period, the cells were marked for flow cytometry. A decrease in the frequency of T cells, CD4 and CD8, producing TNF and CD8 producing IL10 specific to ENV D1, was observed during the febrile phase of dengue fever. There was an increase in the frequency of CD4 cells producing IL4/IL13 specific to ENV D1 in defervescence. During the convalescence period, an increase in the frequency of IL10-producing CD8 cells specific to ENV D1 was found. There was no difference in the frequency of T cells producing IFNg, TNF, IL10, IL17a, IL4 and IL13 in the presence of the Nucleocapsid. Therefore, we conclude that during infection or exposure to the dengue virus, it does not generate T lymphocytes with cross-reactivity capacity to SARS-CoV-2.

Financial support: CNPq.



SYNTHESIS AND USE OF ZIKA VIRUS CHIMERIC PROTEINS TO ELISA PLATFORMS

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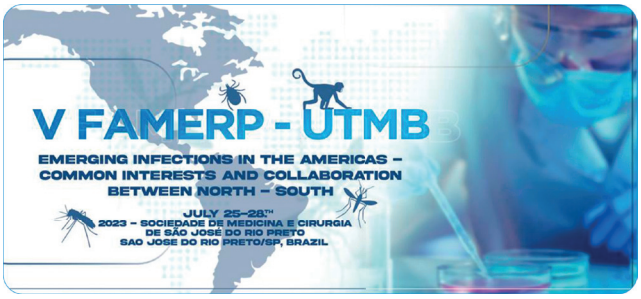
ABSTRACT

Arboviruses constitute a group of viruses transmitted primarily by mosquitoes. They represent a major public health problem worldwide. The emergence of Zika in Brazil (2015) drew attention due to the severe neurological manifestations, congenital complications, and Guillain-Barré syndrome observed in some individuals despite its acute and self-limiting nature. . The great antigenic similarity between flaviviruses makes diagnosis difficult, creating a pressing need for the developmentof new diagnostic tools. Therefore, this work aimed at the generation of a chimeric proteins capable of differentiate ZIKV or DENV infections in serological samples. The recombinant proteins (ZIKV-1, ZIKV-2, ZIKV-3) were designed, and their respective genes, subcloned into a pET21a expression vector. The recombinant proteins were expressed and purified and antigenicity was validated. Purified proteins were tested as solid-phase antigens in standard ELISA protocols for the detection of anti-ZIKV IgG antibodies. The results obtained after standardization are promising, and the tests elaborated with the recombinant antigens showed high sensitivity and specificity, in addition to low crossreactivity with the interferent. The ZIKV-1 protein showed 91% sensitivity and 97% specificity, the ZIKV-2 protein showed 95% sensitivity and 96% specificity, and the ZIKV-3 protein showed 66% sensitivity and 84% specificity. Regarding the DENV interferent, there was 10% cross-reactivity for ZIKV-1 and 19% for ZIKV-3, ZIKV-2 did not show cross-reactivity. The assays were validated at the Laboratório de Pesquisa em Virologia (FAMERP), with similar results, confirming the reproducibility and robustness. The results obtained for ZIKV-1 and ZIKV-2 show great potential for the development of a specific diagnostic test for detecting IgG antibodies to ZIKV, using the produced chimeric proteins.

Financial support: UFMG, CAPES, CNPQ and FAPEMIG.



STUDY OF COINFECTION WITH DISTINCT LINEAGES OF SARS-COV-2 IN THE MUNICIPALITY OF SÃO PAULO

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ABSTRACT

Introduction: With the spread of the coronavirus, the emergence of mutations in the genome of SARS-CoV-2 can alter its pathogenic potential, and these mutations can combine with several others, giving rise to different variants of the virus. These recombinations are more common within the gene that encodes the spike protein. According to the World Health Organization, the variants are classified as variants under monitoring (VUM), variants of interest (VOI), and variants of concern (VOC). Among the VOCs are B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma), B.1.617.2 (Delta), and B.1.1.529 (Omicron). Considering the transition in the occurrence of these variants in the population, recent studies have started to identify cases of coinfection with distinct lineages of SARS-CoV-2, leading to reflections on issues such as virus transmissibility, the frequency at which these coinfections can occur, and possible recombination events. Objective: To identify the occurrence of these coinfections and their frequency in the municipality of São Paulo, as well as to seek the standardization of protocols capable of detecting these events and ultimately analyze possible recombination events. Methods: One possible approach to analyze coinfection would be through the digital PCR method (RT-dPCR), which will be tested in this project. Subsequently, the samples will be sequenced using nanopore technology (ONT - Oxford Nanopore Technologies) to confirm the occurrence of coinfection and analyze possible recombination events that contribute to the understanding of the phenomena leading to the emergence of new lineages. (National Commission of Research Ethics - protocol number CAAE 30127020.0.0000.0068). Preliminary results: A total of 6,097 samples obtained from the Health Department of the Municipality of São Paulo were tested using RT-qPCR, and from this test, we selected 277 samples positive for coinfection with distinct lineages of SARS-CoV-2. From these selected samples, we conducted a test on 16 samples using digital PCR, where 7 samples indeed showed a positive result for coinfection. These results will be compared with others obtained through sequencing, where we are testing a methodology that can effectively detect coinfection with distinct lineages of the same virus. In addition, we tested different dilutions for the application of the sample in RT-dPCR, as it is a sensitive method capable of absolute quantification of the target under study. Conclusion: The preliminary results of this study highlight the occurrence of coinfections with distinct lineages of SARS-CoV-2 in the municipality of São Paulo. The use of methods such as digital PCR (RT--dPCR) and sequencing by nanopore technology (ONT- Oxford Nanopore Technologies) allows for the confirmation of detecting these coinfections, as well as the analysis of possible recombination events. The findings from this study will be relevant to understanding the dynamics of the virus, particularly in terms of transmissibility and genetic evolution. The knowledge gained from this study can be applied not only to SARSCoV-2 but also to other emerging viruses with epidemic and/or pandemic potential.

Keywords: SARS-CoV-2. Coinfection. RT-dPCR. Nanopore

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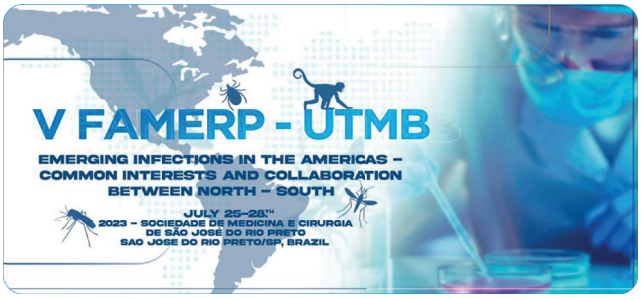

REGULATION MECHANISMS OF THE IMMUNE RESPONSE IN HUMAN DENGUE

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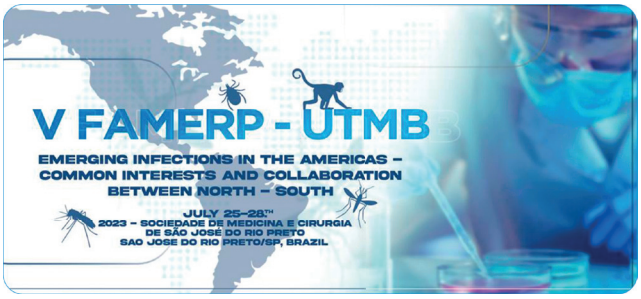
ABSTRACT

Dengue is a viral infection that can be classified into mild dengue, dengue with warning signs (sa+) and severe dengue (sa+/severe). Severe dengue manifestations result from exacerbated activation of the immune response. The importance of regulatory mechanisms promoted by anti-inflammatory molecules and cytokines of multifunctional T cells and regulatory T cells (Tregs) in controlling inflammatory responses is evident in many infectious diseases. Our objective was to investigate the presence of these mechanisms in different clinical forms of dengue infection. PBMCs from dengue patients were cultured with the peptide library of the viral envelope proteins (ENV) or NS3 of DENV1. Patients with mild dengue had higher plasma levels of IFNγ, TNF and IL12p70 when compared to the sa+/severe. The frequencies of DENV-specific CD4 or CD8 T cell producers single or double (TNF or IL10 or IFNγ/TNF or IFNγ/IL10) were higher in mild dengue than in sa+/severe. Patients with mild dengue had higher frequencies of NS3- specific T cells triple producing IFNγ, TNF, and IL10 than sa+/severe. Among the regulatory molecules evaluated, patients with dengue increase the frequencies of Teff GITR+ or LAP+ or PD1+ cells, and Tregs CD226+ and CTLA4+ cells. Patients with mild dengue showed high frequencies of Tregs CD200+ and, importantly, high frequencies of Tregs GITR+ that produce IL10 specific to DENV. Furthermore, using epitope mapping of the DENV1 ENV peptide library, we identify peptides associated with IL10 production by Tregs. The clinical evolution of dengue seems to be related to regulatory mechanisms of the immune response. Tregs from individuals with sa+/severe dengue showed an important deficiency in activation markers, suggesting a dysfunctional phenotype, and the production of IL10 by multifunctional T cells and by Tregs GITR+ cells is associated with the clinical presentation of mild dengue, suggesting that these regulatory mechanisms are important to limit dengue immunopathology.

