IV FAMERP – UTMB

Emerging infections in the Americas – common interests and collaboration between Brazil and USA

Book of Abstracts

December 5-7th, 2019

São José do Rio Preto, SP, Brazil

MEETING

Over the last 10 years, the LPV FAMERP has developed a strong collaboration with the renowned Department of Pathology, University of Texas Medical Branch (UTMB), which is a center of excellence in arboviruses research. From this collaboration, we held three events. The I and II FAMERP-UTMB Meeting, held in 2012 and 2016, brought researchers and students from various institutions from Brazil and abroad to São José Rio Preto, with approximately 200 participants in each event, performing posters, talks, conferences and round-table presentations. The III FAMERP-UTMB Meeting occurred in 2017 together with the São Paulo School of Advanced Science in Arbovirology, a unique theoretical and practical course that provided the students with a critical and comprehensive view on the state of the art of arboviruses. Due to the great feedback we received from the public, in addition to several requests to repeat the event , we resolved to continue our biennial-basis meeting with the **IV FAMERP-UTMB: Emerging infections in the Americas - common interests and collaboration between Brazil and USA**, to be held on December 4 to 7, 2019 in the city of São José do Rio Preto ,SP, Brazil.

Throughout the **IV FAMERP-UTMB**, we will have keynote lectures, short talks and round-tables with renowned specialists and researchers that will discuss the bases and state of the art of research about several vector-borne diseases, including arboviruses, rickettsia, and malaria among others. Many aspects of the research about vector-borne diseases will be our discussion topics, as classical virology; serological and molecular methods; phylogeny and evolution; entomology and methods of field study; the current situation of epidemiology / clinical aspects of arboviruses research in Brazil; the interplay between the virus, the host and their immune system; and the virus-vector interaction and its relation with the dynamics and control of these diseases transmission.

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ORGANIZING COMITTEE

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Nikos Vasilakis (UTMB), PhD

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PROGRAMME

4th December		
14:00 – 18:00	Registration	
13:30 – 17:30	Parallel Meeting	REDe Evaluation Seminar
18:30 – 19:00	Opening ceremony	FAMERP, UTMB, and FAPESP representatives
19:00 – 20:00	Keynote speaker	Mike S. Diamond (WUSM) - *New Insights into Pathogenesis of Emerging Arthropod-Transmitted Viruses*
20:00 – 22:00	Cocktail	
5th December		
08:00 – 09:00	Lecture	Scott C. Weaver (UTMB) - *Mechanisms of Urban Arbovirus Emergence*
09:00 – 10:30	Round Table	Shannan L. Rossi (UTMB) - *Pathology and Immunology of Chikungunya in Animal Models*
		Danielle G. Souza (UFMG) - *Mechanisms Associated to Inflammatory Pain Induced by Chikungunya Virus*
		Jean P. S. Peron (USP) - *Experimental Models for Zika Virus Congenital Syndrome*
10:30 – 11:00	Coffee break	
11:00 – 12:00	Lecture	Nikos Vasilakis (UTMB) - *Prospects of a sylvatc zika transmission cycle in the Americas*
12:00 – 14:00	Lunch	
14:00 – 15:30	Round Table	Luciana B. Arruda (UFRJ) - *How Flavivirus Can Cross the Blood-Brain Barrier*
		Cybele C. Garcia (Universidad de Buenos Aires) - *AHR is a flavivirus host factor and a target for antiviral therapy*
		Irma (Lisa) Cisneros (UTMB) - *Opioid use disorder: Orchestrating antiviral and neuroinflammatory networks during viral CNS infections.*
15:30 – 16:00	Coffee break	
16:00 – 17:00	Lecture	David Walker (UTMB) - *Rickettsial, Oriential, and Ehrlichial Diseases of Potential Importance in Brazil*
17:00 – 18:00	Oral presentations	1. Pierina Lorencini Parise - *Modulation of innate immune response in endothelial cells during Oropouche virus infection* 2. Izabela Maurício de Rezende - *Late relapsing hepatitis after yellow fever* 3. Pedro H. Carneiro - *Investigation of the interaction between human apoliprotein A1 and dengue virus NS1 protein* 4. Ana Carolina de Carvalho - *Establishment of models of Ilhéus virus infection in vitro and in vivo and investigation of the 2'c-methylcytidine treatment over disease* 5. Ingredy Passos - *Therapeutic treatment of dengue virus infection using an antiviral peptide*
6th December		
08:30 – 09:30	Lecture	Louis Lambrechts (Pasteur Institute) - *Dissecting natural variation in mosquito-arbovirus interactions*
09:30 – 10:30	Round Table	Maurício L. Nogueira (FAMERP) - *Interplay between different flavivirus in an endemic area*
		Betânia P. Drumond (UFMG) - *Yellow fever epizootics in urban areas of Minas Gerais, 2017-2018.*

10:30 – 11:00	Coffee break	
11:00 – 12:00	Round Table	Daniela Weiskopf (La Jolla Institute for Allergy & Immunology) - *T cell cross-reactivity between Flaviviruses*
		Mauro M. Teixeira (UFMG) - *Inflammation, infection and host-parasite interactions*
12:00 – 14:00	Lunch	
14:00 – 15:30	Round Table	Fabio Trindade M. Costa (Unicamp) - *Adhesiveness in the biology of Plasmodium vivax: How sticky is this idea?*
		Daniel M. Aguiar (UFMT) - *Rickettsiosis in Brazil*
		Luiz C. Mattos (FAMERP) - *Ocular Lesions in Toxoplasmosis*
15:30 – 16:00	Coffee break	
16:00 – 17:00	Sponsored Lecture	Amilcar Tanuri (UFRJ)
17:30 – 20:00	**Poster presentations**	
7th December		
08:00 – 08:30	Technical Lecture	Felipe Lorenzato (Takeda Pharmaceutical Company) - *Efficacy of a Tetravalent Dengue Vaccine in Healthy 4 to 16 Years Old Children*
08:30 – 09:00	Lecture	Arturo Reyes-Sandoval (University of Oxford) - *Viral-Vectored Vaccines Against Chikungunya Virus*
09:00 – 09:30	Lecture	Marcus V. G. Lacerda (FMT-HVD) - *What do we know about coinfections?*
09:30 – 10:00	Coffee break	
10:00 – 10:30	Lecture	João Pessoa Araújo Junior (UNESP) - *Application of NGS/HTS in clinical samples with low viral copy number (Zika virus)*
10:30 – 11:00	Lecture	Carlos Sariol (University of Puerto Rico) - *Zika Virus Pathogenesis in Non-Human Primate Model*
11:00 – 12:00	Oral presentations	1. Vidyleison N. Camargosa - *In-depth characterization of congenital zika syndrome in immunocompetent mice: antibody-dependent enhancement and an antiviral peptide therapy* 2. Marcilio Jorge Fumagalli - *Previous CHIKV exposure induces partial cross-protection against secondary MAYV infection in mice* 3. Lilian Gomes de Oliveira - *The role of TAM receptors, and their ligand, GAS6, in resistance and susceptibility during ZIKV infection* 4. Daniel Gavino Leopoldino - *Zika virus replication in skeletal muscle contributes to amplification of peripheral viral load and dissemination to central nervous system.* 5. Darlan da Silva Candido - *Genomic history and dynamics of the Chikungunya virus East-central-south African lineage in the Americas*
12:00 – 14:00	Lunch	
14:00 – 14:30	Lecture	Daniel Martins-de-Souza (Unicamp) - *Neuroproteomics on zika-infected human neurospheres*
14:30 – 15:00	Lecture	Nuno Faria (Oxford University) - *Phylogeny of emerging arboviruses in Brazil*
15:00 – 16:00	Closing Conference	Jônatas Abrahão (UFMG) - *Trapping the Enemy: Vermamoeba vermiformis Circumvents Faustovirus Mariensis Dissemination by Enclosing Viral Progeny inside Cysts.*
16:00 – 16:15	**Concluding remarks**	
20:00	**FAMERP LPV Party**	*Admission should be paid separately*

ABSTRACTS

Abstracts Index

Abstract #001

YELLOW FEVER VIRUS DETECTION BY RT-QPCR IN *AEDES SCAPULARIS* MOSQUITO, SÃO PAULO, BRAZIL

Cunha MS[1], Faria NR[2], Caleiro GS[1], Candido DS[2], Hill S[2], Tubaki RM[3], Menezes RMT[3], Nogueira JS[1], Maeda AY[1], Vasami FGS[1], Morales I[4], da Costa AC[4], Mucci LF[5], Sabino E[4]

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Yellow fever (YF) is an arboviral disease endemic to tropical regions of Africa and South America caused by the yellow fever virus (YFV), a single-stranded, positive sense 11kb RNA virus member of the *Flavivirus* genus (1). In the Americas YF spread can occur through an "urban cycle" involving transmission between susceptible humans and *Aedes aegypti* mosquitos, and through a "sylvatic cycle", involving transmission between non-human primates (NHP), mainly *Alouatta* sp., *Sapajus* sp., and *Callithrix* sp, and the canopy-breeding *Haemagogus* sp. and *Sabethes* sp. mosquitoes. Occasional spillover to non-vaccinated humans may occur when these encroach forested areas. Beginning in late 2016 Brazil faced the worst outbreak of Yellow Fever in recent decades. In São Paulo State, the first epizootic events caused by YFV were detected in a previous enzootic area in the North region (São José do Rio Preto and Ribeirão Preto mesorregions) in mid-2016. Mosquitoes (Diptera: Culicidae) were then collected by Superintendência de Controle de Endemias (SUCEN) in 15 municipalities with ongoing epizootic events and in adjacent cities in 2016. Mosquitoes were identified and separated into pools (*Aedini* and *Sabethini*) according to specie, and sent to Núcleo de Doenças de Transmissão Vetorial, Adolfo Lutz Institute, for YFV detection by a probe-based RT-qPCR and/or viral isolation in C6/36 cell lines followed by immunofluorescence assay (IFA). One pool containing 1 female individual of *Aedes scapularis* mosquitoes collected in Urupês on November 2016 in an agricultural area was positive for YFV. Interestingly, no epizootic event was detected in this city. A full-length genome was obtained using the portable Oxford Nanopore minION technology. Phylogenetic analysis revealed that this YFV belongs to South American genotype 1. However, little is known regarding YFV vector competence on *Aedes* spp mosquitos in Brazil. It is believed that *Aedes scapularis* may act as a bridge vector. More studies must be addressed in order to evaluate different Brazilian *Aedes* species in the YFV cycle.

Finnancial support: SES, FAPESP.

Abstract #002

ANIMAL MODEL FOR EVALUATING OXIDATIVE STRESS IN THE HEPATIC PATHOLOGY INDUCED BY THE MAYARO VIRUS

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Mayaro virus (MAYV), a mosquito-borne alphavirus, causes Mayaro fever disease in humans. This febrile illness is characterized by rash, headache, myalgia and arthralgia. Arboviruses such as MAYV have the potential to establish an epidemic scenario, similar to what happened with zika and chikungunya viruses. Although MAYV has the potential to reemerging, its pathophysiological mechanism remains unclear. Recent evidences suggest that oxidative stress plays trigger a pathological mechanism in virus infections, contributing to progression of liver injury. We aimed to study an animal model of the development of oxidative stress and liver injury after infection by MAYV. 3-4-weeks old BALB/c mice were infected by sub-cutaneous injection with 10^5 p.f.u. of MAYV. 4 groups containing 18 animals each (9 infected and 9 uninfected) were monitored daily. MAYV-infected mice revealed lower weight gain at 1 and 3 dpi. Viremia and liver viral load were measured in all the infected animals. Histological changes, hepatic function and redox homeostasis markers were analyzed at 1, 3, 7, and 10 days post-infection (dpi) in all the animal's liver. We measured malondialdehyde (MDA) and carbonyl protein levels as indicators of oxidative hepatic damage in lipids and proteins, respectively. We also evaluated Myeloperoxidase (MPO) activity in the liver as an indirect index of neutrophil activation. Antioxidant defense mechanisms were analyzed, including superoxide dismutase (SOD) and catalase (CAT) enzimes enzymes activity and the glutathione system. Our data showed that the liver is a site of MAYV replication, and the infection causes hepatic histopathological changes with significant increase of the inflammatory infiltrate and redox homeostasis imbalance. We reported at the first time the involvement of oxidative stress on hepatic pathology in BALB/c mice infected by MAYV. Future studies are warranting the therapeutic strategies for Mayaro fever, such as those based on antioxidant compounds.

Finnancial support: UFOP, CNPq, FAPEMIG, CAPES.

Abstract #003

ISOLATION AND CHARACTERIZATION OF ILHEUS AND IGUAPE VIRUSES FROM *ANOPHELES* SPP. MOSQUITOES IN SOUTHEAST BRAZIL

Ribeiro GO[1], da Costa AC[2], **Luchs A**[3], dos Santos FCP[4], Komninakis SV[5,6], Marinho RSS[6], Witkin SS[2,7], Villanova F[1], Deng X[8,9], Sabino EC[2,10], Delwart E[8,9], Nogueira JS[11], Maeda AY[11], de Souza RP[11], Nogueira ML[12], Maiorka PC[13], Leal E[1], Cunha MS[11]

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Flavivirus are widespread in Brazil and are a major public health concern, including Zika and Dengue viruses. Other flaviviruses of medical importance have also been detected in the country, including Saint Louis encephalitis virus, Rocio virus, Ilheus virus (ILHV) and Iguape virus (IGUV). The aim of the present study was to describe the isolation and whole genome characterization of two rarely reported *Flavivirus:* ILHV and IGUV. Brazilian flavivirus strains described here were obtained from *Anopheles* spp. mosquitoes pools collected in 1994 in Sao Paulo State, during routine entomological surveillance. A total of 14 pools were isolated in C6/36 cells, however remained unidentified due to lack of available diagnostic tools at the time. The stored samples were tested for *Flavivirus* genus using RT-qPCR assay. Whole genome sequencing was conducted in positive qRT-PCR samples in order to identify the strains, following by phylogenetic analysis. Five stored samples (5/14) tested positive for *Flavivirus* by qRT-PCR. One nearly full-length ILHV genome (SPAR158517 strain) was acquired from *Anopheles triannulatus* pool, and four IGUV genomes (SPAR158470, SPAR758482/02B, SPAR158482 and SPAR158495 strains) were obtained from *Anopheles cruzii* pools. SPAR158517 ILHV strain showed high nucleotide similarity to Brazilian strains Original (99%) and BrMS-MQ10 (95%) isolated in 1944 and 2010, respectively. The four Brazilians IGUV strains detected here displayed 96% of nucleotide identity to Brazilian SPAn71686 strain isolated in 1979. Phylogenetic analysis phylogenetic analysis showed that Brazilian ILHV strains are highly conserved geographically and temporally. Conversely, IGUV strains are very divergent from the reference strain. PCR-based detection is a powerful tool used both in epidemiological surveillance and genetic and ecological studies. This requires the ability to obtain robust genomic data. The availability of additional genomics and phylogenetic analysis will increase our evolutionary knowledge of these viruses as well as improve their molecular diagnosis.