Financial support: CNPq, FAPEMIG, INCT-Vacinas, the Program for Technological Development in Tools for Health–PDTIS-FIOCRUZ (Flow Cytometry Platform) and Flow Cytometry core facility of the Pharmacy School of UFMF.



PRESENCE OF DIFFERENT RESPIRATORY VIRUSES AMONG PATIENTS WITH ACUTE RESPIRATORY DISEASE DURING THE COVID-19 PANDEMIC IN THE SOUTHEAST REGION OF BRAZIL

Marissa da Silva Lima[1,2] , Maria Cássia Jacintho Mendes Pereira[1,2], Ester Cerdeira Sabino[1,2] , Marina Farrel Cortês[1,2] , Silvia Figueiredo Costa[1,2] , Alessandra Luna[1] , Heuder Paião[1,2], Bianca Silva Costa[1,2], Amanda Miyuki Hidifira[1,2] Pablo Andres Munoz Torres1

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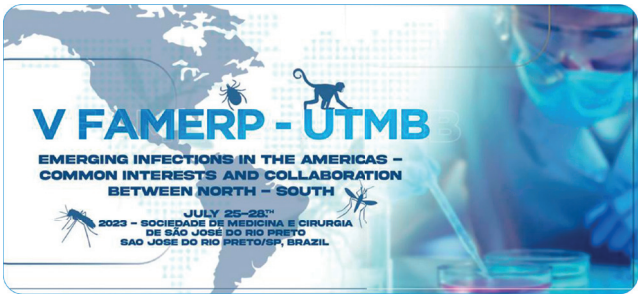
ABSTRACT

Background: Other respiratory diseases can affect the clinical management, progression and outcomes of patients with Covid-19. During the pandemic, investigation of other viral agents and co-infection was limited due to the priority of controlling the circulation of SARS-CoV-2. With the weakening of sanitary restrictions, circulation of seasonal respiratory viruses is expected to resume, offering the opportunity to map their interactions in severe respiratory illnesses. Methods: This is an observational, crosssectional study to be carried out on samples of patients with acute respiratory symptoms, collected between March 2020 and November 2021, treated at health services in the cities of São Caetano do Sul and São Paulo, in a convenience sample. Samples included in the study were tested for the presence of SARS-CoV-2. For the investigation of respiratory viruses, 613 samples were evaluated using the commercial platform Kit XGEN MULTI PR21 and 365 samples were tested using the Filmarray BioFire System. Results: Among the 978 patients in this study, the median age of the patients was 45 years; 60.32% identified as female, 39.67% identified as male. Of these, 539 (55%) patients tested positive for SARS-CoV-2 and 181 (18.5%) were positive for one or more additional pathogens. Twelve different respiratory viruses was detected among the 181 investigation of respiratory viruses, and 61 of those specimens tested positive for more than one additional respiratory virus. The most common pathogens include rhinovirus/enterovirus (n= 80, 44.19%). Interestingly, there were (n=23, 2.35%) of SARS-CoV-2 coinfections with others respiratory viruses. Conclusion: Our findings are likely to lead to further investigations of SARS-CoV-2-associated infections, and these viral factors may prompt public health officials to improve seasonal respiratory pathogen surveillance practices and address the risk of disease severity.
Keywords: Acute respiratory syndrome, Covid-19, viral painel.

Financial support: Centro Brasil-Reino Unido para Descoberta, Diagnóstico, Genômica e Epidemiologia de Arbovírus-CADDE



SURFACE FIBRILS ORGANIZATION AS SPECIFIC TRADEMARKS OF DIFFERENT MIMIVIRUS LINEAGES

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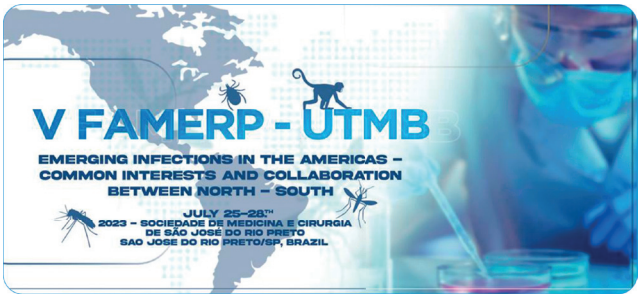
ABSTRACT

Among the most intriguing structural features in the known virosphere, mimivirus surface fibrils fuel debates on evolutionary traits of viral gigantism. Fibrils are approximately 150 nmlong highly glycosylated proteinaceous filaments, covering the mimivirus capsid surface, which expand the viral particle up to 750 nm diameter and promote particle adhesion to host cell. Although mimiviruses are abundant in a plethora of worldwide biomes, there is no comparative analysis on fibrils organization and abundance among distinct mimiviruses lineages. Here, by combining a set of methods and analyses, including electron microscopies, image processing and genomic sequencing, we describe, for the first time to our knowledge, lineage-dependent profiles of fibrils organization among mimiviruses. Microscopical analyses of 15 mimiviruses revealed at least three profiles of isolates based on fibrils organization: i) those with abundant fibrils, distributed homogeneously on the capsid surface, as previously described; ii) isolates with particles almost fiber-less; and iii) isolates with particles containing fibrils in abundance, but organized as clumps. Hallmark genes sequencing revealed that those well-defined fibrils organization profiles correspond to evolutionary-related viruses, belonging to lineages A, B and C, respectively. To demonstrate that fibrils appearance is not due microscopy artifacts, isolates were analyzed concomitantly in pairs or trios, reinforcing the aforementioned diversity on fibrils organization. The analyses of the most abundant protein described in fibrils, the GMCoxidoreductase, revealed lineage B (almost fiber-less) as the most divergent among mimiviruses. At last, virological assays demonstrated that lineage A mimiviruses are more efficient to trigger phagocytosis in amoebas then lineage B and C mimiviruses. Taken together, our data suggest a notable diversification on mimiviruses fibrils organization after lineages radiation.

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



SEROLOGICAL EVIDENCE OF CONTINUED CIRCULATION OF YELLOW FEVER VIRUS IN CALLITHRIX PENICILLATA IN MINAS GERAIS STATE

Matheus Soares Arruda [1,5] , Thaís Alkifeles Costa[1,5] , Daniel Jacob da Circuncisão[1], Mikaelly Frasson Testa[2], Ana Maria de Oliveira Paschoal[2], Daniel Ambrozio da Rocha Vilela[2], Nikos Vasilakis[3,5] , Kathryn A. Hanley[4,5] , Betânia Paiva Drumond[1,5]

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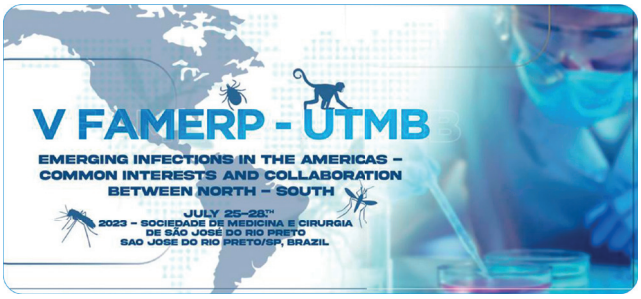
ABSTRACT

Yellow fever virus (YFV), an arbovirus maintained in Brazil in a sylvatic, enzootic cycle involving Haemagogus and Sabethes spp mosquitoes and non-human primates (NHP) Human infections occur via spillover from the sylvatic cycle in forested areas where hosts and vectors co-occur; such infection can result in yellow fever (YF), an acute febrile illness that can lead to death. Between 2017 and 2018, a massive YF outbreak took place in Minas Gerais (MG). Since 2020 YF epizootics have been confirmed in the state, and in 2023 one case of human infection was confirmed revealing continued virus circulation. The aim of this study is to quantify YFV in 90 specimens of Callithrix sp. in urban or peri-urban areas of Belo Horizonte MG. In this work, we will use serum samples from free-living NHP sent to the Wild Animal Screening Center (CETAS/BH) and from NHP captured in parks or other green urban areas of BH (permits SISBIO 77400, CEUA 98/2017). Morphometric measurements and teeth characteristics were used for age estimation. To date, seven Callithrix penicillata from MG, including urban areas of the Metropolitan region of Belo Horizonte, four of which were < 1.5 years and three of which were ≥ 1.5 years old, have been obtained for this study. Serum from each animal was tested by plaque reduction neutralization test (PRNT) against YFV. Immunochromatography tests (IgM and IgG) for YFV (EcoDiagnóstica) were also used. Sera were diluted 1:20 up to 1:80 and used in a PRNT test. Three samples presented plaque reduction in the dilution 1:20 indicating virus neutralization ranging from 57% - 67% and were positive for IgM against YFV (two young and one adult NHP). The detection of IgM antibodies in NHP, including young ones, indicates the continued circulation of YFV in NHP in MG, including the Metropolitan region of Belo Horizonte. These results reinforce the need for studies to understand the host-vector networks that could be related to YFV transmission in urban settings in Brazil.