Finnancial support: FAPESP (#2017/00021-9, #2016/01735-2, #2012/23645-4, #2015/12944-9), CNPq (#400354/2016-0).

Abstract #004

ANALYSIS OF THE INTERACTION BETWEEN DENGUE VIRUS 2 NS1 PROTEIN AND HUMAN CD14 PROTEIN IN MONOCYTES

Carneiro PH[1], Coelho DR[1], Andrade YS[1], Matassoli FL[2], Meuren LM[2], Conde JN[1], de Arruda LB[2], Mohana-Borges R[1]

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NS1 protein is essential for Dengue virus (DENV) and can be secreted into the serum of infected patients. NS1 protein can activate monocytes and macrophages, by a mechanism not yet fully elucidated. Our group has identified that the NS1 protein is capable of interacting with hepatocytes' proteins, among them, CD14 protein, which is a membrane receptor found mainly in monocytes. In these cells, CD14 and the TLR4 membrane receptor are responsible for recognizing bacterial products, such as LPS. In this context, it is interesting to evaluate the role of NS1 protein in modulating signaling by CD14, as it may reveal a new target for dengue treatment. To confirm the interaction in vitro, an enzyme-linked immunosorbent assay (ELISA) and co-immunoprecipitation (Co-IP) were realized using the purified CD14 and NS1 proteins. Fluorescence optical microscopy assays with primary monocytes infected with the DENV2 virus or treated with purified NS1 were used to verify the colocalization of NS1 and CD14 in primary monocytes. To verify the interaction by molecular docking, using the three-dimensional models of the NS1 and CD14 proteins obtained in the Protein Data Bank. An immunophenotyping analysis of monocytes treated with NS1 and LPS-RS (TLR4-signaling antagonist) was performed for 48h, seeking to verify the cell activation by flow cytometry. Further, the supernatants of these monocytes were analyzed by capture ELISA for cytokine analysis. The interaction between NS1 of DENV2 and CD14 was confirmed by all interaction assays. Molecular docking also suggests that NS1 and LPS share the same site of interaction with CD14. Monocytes treated with NS1 expressed more CD14. There was also a 50% increase of the HLA-DR molecule, responsible for the presentation of antigen and LPS-RS rescued the phenotype caused by NS1. NS1 also induced secretion of IL-8 and IL-6 cytokines. As perspectives, we aim to perform other approaches to confirm the contribution of these receptors.

Finnancial support: FAPERJ, CNPq, CAPES, ICGEB.

Abstract #005

PRODUCTION OF *ZIKA VIRUS* RECOMBINANT PROTEIN E, USING *E. COLI* EXPRESSION SYSTEM AND EVALUATION OF ITS ANTIGENICITY

Moraes TFS[1,2], Bagno FF[2], Sergio SAR[2], Salazar N[2], Figueiredo MM[2], Fonseca MSP[2], Versiani AF[3], Fonseca FG[1,2]

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For decades, infections caused by *Zika virus* (ZIKV) were neglected in function of your usual mild symptoms and limited number of cases. In recent years, however, the fast dissemination of ZIKV and its unexpected link to neuropathies such as congenital microcephaly and Guillain-Barré syndrome turned the virus into an emerging public health problem of global concern. The ZIKV-E protein is the main component of the viral surface, and the major target for the induction of a protective immune response, probably based on neutralizing antibodies. The present work shows the standardization of the heterologous expression, purification and evaluation of the antigenicity of the ZIKV-E protein as vaccine immunogen or diagnostic tool component. For this, pET-21 vector containing the C-terminally truncated coding sequence of ZIKV-E was used to transform *E. coli* BL21. Transformant clones were induced by IPTG at 37°C for three hours or 18°C overnight. Additional tests were performed to evaluate the solubility of the protein in different fractions of urea, followed by purification in the Akta prime system by affinity columns. Western blot assays were performed using commercial antibodies (Anti-His e Panflavi 4G2) and sera from mice infected with ZIKV. The purified protein was stable, free of important contaminants and readily recognized by commercial Anti-His e Panflavi 4G2 monoclonal antibodies as well as by pools of sera from mice infected with ZIKV. No cross reactions to sera from ZIKV-seronegative mice were detected. Our preliminary results confirmed the antigenicity of the protein and suggest its potential to be used either in vaccine formulations or in diagnostic platforms.

Finnancial support: This work was supported by Capes, Fapemig and through a Finep-Zika Institutional grant.

Abstract #006

EVALUATION OF ANTIVIRAL ACTIVITY OF EXTRACT AND ISOLATES OF *PSYCHOTRIA* SP. AGAINST *MAYARO VIRUS*

Moraes, T. F. S.[1, 2]; Ferraz, A. C.[1,3]; Cruz, W. S.[1]; Vieira Filho, S. A.[3]; de Brito Magalhães, C.L.[3]; Magalhães, J. C.[1]

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Mayaro virus (MAYV) is an emergent arbovirus that causes Mayaro fever, characteristic to induce an incapacitating polyarthralgia for which have been reported sporadic cases and small epidemics in South American countries. To date, there are no antiviral drugs or vaccines against this virus and antiviral research becomes relevant. The genus *Psychotria* has species for which bioactive extracts have been reported with antimicrobial activity. Thus, the goal was to evaluate the antiviral activity in the extract (D4) of *Psychotria* sp and their isolates DX and TX. We determined the cytotoxic concentration for 50% of the cells (CC50), the effective protective concentration for 50% of the infected cells (EC50) and the mechanisms of action. No cytotoxic action was detected for the D4 until a greater value of 750 ug/mL, for DX and TX, the CC50 values were 119 and 349 μg/mL, respectively. For the moi 0.1 virus/cell, D4, DX and TX presented antiviral action at concentrations of 22, 28 and 93 μg/mL, respectively. When increasing by up to 100 times viral load, extract and isolates were able to block the infection increasing only 4x the concentration of D4, 3x for DX and 2x for TX. In the mechanisms of action, the D4 showed a potent virucidal effect from 16 μg/mL, while the isolates were not able to inhibit the infection. The D4 at 250 μg/mL, also affected the adsorption and penetration viral, not observed for isolates. By evaluating the effects of the compounds after viral penetration, the results showed that extract and isolates are efficient to inhibit the production of viral load in up to 6 log units, if added up to 24 hours after infection (hpi). Thus, this study showed that the plant has antiviral properties against MAYV, it is presumed the existence of other active principles in the extract, besides DX and TX, that have unleashed the potent virucidal action observed.

Financial support: FAPEMIG, UFSJ.

Abstract #007

YELLOW FEVER EPIZOOTICS IN URBAN AREAS IN MINAS GERAIS, BRAZIL (2018): A POTENTIAL RISK FOR URBAN TRANSMISSION CYCLE?

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Yellow fever (YF) is an endemic disease in the Brazilian Amazon basin, with sporadic cases reported in other regions during warm/wet season. The urban cycle is considered eradicated in Brazil, since 1942, and YF virus (YFV) has been maintained through a sylvatic cycle involving non-human primates (NHPs), and wild mosquitoes. Neotropical NHPs are susceptible to YFV infection, developing the disease and often dying. The greatest sylvatic YF outbreaks in the country recently occurred between 2016/2018, mainly in the Southeast region, causing thousands of NHP and human cases and deaths. We analyzed samples from NHP carcasses collected in 2018 from different urban/periurban areas of Minas Gerais (MG). Total RNA was extracted from liver and used for 1-Step RT-qPCR using specific primers and TaqMan® probe targeting the 5'-NC region of YFV genome. From positive samples, cDNA was synthesized and used in PCR, with primers targeting CprM/envelope. After sequencing, phylogenetic analysis was performed. From 233 liver samples from NHP carcasses collected during January to December/2018 in urban/periurban areas, YFV was detected in 29.6% of the samples (69/233) from *Alouatta* spp. (1/2), *Callithrix* spp. (67/226), and non-identified NHPs (1/5). YFV was detected in samples from 42 municipalities, mainly in the Metropolitan region of Belo Horizonte (66.6%, 46/69). We observed the predominance of *Callithrix* spp. in the individuals collected. The extensive occurrence of YF in MG, the presence of YFV positive NHP inside the cities, with proximity to humans associated with the presence of competent vectors, could be a risk that can contribute to the re-urbanization of YF in Brazil. To date there is no epidemiological evidence of the urban transmission cycle in Brazil in this YFV outbreak. Nevertheless, further studies should be conducted to investigate the competency of urban and periurban vectors to transmit YFV and the possible risks for the occurrence of YF in urban centers.

Financial support: FAPEMIG, CNPq, MCTIC, CAPES, MEC-Decit, SCTIE-MS, SES-MG, SEPLAG-MG, UFMG.

Abstract #008

DEVELOPMENT OF REAL-TIME POLYMERASE CHAIN REACTION WITH HIGHT RESOLUTION DISSOCIATION CURVE (HRM) FOR DETECTION AND DIFERENTIATION OF LINEAGES I AND II OF DENGUE VIRUS TYPE 2 CIRCULATING IN BRAZIL

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Dengue viruses are a serious public health problem. Belonging to the *Flaviviridae* family and *Flavivirus* genus, Dengue virus type 2 is responsible for major epidemics and severe cases of the disease in Brazil. Phylogenetic analysis studies revealed the circulation in the country of two distinct strains of the Southeast Asian genotype, named lineages I and II. In 2008, DENV-2 lineage II was responsible for the country's most serious epidemic, reaching mainly young people up to 15 years of age. The objective of this study is to develop and standardize a "lineage typing" protocol for rapid and inexpensive differentiation of DENV-2 lineages, using the polymerase chain reaction (PCR) technique in real-time by analysis of the High-Resolution Melting (HRM) high-resolution dissociation curve tool. Methodology starts with the identification of the genome region where the single nucleotide polymorphism is located and primer design for amplification of this region. Thermocycling by Applied Biosystems Melt Doctor HRM Master Mix and subsequent analysis of the high-resolution dissociation curve will follow. Serum samples previously diagnosed as positive for DENV-2, containing genotype and lineage information will be used, as well as samples positive for DENV-2 without lineage information. Finally, Sanger sequencing will confirm the identity of the lineage characterized by PCR-HRM. Preliminary results lead to the identification of optimal primers for the PCR. Experiments are still ongoing, and we expect that the melting curve analysis will be able to identify and differentiate the amplified fragments, according to the dissociation temperature inherent to each product. After validation, we expect that the method will become a new rapid and inexpensive tool for characterization and genomic surveillance of DENV, that could be an alternative to the genomic sequencing approach.

Financial support: INOVA 2019/FIOCRUZ.

Abstract #009

MORPHOLOGY AND MORPHOGENESIS OF ARBOVIRUSES CIRCULATING IN BRAZIL: DENGUE, YELLOW FEVER, ZIKA AND CHIKUNGUNYA

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The epidemic of Zika virus (ZIKV) in 2015 in the Americas it provided evidence for features of pathogenicity that had not been observed previously in infections by *Flaviviruses*. Studies *in vitro* shows that part of the ZIKV morphogenesis may occur within viroplasm-like structures, never seen in other flaviviruses. In this work C6/36 and Vero cells were inoculated with samples of the main circulating arboviruses in Brazil (dengue virus serotype 1 [DENV-1], DENV-2, DENV-3, DENV-4, ZIKV, yellow fever virus [YF] and chikungunya virus [CHIKV]) and the infection was characterized by transmission electron microscopy. In all cells cultures inoculated, virus particles and replication was observed. The particle morphology was similar between the four serotypes of DENV, YF and ZIKV, with differences only in diameter. The CHIKV particles showed spherical format with an approximate diameter of 50-60nm and with the envelope structure enough evident. The cells infected with DENV-1, -2, -3 and -4 and ZIKV showed tubular structures associated with rough endoplasmic reticulum with virus-like particles inside. We also observed these tubular structures vesicle in cells infected with YF virus. In cells infected with ZIKV, large viroplasm-like compartments localized in the perinuclear area together with peripheric rough endoplasmic reticulum, mitochondria and microtubules, were verified. In cells infected with CHKV it was observed that the particles are internalized mainly by clathrin-mediated endocytosis. Virus particles was observed in endocytic vesicles and in compartments formed by unitary membranes where nucleocapsids were observed. The release of CHKV particles was by budding. The susceptibility of C6/36 and Vero cells to infection with a DENV-1, DENV-2, DENV-3, DENV-4, ZIKV, YF and CHIKV samples isolated from Brazilian patients was confirmed in the present study. The data presented in this study are important for use in the development of model systems to evaluate therapeutic approaches.

Financial support: CNPq.

Abstract #010

ZIKA VIRUS INVESTIGATION IN CAPYBARAS, MATO GROSSO DO SUL, BRAZIL

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In 2014, an epidemic of acute exanthematous disease caused by zika virus (ZIKV) spread to Brazil and other countries of the Americas. Despite ZIKV being zoonotic in origin, information about its potential circulation in wildlife species in the Americas is scarce. Considering the large geographic distribution and synanthropic characteristics of capybaras (*Hydrochoerus hydrochaeris*) in Brazil, we conducted an active surveillance to determine the exposure of free-ranging capybaras to ZIKV in the state of Mato Grosso do Sul, West-central region of Brazil. From February 2017 to March 2018, 21 free-ranging capybaras were captured using tranquilizer darts in urban parks of Campo Grande, state's largest city with a current population of 840,000 residents. Seeking active infection, capybara whole blood samples were screened for ZIKV nucleic acid through a pan-flavivirus real time RT-PCR followed by ZIKV specific RT-PCR and sanger nucleotide sequencing. Seeking previous exposure to ZIKV, we also tested capybara plasma samples for ZIKV neutralizing antibodies by plaque reduction neutralization test (PRNT90). Considering monotypic reactions to be the most reliable with no indication of cross-reaction, plasma samples that were ZIKV-seropositive were also tested for dengue, yellow fever and west nile viruses to discard heterologous reactions. All whole blood samples tested negative for ZIKV by RT-PCR, and three plasma samples (14%) showed monotypic reaction in low titers for ZIKV. Results presented here suggest no active transmission of ZIKV in capybaras between 2017 and 2018, and also low evidence of potential spillover exposure of capybaras to ZIKV in Mato Grosso do Sul.

Financial support: Centers for Diseases, Control and Prevention; Conselho Nacional de Desenvolvimento Científico e Tecnológico, Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro, Fundação Oswaldo Cruz.