Financial support: CREID/NIAID/NIH - U01 AI151807; CAPES

BIOMARKER SIGNATURE IN PATIENTS WITH ARTHRALGIA DURING



ACUTE CHIKUNGUNYA VIRUS INFECTION: IFN-G, IL-6 AND IL-10 AS UNIVERSAL HALLMARKS OF DISEASE OUTCOME

Caio Wilker Teixeira[1,2]; Jonai Pacheco Dia[3] ; Lizandra Morgado-Santos[2] ; Sarah Giarola-Silva[2] ; Ismael Artur da Costa-Rocha[2] ; Ana Carolina Campi-Azevedo[2] ; Andréa Teixeira-Carvalho[2] ; Sheila Maria Barbosa de Lima[2] ; Adriana de Souza Azevedo[2] ; Olindo Assis Martins-Filho[2] *; Josélio Maria Galvão de Araujo[3]; Jordana Grazziela Alves Coelho-dos-Reis [1,2]

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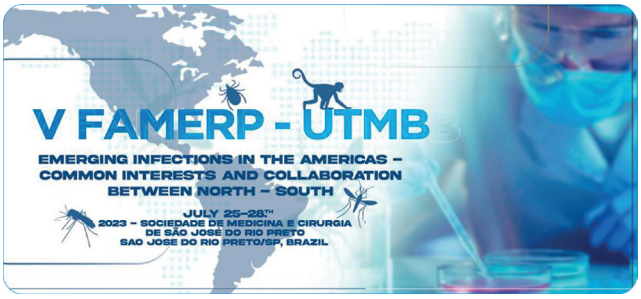
ABSTRACT

Chikungunya fever is currently an eminent public health threat in Brazil. Chikungunya virus (CHIKV) is capable of causing a viral disease characterized by fever and severe joint pain. Arthralgia after several months of infection is a unique feature of this viral infection that is associated with the interaction between the virus and the host. CHIKV induces a severe systemic and local inflammatory response in joints even in the absence of viremia, and the mechanisms and molecules associated with this tissue damage and arthralgia are still unclear in humans. Therefore, the present study aimed to evaluate serum biomarkers in serum during acute Chikungunya virus infection according to age subgroups and days of infection and their association with arthralgia. For this, patients with Chikungunya fever from the state of Rio Grande do Norte, where it has caused recurrent outbreaks, were investigated. A total of 76 patients (25 men and 51 women) testing positive for CHIKV by RT-qPCR were included in the study and serum samples were collected within the first few days of symptom onset. The highest viral load was found on the first day of infection. Healthy controls matched for age and sex (n=161) were also included in the study. The Luminex assay was performed for the detection of chemokines, pro-inflammatory cytokines, regulatory cytokines and growth factors. Our results demonstrated a robust cytokine storm composed of pro-inflammatory response with absence of the regulatory cytokine axis. IFN-g, IL-6 and IL-10 were universal hallmarks of chikungunya fever. Analysis of system biology reveals unique patterns of CHIKV infection that may reveal important biomarkers of arthralgia. With that said, these observations suggest distinct signatures of CHIKV patients that may be essential for establishing putative targets for immunomodulatory therapies.

Financial support: CNPq; CAPES; IRR-FIOCRUZ/MG; UFMG.



A PEPTIDE FORMULATION BASED ON ANNEXIN A1 POTENTIATES ITS ANTIINFLAMMATORY AND PRO-RESOLVING EFFECTS AGAINST DENGUE VIRUS-INDUCED PATHOLOGY

Martins, J. R.[1] ; Batista, V. L.[2] ; Santos, A. L. C.[3] ; Fonseca, T. C M.[4] ; Queiroz-Junior, C. M.[1] ; Guimarães, P. P. G.[3] ; Teixeira, M. M.[5] ; Costa, V. V.[1]

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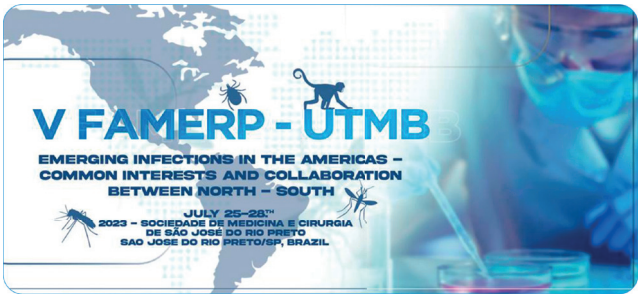
ABSTRACT

Severe dengue (DG) is characterized by excessive inflammation, characterized by a "cytokine storm", causing vascular leakage, hemorrhage, and hypovolemic shock. Innate immune cells and their products, such as neutrophils, mast cells, and macrophages, contribute to these inflammatory processes by releasing TNF, PAF, and MCPT-1. Currently, there are no approved drugs targeting host molecules for dengue, emphasizing the urgent need for drug development or repurposing. Our research focuses on Annexin A1 (AnxA1), a protein involved in the resolution of inflammation, which is mediated by FPR2/ALX receptor engagement expressed on various cells. We found that AnxA1 knockout (AnxA1KO) mice are more susceptible to and experience delayed resolution of DG. Conversely, treatment of mice with the AnxA1 mimetic peptide (Ac2-26) improves disease outcomes without compromising the host's ability to control infection. However, systemic administration of proteins like AnxA1 is challenging due to their rapid degradation. To address this issue, we developed a cyclodextrin (CDX) formulation to enhance stability, bioavailability, and in vivo effects of Ac2-26. In vitro studies using Vero CCL-81 cells treated with different concentrations of CDX (HP-BCD), either infected or not with DENV-2, demonstrated preserved cell viability and absent antiviral effect. A129 were infected with DENV-2, received intraperitoneal treatment of pure or formulated Ac2-26, B.I.D and were euthanized at 3dpi. Administration of pure or formulated Ac2-26 reversed clinical scores and thrombocytopenia induced by DENV infection. Meanwhile, only the formulated Ac2-26 reduced plasma levels of MCPT-1 and spleen levels of CXCL1, INFy, CCL2, and CCL5. It also mitigated mast cell degranulation and inflammatory infiltrate in the liver upon DENV-2 infection. Overall, peptide formulation based on AnxA1 potentiates its anti-inflammatory and pro-resolving effects likely due to improved stability and bioavailability in the host.

Financial support: CNPq, CAPES, FAPEMIG, FINEP, INCTdengue and Host Microorganism Interaction. Para mulheres na Ciência, L'Oreal e Unesco.



MOLECULAR INVESTIGATION OF CHIKUNGUNYA VIRUS IN NON-HUMAN PRIMATES IN THE STATE OF MINAS GERAIS IN 2017

Thais Alkifeles Costa[1] , Matheus Soares Arruda[1] , Gabriela Fernanda Garcia Oliveira[1] , Gabriel Dias Moreira[1] , Érica Munhoz de Mello[2], Nikos Vasilakis[3] , Kathryn Hanley4 , Betânia Paiva Drumond[1]

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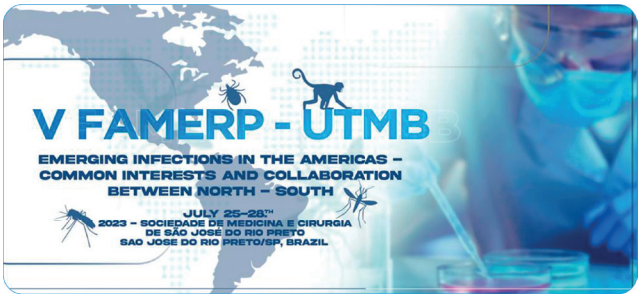
ABSTRACT

Chikungunya virus (CHIKV) was introduced in Brazil in 2013, and has caused recurrent outbreaks in the country ever since. Although CHIKV is transmitted by Aedes aegypti to humans in urban centers, there is a risk of the establishment of a sylvatic enzootic cycle maintained in non-human primates (NHP) and competent vectors in urban and peri-urban areas, like Aedes albopictus. The objective of this study was to investigate the presence of CHIKV RNA in liver samples from NHP carcasses collected in Minas Gerais state in 2017. Carcasses of NHP were sent to the Zoonoses Laboratory of Belo Horizonte by the Yellow Fever (YF) Surveillance Program and liver samples were forwarded to Laboratório de Vírus/UFMG. Out of the 184 samples tested, 66 were from rural areas, 101 from urban areas, and 17 from peri-urban areas. Of the NHP genera sampled from different parts of Minas Gerais state, 135 were Callithrix, 25 Alouatta, 23 Callicebus and 1 Sapajus. The samples were subject to total RNA extraction and RT-PCR with primers targeting a 167nt long amplicon in the CHIKV E gene. Host β-actin gene was used as endogenous control in RT-PCR. All samples were positive for the presence of control gene and no CHIKV RNA was detected in the samples tested. While it is possible that CHIKV RNA detection may have been undermined by the short viremia in CHIKV infection and the degradation of RNA in samples from carcasses. This same sample set yielded 117 samples positive for YFV, which has a similarly short viremia and RNA genome. The molecular investigation is crucial for understanding the dynamics of viral circulation, especially given the current epidemiological and outbreak situation of CHIKV in Brazil and neighboring countries, with a high number of cases of the disease in urban centers. Despite the absence of CHIKV RNA in the animals studied here, other researchers have detected serological evidence of CHIKV in NHP in Brazil, reinforcing the relevance of surveillance in these hosts.