Abstract #011

TROPISM OF DENGUE VIRUS TYPE 4 IN A BALB/C MURINE MODEL: EXPERIMENTAL INFECTION AND ANALISYS OF MORPHOLOGICAL ASPECTS

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Dengue is an emerging human disease, caused by the arthropod-borne virus named Dengue Virus (DENV). DENV is categorized in 4 genetically distinct individual serotypes (DENV-1 through -4). According to the CDC, around 2.5 billion people live in areas where there is risk of dengue transmission. Ever since its introduction in Brazil, in 1981, DENV-4 has remained absent from the national epidemiological scene for almost 25 years, until its reintroduction in 2010. To this day, the mechanisms associated with the immunopathogenesis of DENV are not yet fully understood. This is mostly due to the absence of an experimental animal model that adequately replicates the DENV infection as it is observed in humans. This simple fact brings about the greatest difficulty faced when studying the interaction between virus and host, as well as the development and production of effective drugs and vaccines against DENV. Currently proposed models utilize immunodeficient animals, very invasive inoculation routes, and neuroadapted viral inocula, conditions that do not reproduce the disease progression as it happens in human cases. The present study aims to analyze the potential tropism of DENV-4 for hepatic, pulmonary and cardiac tissue and evaluate the morphological and ultrastructural alterations caused by the virus in said organs. To achieve this goal, immunocompetent mice of the BALB/c line were inoculated via intravenous route with non neuroadapted doses of DENV-4 isolated from human case. Alterations observed in the analyzed tissue presented similar profile to that shown in human cases of Dengue, with intense presence of activated inflammatory cells, wide areas of hemorrhage, and alterations in the intercalated disc structure. Particular findings, such as the vast presence of inflammatory cells in cardiac tissue, and alterations on the intercalated disc's morphology – alterations not commonly described in heart – suggest that DENV-4 could have a stronger affinity with this organ.

Financial support: CNPq, IOC/Fiocruz.

Abstract #012

ZIKA VIRUS INDUCES OXIDATIVE STRESS IN LIVER AND BRAIN OF C57BL/6 MICE

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Zika disease is caused by *Zika virus* (ZIKV). In 2015, the first cases of ZIKV infection in the American continent, especially in Brazil, were reported. Fever, headache, rash and conjunctivitis are some symptoms of this disease. Also serious neurological damage such as microcephaly has been reported to this infection. Despite the importance many aspects of ZIKV pathogenesis remain unknown. Recent evidence suggests that oxidative stress could play an important role in the pathogenesis of viral diseases. The oxidative stress is established when there is a disruption/dysregulation of signaling and redox control caused by the increase of "Reactive Oxygen Species" and/or a reduction in the antioxidant defense system. Since previous studies have suggested that oxidative stress might play an important role in the pathogenesis of ZIKV *in vitro*, we investigated whether ZIKV infection causes this event in C57BL/ 6 mice. Ten-days-old C57BL/6 mice were infected intraperitoneally with 107 PFU of ZIKV and 8 days pi the animals were euthanized and serum, liver and brain obtained for dosages. All inoculated mice were susceptible to infection and exhibited clinical signs within 3 days p.i. manifested by prostration, ruffled hairs, shaking, curved body and a weight loss. The aspartate and alanine aminotransferases in the serum were significantly increased in infected animals suggesting notable involvement of hepatic injury. Lipid peroxidation and protein oxidation levels were measured by the thiobarbituric acid (TBARs) and protein carbonyls, respectively. ZIKV infection resulted a significant increase in the TBARs and protein carbonil levels in both organs. Since TBARs is a by-product of lipid peroxidation and protein carbonil is an oxidative modification in proteins marker, we believe that oxidative stress occurred during ZIKV infection. Although, we still intend to perform more experiments to better understand the relationship between ZIKV pathogenesis and oxidative stress.

Financial support: UFOP, FAPEMIG, CNPq, CAPES.

Abstract #013

ANTI-MAYARO VIRUS ACTIVITY DETECTED IN LEAVES AND BRANCHES OF *MAYTENUS SP.*

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The Mayaro virus (MAYV) is an arbovirus known to be endemic to the Amazon region and recently, after confirmation of its possible transmission by *Aedes aegypti* and emergence of cases in different regions of Brazil, the MAYV was classified as an emerging virus. Mayaro fever induces a disabling polyarthralgia, and there is no therapy or vaccine available against this virus. Therefore, the search for antivirals is important, highlighting the molecules of the plant kingdom, such as those originating from the genus *Maytenus* that present compounds for which biological activities have already been described, making the species of the group candidates for the prospection of antivirals. This proposal aims to investigate the presence of antiviral activity against the MAYV in extracts of *Maytenus sp*. Initially, four extracts of *Maytenus sp.* leaves (M7 to M10) were submitted to determination of the cytotoxic concentration to 50% of the cells (CC50). Then, *in vitro* antiviral assays were performed to evaluate the effective/protective concentration for 50% of the MAYV infected cells (EC50), with emphasis on the M9 extract that obtained an EC50 of 12.03 μg/mL, and a selectivity index (IS) of 80. When the plaque reduction capacity was evaluated at a moi 0.1, a significant reduction was observed in the number of plaque forming units (PFU) at concentrations 25 and 12, 5 μg/mL and total viral clearance at 50 μg/mL. When the action of M9 in the moi 1 and 5 it was observed that the extract still showed protection of the cells, with an EC50 of 40 μg/mL (moi 1) and 178 μg/mL (moi 5). The observation of the cell morphology also corroborates the results that M9 protected the cells, presenting cytopathic effects only in the concentrations below the EC50, in the three different viral charges evaluated. Thus, the results obtained from the *in vitro* tests indicates that M9 can prevent the evolution of the MAYV infection, which may lead to the development of anti-Mayaro drugs.

Financial support: CNPq, CAPES, FAPEMIG, UFSJ.

Abstract #014

ANALYSIS OF THE INTERACTION BETWEEN DENGUE VIRUS NS1 PROTEIN AND HUMAN PLASMINOGEN

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Dengue virus belongs to the *Flaviviridae* family and its genome encodes 3 structural and 7 nonstructural proteins. Amongst the latter, NS1 glycoprotein stands out, being the only one secreted by the infected cell. Previously, the interaction between NS1 and several liver proteins was mapped, including plasminogen. Once active, plasminogen acts on the coagulation cascade, cell migration and complement system, being essential for homeostasis.Thus, the objective established for this work is the investigation of this interaction to understand its effects in the context of a DENV infection. Firstly, purified plasminogen was incubated with serial dilutions of NS1 and labeled with antibodies against the viral protein so that interaction could be confirmed. The experiment was repeated, this time incubating NS1 with normal human serum and adding antibodies against plasminogen. In other assay, NS1 was incubated with pre-activated plasmin or serum and reaction samples were collected during 4 hours of incubation. Then, by Western Blotting, cleavage over time was verified by adding anti-NS1 antibodies. ELISA was also performed, but with the adsorption of uPA, to evaluate whether the viral protein could interact with plasminogen regulatory proteins. Next, BHK-21 cells were treated with NS1 for 1 hour and pre-activated plasmin was added to the culture. Afterwards, by flow cytometry, the enzyme's potential to remove the viral protein from the cell membrane was evaluated, as its presence in non-infected cells may be associated with facilitating the entry of viral particles into them. Thus, it was demonstrated that NS1 interacts with plasminogen and can be cleaved by its active form, but only in the absence of serum, suggesting its interaction with regulators, as confirmed for uPA. Also, plasmin is able to remove NS1 attached to cell membranes, which may result in a pro-host mechanism. Therefore, our group plans to elucidate the role of this interaction during DENV infection.

Financial support: FAPERJ, CNPq, CAPES, ICGEB.

Abstract #015

ORIGIN AND DISPERSION OF DENGUE VIRUS SEROTYPE 4 IN VITÓRIA, ESPÍRITO SANTO STATE, BRAZIL

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Dengue virus serotype 4 (DENV-4) was first reported in Vitória, Espírito Santo State, Brazil, in 2012. Due to its introduction, in 2013 occurred an epidemic with a record of 19,449 suspected cases. This study presents the origin of the DENV-4 and the patterns of disease spread during this epidemic. Ten sequences of NS1/envelope genes obtained from blood samples were used for the phylogenetic analysis. Secondary data from the System for Notifiable Diseases were used for the assessment of spatial variation in temporal trends using SaTScan™ and included 18,861 cases with address information reported from September 2012 to June 2013. DENV-4 genotypes I and II were identified. The genotype I was closely related to a strain from Bahia state collected in 2011. The genotype II was closely related to strains from Roraima state identified in 2010, Mato Grosso State in 2012, and São Paulo state in 2015. The introduction was originated from Venezuela and Colombia and spread to the Brazilian States between 4.7 to 12.1 years before 2015 (mean = 8.2 years). During the epidemic, eleven space-time clusters were detected. The Time Trend Increase (TTI) in the overall territory was 635.85%. Five space-time clusters presented a value lower than this (TTI range = 42.91%-356.62%), and six of them a value higher than this (TTI range = 1,238.95%-3,967.54%). Clusters with lower TTI presented higher relative risk (median = 1.48 (interquartile range = 1.48-1.87)) and lower-income (median = R$ 620.00 (interquartile range = 538-652)) than clusters with higher TTI (relative risk median = 0.93 (interquartile range = 0.54-1.19), income median = R$ 1,450.00 (interquartile range = 1,136-2,260)) (p-value >0.02). DENV-4 suffered a local evolution in Brazil, reaching Vitória from other States. Then, the transmission in low-income areas was explosive, with a fast reduction due to population herd immunity. These areas played a role of hotspots, dispersing dengue to near areas.

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Abstract #016

MODULATION OF INNATE IMMUNE RESPONSE IN ENDOTHELIAL CELLS DURING OROPOUCHE VIRUS INFECTION

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Oropouche orthobunyavirus (OROV) is an emerging arbovirus associated with a fever illness called Oropouche fever in the Amazon region of South and Central America. Moreover, OROV can cross the blood-brain barrier and cause central nervous system infection in humans and others vertebrates, such as golden hamster and neonate mice. However, the pathogenic mechanisms associated with the blood-brain barrier breakdown is not fully understood. Thus, to characterize the OROV infection and gene modulation in endothelial cells, we infected human (HBMEC) and murine (BEND3) immortalized endothelial cells with different Multiplicity of Infections (MOIs) and determined the viral load, cell viability and the level of expression of genes genes related with innate immune response and endothelial adhesion during 1, 4, 12, 24, 48 and 72 hours post infection by focus forming assay, MTT and qRT-PCR, respectively. Immunofluorescence was also performed to analyze the structure of endothelial tight junctions using antibodies against ZO-1 and OROV. Interestingly, although OROV is able to replicate in both endothelial cells, the morphological alterations induced by this infection are different in HBMEC and BEND3 cells. While HBMEC cells are lysed during OROV infection, no death was observed in BEND3 cells until 72 hpi. The OROV infection induced a strong antiviral response in BEND3, with increased expression of TLR7, IRF5, IFN-b, IFIT-1, OAS1L and MX-1. In addition, the OROV replication in BEND3 cells induced a expression of adhesion endothelial factors such as ICAM-1. In contrast, nor Interferon Induced Genes (ISGs), TLR7 and IRF5 nor adhesion endothelial factors were induced in HBMEC cells after OROV infection. Consequently, endothelial Tight-Junctions were not disrupted during OROV infection as seen by anti-ZO-1 staining assay. Thus, we can speculated that OROV is able to antagonize antiviral response in human cells but not in mouse endothelial cells.

Financial support: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Abstract #017

THE INNATE IMMUNE RESPONSE AND ANTIBODY PRODUCTION BY B CELLS ARE ESSENTIAL FOR RESTRICTION OF OROPOUCHE VIRUS PRIME-INFECTION

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Several arboviruses have emerged or reemerged and dispersed globally in the last years, such as Zika, West Nile and Chikungunya viruses. Brazil has the largest virus diversity in the world and Oropouche (OROV) and Mayaro (MAYV) viruses are pointed as possibly responsible for new outbreaks of arthropod-borne viral diseases in others regions of the world. Oropouche infection is associated with a febrile illness, characterized by symptoms such as rash, photophobia, myalgia, and polyuria. Moreover, some patients have neurologic complications after OROV infection, as encephalitis and meningitis. However, the pathogenic determinants associated with neurological involvement is not fully understood. Thus, the aim of this study was to investigate the role of B cells for protection against neuroinvasion by OROV in a mouse model. For this, we determined morbidity, mortality and viral tropism of 4-5 weeks old C57BL/6 WT mice (n=10), Rag1 KO mice (lacking both mature B and T cells, n=10), μMT mice (lacking only mature B cells, n=10), and TCRbd mice (lacking only mature T cells, n=10) after infection of OROV by subcutaneous route. Interestingly, while WT and TCRbd mice were resistant to infection, Rag1 KO and μMT mice were vulnerable and died with signs of neurologic involvement. The viral load determined by focus forming assay showed a liver and brain tropism as soon as three days after infection. In addition, sera harvested from day 6 post-infection could prevent Rag1 KO mice from neurologic disease, while sera from WT uninfected mice were not able to protect Rag1 KO mice. In the end, $CD19^{Cre}$-$MyD88^{flox/flox}$ were partially vulnerable to OROV infection. In short, our results suggest that antibody production within 6 days, possibly IgM isotype, and the innate immune response by B cells are essential to restrict OROV replication and neuroinvasion in mice.

Financial support: São Paulo Research Foundation (FAPESP).

Abstract #018

IDENTIFICATION OF DIFFERENTIALLY EXPRESSED miRNAS IN HUMAN PROSTATIC CELLS INFECTED WITH ZIKV

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Zika virus (ZIKV) is a virus transmitted mainly by *Aedes aegypti* mosquitoes and infection with distinct ZIKV strains in some models *in vitro* and *in vivo* has demonstrated that the host's response to infection is strain-dependent. Recent findings suggest that this virus deregulates host miRNA profile and that this is an important event throughout the course of the infection. Herein, we evaluated the susceptibility, the permissiveness and the cellular miRNA profile of human prostatic epithelial cells (PNT1A) to two different strains of ZIKV, a classical African strain, MR766 ($ZIKV^{MR766}$) and a Brazilian strain, $ZIKV^{BR}$. So, we infected PNT1A cells with ZIKV strains and performed an indirect immunofluorescence assay for protein envelope; monitored infectious viral particles production and RNA viral copies by plate assay and qPCR, respectively, and analyzed the miRNA cellular profile by PCR array. Our results demonstrated that human prostate cells are susceptible and permissive to ZIKV infection and did not present any imposition regarding infection by distinct strains of this virus. The strains did not differ in the kinetics of replication in prostate cells, but presented differences in miRNA's cell expression modulation. After infection, 16 miRNAs were modulated in prostate cells, a small group of 6 miRNAs were modulated by both strains while a set of 10 miRNAs showed to be modulated exclusively by $ZIKV^{BR}$. *In silico* analyses predicted that the miRNA upregulated exclusively by the infection by the Brazilian strain may regulate genes and pathways associated to inflammation, immunity, cell survival and cell proliferation. Taken together, our results indicate that prostate may be an important role in the sexual transmission of ZIKV and highlights that different strains of ZIKV may induce a differential host miRNA expression which may influence the differences in the physiopathology presented after the infection by different strains.

Financial support: FAPESP.

Abstract #019

CIRCULATION OF DIFFERENT DENGUE VIRUS SEROTYPES IN *AEDES AEGYPTI* FEMALE MOSQUITOES IN GOIÂNIA-GO

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Aedes aegypti is the main vector associated with dissemination of dengue virus (DENV) throughout the Americas. In Brazil, DENV is a major public health problem, especially due to mosquito infestation and proper environmental and urban conditions. In the central-western region of the country, Goiânia is one of the most affected cities, where dengue outbreaks are frequent and epidemiological surveillance is constant. Although there are several services attempting to monitor vector infestation, virological surveillance in mosquitoes is not yet established. In this sense, this study investigated the occurrence of dengue virus in *Aedes aegypti* mosquitoes from Goiânia, Goiás. Specimens were collected during the first semester of 2017, in peridomicile areas of neighborhoods located in North, South, Center, East, West, Northwest and Southwest regions of the city. After sorting for species and sex identification, female specimens were packed in cryotubes and transferred to laboratory. Pools of 10 to 14 mosquitoes were prepared by maceration and suspension in PBS buffer, followed by extraction of viral RNA using a commercial kit and DNAse treatment. Molecular investigation and serotyping through conventional RT-PCR followed by Semi-Nested PCR protocol were performed and amplification products were analyzed in agarose gel electrophoresis. A total of 1,011 mosquitoes distributed in 84 pools were analyzed, 12 pools from each region. A global positivity index of 6.0% (5/84) was observed. DENV-1 (3.6%), DENV-2 (1.2%) and DENV-4 (1.2%) were detected in the North, South, West and Southwest regions. According to epidemiological data, these were the same serotypes circulating in Goiânia in 2017. Considering vector control as the only effective method for dengue prevention, establishment of circulating serotypes and areas with infected mosquitoes is relevant for determining hotspots for transmission and directing prevention and control efforts.

Financial support: Fundação de Amparo à Pesquisa do Estado de Goiás (FAPEG).

Abstract #020

OCCURRENCE OF DENGUE VIRUS SEROTYPE 2 IN GOIÂNIA-GOIÁS DURING THE 2018 EPIDEMIC PERIOD

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Dengue is the most important arboviral disease in the world, considered endemic in more than 100 countries and mainly affecting the American continent. Brazil has been suffering the consequences of successive dengue fever epidemics for more than 30 years, as the presence of widely populated urban centers infested by main vector, *Aedes aegypti*, has given the ideal conditions for virus dissemination. Since DENV-1 introduction in Goiás, the scenario has consisted of successive epidemics with alternation in serotype predominance. Thus, identification of circulating serotypes is relevant information for disease surveillance. In this sense, this study aimed to investigate the occurrence of dengue virus in serum samples from patients attended at public health facilities, presenting 1 to 7 days of classical symptoms for dengue fever, during the 2018 epidemic period in the city of Goiânia-Goiás. Serum samples collected were submitted to viral RNA extraction by commercial kit, following manufacturer's instructions. Molecular investigation by real time RT-PCR and serotyping through conventional RT-PCR followed by Semi-Nested PCR protocol were performed. A total of 58 samples were analyzed, and a general positivity index of 32.7% (19/58) was observed. DENV-2 was the only serotype identified. The results corroborate with data provided by the Municipal Government for the same sample period, when DENV-2 was the only serotype circulating in Goiânia. By the end of the year, DENV-1, DENV-2 and DENV-4 were notified, an epidemiological profile that has been in place since 2014. This study contributes with relevant data to the monitoring of DENV epidemics and reinforces the importance of constant surveillance for dengue virus in such endemic areas, as the identification of circulating strains is crucial for elucidation of dispersion and virulence patterns of disease.

Financial support: Fundação de Amparo à Pesquisa do Estado de Goiás (FAPEG).

Abstract #021

SILYMARIN INHIBITS OXIDATIVE STRESS INDUCED BY MAYARO VIRUS INFECTION IN LIVER OF BALB/C MICE

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Although little known to the population, Mayaro virus (MAYV) is the cause of an old disease. In Brazil, Mayaro fever was endemic in the Amazon region, however, in recent years outbreaks of MAYV have increased considerably in the metropolises, and their circulation in Southeastern Brazil has recently been identified. In addition, the potential of *Aedes aegypti* in transmit MAYV contributes to the virus being able to install an urban cycle in the near future. Clinical manifestations begin with a nonspecific acute fever, characterized by highly debilitating arthritis/arthralgia that may persist for months, just like in Chikungunya. The treatment is symptom-based and there are no vaccines or antiviral drugs available. Recently we demonstrated that oxidative stress plays an important role in the MAYV infections, and compounds capable of inhibiting oxidative stress could represent a novel therapeutic approach in modulating MAYV-associated oxidative cellular damage. In this context, we show that silymarin has promising antiviral and antioxidant actions against MAYV *in vitro*. Thus, we evaluated whether silymarin would be able to reduce the damages caused by oxidative stress induced by MAYV infection, in three-week BALB/C mice, infected subcutaneous with 10^7 PFU, and treated with 100 mg/kg/day silymarin administered by gavage. With 5 dpi the animals were euthanized, the liver and blood were obtained for dosages. Weight loss, increased serum levels of hepatic transaminases (ALT and AST), increased hepatic levels of oxidative stress biomarkers (lipid peroxidation and protein oxidation), thrombocytopenia, leukopenia, red blood cell and hemoglobin reduction were observed in infected animals. After silymarin treatment all these parameters were reversed significantly. Furthermore, RT-qPCR analyzes of the liver exhibited a drastic reduction in MAYV genome detection after treatment, showing the promising antioxidant and antiviral potential of silymarin in MAYV infection *in vivo*.

Financial support: Capes, Fapemig, UFOP.

Abstract #022

EVALUATION OF THE ANTI-VIRAL EFFECT OF *MAYTENUS* SP. BRANCH EXTRACTS ON *ZIKA VIRUS*

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Zika virus (ZIKV) is an emerging arbovirus in Brazil and became a public health problem in 2015, when its infection was associated with an increase in the number of cases of children born with microcephaly. Currently, there is no vaccine or specific treatment for this disease. Aiming promising sources for effective treatments against ZIKV and other arboviruses we are investigating plants that are rich in secondary metabolites that can have varying biological effects. The plants of the genus *Maytenus*, have proven effect antitumor, antibacterial and anticholinesterase. Thus, the aim of this study was to evaluate the antiviral effect of *Maytenus* sp. branch extracts on ZIKV. First, the cytotoxicity assay was performed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric method, using the Vero mammalian cells. After 48 hours of treatment with hexane, chloroform, ethyl acetate and methanol extracts, at concentrations of 7.8 to 1000 μg/mL, the toxic dose for 50% of cells (CC50) was determined. Therefore, concentrations lower than CC50 were used for the global antiviral assay, also by the MTT method. Vero cells and ZIKV (moi 0.1) were treated with differents extracts for 30 minutes and soon after, pooled and kept in treatment for 48 hours. Thus, the effective concentration to protect 50% of infected cells (EC50) was determined and the selectivity index (SI) of the extracts was calculated by the ratio CC50/CE50. The chloroquine was used as a positive control. Among the four extracts tested, the ethyl acetate extract presented the best results with 1000 μg/mL CC50 and 55.6 μg/mL EC50, reaching an SI of 18. Thus, it can be inferred that *Maytenus* sp. ethyl acetate extract had a potent antiviral effect against ZIKV. Fractionation of the extract is necessary to separate its compounds and identify which is responsible for the antiviral effect.

Financial support: CAPES, UFSJ, FAPEMIG, CNPq.

Abstract #023

ANALYSIS OF ANTIVIRAL EFFECTS OF MEK/ERK INHIBITOR ON ZIKV INFECION IN AN ANIMAL MODEL

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Zika virus caused, between 2015 and 2016, epidemics in America, Africa, Pacific and southeast of Asia, and has been declared a world public health concern by WHO due the disease association with neurological problems and severe newborn sequelae. Our objective was to evaluate the efficiency of the MEK inhibitor Mekinist (TRA) in the treatment of ZIKV infection. First, we establish an infection model using IFNAR -/- SV 129 mice through an experiment with two different infective doses, 10^3 and 10^2 PFU/animal and evaluate the disease signs, body weight and mortality. We observed five main signs: piloerection, dorsal arching, locomotion problems, conjunctivitis and paralysis. After 14 days, we observed 25 and 75% of mortality with the infective doses of 10^2 PFU and 10^3 PFU, respectively. We then tested the efficiency of the inhibitor in the treatment, using the dose of 10^3 PFU. The treatment was done with a dose of 2mg/kg of TRA diluted in CMC 0,5% once a day, between the first and the tenth day post infection (d.p.i.) In the eight d.p.i. animals that presented locomotion problems and paralysis were euthanized and the liver, spleen, brain and testicles were collected. The viral load (qPCR) and the viral titers (pfu/ml) were then determined. We observed that in the group of treated animals some of the signs were delayed, and for the females there was an increment on the survival rate of 40% at 14 d.p.i. Alterations in the viral load were verified only in the brain, with a reduction of 20 fold (genomic equivalents) and 80 fold (viral titers). Next, lower infective doses (10 and 50 pfu/animal) will be tested with groups of infected and either TRA-treated or untreated mice and evaluate the survival rate and body weight of the mice during the course of the infection.

Financial support: CNPq, CAPES, FAPEMIG/PPSUS, PRPq-UFMG, Ministry of Health and Health Department of the State of Minas Gerais.

Abstract #024

MAYV CHARACTERIZATION AND ANALYSIS OF ANTIVIRAL EFFECTS OF SIGNALING PATHWAY INHIBITORS

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MAYV belongs to the genus *Alphavirus*, family *Togaviridae* that also includes the CHIKV. MAYV is found mainly in Central and South America causing a febrile illness followed by arthralgia that can persist for long periods of time. MAYV fever is considered a neglected disease due the few epidemiologic data and in Brazil is a potential public health hazard with the number of suspicious cases increasing every year. The first aim of this work was to characterize our MAYV sample. Accordingly, we started with multiplication curves in VERO cells with different MOIs followed by transmission electron microscopy to the analysis of the multiplication cycle. For the genetic approach we used self-designed primers to sequence the sample through the E1 gene. We also did initial studies with various pharmacological inhibitors looking for possible antiviral targets through plaque assay in VERO cells. At least we did a mortality curve with IFNAR -/- SV129 mice and two different infective doses 10^3 and 10^2 PFU. We observed a fast multiplication cycle with a short eclipse phase of 4 h.p.i. followed by the exponential phase with the peak of multiplication occurring at 18 h.p.i. with all cell monolayer destroyed at 24 h.p.i. In the antiviral tests, we observed that the MEK/ERK and JNK inhibitor reduced at more than 90% the viral load at 15 h.p.i. In mice we observed 100 % of mortality at 3 d.p.i. for 10^3 PFU and 100% at 4 d.p.i. for 10^2 PFU. The next steps of this work will be the analysis of sequencing and TEM, continuing of the biological characterization and animal studies.

Financial support: CNPq, CAPES, FAPEMIG/PPSUS, PRPq-UFMG, Ministry of Health and Health Department of the State of Minas Gerais.

Abstract #025

THE IMPACT OF TWO DENGUE VIRUS TYPE 2 LINEAGES ON HEPATORENAL AXIS IN IMMUNOCOMPETENT MOUSE MODEL

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Dengue is the leading cause of disease and death in tropics and subtropics and represents a serious economic burden. Around 40% of the world's population are at risk of infection. Dengue is caused by one of the four distinct serotypes of dengue virus (DENV-1, -2, -3 and -4) and presents a broad spectrum of manifestations, with unpredictable clinical evolution/outcome and involvement of different organs is observed. The most frequently affected organ is the liver, however, DENV has been detected in kidney samples, and although DENV-induced renal dysfunction is not often observed, increase of creatinine level and proteinuria have been reported as well as histopathological alterations and acute renal failure. Therefore, the purpose of this study is investigating the impact of infection of two DENV-2 Lianeages on the hepatorenal axis using an immunocompetent mouse model. Ten BALB/c mice were infected with each DENV-2 Lineage. The mice were euthanized 72 hours post-infection and samples were processed for histopathological analysis. Results show that infection outcome is similar for both Lineages. Liver samples showed inflammatory infiltrate, sinusoid capillary dilation, micro and macrovesicular steatosis, vascular congestion, enlarged nucleus and other nuclear atypia and hemorrhage. There was a slight increase in number of binucleated hepatocytes in infected samples, which also presented inferior number of this cell population when compared to negative control. Concerning kidney, the observed alterations were tubular necrosis, cytoplasmic inclusions, glomerular atrophy, obliteration of urinary space and hemorrhage. Moreover, it was observed that the mean weight of infected kidneys was superior when compared with negative control. Besides the liver, which is a well known target of DENV, kidneys are also involved during the course of infection, thus the role played by hepatorenal axis in dengue immunopathogenesis should be further investigated.

Financial support: Instituto Oswaldo Cruz, CNPq.

Abstract #026

INTRACELLULAR SIGNALING AND METABOLIC PATHWAYS TRIGGERED DURING THE ANTIBODY-SECRETING CELL DIFFERENTIATION UPON THE DENGUE VIRUS INFECTION

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Dengue is a mosquito-borne viral disease that affects annually about 400 million people worldwide. Caused by four Dengue virus (DENV) serotypes, it ranges from asymptomatic to life threatening forms. Curiously, Dengue patients present an exacerbated and transient blood antibody-secreting cell (ASC) response around seven days after the symptoms onset. The frequency of those DENV-induced ASCs represents more than 50% of all circulating blood B cells, which is greater than found for other infections, immunizations and plasma cell-derived leukemia. Moreover, the magnitude of that response directly correlates with disease severity. Thus, how does the DENV infection induce this enormous response? To answer this question, we initially cultured peripheral blood mononuclear cells (PBMCs) from healthy individuals with DENV TVP/360 particles. Upon the DENV or mitogen (positive control) stimulation, PBMCs displayed enhanced number of cells with ASC phenotype and function in comparison to mock. The full DENV-mediated ASC differentiation seem to be dependent on the presence of other cells contained in the PBMC as well as cell-cell contact. In contrast, the DENV- and mitogen-mediated ASC differentiation clearly differed among each other regarding their intracellular signalling and metabolism pathways. Public transcriptome data obtained from DENV patients support our *in vitro* findings. Furthermore, the Zika virus infection stimulate a lower activation of the DENV-specific pathways and does not elicit such robust ASC responses. Therefore, our data suggest the involvement of novel pathways in the massive ASC differentiation process observed in Dengue patients.

Financial support: CAPES, CNPq and FAPESP.