Financial support: CREID/NIAID/NIH - U01 AI151807; CAPES



MOLECULAR AND SEROLOGICAL INVESTIGATION OF ARBOVIRUSES IN SMALL WILD MAMMALS FROM A SYLVATIC-URBAN AREA OF BELO HORIZONTE, MINAS GERAIS

Ana Gabriella Stoffella Dutra[1]; Pedro Henrique Bastos e Silva[1]; Karolina Lopes Dias[1]; Gabriela Ribeiro[1]; Ana Luiza Campos Cruz[1]; Bruna Hermine de Campos[2] ; Nadja Simbera Hemetrio[3] ; Lara Ribeiro de Almeida[4] ; Betânia Paiva Drumond[1]; Marcelo Pires Nogueira de Carvalho[2] ; Adriano Pereira Paglia[5] ; Giliane de Souza Trindade[1]

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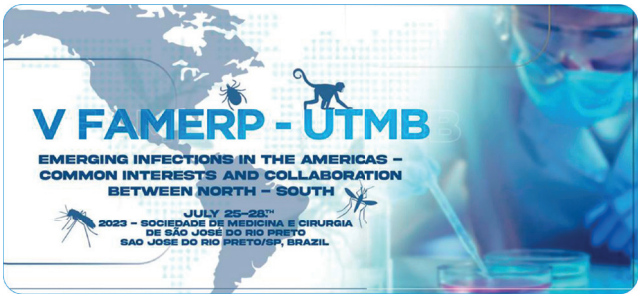
ABSTRACT

Emerging infectious diseases (EID) represent a worldwide threat. In this context, 70% of pathogen outbreaks are linked with a sylvatic origin, with viruses being the second largest cause of EID. In Brazil, the overlap of EID has a major impact on public health, as an example of arboviruses. Considering the Brazilian epidemiological context, YFV, DENV, ZIKV, and CHIKV viruses are responsible for significant epidemic events. The aim of this work is to investigate arboviruses circulation in small mammals present in urban parks of Belo Horizonte, MG. The collection of mammals was approved by Ethics Committee and was carried out in two urban parks, in 2018, 2019 and 2021. Were collected biological samples from rodents (n=125), marsupials (n=3), and coatis (n=40). For molecular investigation, liver and kidney samples (n=256) from rodents and marsupials, and swabs (oral/anal) from coatis (n=40) were submitted to nucleic acid extraction using the RNeasy Mini Kit and QIAmp Viral RNA Mini Kit (QIAGEN). The obtained total RNA samples were submitted to RT-qPCR using specific primers and probes to detect each viral species. For endogenous control, a qPCR for the β-actin gene was also performed. Considering all samples analyzed, no evidence of arboviruses RNA was found. Since the epidemics of sylvatic YF (2016-2019) that affected mainly southeast Brazil, the YFV surveillance is significant, especially in these parks that confirmed epizootic cases in recent years. Thus, to access the possible pre-exposition of coatis to YFV, serum samples (n=40) were submitted to Plaque Reduction Neutralization Test (PRNT). So far, our results, show no evidence of anti-YFV neutralizing antibodies, however, some samples are still being tested. The arboviruses investigation in small mammal species contributes to better explaining the circulation dynamics of these viruses in Brazil. Also, the possible occurrence of enzootic cycles near to sylvatic-urban areas points to the risk of new emergency events. Keywords: Arboviruses, Emerging infectious diseases, urban parks, molecular investigation, serological investigation, small mammals.

Financial support: CNPq, FAPEMIG, CAPES



ANALYSIS OF LABORATORY PARAMETERS OF PATIENTS WITH COVID-19

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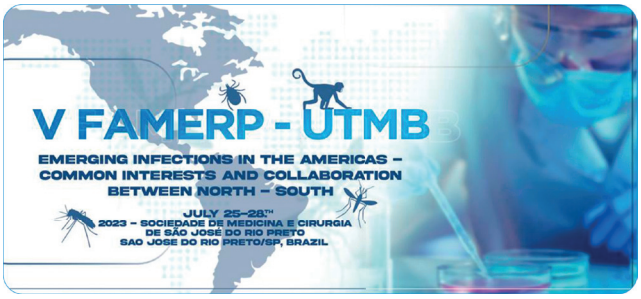
ABSTRACT

The pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) is an emerging crisis affecting the public health system. Some hematological and biochemical parameters can help in predicting the clinical outcome of patients with COVID-19. Therefore, the objective of this study was to analyze hematological and biochemical parameters of patients diagnosed with COVID-19 at Risoleta Tolentino Neves Hospital in Belo Horizonte, Minas Gerais. Eighty-one patients with a diagnosis of SARS-CoV-2 infection confirmed by RT-PCR were included in the study. Biochemical and hematological dosages were collected at three times (0, 7 and 14 days of hospitalization). The GraphPad Prism 8.0 software (GraphPad Software Inc.) was used for statistical analysis of the data for comparison between groups. Among the parameters evaluated, those that presented performance greater than or equal to 70% were considered as possible biomarkers to define severity of the patient with COVID-19. They are: global leukocyte, neutrophil, sodium, chloride, lactate, and urea. These parameters had their values increased in critically ill patients mainly at the third collection time, especially urea, which showed a performance of 84% in distinguishing discharge and death. These results indicate the importance of laboratory monitoring allied to the clinical aspects of patients with COVID-19.

Financial support: CAPES, CNPq and FAPEMIG.



VIRAL ETIOLOGIES OF ACUTE UNDIFFERENTIATED FEBRILE ILLNESS AT TERTIARY CARE HOSPITAL IN SÃO JOSÉ DO RIO PRETO, BRAZIL

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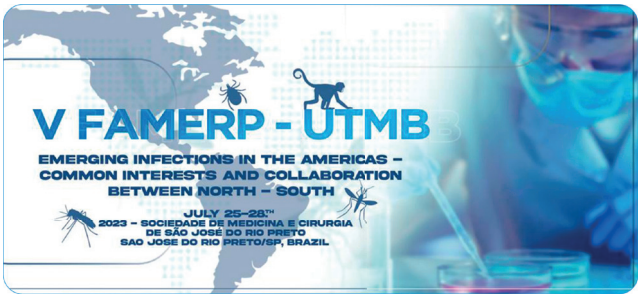
ABSTRACT

Acute undifferentiated febrile illness (AUFI) is caused by several pathogens, mostly viruses, with significant morbidity and mortality in low- and middleincome countries, particularly children. Fever can be fatal if the etiology is not confirmed and appropriately treated. Causes of AUFI often remain undetermined due to the overlap of symptoms and limited available diagnostics. Oral fluid sampling might be a suitable, non-invasive, easy-to-use alternative for the diagnosis of AUFI infections. The present study aimed to assess the etiology of AUFI in children ≤ 15 years old in a tertiary care hospital in São José do Rio Preto, São Paulo, Brazil. The observational prospective study is being conducted from December/2021. So far (May/2023), a total of 220 oropharyngeal swab samples were screened for enterovirus (EV), adenovirus (AdV), and SARS-CoV-2 using RT-qPCR. Of the 220 patients tested, ages ranged from 0 to 14 years old, being 145 (66%) children ≤ 5 years old, reinforcing that AUFI is an important pathogen in childhood diarrhea. Of this total, 57 (39%) were males and 88 (61%) were females. The etiology of AUFI was identified for 17% (38/220) of the patients enrolled in the study. EV was the most prevalent agent (10%, 23/220), followed by AdV (5%, 9/220) and SARS-CoV-2 (2%, 6/220). Co-infections were also identified (1.4%, 3/220), including EV/AdV (0.9%, 2/220) and AdV/SARS-CoV-2 (0.4%, 1/220). Data obtained here indicate that oral fluid samples are a suitable public health alternative for AUFI diagnosis, potentially lowering the barriers for sampling. It is important to note that the etiologies in the majority (83%, 182/220) of AUFI remained unknown, suggesting that an expanded viral screening panel could be applied. Enhanced understanding of common causes of AUFI in São José do Rio Preto will provide appropriate treatment for the patients and might assist in the public health care planning in infectious diseases emergencies.

Financial support: CREATE-NEO (NIH grant 1U01Al151807), FAPESP, NIH.



YELLOW FEVER VIRUS MOLECULAR INVESTIGATION IN NON-HUMAN PRIMATES: FINDINGS FROM A STUDY IN MINAS GERAIS, BRAZIL (2021-2023)

Gabriela Fernanda Garcia Oliveira[1], Gabriel Dias Moreira[1], Anna Catarina Dias Soares Guimarães[1], Thais Alkifeles Costa[1], Matheus Soares Arruda[1], Erica Munhoz de Mello[2], Nikos Vasilakis[3], Kathryn Hanley[4], Betânia Paiva Drumond[1],

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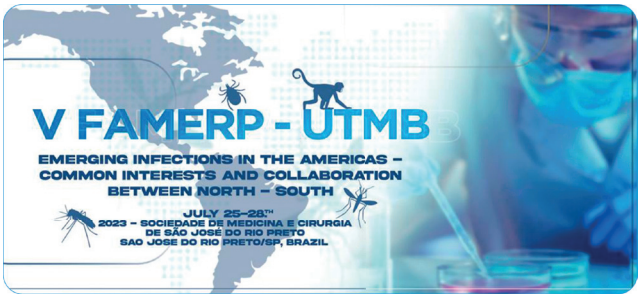
ABSTRACT

Yellow fever virus (YFV; genus Orthoflavivirus, family Flaviviridae) infection causes yellow fever (YF), a disease endemic in Africa and South America. In Brazil, the Amazon Basin is a YFV endemic region where the virus circulates in a sylvatic cycle involving Haemagogus sp. and Sabethes sp. mosquito vectors and non-human primates (NHP) hosts. In 2017 and 2018, Brazil faced a major YF outbreak with over 1500 human and 2000 epizootic confirmed cases, mainly in Minas Gerais state (MG); since 2019 human cases and epizootics have continued to be reported, albeit at a much lower frequency. Due to their role as sentinels of YF, investigation of NHP deaths is mandatory. This study aimed to detect YFV infection in NHP samples collected from 2021 to 2023 in MG using an established RT-PCR protocol. A total of 282 liver and lung samples from 141 NHP carcasses collected across different regions of MG were tested. Total RNA was extracted from 20 to 30 mg tissue fragments using the QIAGEN RNeasy mini kit. RT-PCR was performed on the extracted RNA, targeting the β-Actin gene as an endogenous control and a region of the YFV 5'UTR for viral genome detection. The samples had a 12.8% positivity rate (n=18), YFV genome was detected in 10 out of 54 NHPs from 2021, 8 out of 70 NHPs from 2022, and 0 of the 17 NHPs from 2023. They were mainly from the genus Callitrhix sp. (n=16) and collected from six out of 12 mesoregions from MG. Most samples came from urban areas (n=13), with two samples lacking identification and collection site. These results confirm the continued circulation of YFV in NHPs during 2021 and 2022 in urban areas of MG, outside the Brazilian endemic region. Expansion of YFV to this extent has not been observed in the country since the end of urban yellow fever in Brazil in 1942. Furthermore, our data reveal the risk of the re-establishment of the urban cycle and the importance of monitoring NHP for YFV surveillance and outbreak prediction.

Financial support: CREID/NIAID/NIH -U01 AI151807 and CAPES



AMAZON COMPOUNDS AS A SOURCE OF ANTIVIRAL MOLECULES AGAINST THE MAYARO VIRUS

Mikaela dos Santos Marinho[1] ; Igor Andrade Santos[1] ; Natasha Marques Cassani[1] ; Kidney Gomes de Oliveira Neves[2] , Yana Araújo Alcantara[2] , Marcos Batista Machado[2] ; Edinilze Souza Coelho Oliveira[2] ; Ana Carolina Gomes Jardim[1,3] .

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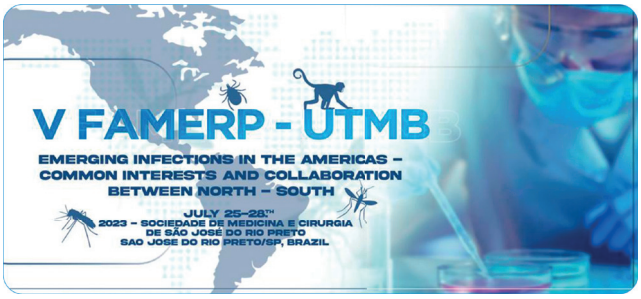
ABSTRACT

Introduction: Mayaro virus (MAYV) is the etiological agent of Mayaro fever, a disease characterized by fever, rashes, myalgia, and arthralgia, that can progress to a chronic condition, resulting in long-term polyarthritis and polyarthralgia. Currently, there are no antiviral drugs for the management of Mayaro fever. In this context, the Amazon Forest demonstrates a potential source of molecules to be investigated as antiviral candidates to combat MAYV. Methods: Vero E6 cells were treated with 23 different Amazon compounds at the highest non-cytotoxic concentrations, previously determined by MTT viability assay, along with the infection with MAYV expressing the nanoluciferase (MAYV-nanoluc) at a multiplicity of infection (MOI) of 0.1. After 24h, the cells were harvested using Renilla-luciferase lysis buffer, and virus replication levels were quantified by measuring nanoluciferase activity through the Renilla-luciferase assay system. Effective concentration of 50 % (EC50) and cytotoxic concentration of 50 % (CC50) were calculated by performing a dose-response curve for compound 7 with concentrations ranging from 3.1 to 400μM in a two-fold serial dilution, with the absence or presence of MAYV-nanoluc infection. Time of drug-addition assay was also performed to investigate the effects of the compound on different stages of MAYV cycle. Results: Among the tested molecules, compounds 7, 20, and 21 inhibited MAYV infection in 49%, 43%, and 36%, respectively. Since the compound 7 presents the best antiviral ratio, EC50 and CC50 were determined, with values of 87 μM and 219.1, respectively, resulting in the selectivity index (SI) of 2.52. The molecule exhibited a virucidal activity, inhibiting the entry, and reducing MAYV infection by over 79%. Conclusion: Compound 7 demonstrates potential as a template for the design of novel antiviral drugs against Mayaro fever, requiring further studies to investigate its mode of action.

Financial support: CAPES, FAPEMIG, CNPq.



REPURPOSING POTENTIAL OF AMINOADAMANTANE DERIVATIVES AGAINST MAYARO AND ZIKA VIRUS

Mikaela dos Santos Marinho[1] ; Natasha Marques Cassani[1] ; Igor Andrade Santos[1] ; Anna Karla dos Santos Pereira[2] , Pedro P. Corbi[2] , Ana Carolina Gomes Jardim[1,3] .

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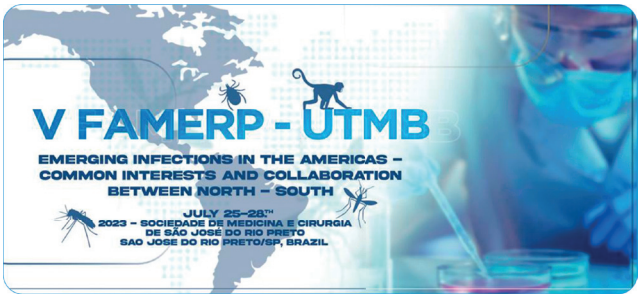
ABSTRACT

Introduction: Arboviruses cause emerging diseases with wide distribution in Brazil, presenting an endemic character in some regions, in addition to originating outbreaks. Among them, Mayaro (MAYV) and Zika virus (ZIKV) have drawn the attention for their ability to cause febrile illnesses, clinical complications, and chronic manifestations. Furthermore, there is no licensed antiviral therapy to treat Mayaro and Zika fevers. In this context, adamantane derivatives exhibit interesting physical-chemical and biological properties, in addition to presenting previously reported inhibitory activity against some viruses. Here we assessed the antiviral potential of the aminoadamantane (1), an adamantane derivative, against MAYV and ZIKV in vitro. Methods: Vero E6 cells were treated with aminoadamantane (1) in a two-fold serial dilution ranging from 2 to 400μM, along with the infection with MAYV expressing nanoluciferase (MAYV-nanoluc) at a multiplicity of infection (MOI) of 0.1 for 24h, or with ZIKVPE243 at an MOI of 0.01 for 72 hours. MAYV replication levels were quantified by measuring nanoluciferase activity through the Renilla-luciferase assay system, and ZIKV titers were evaluated by focus formation units using immunofluorescence assay. Results: Aminoadamantane (1) presented a dose-dependent antiviral activity, with an EC50 of 80.3 μM, CC50 of 194.2 μM, and selectivity index (SI) of 2.42 for ZIKV. As for MAYV, EC50 and CC50 values were 88.5 μM and 291.9 μM, respectively, presenting a SI of 2.3. Conclusion: Our findings demonstrate that the aminoadamantane (1) is an interesting derivative and could be repurposed for the treatment of Mayaro and Zika fever, which may further stimulate its potential as a broad-spectrum antiviral drug.

Financial support: CAPES, FAPEMIG, CNPq.