Abstract #027

CHARACTERIZATION OF TIM AND TAM RECEPTORS EXPRESSION IN PLACENTA OF PREGNANT WOMEN WITH ZIKA VIRUS INFECTION DURING 2016 OUTBREAK IN CAMPINAS/SP

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The aim was to characterize TIM and TAM receptors expression in placenta of pregnant women naturally infected with Zika virus (ZIKV). For this, fragments of placenta were obtained of 17 women who presented symptoms compatible with arbovirus infection during pregnancy following a systematic protocol for placental sampling. 04 fragments of five different regions, umbilical cord, chorionic villus, chorionic plate, basal plate and amniotic membrane, were collected. The acute phase symptoms occurred during first trimester in 03 women, second trimester in 06, and during third trimester in 08. Two of the 17 newborn presented Congenital Zika Syndrome (CZS), evolving either to neonatal death. 09 pregnant women had positive detection to ZIKV in the acute phase samples, such as urine, blood and serum samples. Of the total placentas collected, 14 presented ZIKV positivity in different regions by RT-qPCR assay. Therefore, 05 pregnant women were misreported during acute phase of ZIKV infection based on the data obtained by placental detection. There are no significant differences in the TYRO3, AXL (TAM receptors) and TIM1 (TIM receptor) expression comparing infected and uninfected placentas by RT-qPCR assay. Thus, apparently, ZIKV infection does not modulate TIM and TAM receptors expression in different placental regions in humans. In addition, the results indicate that ZIKV can infect different regions of placentas of naturally infected pregnant women, and its detection after several weeks or months of the initial symptoms suggests that this organ can be site for viral persistence during pregnancy.

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Abstract #028

LATE RELAPSING HEPATITIS AFTER YELLOW FEVER

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After two months of yellow fever (YF) onset, one patient presented hyporexia, asthenia, adynamia, and jaundice, with laboratory tests indicating rebound in transaminases (ALT>AST), total and direct bilirubin levels. The patient was followed up and monitored, and tests were run to investigate the case. Tests discarded autoimmune hepatitis, other inflammatory liver diseases, metabolic liver disease, or new infections caused by hepatotropic agents. IgM and neutralizing antibodies were detected, but no viremia for yellow fever virus (YFV). A liver biopsy was collected three months after the onset of yellow fever and tested positive for the presence of wild-type YFV RNA (364 genomic copies/gram/liver), also presenting continued cell damage and YFV antigens, mostly in zones 2 and 3 of the hepatic acini. Transaminases and bilirubin levels remained elevated for five months, and the arresting of symptoms was reported for six months after the onset of YF. Several serum chemokines, cytokines, and growth factors were measured, and a similar immune response profile was observed when earlier phases of convalescence were compared (weeks 5 and 11 after YF onset), but more pronounced changes were observed in later stages (week 28 forward), coinciding normality of transaminases. The results indicate viral persistence in the liver, after three months of YF onset, and reinforce the need of extended follow up of YF patients and further studies to investigate the role of a possible viral persistence and the immune response causing relapsing hepatitis following yellow fever.

Financial support: CNPq, Capes, FAPEMIG, PRPq-UFMG, Ministério da Saúde-DECIT.

Abstract #029

THE IMPACT OF MODELING AND DATA ANALYSIS ON PUBLIC HEALTH PRACTICAL INTERVENTION: DENGUE FEVER, A CASE STUDY

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In recent years, mathematical modelling became an important tool for the understanding dengue fever epidemiology and dynamics, acting as a possible tool to understand, predict and develop strategies to control the spread of disease and used to evaluate the introduction of intervention strategies like vector control and vaccination. Dengue fever epidemiological dynamics shows large fluctuations in disease incidence, and several mathematical models describing the transmission of dengue viruses have been proposed to explain the irregular behavior of dengue epidemics. In this talk, we present a set of models motivated by dengue fever epidemiology and compare different dynamical behaviors originated when increasing complexity into the model framework, anticipating that temporary cross-immunity and difference between primary and secondary infections appear to be the key factors determining disease transmission, outcome of infection and epidemics. The extended models show complex dynamics and qualitatively a very good result when comparing empirical data and model simulations. The models are parametrized on the official notification dengue data from Thailand. Three real scenarios will be evaluated and the impact of empirical data analysis on public health practical intervention will be discussed.

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Abstract #030

HEPATORENAL AXIS INVOLVEMENT DURING INFECTION BY DENGUE VIRUS SEROTYPE 3 IN IMMUNOCOMPETENT MURINE MODEL

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Tissue changes during dengue are commonly found in the liver, corroborating the hypothesis that this organ is one of the main targets for DENV. Nevertheless, renal involvement has been described and seems to play an important role in mortality caused by infection. The aim of this study was to evaluate the involvement of the hepatorenal axis in the course of DENV-3 infection through morphological and biochemical analyzes. Male BALB/c mice were intravenously infected with DENV-3 and euthanized in 72 h.p.i (hours post infection), 7 and 14 d.p.i (days post infection). A significant increase in kidney weight at all times of infection was an unexpected finding. A decrease in transaminases and urea BUN levels and an increase in serum creatinine values were observed at all times of infection. Morphological analysis of liver fragments of BALB/c 72 h.p.i mice demonstrated dilation of sinusoid capillaries, increase in amount of sinusoidal cells, vascular congestion, cellular infiltrates and steatosis. Signs of nucleus enlargement, alteration in the chromatin distribution pattern and presence of atypical nuclear inclusions were observed. In the kidneys, morphological analysis revealed glomerular atrophy, congestion, infiltrating cells, presence of cytoplasmic inclusions in proximal convoluted tubule epithelial cells, areas of absence of glomeruli and increase of proximal convoluted tubules microvilli density. Presence of pycnotic nuclei, endothelial adhesion of platelets, signs of intense protein synthesis activity and presence of dengue virus like particles in interstitial cells were observed. The results described show that, although the liver is described as the main target organ during the course of DENV infection, renal involvement seems to play a fundamental role in the pathophysiology of the disease, and further research on this axis is needed.

Financial support: IOC, CNPq.

Abstract #031

DENGUE SEROTYPE 3 VIRUS INFECTION IN BALB/C MICE: CLINICAL CHANGES, MACROSCOPY, VIRAL DETECTION AND HEMOGRAM

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Several epidemiological studies have correlated DENV-3 infection with severe conditions, and, in Brazil, this serotype shows a greater association with severer signs and symptoms, such as shock, abdominal pain and exanthema, compared to other serotypes. The establishment of animal models for studies of DENV infections is of great relevance for research on pathogenesis, immunity, development and testing of drugs and vaccines. However, said studies have met numerous challenges, since circulating epidemic viruses do not naturally infect nonhuman species. In this scenario, the main goal of this study was verifying clinical and macroscopic alterations in BALB/c mice experimentally infected with DENV-3, as well as quantifying the viral load in organ, serum and saliva samples and possible infection-induced blood count changes. Male BALB/c mice, 2 months old, were intravenously infected with DENV-3 and euthanized in 72 h.p.i (hours post infection), 7 and 14 d.p.i (days post infection), according to the analyzes to be performed. During the animal experimentation stage, no mice died and all animals were euthanized. Five animals (20%) showed hair raising at 72 h.p.i. Rectal temperature increase (0.7ºC $p = 0.013$) was observed in 72 h.p.i mice compared to the control group. No variations in body weight were observed between 72 h.p.i and control mice. Macroscopic analyzes of the cranial vault of BALB/c 72 h.p.i mice showed focal accumulation of blood in the anterior region of the dura mater. Blood count analysis showed a significant increase in hematocrit ($p=0.008$) in 14 d.p.i, a reduction in leukocyte count at all times of infection and a significant reduction in platelet count in 7 d.p.i ($p=0.002$) and 14 d.p.i ($p=0.003$). Although low viremia was observed in 72 h.p.i, the viral genome was detected in at least one of each organs tested, as well in saliva samples, with titers higher than those used in experimental inoculation. These results demonstrate the susceptibility of BALB/c mice to DENV-3 infection.

Financial support: IOC, CNPq.

Abstract #032

SEROPREVALENCE OF CHIKUNGUNYA VIRUS IN SYMPTOMATIC INDIVIDUALS IN GOIANIA-GOIAS

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Vector-borne diseases are an important problem of the public health worldwide. Chikungunya fever is an emergent arbovirus disease caused by chikungunya virus (CHIKV), a pathogen of the Alphavirus genus, Togaviridae family. The virus is transmitted mainly through the bite of female mosquitoes of the genus Aedes. Acute fever and polyarthralgia are highly indicative of an infection, with arthralgia appearing in 30–90% of cases. The seroprevalence rates for CHIKV vary between 10.2% and 75% in different countries. In Brazil, particularly in the Central-West region, studies that evaluate the seroprevalence CHIKV are scarce. In this context, this study aimed to investigate the seroepidemiological profile of chikungunya virus infection in the population from Goiânia-Goiás, during the years 2016 and 2018. Serum samples were collected from 174 individuals with clinical manifestations suspected of CHIKV infection, attended at public health facilities, during the months of February to August 2016 and from April to May 2018. The serum samples were submitted to the enzyme-linked immunosorbent assay (ELISA) for specific antiCHIKV-IgG antibodies detection, following the manufacturer's instructions. Of the total samples analyzed, it was observed a global detection rate of 2.3% (4/174). Analysis considering the year of collection and the age of the individuals revealed higher positivity for the sample group of 2018 and among individuals over 30 years, although without statistical difference. Regarding gender, a statistically higher positivity index was observed among males (8.0%) ($p < 0.05$). In all the positive cases, the individuals sought medical attention reporting fever, myalgia and multiple arthralgias between two to five days. The results obtained in the present study help to understand the epidemiological and temporal factors associated with CHIKV and demonstrate the need for continuous monitoring and investigation of the circulation of this agent in the region.

Financial support: Fundação de Amparo à Pesquisa do Estado de Goiás (FAPEG).

Abstract #033

INVESTIGATION OF THE INTERACTION BETWEEN HUMAN APOLIPROTEIN A1 AND DENGUE VIRUS NS1 PROTEIN

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Dengue is the most important neglected tropical disease caused by arbovirus. Nearly half of the world's population lives in endemic areas. Each year about 390 million infections are estimated, leading to more than 20 thousand deaths, and costs charged for billions of dollars worldwide. Dengue virus (DENV) belongs to the *Flaviviridae* family, and it is transmitted by Aedes mosquitoes. DENV have four different serotypes, which is a major challenge for vaccine production. Symptomatic dengue is characterized by fever, aches, and rash. Some cases may progress to severe dengue, showing signs of hemorrhage, plasma extravasation and shock. There is no specific treatment for dengue, but oral or intravenous hydration. Low levels of HDL are also related to severe dengue. HDL is responsible for reverse cholesterol transport (RCT) and regulation of cholesterol levels on peripheral tissues. Apolipoprotein A1 (ApoA1) is the major protein component of HDL, and here we describe its interaction with the DENV nonstructural protein 1 (NS1). NS1 is secreted by infected cells and can be found circulating in the serum of patients since onset of the symptoms. NS1 concentration in plasma is related to dengue severity, attributed to immune evasion and harmful inflammatory response. We show here that NS1 protein induces the increase of cholesterol rich domains (lipid rafts) on non-infected cell membrane and enhances further DENV infection. We also show that ApoA1-mediated lipid raft depletion inhibits both DENV infection and replication. In addition, ApoA1 was also able to neutralize NS1-induced cell activation, and to prevent NS1-mediated enhancement of DENV infection. Furthermore, we show that D4F mimetic peptide, originally developed for treatment of atherosclerosis, is also capable of mediating lipid raft depletion in order to control DENV infection. Taken together, our results suggest the potential of RCT-based therapies for dengue treatment. However, in vivo studies are still needed to assess the importance of RCT in dengue infection.

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Abstract #034

STUDY ON THE VIABILITY OF CHIKUNGUNYA AND MAYARO VIRUSES IN BLOOD PRODUCTS

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Arboviruses are cause of many human diseases in tropical and subtropical countries. Their diseases involve clinical manifestations ranging from asymptomatic infection, acute febrile illness to hemorrhagic fever, and encephalitis. Moreover, these arboviruses are usually underdiagnosed. Thus, due to the high number of asymptomatic infections and their high viremias, blood donors can transmit these viruses via transfusion. Chikungunya (CHIKV) and Mayaro (MAYV) viruses have caused epidemics in different regions of Brazil. These outbreaks are probably related to environmental factors such as climate and in the case of CHIKV, the high urban infestation by *Aedes* mosquitoes. During outbreaks of CHIKV and MAYV, infected viremic donors become a serious risk to the safety of blood products. We show here an evaluation on the viability of CHIKV, MAYV in blood products, assessing the risk of transfusion transmission of these viruses. Blood bags that would be discarded after undergoing evaluation and certification of any type of infection were artificially infected with previously quantified MAYV and CHIKV. The technique for infection injecting the virus into the tubular portion of the whole blood bag of the whole blood bags was standardized. After infection, the bags were incubated and homogenized for one hour at room temperature for virus adsorption. Blood products (red blood cells concentrate, platelet concentrate and fresh frozen plasma) were obtained by centrifugation of these infected blood bags. Subsequently, CHIKV or MAYV infections were searched in the blood products by indirect immunofluorescence, plaque assays for virus quantification and viability of the virus and also, RNA has been extracted from the samples for virus genome detection using RT-PCR techniques.

Financial support: CNPq and FAPESP.

Abstract #035

ESTABLISHMENT OF MODELS OF ILHÉUS VIRUS INFECTION *IN VITRO* AND *IN VIVO* AND INVESTIGATION OF THE 2'C-METHYLCYTIDINE TREATMENT OVER DISEASE DEVELOPMENT

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Ilhéus virus (ILHV) is a mosquito-borne flavivirus of the Japanese Encephalitis serocomplex, which comprises relevant viruses such as St. Louis Encephalitis, West Nile and Japanese Encephalitis viruses. As a neglected tropical arboviral disease circulating in Latin America, ILHV infection poses a significant treat for vulnerable populations, causing a febrile disease with symptoms ranging from mild fever to severe neurological disease. Little is known about the mechanisms of disease development and no vaccines or treatments are available. Our objective was to establish parameters for study of ILHV infection *in vitro* and *in vivo* and delineate possible therapeutic strategies against this virus. For the *in vivo* models, we assessed lethality, inoculation route, symptoms development, inoculum-response, affected organs and variations of disease development regarding sex in immunodeficient (A129) and wild-type (FVB) mice. We established ILHV infection models in cultures of monkey kidney cells (VERO) and neuroblastoma cells (SH-SY5Y). Three different classes of compounds were selected for testing: galanin, itaconic acid (IA) and 2'C-methylcytidine (2'CMC). Only 2'CMC was able to significantly reduce viral load and investigation of its role over ILHV infection has progressed to determination of CC_{50} and EC_{50}, time-of-drug-addition and the survival experiments *in vivo*, in which ILHV-infected mice were treated with 2'CMC or vehicle. Our results show that treatment with 2'CMC *in vitro* in concentration as low as 30µM significantly reduced the viral load of ILHV and cytotoxicity wasn't observed until 100µM, lying within a safe concentration interval regarding the CC_{50} described for 2'CMC in literature. Administration of 2'CMC *in vivo* resulted in prolonged survival and delayed disease onset in a highly susceptible model, thus pointing 2'CMC as a possible treatment for ILHV infection.