ANTIVIRAL IN VITRO AND EX VIVO EFFECT OF BROMELAIN COMBINED WITH N-ACETYCYSTEIN: EVIDENCE OF ANTIVIRAL ACTIVITY IN COVID-19 PATIENT TRACHEAL ASPIRATE SAMPLES

Erik Vinicius de Sousa Reis[1] , Linziane Lopes Ferreira[1] , Felipe Alves Clarindo[1] , Geovane Marques Ferreira[1], Leonardo Camilo de Oliveira[3] , Luciana Debortoli de Carvalho[4], Flávio Guimarães da Fonseca[1,2], Sarah J Valle[5], David L. Morris[5] , Jordana Grazziela Alves Coelhodos-Reis[1]

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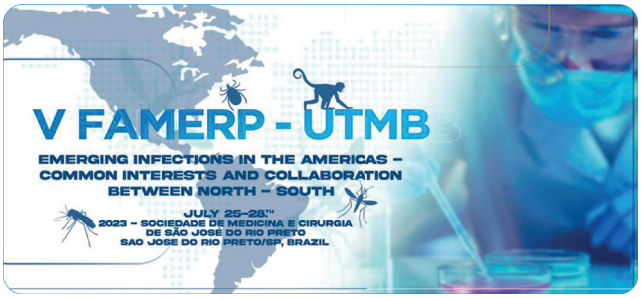
ABSTRACT

COVID-19 represented one of the biggest challenges of modern civilization and is yet to be eradicated. SARS-CoV-2 is a very resilient virus that is able to mutate, escape antibodies generated by either viral exposure or vaccination and, therefore, SARS-CoV-2 remains as an eminent public health threat. Due to the varied symptoms and the large number of infections and deaths caused by COVID-19, it is extremely important to study alternative approaches to contain future impact of the disease to society caused by newly formed variants of concern. Currently, there is no 100% effective antiviral treatment for the severe form of COVID-19, and although treatment with dexamethasone and mechanical ventilation is the standard procedure to treat the disease, many patients still succumb to infection. In this regard, BromAc® is a combination of Bromelain and N-acetylcysteine, that has shown important mucolytic effect as well as anti-inflammatory properties. Therefore, in the present study, we have performed in vitro and ex vivo analysis to access the effect of Bromelain combined with N-Acetycystein in containing virus at different levels. Here, evidence of virucide activity of the combined compounds in vitro in Vero-ACE2/TMPRSS2 cell line infected by Omicron variant. In addition, the combined compounds can abrogate RNA genomic copies of SARSCoV-2 virus in tracheal aspirate samples from critically ill COVID-19 patients. In addition, atomized BromAc® promotes cleaveage of S1 Spike subunit in tracheal aspirate samples which brings novel evidence of antiviral activity in COVID-19 patient tracheal aspirate samples, observed, both by flow cytometry and plaque forming units assay. All in all, these results bring to light evidence of ex vivo antiviral activity of the combination of Bromelain and N-Acetycystein, which suggests its potential as a nebulized approach for the treatment of COVID-19.

Financial support: CAPES, CNPq, FAPEMIG, and Mucpharm



EXTENSIVE LUNG INVOLVEMENT IN A MURINE MODEL OF SEVERE DENGUE: MORPHOLOGICAL ASPECTS AND VIRAL LOAD

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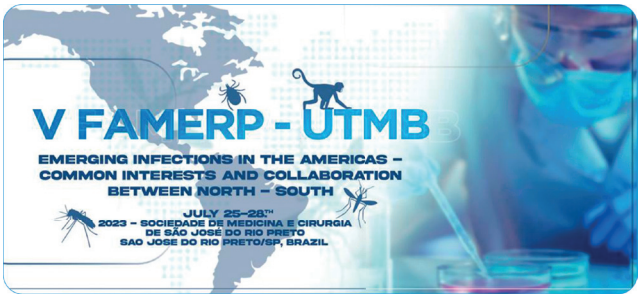
ABSTRACT

Recent epidemiological evidence has reinforced that the increased risk for severe dengue (SD) is associated with secondary infection by a heterologous serotype. During the course of SD, pulmonary hemorrhage is severe and multifactorial, involving vasculopathy, platelet dysfunction and coagulation disorders. The objective of this work is to evaluate the pulmonary involvement in a model of secondary dengue and to correlate the morphological findings with the viral load in this organ. For this, AG129 mice were inoculated with a strain of DENV-3 and, after 8 weeks, inoculated with a strain of DENV2. Lungs were collected 24, 48, 72 and 96hpi and processed for microscopy analysis and for viral genome detection. Macroscopy revealed areas of right lobar consolidation with hypocrepitation and wine coloration at 24, 48 and 72hpi. At 24hpi, the histopathological analyzes showed diffuse neutrophilic inflammatory infiltrate, associated with septal thickening and alveolar collapse. Diffuse hemorrhage was present, with areas of impairment of the usual architecture and endothelial activation, which sometimes accompanied intravascular neutrophilic margination. At 48hpi, a change in the profile of the inflammatory infiltrate is observed, with an influx of mononuclear and polymorphonuclear cells. At 72hpi, a predominance of neutrophils was observed, similarly to the 24hpi group. In the 48 and 72hpi groups, inflammation was accompanied by congestion, edema, hyperinflation and hemorrhage with a multifocal to coalescent distribution. At 96hpi, the neutrophilic inflammatory infiltrate remains, this time markedly diffuse. Hemorrhage is discreet in this group. The viral genome was detected in all tested lungs, ranging from 104.7 to 105.4 RNA copies/mL. From the results described, it can be stated that the model can be used to deepen studies on the pathophysiological involvement of the lung in SD and that changes in this organ can play a key role in the context of expanded dengue syndrome.

Financial support: CAPES and CNPq,



NANOPORE SEQUENCING FOR UNIQUE IDENTIFICATION OF CULICIDAE SPECIES, BLOOD MEAL AND PATHOGENIC VIRUSES

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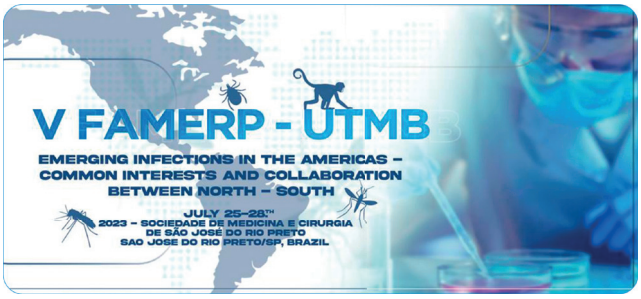
ABSTRACT

Arbovirus transmission is a complex process that involves interactions between virus, vector and host. Therefore, investigating the behavior of arthropods, such as feeding preferences,is important to understand and control virus transmission. This study aimed to design a protocol capable of identifying the source of blood meals in female mosquitoes through sequencing on Nanopore platforms, with simultaneous identification of the mosquito species and any pathogenic viruses. Females of Aedes aegypti and Aedes albopictus mosquitoes were artificially fed with bovine and human blood infected with Zika virus. These samples served as controls, as the blood sources, mosquito species and virus were known. To target mitochondrial DNA of the mosquito and its blood source, cytochrome B and cytochrome C oxidase I primers were used for amplification. As for viruses, 9N random primers from SMART-9N metagenomic technique were used to amplify the RNA of any virus. All products were sequenced on MinION nanopore platform, generating reads in real-time. Using Minimap2 software, the sequences were mapped to Culicidae and vertebrate databases from GenBank and Barcode of Life. They were also compared with MiniKraken2 database for detection of known pathogenic organisms. It was possible to successfully identify both mosquito species and their respective blood meal, as well as the Zika virus genome in the infected females. The results show that a single nanopore sequencing library is able to detect three organisms involved in the dynamics of virus transmission. This approach can facilitate a rapid identification of potential and known vectors as well as potential viral reservoirs. To increase its sensitivity and specificity, further tests should be performed using more comprehensive databases and multiple markers, including other arthropod species and arboviruses.

Keywords: Culicidae, Hosts, Arbovirus, Nanopore, Metagenomics.

Financial support: CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), CADDE (Centre for Arbovirus Discovery, Diagnosis, Genomics and Epidemiology) and FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) Grant number: 18/14389-0.