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Abstract #036

DEVELOPMENT OF A RECOMBINANT CHIMERIC VACCINE AGAINST ZIKA VIRUS

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Flaviviridae family at *Flavivirus* genus, includes several arboviral species that are important for public health in Brazil, as Yellow Fever virus (YFV), Dengue virus (DENV) and Zika virus (ZIKV). Flaviviruses present a genome that encodes a single polyprotein, which is further cleaved to form the structural (capsid, precursor membrane and envelope) and the non-structural (Ns1-Ns5) proteins. ZIKV was introduced in Brazil in 2015, producing a dengue-like epidemic that was further associated with a congenital Zika syndrome, which affects the central nervous system and may result in microcephaly to the newborns. Furthermore, ZIKV infections are associated with cases of flaccid paralysis by Guillain-Barré syndrome. Thus, the development of a protective vaccine against Zika virus infections is of high importance for Brazil. The present study aims to develop a chimeric vaccine against ZIKV. For that, recombinant virus has been engineered and its whole genome has been cloned into a plasmid vector under T7 promoter control. The viral construct includes the backbone of YFV strain 17DD with its structural prM and E genes exchanged by the respective genes of ZIKV. Attempts of YFV17DD/ZIKV viral rescue have been performed by lipofectamine transfection on BSR/T7 cells. To confirm the chimeric virus propagation, immunofluorescent assay, RT-PCR, and plaque assay have been performed. Future experiments will include determination of the viral curve of replication, analysis of plaque morphology and viral tropism studies. Furthermore, it will be determined the 50% lethal doses (DL50) of ZIKV in 8 weeks old mice of A129 strain. The vaccine efficiency will be evaluated by intraperitoneally (i.p.) inoculation of recombinant YFV17DD/ZIKV in 4 weeks old A129 mice, that will be later challenged with ZIKV DL50 and animals will be monitored for disease clinical score, weight loss and death. Blood will be collected at different time points post-challenge for analysis of viremia and for serology.

Financial support: CNPq and FAPESP.

Abstract #037

YELLOW FEVER (YF) VACCINATION DOES NOT INCREASE DENGUE SEVERITY: A RETROSPECTIVE STUDY BASED ON 11,448 DENGUE NOTIFICATIONS IN AN YF AND DENGUE ENDEMIC REGION

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We study the association between prior yellow fever immunization and clinical outcomes of dengue infections in individuals of varying sexes and ages. Serological interactions between dengue virus and other flaviviruses could drive antibody dependent enhancement, which is associated with disease severity in dengue infections. This effect may influence disease severity in individuals subsequently affected by related flaviviruses, such as dengue. We compare the severity of dengue episodes between patients vaccinated and non-vaccinated against yellow fever. We evaluated the severity of 11,448 lab-confirmed dengue cases reported in São José do Rio Preto, Brazil, in 7370 YF vaccinated patients compared to 4043 unvaccinated patients. We regressed dengue severity against YF vaccine status and a number of demographic, clinical, and laboratory variables as controls. We also evaluated the association between YF vaccination status and the clinical and laboratory symptoms of dengue patients. We did not find any evidence of increased risk for severe dengue in patients vaccinated against YF (odds ratio = 1.00; 95% confidence interval = 0.87-1.14). Most of the variables analyzed did not have a statistically significant association with YF vaccination status. We found no evidence that YF vaccination in dengue-endemic areas increases the risk of severe dengue fever.

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Abstract #038

THERAPEUTIC TREATMENT OF DENGUE VIRUS INFECTION USING AN ANTIVIRAL PEPTIDE

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Dengue virus (DENV) is an arbovirus transmitted in urban areas by the hematophagous mosquito *Aedes aegypti*, which is associated with health and socioeconomic impacts worldwide. Infection of humans by one of the four serotypes (DENV 1-4) is associated with a wide spectrum of disease manifestations ranging from: asymptomatic or mild manifestations similar to other viral infections, which are self limited to hemorrhagic fever or dengue shock syndrome. The development of therapies is indispensable for disease control, since the current measure (vector control) is inefficient. In a study by our group, it has been shown that the synthetic peptide AH-D, administered to *Zika virus* (ZIKV) infected mice, has significantly reduced the clinical signs and mortality caused by this pathogen, even protecting against neurological damage. Like ZIKV, DENV has a lipid envelope, which is essential for its structural integrity. Therefore, the objective of our study was to evaluate the activity of the peptide against DENV infection. For that, VERO cells were infected with clinical isolate DENV-3 and treated with different concentrations of AH-D peptide. In parallel A129-/- mice, which lack the type I Interferon receptors (α/β), were infected with a lethal inoculum of a clinical isolate of DENV-3 and AH-D treatment performed intravenously (at different concentrations 25, 12,5 and 6,25 mg/kg, B.I.D), from the 2nd day of infection until day 5.. On day 5, morbidity and mortality were evaluated, as well as inflammatory and virological parameters in blood and various tissues of mice. Our results demonstrate that AH-D peptide treatment induced a significant reduction in viral loads (*in vitro* and *in vivo*) as well as prevention of body weight loss and lethality rates. Indeed, AH-D was able to prevent the DENV-induced thrombocytopenia, which is a hallmark of the dengue disease. Overall, AH-D treatment is able to prevent the major disease manifestations induced by DENV infection.

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

Abstract #039

SCREENING THE BRAZILIAN FLORA FOR NEW ANTI-ZIKA COMPOUNDS

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Zika virus (ZIKV) emerged as a global health threat due to its association with severe outcomes in humans, including microcephaly and other neurological complications. ZIKV replication and induction of neuronal death are considered key factors for severe ZIKV-induced disease. Currently, there is no approved vaccine or drug available to treat ZIKV Infection. Within this context, plant compounds might represent a source of new anti-ZIKV agents. We herein report an *in vitro* screen of 32 plant extracts against ZIKV. The species were selected based on a chemosystematic approach; i.e. the occurrence of triterpenes, steroids and polyphenols, as well as on their previously reported anti-inflammatory properties. Extracts initially have their cytoxicity assessed in Vero cells by the MTT and LDH (lactate dehydrogenase) methods. All tested extracts did not reduce cell viability at 30 µg/mL. Next, the antiviral ability of the 32 extracts was tested *in vitro* against a Brazilian ZIKV strain isolate. For that, Vero cells were infected with ZIKV and treated or not with three different concentrations (30, 10 and 3 µg/mL) of the 32 extracts, followed by viral loads analysis by plaque reduction assay. Results revealed that eight extracts inhibited significantly ZIKV replication, in a concentration-dependent manner. The active extracts were then submitted to dereplication studies by UPLC-ESI-MS, aiming to identify some constituents. The plants are mainly composed by polyphenols, including flavonoids, catechins and proantocyanidins. One of the active species, *Ouratea spectabilis*, was shown to be a source of unusual new dimeric flavanones, as disclosed by a recent work of our group. The active extracts are now being tested in ZIKV infected human neuroblastoma cells (SH-SY5Y) and the best candidates will be further tested *in vivo*. Overall, our results suggest that plant extracts could be a novel source of anti-zika compounds.

Financial support: INCT-Dengue, CAPES.

Abstract #040

IDENTIFICATION OF THE PRESENCE OF DENGUE VIRUSES IN URINE SAMPLES FROM INDIVIDUALS OF THE BASIC HEALTH UNIT IN THE CITY OF MIRASSOL-SP

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Dengue virus, family *Flaviviridae* and genus *Flavivirus*, is one arbovirus transmitted by *Aedes aegypti* with greater global importance affecting, thousands of people worldwide every year. It has been the cause of several serious cases, leading many patients to death. The present study aimed to identify the presence of dengue viruses in urine samples from symptomatic patients who were attended at the Emergency Care Unit in the city of Mirassol- SP (UPA– Mirassol) from 2018 to 2019. One hundred and thirteen urine samples, ranging from 3 to 83 years old, with arbovirosis symptoms were collected. The presence of characteristic symptoms of arboviruses was determined by the attending physician at the UPA. Viral RNA was extracted from urine samples using Trizol and tested for DENV2 by One-Step qPCR. The symptoms presented by the patients were evaluated by questionnaires. The results of real time qPCR assay revealed from the 113 symptomatic patients evaluated 34 (30.1%) were positive. Among the positive cases, 58.8% were female and 41.2% male. The age range with the highest number of cases was 41 to 50 years. Co-infection with ZIKV was also evaluated corresponding to 11,7% of the DENV2 infected patients. The most common symptoms reported by patients positive for DENV2 were muscle pain, fever, retro-orbital pain, itchy skin, exanthema and arthralgia, followed by nausea, red eyes, vomiting and intolerance to light, which appeared in a smaller number of cases. The study results contributed to a better understanding of dengue viruses activity in the city of Mirassol-SP. Urine testing proved to be important for detection of DENV2 on days 3-13 after onset of symptoms, extending virus detection time, because in sera samples the viremia period varies from day 2 to 6 after onset of symptoms.

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Abstract #041

ZIKA VIRUS INHIBITION BY COPAIBA (*COPAIFERA OFFICINALIS*) OIL NANOEMULSION

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Since the 2015 outbreak, Zika virus (ZIKV) has spread all over the world. It has become a major global health issue due to the neurological complications related to ZIKV infection such as Guillain–Barré Syndrome and Zika virus Congenital Syndrome. The virus is transmitted by *Aedes* mosquitoes but also by blood transfusion and sexually which allows the transmission of the virus in vector-free environments. So far, there are no vaccines or specific treatments for ZIKV infection, which makes important to develop specific therapies for its treatment. Here we evaluated the ability of a copaiba oil nanoemulsion to inhibit ZIKV. For the *in vitro* assays, first, we defined the highest non-cytotoxic concentration of the copaiba-based nanoemulsion in Vero cells by MTT assay. A concentration of 180 µg/mL was chosen since it maintains 100% cell viability up to 96h after treatment. Vero cells were infected and simultaneously treated with copaiba oil nanoemulsion at the highest non-toxic concentration. After 96h, results were evaluated by plaque assay revealing a viral inhibition of 80% ($p<0,05$). In order to understand in which steps of the viral life cycle the drug is acting on, we performed time-of-addition experiments and analyzed viral RNA by qPCR after 48h. Preliminary results show that the copaiba oil nanoemulsion has virucidal effect inhibiting 92.5% ($p<0,05$) of virus release. It also showed an effect in post-entry steps inhibiting 99.1% ($p<0,05$) of ZIKV intracellular RNA, when compared to the control. Additional experiments are being performed to confirm the preliminary results and also to understand the mode of action of the copaiba oil nanoemulsion in inhibiting Zika virus infection.

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Abstract #042

EARLY DETECTION OF ZIKA VIRUS IN MALE REPRODUCTIVE TRACT OF AG129 MICE

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Zika virus (ZIKV), an important arbovirus, is transmitted mainly by the bite of mosquitoes from the *Aedes* genus. ZIKV infection has been associated to neurological diseases, like Guillain-Barré syndrome in adults and Congenital Zika Syndrome in fetuses of pregnant women infected by this virus. Cases of sexual transmission also have been reported around the world and the persistence of this virus in semen raises several questions about how and where it circulates in the male reproductive tract (MRT). We analyzed e ZIKV infection in MRT of nine AG129 mice at 2 days post-infection (dpi), peak of viremia. Total RNA viral loads were detected by Real Time PCR in organs and the averages obtained were: $7{,}6x10^8$ RNA copies in testicles, $6{,}13x10^8$ in epididymis and $4{,}41x10^7$ in prostatic complex (prostate and seminal vesicle). In brain, the RNA levels were $6{,}76x10^8$ and $9{,}41x10^{10}$ in serum. Immunohistochemical (IHC) analyses, based on the envelope protein, showed an early infection in organs of MRT, since ZIKV positive antigens were detected in cells within or surrounding blood vessels, in interstitials regions or connective tissue of the organs and in epithelial cells of tissues. A more robust infection was seen in the testicles and epididymis at 2 dpi, in prostate it was also possible to observe the viral presence. Positive antigens for NS5 protein, the virus RNA-dependent RNA polymerase, were also detected by IHC, suggesting that the virus replicates in these organs. Zika virus was found in a very initial stage of infection (2 dpi) on MRT, infecting all analyzed organs in male reproductive tract at 2 days post-infection and may be replicating in testicles, epidydimis and prostate complex.

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Abstract #043

MOLECULAR INVESTIGATION OF ENTERIC VIRUSES IN THE ETIOLOGY OF THE CENTRAL NERVOUS SYSTEM VIRAL INFECTIONS IN CEREBROSPINAL FLUID SAMPLES FROM THE HOSPITAL DE BASE OF SÃO JOSÉ DO RIO PRETO - SP, 2016-2017

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Enteroviruses (EVs) are commonly associated with central nervous system (CNS) syndromes. Recently, gastroenteric viruses have also been associated with CNS neurological disorder. The aim of the present study was to screen cerebrospinal fluid samples (CSF) for EV, Rotavirus (RVA), Norovirus (NoV) and Astrovirus (AstV) in order to ascertain the diagnostic in CNS infection cases with unknown etiology. This study was conducted with convenience clinical samples collected from patients at Hospital de Base of São José do Rio Preto-SP during 2016-2017. A total of 289 CSF samples were tested for the presence of EV and NoV by real-time (q)RT-PCR, AstV by conventional RT-PCR, and RVA by ELISA. NoV, RVA and AstV were not detected. EV infection was detected in 5.9% of samples (17/289), and submitted to RT-PCR for genetic characterization. Positive EV samples were also inoculated in RD and HEp2 cell lines to prove virus infectivity, and potentially identify EV strains that failed to direct molecular methods. Five samples (5/17) were successfully amplified and sequenced. Three different serotypes were identified: Echovirus 3 (E3) (1/5), Coxsackie virus A6 (CVA6) (1/5) and Coxsackie virus B4 (CVB4) (3/5). CVB4 are commonly associated to EV infections. In Brazil, limited data are available regarding CVB4 incidence and its association with CNS infections. E3 was frequently detected in 70´s; but after the 90´s has become very uncommon. There are few reports of its detection in Brazil. CVA6 has recently emerged as a major cause of hand, foot and mouth disease worldwide. The surveillance proposed here offered an opportunity to identify for the first time in Brazil CVA6 associated to CNS infection. Identifying circulating EVs can help to improving our understanding of their potential in health burden and enabling a prompt response in case of outbreaks. Laboratory studies on EV-associated CNS infections in Brazil need to be intensify.

Financial support: FAPESP.