DETECTION OF FLAVIVIRUSES AND INSECT-SPECIFIC VIRUSES IN MOSQUITOES COLLECTED IN URBAN AND FOREST FRAGMENT AREAS OF A DENGUE ENDEMIC AREA IN NORTHWEST OF SÃO PAULO STATE, BRAZIL

Igor da Silva Teixeira[1] , Victória Bernardi Ciconi[1] , Victor Miranda Hernandes[1] , Maisa Parra[1] , Margareth Regina Dibo[2] , João Trindade Marques[3] , Nikos Vasilakis[4] , Maurício Lacerda Nogueira[1,4], Lívia Sacchetto[1]

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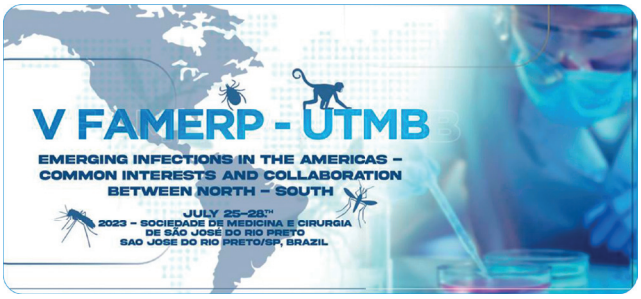
ABSTRACT

Insect-specific viruses (ISVs) are a group of RNA viruses that infect insects and insect cells. ISVs may influence arbovirus vector competence and can potentially be used as biological control agents or vaccine delivery platforms. Methods: From March 2022 to March 2023, 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) of São José do Rio Preto (SJdRP), São Paulo, Brazil. We have submitted 262 pools for viral isolation and molecular investigation for medically important flaviviruses and alphaviruses using RT-qPCR with specific and generic primers and for ISVs using PCR and specific primers. Results: We detect Humaita-Tubiacanga virus (HTV) in 39 pools (15 %) of mosquitoes from Aedes, Culex, and Sabethes genera. Phasi Charoen-like virus (PCLV) was detected in 51 pools (19%) of mosquitoes from Aedes and Culex genera. Culex flavivirus (CxFV) was detected in 23 pools (9%) of Culex genus. We detect Guapiaçu virus (GUAPV) in three pools (1%) of mosquitoes from Limathus, Culex, and Psorophora genera. Additionally, 92 pools (35%) of mosquitoes from Aedes and Culex genera tested positive in a pan-Flavivirus PCR assay. All PCR products were confirmed by Sanger sequencing. In addition, we have some co-infections: 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 (DENV), Zika (ZIKV), Yellow Fever (YFV), Mayaro (MAYV), Oropouche (OROV) and chikungunya (CHIKV) viruses. We are in the process of fully characterizing the viruses detected. Conclusions: Our results demonstrate the viral diversity in mosquitoes sampled in various ecotypes in SJdRP and warrant further studies assessing their contribution to vector competence and their interactions with circulating arboviruses in the region.

Financial Support: CAPES, CREATE-NEO (NIH grant 1U01AI151807)



ANTIVIRAL ACTIVITY OF A PEPTIDE ISOLATED FROM WASP VENOM AGAINST CHIKUNGUNYA VIRUS

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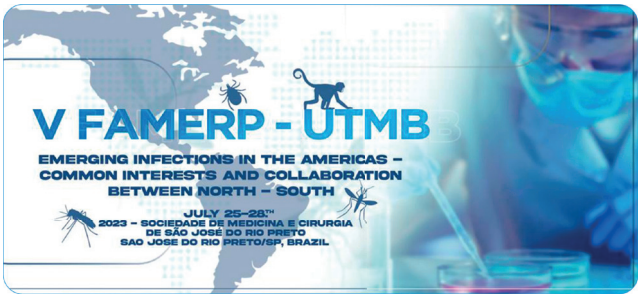
ABSTRACT

Introduction: Chikungunya virus (CHIKV), the causative agent of Chikungunya fever, is mainly transmitted by the bite of Aedes genus mosquitoes. The disease is characterized by painfully arthralgias, representing a great impact on public health, mostly in the tropical and subtropical areas. CHIKV infection can progress to a chronic condition that may last for years. Up to date, there are no available antiviral drugs against Chikungunya fever, being the treatment of infected patients based on controlling the symptoms with analgesics and anti-inflammatory drugs. In this context, peptides isolated from the venom of wasps have been reported for their biological properties, as antitumoral, antimicrobial, and antiparasitic activities. In this study we investigated the antiviral activity of the peptide (P1), synthesized based on proteins isolated from the venom of the solitary Eumenine wasp (Oreumenes decoratus), on the CHIKV infection in vitro. Materials and methods: The effects of P1 on the CHIKV replicative cycle were evaluated employing CHIKV-nanoluciferase, a viral construct inserted of a nanoluciferase reporter gene (- nanoluc), to infect baby hamster kidney cells (BHK-21). Cell viability and viral infectivity analysis were evaluated through MTT and luminescence assays, respectively. Results: P1 was capable of inhibiting CHIKV replication in 69%, assessed by the antiviral results, and there was no citotocixity presented at the tested concentration (5µg/mL). Conclusions: Our data demonstrate that P1 acts as an inhibitor of CHIKV replication, representing a candidate for the development of future treatments against Chikungunya fever. Additionally, the results presented here highlight the antiviral potential of molecules isolated from the venom of wasps.

Keywords: Chikungunya virus; Chikungunya fever; Wasp venom; Antivirals.

Financial support: FAPEMIG, CNPq, CAPES, FAPESP



PERSPECTIVES ON THE ANTIVIRAL POTENTIAL OF MOLECULES ISOLATED FROM THE AMAZONIAN FOREST: CPR01 AS A DRUG CANDIDATE AGAINST CHIKUNGUNYA VIRUS

Uriel Enrique Aquino Ruiz[1] , Shiraz Feferbaum Leite[1]1 , Igor Andrade Santos[1], Leonardo Cavalcante Queiroz[2] , Yana Araujo Alcantara[2] , Marcos Batista Machado[2] , Ana Carolina Gomes Jardim[1,3]

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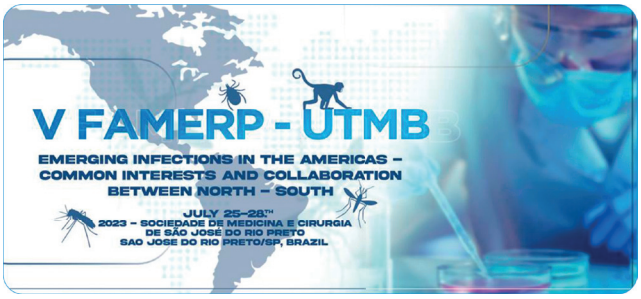
ABSTRACT

Introduction: Chikungunya virus (CHIKV) is the causative agent of Chikungunya fever, a disease characterized by painfully arthralgias. CHIKV infection can progress to a chronic condition that may last for years, representing a great impact on public health, mostly in the tropical and subtropical areas. Up to date, there are no available antiviral drugs against Chikungunya fever, being the treatment of infected patients based on controlling the symptoms with analgesics and anti-inflammatory drugs. In this context, the Brazilian Amazonian flora represent a vast, largely untapped, resource of potential antiviral compounds to be investigated. Here we evaluated the antiviral potential of natural compounds isolated from native and endemic Amazonian plants against CHIKV. Materials and Methods: The effects of 19 Amazonian compounds on the CHIKV replicative cycle were evaluated employing CHIKV-nanoluciferase, a viral construct inserted of a nanoluciferase reporter gene (-nanoluc), to infect baby hamster kidney cells (BHK-21). Cell viability and viral infectivity analysis were evaluated through MTT and luminescence assays, respectively. Taking these preliminary results, we selected CPR01 as the strongest candidate to inhibit de CHIKV replication. Therefore, the antiviral potential of CPR01 were assessed by performing EC50 and CC50 assays, and by evaluating different stages of infection employing time of drug-addition tests such as pre-treatment, entry, post-treatment and virucidal tests. All experiments were performed in triplicates and were performed a minimum of three times. Results: We found that 8 compounds at non-cytotoxic concentrations inhibited over 50% of virus infection (ranging from 52 to 90%). Nevertheless, the highest activity was identified for CPR01, and therefore, further antiviral analysis was performed with this compound, which demonstrated the selectivity index (SI = ratio between cytotoxicity and infectivity) of 18.3. Then, we carried out time of drug-addition tests and found that CPR01 inhibited all viral replicative stages analyzed (pre-treatment = 49%; viral entry = 71%; post-entry = 72%; viricidal = 60%). Conclusions: Our data demonstrate that CPR01 acts as a strong inhibitor of CHIKV replication, representing a candidate for the development of future treatments against Chikungunya fever.

Keywords: Chikungunya virus; Chikungunya fever; Natural Compounds; Antivirals; Amazonian Biome.

Financial support: FAPEMIG; FAPEAM; CNPq; CAPES.



CHILDREN WITH SEVERE ACUTE RESPIRATORY SYNDROME (SARS): PROCESS FOR IDENTIFICATION OF SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) ON THE TLR7 GENE

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ABSTRACT

The COVID-19 pandemic led to the death of 17,400 children worldwide, and 3,500 children in Brazil. In the severe form, the lower respiratory system is impaired, culminating in SARS, sequels, or death. Despite severe cases of COVID-19 being associated with comorbidities, severe childhood cases occur without comorbidities. TLR7 is an endosomal toll-like receptor that recognizes viral RNA (ss). In SNPs, one nucleotide is replaced, which may lead to loss of biological function of the protein. The goal of this work is the frequency identification of the rs179008 (A/T) genotype in the TLR7 in children who had SARS (COVID+). For the feasibility of this work, we relied on the medical team of Hospital João Paulo II. Once we got the samples, we did DNA extraction, PCR, PCR purification, sequencing, and electropherogram analysis. Seventy samples from children with SARS were obtained. The allelic frequency of the rs179008 was 0.09. According to Global ALFA databases of human genetic variations, the presence of the rs179008 genotype is 0.20. Thus, our data indicate a relation between the presence of the rs179008 SNP to a milder COVID. To understand this data result and guide future in vitro studies, we modeled the sequences to identify the possible impacts of the DNA A/T mutation on the protein. The mutation led to a replacement of GLN11LEU in the signal peptide region, leading to a change in the hydrophobicity of the molecule and perhaps interfering with correct location signaling. Our hypothesis, although we have not yet worked with TLR9 SNP, is that in the absence of TRL7, TRL9 could make up for the deficiency in recognition and production of IFN I adequately and with less induction of other cytokines than with TLR7 induction, reducing the risk of the cytokine storm. We expected not only to contribute to the identification of possible host SNPs related to severe COVID-19 but also to identify possible markers for severe cases.