Abstract #044

ANNEXIN-A1 AS A NOVEL PRO-RESOLVING MOLECULE AGAINST CHIKUNGUNYA VIRUS INFECTION

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Annexin A1 (AnxA1) is a protein that has anti-inflammatory and pro-resolving actions. Here, we investigated the role of AnxA1during *Chikungunya* (CHIKV) infection. AnxA1 KO mice (AnxA1$^{-/-}$) or WT (*background* BALBc) were infected with 1x10^6 PFU of CHIKV through intraplantar route and the following analysis performed at 1, 3, 7 or 14 days upon infection: viral loads in different organs, total and differential blood leukocyte counts, indirect macrophage and neutrophil recruitment to target organs (NAG and MPO analysis, respectively), chemokines and cytokine levels, histopathological evaluation, and paw edema and mechanical hypernociception, which were evaluated until day 21 post-CHIKV inoculation. Our results shown that KO mice showed significantly lower viral titers in the paw, ankle joint, quadriceps muscle, spleen and liver when compared with infected-WT mice. Hematological analysis revealed that KO mice presented late leukocytosis (3rd day), while in WT mice, leukocytosis occurred on the 1st day of infection. In addition, NAG and MPO levels were significantly higher in the paw of KO mice, results that are in accordance with the increased of CXCL1 and IL-6 mediators in this organ. Meanwhile, levels of TNF-α, CCL2 or IL-10 were similar in paw tissue, when compared with infected-WT mice. Finally, histopathological scores were more intense in KO mice, as shown by an intense inflammatory infiltrate, congestion of blood vessels and loss of tissue architecture. In contrast, paw edema was significantly higher in WT mice, at day 1 post-infection while, similar hypernociception values were found in both groups during the kinetic of infection. In conclusion, our results suggests that the AnxA1 plays a protective role in the pathogenesis of CHIKV infection by inducing a rapid and more effective immunoinflammatory response, which contributes to early viral clearance. Further studies are needed to investigate the cellular and molecular mechanisms involved in this protection phenotype.

Financial support: CNPq, CAPES, FAPEMIG, Instituto Nacional de Ciência e Tecnologia em Dengue (INCT em Dengue).

Abstract #045

ACTIVATION OF PLATELET-ACTIVATING FACTOR RECEPTOR OR THE 5-LIPOXYGENASE PATHWAY DOES NOT IMPACT THE COURSE OF DISEASE INDUCED BY CHIKUNGUNYA VIRUS

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Chikungunya virus (CHIKV) is associated with outbreaks of infectious rheumatic disease in humans, which can last for months to years. Treatment is supportive. Inflammatory responses unleashed by CHIKV during the acute phase of the disease is characterized by production of a variety of inflammatory mediators including, protein and lipid mediators. Platelet-activating factor (PAF) is a potent lipid mediator of inflammation that acts on its receptor (PAFR) expressed especially in leukocytes, platelets, and endothelial cells. Similarly, arachidonic acid cascade metabolites such as leukotrienes, especially LTB4, are also involved in arthritic conditions, such as in rheumatoid arthritis. Here, we investigated the role of PAFR and the 5-LO enzyme in the pathogenesis of CHIKV infection. PAFR knockout mice PAFR$^{-/-}$ or WT (*background* C57BL/6) and 5-Lypoxygenase KO mice (5-LO$^{-/-}$) or WT (Sv129) were infected with 1x 10^6 PFU of CHIKV through intraplantar route and several analysis performed on days 1, 3, 7 or 14 post-infection. Viral loads, macrophage and neutrophil recruitment to target organs measured by (NAG and MPO analysis, respectively), chemokines and cytokine levels, paw edema and mechanical hypernociception were evaluated. Results revealed that both PAFR$^{-/-}$ and 5-LO$^{-/-}$ mice presented similar, viral loads in the paw, popliteal lymphonode, plasma, quadriceps muscle, and liver as compared to infected-WT littermates. When the paw of PAFR-/- mice was analyzed, MPO levels were significantly higher at days 1, 3, and 7 post- infection, however, no differences in cytokine levels (IL-6, IL-1β, TNF-α or IL-10) were observed when compared to infected-WT mice. Finally, similar levels of paw edema and mechanical hypernociception were found in both PAFR$^{-/-}$ and 5-LO$^{-/-}$ mice when compared with its infected controls. In summary, our results suggest that PAFR activation as well as the 5-LO enzyme does not contribute to the outcomes of CHIKV.

Financial support: CNPq, CAPES, FAPEMIG, Instituto Nacional de Ciência e Tecnologia em Dengue (INCT em Dengue).

Abstract #046

EVALUATION OF A RECOMBINANT PROTEIN IN ELISA AND RAPID TEST FOR DIAGNOSIS AND SURVEILLANCE OF CHIKUNGUNYA

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Chikungunya virus (CHIKV, family *Togaviridae*), is a mosquito-borne pathogen that causes a disease characterized by acute onset of fever accompanied by arthralgia and intense joint pain. Clinical similarities and cocirculation of other arboviruses in Brazil highlight the necessity of accessible diagnostic tools. Point of care tests (POCT) currently available to detect CHIKV infections have been associated with low accuracy. Moreover, there is no commercial kit registered by ANVISA which was entirely produced in Brazil. The antigens are mostly imported from other countries. The aim of this work is to generate an antigen able to detect CHIKV infections in serological assays and provide national diagnostic devices. Following computational design, the gene coding for the recombinant CHIKV protein (rCHIKVp) was synthesized and the protein was purified by affinity chromatography. Antigenicity of the protein was initially confirmed by western-blot using sera from CHIKV infected mice. Additionally, the seroreactivity of r-CHIKV protein has been evaluated using a panel of human sera, by indirect IgG Enzyme-Linked Immunosorbent Assays (ELISA) and Immunochromatographic tests (ICT). ICTs are being carried out on two formats. Firstly, rCHIKVp is conjugated to colloidal gold and assays are performed against two test lines (α-IgM e α-IgG). In this case, the control line will be a polyclonal antibody produced in rabbits immunized with rCHIKVp. The other format is composed by rCHIKVp in the test line and protein A in the control line. The performance of rCHIKVp in ELISA showed sensitivity of 100% (49/49) and specificity of 96% (52/54). Additionally, no cross-reactivity was found against sera of Zika and Dengue positive patients. Preliminary results of ICTs also have shown the ability of the antigen to recognize CHIKV infections by POCT. These results indicate that this protein is a potential candidate to be used in serological tools to detect CHIKV infections.

Financial support: CNPq, CAPES, FAPEMIG.

Abstract #047

TREATMENT WITH SILYMARIN IMPROVES SURVIVAL OF ZIKA VIRUS INFECTED C57BL/6 MICE

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Zika virus (ZIKV) is an arbovirus belonging to the *Flaviviridae* family and genus *Flavivirus*. It is the etiological agent of the Zika fever, harboring symptoms common to most arboviruses: fever, headache, joint pain or rash and conjunctivitis. Additionally, scenarios with serious damage to the nervous system such as microcephaly associated with this pathology have been reported for this virus. ZIKV was first isolated in 1947 in the Uganda and in 2015 the spread of this virus was reported in Latin American countries, including Brazil, resulting in major outbreaks and epidemics with numerous cases of human infection. Still, despite its importance, there is no antiviral treatment or vaccine available so far. Thus, studies have pointed to the promising use of natural substances with antiviral activity. Silymarin, a set of flavonoides extracted from the milk thistle plant (*Silybum marianum*) has gained prominence in the past few years on scientific research due to its antioxidant, hepatoprotective and antiviral properties. Also, we found anti-ZIKV activity of silymarin in vitro. Therefore, the aim of this study was to evaluate whether silymarin presents antiviral activity in ZIKV-infected C57BL/6 mice. For this, ten-days-old C57BL/6 mice were intraperitoneally infected with 107 PFU of ZIKV and afterwards intraperitoneally treated with silymarin at a dosage of 100 mg/kg/day. Administration was started one day prior infection and ended one day before euthanasia. The animals were monitored for 21 days being evaluated for survival ratios and weight loss. Silymarin treatment ensured the survival of approximately 60% of the animals, compared with the untreated group, however both treated and untreated animals presented impairment on weight gain. Overall, these results are preliminary but suggest that silymarin treatment can have anti-ZIKV activity *in vivo* and opens new perspectives for the use of natural compounds in the treatment of Zika disease.

Financial support: UFOP, FAPEMIG, CNPq, CAPES.

Abstract #048

BEYOND MEMBERS OF THE FLAVIVIRIDAE FAMILY, SOFOSBUVIR ALSO INHIBITS CHIKUNGUNYA VIRUS REPLICATION

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Chikungunya virus (CHIKV) causes a febrile disease associated with chronic arthralgia, which may progress to neurological impairment. Chikungunya fever (CF) is an ongoing public health problem in tropical and subtropical regions of the world, where control of the CHIKV vector, *Aedes* mosquitos, has failed. As there is no vaccine or specific treatment for CHIKV, patients receive only palliative care to alleviate pain and arthralgia. Thus, drug repurposing is necessary to identify antivirals against CHIKV. CHIKV RNA polymerase is similar to the orthologue enzyme of other positive-sense RNA viruses, such as members of the *Flaviviridae* family. Among the *Flaviviridae*, not only is hepatitis C virus RNA polymerase susceptible to sofosbuvir, a clinically approved nucleotide analogue, but so is dengue, Zika, and yellow fever virus replication. Here, we found that sofosbuvir was three times more selective in inhibiting CHIKV production in human hepatoma cells than ribavirin, a pan-antiviral drug. Although CHIKV replication in human induced pluripotent stem cell-derived astrocytes was less susceptible to sofosbuvir than were hepatoma cells, sofosbuvir nevertheless impaired virus production and cell death in a multiplicity of infection-dependent manner. Sofosbuvir also exhibited antiviral activity *in vivo* by preventing CHIKV-induced paw edema in adult mice at a dose of 20 mg/kg of body weight/day and prevented mortality in a neonate mouse model at 40- and 80-mg/kg/day doses. Our data demonstrate that a prototypic alphavirus, CHIKV, is also susceptible to sofosbuvir. As sofosbuvir is a clinically approved drug, our findings could pave the way to it becoming a therapeutic option against CF.

Financial support: CNPq, FAPERJ, CAPES, FIOCRUZ.

Abstract #049

ANTIVIRAL EFFECT OF AN ANTIHISTAMINE DRUG AGAINST *ZIKA VIRUS*

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Zika virus (ZIKV) is an arbovirus member of Flaviviridae family which is mainly transmitted by *Aedes aegypti* mosquitoes. In 2015–2016, Brazil was affected by 440,000 and 1.3 million people ZIKV infections and congenital neurological malformations, such as microcephaly, and other pregnancy outcomes were also associated to ZIKV infections. In addition, there are currently no antiviral drugs or licensed vaccines against ZIKV. Repositioning drugs is an important tool as it can reduce the time and cost of finding new therapies. Piperazine antihistamines are drug which treat allergies and, currently, studies have reported their repurposing as antivirals. Thus, this study aimed to evaluate the anti-ZIKV effect of a piperazine antihistamine drug. First, cytotoxicity concentration for 50% of Vero cells (CC_{50}) was performed by the MTT method. The antihistamine was tested at different concentrations (3.125 to 200 μg/mL). In the global activity antiviral assay, Vero cells and virus (MOI 0.01) were treated 30 minutes prior to infection at non-toxic concentrations to determine the effective concentration for 50% of the infected cells (EC_{50}) by the ZIKV also by the MTT method. Selectivity index (SI) was calculated by the ratio of CC_{50} to EC_{50}.The anthistamine drug reduced cytopathic effect and showed CC_{50} of 39.15 μg/mL and EC_{50} of 20.8 μg/mL (IS of 1.9). Ribavirin showed CC_{50} of 31.2 μg/mL, EC_{50} of 4.16 μg/mL (IS of 7.5). Therefore, these results indicate this piperazine anthistamine as a promising antiviral effect. Studies has been conducted in order to characterize the antiviral effect during ZIKV multiplication cycle.

Financial support: CAPES, UFSJ, FAPEMIG, CNPq.

Abstract #050

VIRAL COINFECTION IN PATIENTS WITH NEUROLOGICAL DISORDERS IN CAMPINAS, BRAZIL

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Arboviruses are transmitted by hematophagous arthropods and cause millions of infections and thousands of deaths every year. Brazil harbors the most arboviral diversity in the world, including important human pathogens, such as Dengue, Yellow Fever, Chikungunya (CHIKV) and Zika (ZIKV) viruses. This scenario increases the risk of outbreaks and arboviral co-infections, as well as reinforces the importance of the differential diagnosis and monitoring these viruses. Currently, metagenomics is an efficient approach to determine the virosphere in all types of biological samples. In this study, we have applied high-throughput screening (HTS) approach to identify potential co-viral infections in patients that developed symptoms and signs of central nervous system (CNS) diseases during ZIKV outbreak (2016-2017) in Campinas city, Brazil. Thus, the acute-phase serum specimens were collected from the Brazil ZIKV cohort of patients with CNS symptoms admitted in hospitals at Campinas. Viral RNAs were extracted from serum samples and analyzed by qPCR to determine the diagnosis of ZIKV. Subsequently, we applied the HTS approach using HiSeq 2500 (Illumina) to identify the potential co-viral infections in ten patients with CNS manifestation and diagnosis ZIKV positive. The mono-infection by ZIKV in patients with CNS manifestation was predominantly in 9 out of 10 patients analyzed. However, we sequenced a partial genome of CHIKV and the complete genome of a pegivirus (*Pegivirus* genus, *Flaviviridae* family) in a serum sample of a patient of 27 years old with meningitis collected in 2017. Based on phylogenetic analysis, CHIKV strain detected was classified as East-Central South African genotype. On the other hand, the pegivirus strain was classified into genotype 1 of *Pegivirus C* and shared 98% identity in amino acids with strains this same genotype. In summary, we reported a case of one patient with multiple viral infection with ZIKV, CHIKV, and pegivirus.

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Abstract #051

EPIDEMIOLOGICAL AND DIAGNOSTIC ANALYSIS OF ZIKA VIRUS OF MIRASSOL REGION – SP

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Brazil, being a predominantly tropical country with a hot and rainy summer, favors the proliferation of mosquitoes and the consequent spread of arboviruses. Thus, the country presents several arboviruses, being the focus of this work the Zika virus infection (ZIKV). Clinical manifestations include low fever, rash, conjunctivitis, myalgia and arthralgia, as well as malaise and headache. Since these symptoms are common to other arboviruses, the differential diagnosis has been facing difficulties. In addition, there is a wide cross reaction of serological tests by antibodies with other viruses of the same genus (*Flavivirus*), such as Dengue virus. Therefore, this study aims to detect the presence of ZIKV in serum and urine samples from patients in the region of Mirassol - SP with suspicious symptoms for arboviruses, by the extraction of nucleic acid (ARN) from the samples and subsequent Real Time Polymerase Chain Reaction. Also, through a questionnaire applied to patients, make a general approach about the infection, relating the symptoms and epidemiological aspects. Thus, to date, 10.4% of patients (14/134) have been diagnosed with ZIKV, with muscle pain (85.7%), fever (64.3%), arthralgia and eye pain (57.1%), the most frequently reported symptoms. Such symptoms are closer to dengue virus infection, suggesting a co-infection between both viruses. The geographical distribution of positive cases is concentrated in the urban area, contributing as a warning to the municipality, in order to develop programs against the vector, avoiding new infections.