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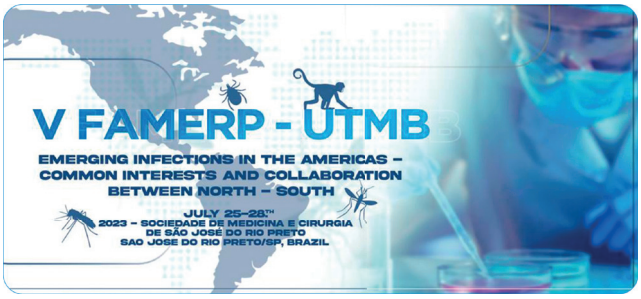

THE EMERGENCE OF POXVIRUSES IN BRAZIL: ADDRESSING CONCERNS OF ZOONOTIC POTENTIAL

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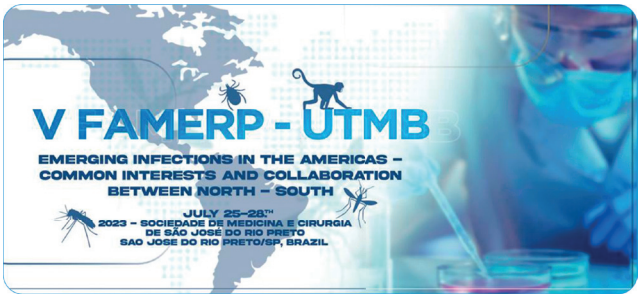
ABSTRACT

Poxviruses play an important role in human history, like smallpox in the past and the recent outbreak of Mpox outside the African continent. In Brazil since 2000, the expansion of cases of a poxvirus called vaccinia virus (VACV) has been observed, causing a neglected disease in humans and animals. Also, we have the economic and social impact of diseases and the environmental concern of the spillover or spillback in animals. Cattle and human outbreaks have been described in southeastern Brazil since 2000 and have occurred in almost half of the territory. Phylogenetic studies have shown high levels of polymorphisms among isolated VACVs, which indicate the existence of at least two genetically divergent clades; this has also been proven in virulence assays in a mouse model system. In humans, VACV infection is characterized by skin lesions, primarily on the hands, accompanied by systemic symptoms such as fever, myalgia, headache, and lymphadenopathy. Seroprevalence, poxvirus eco-epidemiology, and genomic surveillance studies were done by our group showing continuous dissemination of this virus in Brazil. In the Brazilian scenario, we have a unique opportunity to evaluate the monkeypox virus in populations naturally infected with VACVs. These studies will be developed by a group of the recently approved INCTPOX which will lay a basis for advancement in diagnosis, vaccines, and antivirals for poxviruses. Our public health and animal health system needs the support of the research sectors to act to prevent the spread of infections.

Financial support: FAPEMIG, CAPES, CNPq



RESUME: OUTBREAK OF PRIMATE ERYTHROPARVOVIRUS 1 IN INDIVIDUALS WITH ACUTE FEBRILE ILLNESS SUSPECTED OF ARBOVIRAL INFECTIONS IN THE STATES OF RIO GRANDE DO NORTE AND MATO GROSSO DO SUL DURING THE YEARS 2016 AND 2017

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ABSTRACT

The prevalence of Primate Erythroparvovirus 1 (B19V) in the general population has been observed to follow a cyclic pattern of occurrence every 4-5 years, with genotype 1 being predominant. During epidemic periods, the disease can cause a variety of clinical conditions, including erythema infectiosum, arthropathy, transient aplastic crisis, and non-immune hydrops fetalis. However, the majority of B19V infections are asymptomatic or present with mild symptoms, which have a self-limiting clinical course. This characteristic poses significant challenges for performing a differential diagnosis, courtesy of overlapping symptom presentation with other pathogens causing fever and rash, alongside the widespread circulation of arboviruses within population. In this study, we investigated suspected cases of Dengue (DENV), Chikungunya (CHIKV) and Zika (ZIKV) and reported an occult outbreak of B19V during arbovirus epidemics in the states of Rio Grande do Norte (RN) and Mato Grosso do Sul (MS) between 2016 and 2017. A total of 1,873 serum samples obtained from individuals with acute fever were analyzed, including 713 samples from RN and 1,160 from MS. The samples were subjected to molecular testing using RT-qPCR for the detection of DENV, CHIKV and ZIKV, as well as for the Flavivirus, Alphavirus and Enterovirus genera, and qPCR for B19V and Primate Erythroparvovirus 4 (PARV4) species. Genetic analysis was performed using primers designed to cover the complete genome of B19V and PARV4. The results showed that out of the 713 cases investigated in RN, 192 (26.9%) demonstrated a viremia for B19V. Although B19V infection is typical in childhood, we observed that 85% of the infected patients in RN and 71.4% of the infected patients in MS were adults. We performed genome sequencing on 188 individuals, confirming the presence of genotype 1 in all samples. In conclusion, the results obtained through molecular testing demonstrated its effectiveness in confirming the major arboviruses as well as detecting B19V during seasonal epidemics. It is important to emphasize that B19V infection in pregnant women can lead to significant fetal damage, recognizing the occurrence of hydrops fetalis and anemia, making it an important cause of fetal loss. These findings have fundamental relevance to laboratory surveillance as they contribute to increasing awareness of B19V caused epidemics.

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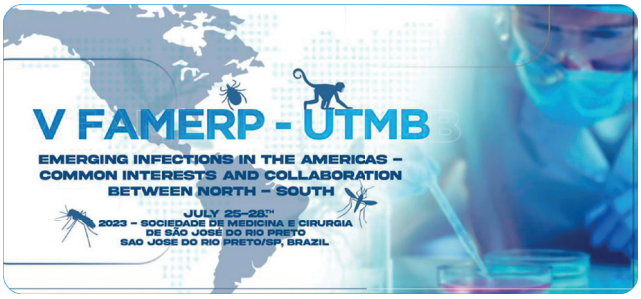

STRUCTURAL AND KINECT CHARACTERIZATION OF MAYARO VIRUS NSP2 PROTEASE

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ABSTRACT

Mayaro virus, (MAYV) is an arbovirus belonging to the Alphavirus genus and the causative agent of the Mayaro fever, a febrile condition that in severe cases causes incapacitating arthralgia that can persist for months. This virus exhibits a sylvatic cycle in many countries of Latin America (including Brazil), where it is transmitted to humans by Haemagogus mosquitoes, but evidence indicates MAYV is capable of infecting Aedes mosquitoes, that also circulate in urban environments and could promote MAYV spread to more densely populated areas where it could cause outbreaks. Currently, there is no treatment or vaccine against Mayaro fever, the lack of information about this virus is an obstacle in the search for therapeutic strategies since most of the information available at a molecular level is based on inferences made from studies of other alphaviruses. The non-structural protein 2 (nsP2) is an 87 kDa nonstructural protein conserved among alphaviruses. During replication nsP2 cleaves the viral polyprotein through a cysteine-protease domain on its C-terminal (nsP2pro), generating the non-structural proteins in their mature and functional state. Due to its conservation and essential role during viral replication the nsp2pro is considered a promising target for the development of antiviral drugs but, unfortunately, the literature about MAYV nsP2pro functionality and 3D structure is scarce. Thus, in this work we aimed to elucidate the tridimensional structure of nsP2pro (41 kDa) using X-rays crystallography and characterizing nsP2 proteolytic activity using an enzymatic assay. We were able to develop protocols for the expression of the nsP2pro from recombinant E. coli cells and a chromatographic protocol for its purification. The characterization assays indicated that the recombinant nsP2pro is in a pure, monomeric, structured, stable, and active form. Key words: Mayaro virus, C9 protease, nsP2pro, x-ray Crystallography

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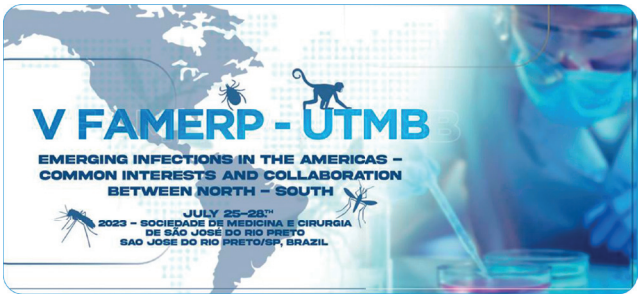
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