Financial support: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

Abstract #052

EVALUATING THE VALIDITY OF DENGUE CLINICAL-EPIDEMIOLOGICAL CRITERIA FOR DIAGNOSIS IN PATIENTS RESIDING IN A BRAZILIAN ENDEMIC AREA

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This study aimed to evaluate the validity (i.e., sensitivity and specificity) of clinical diagnosis compared to laboratory diagnosis of dengue in a large retrospective sample of patients from a dengue-endemic area of Brazil. We evaluated 148,299 reported dengue cases in São José do Rio Preto, Brazil. Of these, 83,506 (56.3%) were diagnosed based exclusively on clinical-epidemiological criteria, and 64,793 (43.7%) also received laboratory confirmation. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of patients' demographic and clinical characteristics were analyzed, and whether thrombocytopenia was present, compared to a laboratory-based dengue diagnosis. We measured the association between these variables and dengue-positive laboratory tests. Logistic regression was undertaken to evaluate the probability of dengue-related signs and symptoms being present in clinical and laboratory diagnosis, compared to clinical diagnosis. We found variability in sensitivity to signs and symptoms (ranging from 0.8 to 81.1 (hematuria and fever, respectively), and in specificity, ranging from 21.5 to 99.6 (fever and metrorrhagia, respectively). Thrombocytopenia exhibited a higher PPV (92.0) and a lower NPV (42.2), and was the only variable showing some agreement with a specific laboratory diagnosis of dengue ($\varphi = 0.38$). The presence of exanthema led to a greater likelihood of concordant clinical and laboratory diagnoses (odds ratio (OR): 4.23; 95% confidence interval (CI), 2.09–8.57), as did thrombocytopenia (OR: 4.02; 95% CI, 1.32–12.27), when using multivariate logistic regression. We found substantial variation in sensitivity, specificity, PPVs, and NPVs, concerning clinical-epidemiologically determined dengue signs and symptoms.

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Abstract #053

ZIKA VIRUS AND OTHER VIRUSES ASSOCIATED TO NEUROLOGICAL SYNDROMES FROM PATIENTS ATTENDED IN THE CLINICAL HOSPITAL OF UNICAMP

Leon LL[1], Lima RG[1], Boffi LC[1], Bindilatti RN[1], Menoni SMF[1], Domingues JC[1], Garlip CR[2], Costa SCB[1], Bonon SHA[1].

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Viral infections of the nervous system are a medical emergency, where human herpesviruses, enteroviruses and arboviruses are most important. These infections can be responsible for many neurological diseases, such as meningitis, encephalitis and meningoencephalitis, and are cause of sequela and death. The traditional investigation of nervous system infection based on the search for other non-virus microorganisms, while very relevant, exposes an investigative gap that should not be overlooked. The choice of RT-PCR, PCR and qPCR is justified by their successful use, providing a fast and reliable investigation in the neurotropic virus. The aim of this study was to detect the presence of viruses that cause nervous system infection in cerebrospinal fluid (CSF) samples from patients with suspected viral infection, belonging to the families: *Flaviviridae* (ZIKV, DENV, YFV, ROCV, SLEV, WNEV, ILHV); *Togaviridae* (CHIKV, WEEV, VEEV, EEEV, AURA and MAYV); *Picornaviridae* (non-polio enterovirus) and *Herpesviridae* (HSV-1, HSV-2, VZV, EBV, CMV and HHV-6). A prospective, descriptive, case series study is being conducted using 544 CSF samples from patients with clinically suspected of acute nervous system viral Infection. PCR, RT-PCR and qPCR tests are being performed to identify the genome of viral etiological agents. The detection of Herpesvirus occurred in 32/544 (5.9%); Enterovirus was detected 37/544 (6.8%) samples; Flavivirus was detected in 87/544 (15.6%) patients (56, 10.3% DENV; 30; 5.5% ZIKV; 3, 0.5% YFV). Information on the co-circulation of these viruses will allow a better understanding of the relationship with acute neurological syndromes in our population, allowing us a better understand this scenario and in the future to ensure better prognosis of these patients, reducing the morbidity and mortality caused by these infections.

Financial support: FAPESP, CAPES.

Abstract #054

MOLECULAR DETECTION OF ZIKA VIRUS IN PATIENTS WITH SUSPECTED INFECTIOUS NEUROLOGICAL SYNDROMES ATTENDED IN THE CLINICAL HOSPITAL OF UNICAMP IN THE PERIOD OF 2017-2018

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The Zika virus (ZIKV) is an arbovirus transmitted by the *Aedes aegypti* mosquito that has gained notoriety with the recent outbreak (2015), presenting clinical features that had not been previously described. This virus was able to cause neurological manifestations, such as microcephaly, meningitis and Guillain-Barré syndrome. With this epidemiological situation, the need to develop and standardize methodologies that was able to acquire fast and reliable diagnosis of this virus was highlighted and encouraged by WHO, especially for differentiation from other arboviruses, such as DENV. The detection by nucleic acid amplification tests (NAAT) are of high sensitivity and specificity, ensuring accuracy when well standardized. Thus, the aim of the study is to identify by qPCR the presence of ZIKV RNA in CSF samples from patients with clinically suspected of viral infection in the nervous system and to evaluate possible new symptoms. Were included 544 patients with symptoms of neurological syndromes of probable viral etiology, treated at the Clinical Hospital of the State University of Campinas (HC/UNICAMP) in the period of 2017-2018. The qPCR for ZIKV demonstrated positivity in 30/544 (5.5%) CSF samples, which were analyzed using a set of primers and probe based on the MR766 strain (CDC), and the mean Cq was 34.5. There were several neurological symptoms presented by these patients, since meningitis and Guillain Barré Syndrome. The circulation of arboviruses in patients with acute neurological syndromes and the date around these cases is of great importance to know better the panorama of ZIKV in our population in order to ensure better prognosis of these patients in the future, increasing knowledge about these emerging arboviruses and reducing the morbidity and mortality caused by these infections.

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Abstract #055

Cell Migration and Virus Spreading After OROV Infection in a Subcutaneous Chamber in Mice

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Oropouche (OROV) is an emerging virus associated with epidemic of febrile disease in Amazonic region of Brazil and other countries of South and Central American. Indeed, more than 30 epidemics of OROV fever have been documented in Brazil with more than 500 thousand reported cases. In addition, OROV is neurotropic in animal models and can cause encephalitis and meningitis in humans. However, little is known about the pathogenic aspects that guide the neurotropism of OROV. Neutrophils are polymorphonucleated cells that acts as one of the first-responders to migrate towards the site of inflammation, being essential to control many viral infections. However, circulatory neutrophils can act as Trojan horse during neuroinvasion of some viruses, driving the development of encephalitis in humans and animals. Therefore, to characterize the role of neutrophils for protection or disease development during immunosuppression, cellular migration, viral replication, cytokine production and mortality of C57BL/6 WT, *Ifnar*-/-, *Rag*-/- e Mrp8 CreiDTR (with neutrophil depletion) mice will be determined after OROV infection in a subcutaneous chamber implanted in these animals. As expected, we were able to detect high viral titers in the exudate present within the subcutaneous chamber implanted in WT mice, with the migration of neutrophils, macrophages and dendritic cells to this site of infection.

Financial support:

Abstract #056

RESEARCH ON ANTIVIRAL ACTIVITY OF PARTIES OF THE SPECIES *BAUHINIA HOLOPHYLLA* ON *ZIKA VIRUS*

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Zika virus (ZIKV) is a flavivirus transmitted by the *Aedes aegypti* mosquito which became a public health problem in Brazil, due to the increase in the number of cases from 2015 and its association to several congenital diseases such as microcephaly. There is no vaccine available and s pecific antiviral drug able to inhibit ZIKV replication are necessary. Currently, the treatment of infections is directed to symptom relief by the administration of analgesics and antipyretics. Several species of *Bauhinia* have been widely related to the treatment of gastrointestinal diseases, diabetes, and inflammation, due to their high composition of flavonoids such as quercetin and rutin. However, there are no studies in the literature to evaluate the antiviral effect of *B. holophylla* against ZIKV. So, the present work aimed to investigate the antiviral activity of the hydroalcoholic extract of *B. holophylla* and three fractions against ZIKV. Bioassays were performed using ZIKV and Vero mammalian cells. First, cytotoxicity concentration for 50% of cells (CC_{50}) was performed by the MTT method. The extract and fractions 26, 38 and 46 were tested at different concentrations (3.91 to 1000 μg/mL). In the global activity antiviral assay, cells and virus (MOI 0.01) were treated 30 minutes prior to infection at non-toxic concentrations to determine the effective concentration for 50% of the infected cells (EC_{50}) by the ZIKV also by the MTT method. Selectivity índex (SI) was calculated by the ratio of CC_{50} to EC_{50}. Ribavirin was used as a positive control. As a result, fraction 38 showed most promising anti-ZIKV effect with EC50 value of 6.01 μg/mL and SI of 22.97. The SI suggests a safe and significant therapeutic window between the cytotoxic and effective dose to inhibit ZIKV infection. Virucidal and mechanism of action assays for fraction 38 will be performed to further studies on identify new leads to anti-ZIKV drug discovery.

Financial support: CAPES, UFSJ, FAPEMIG, CNPq.

Abstract #057

INVESTIGATION OF THE ARBOVIRUSES OCCURRENCE IN PREGNANT WOMEN IN THE REGION OF SÃO JOSÉ DO RIO PRETO-SP

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Dengue (DENV), Zika (ZIKV) and Chikungunya (CHIKV) infection in pregnant women is of great concern for the possible damage caused by these viruses to mothers and fetuses. ZIKV is related to microcephaly and other severe brain abnormalities in neonates. CHIKV infection in pregnant women in the intrapartum period can lead to vertical transmission, with the possibility of worsening in the neonate. DENV infection in pregnant women is not correlated with the occurrence of congenital malformations; however, they are considered a risk group because they have a prognosis of progression to more severe forms and death, increased risk of premature births and the possibility of vertical transmission due to perinatal maternal infection. In this study, a retrospective results analysis of DENV, ZIKV and CHIKV was performed to determine the occurrence of these arboviruses in pregnant women. Between January and June 2019, samples from 384 pregnant women with a history of fever during pregnancy were included in the study. We analyzed the qPCR results for DENV, ZIKV and CHIKV of serum and urine samples from these pregnant women and 18 newborns (RN). The dengue serotype-2 (DENV-2) was detected in 98 (25.5%) of the pregnant women serum samples. ZIKV and CHIKV were not detected. From the total of pregnant women, samples of 18 (4.7%) newborns were also analyzed. Of these, 2 (11%) pregnant were positive for DENV-2 and no newborn sample was positive for the arboviruses tested. During the period, one pregnant woman died due to DENV-2. Regarding age group, the predominance was 25 to 32 years (36%), followed by 33 to 40 years (32%) and 17 to 24 years (29%) presenting no significant difference. Literature data report a positivity similar to that found in this study. Our results confirm the importance of investigate dengue in pregnant women and its vulnerability to aggravation and death from the disease. Early diagnosis of these arboviruses enables adequate care for pregnant women.

Financial support:

Abstract #058

Prevalence of Dengue and Leptospirosis IgM Antibodies in Patients Notified for Both Diseases

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Leptospirosis and dengue are two commonly seen infectious diseases in Brazil and have emerged as an important concern of public health, particularly in tropical and subtropical regions. Dengue fever is an arthropod born viral disease whereas leptospirosis is a zoonotic disease caused by the spirochetes of the genus *Leptospira.* Clinical differential diagnosis of leptospirosis from dengue fever is often difficult due to overlapping clinical symptoms, so laboratorial tests possess a great value in some cases. In this study, a data collection of dengue and leptospirosis Elisa IgM results performed by the Adolfo Lutz Institute from January 2015 to april 2019 were done. The presence of dengue and leptospira IgM antibodies were investigated in patients notified for both diseases at the same period. The data collection showed that 328 patients reported for dengue and leptospirosis were receveid for analysis. Of these, 57 (17.37%) patients were positive for dengue IgM antibodies and 42 for leptospirosis IgM antibodies (12.8%). Regarding co-infections, two patients presented reagent serology for dengue and leptospirosis at the same time, with a prevalence of 0.6%. One was a 19-year-old woman, resident in the city of Catanduva (SP) in February 2015 and the other a 37-year-old man living in the countryside of the municipality of Fernando Prestes in May 2018. In comparison to other studies, our co-infection prevalence are similar, as dengue and leptospirosis cases have been reported with frequency ranging 0.9-8%. This study has limitations, mainly because the results should be confirmed by other methods. We conclude that the investigation of both diseases is very important due to similar symptoms. The laboratorial diagnoses play an important role in these cases, as it helps the clinician to guide the adequate treatment to patients.

Financial support:

Abstract #059

INVESTIGATION OF ARBOVIRUS IN TICKS

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Brazil has a great diversity of arthropods and vertebrate animals which together with the climatic conditions constitute favorable characteristics for the occurrence of arboviruses. Ticks are hematophagous arthropods, obligate parasites, which use a wide range of hosts. As vectors, ticks are capable of transmitting a wide variety of pathogens, and are among the most important vectors of diseases affecting wild and domestic animals and humans. The Municipal Zoo of the city of São José do Rio Preto – SP, is a reference in emergency care and treatment of wild animals in the region. The animals attended at the Zoo often have tick infestation. Investigating the presence of arbovirus RNA in ticks from wild animals is of great importance for the identification of viruses transmitted by these ectoparasites that circulate in wildlife, emphasizing the importance of providing information that contributes to epidemiological surveillance in the region. The aim of this project is to investigate the presence of arbovirus RNA in ticks collected from wild animals received at the São José do Rio Preto Municipal Zoo. The collected ticks are stored in pools according to species and the animal from which they were collected. To date, samples of 18 pools from four different animals have been tested. RNA was extracted from the macerated pools, cDNA was synthesized and viral amplification was performed by NESTED-PCR technique using *Flavivirus* specific primers. NESTED-PCR products were analyzed on 1% agarose gel. Of the samples tested, 17 were negative for the presence of *Flavivirus* RNA and one sample, from *Amblyomma nodosum* ticks collected found in giant anteater (*Myrmecophaga tridactyla*) presented a week amplification of the expected size. Further analyses are being performed to sequence the amplicon and confirm the *Flavivirus* identification.

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Abstract #060

ANALYSIS OF THE CELLULAR IMMUNE RESPONSE IN MICE IMMUNIZED WITH A DNA VACCINE BASED ON THE DENV2 NON-STRUCTURAL 1 PROTEIN

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Dengue fever is an arthropod-borne viral disease that poses as a major threat in terms of public health in tropical and subtropical countries worldwide. The importance of the cellular immune response against dengue has been increasingly highlighted in recent years, especially in regard to vaccine development. Our group has constructed a DNA vaccine based on the non-structural protein 1 (NS1), named pcTPANS1, that was able to induce protection in BALB/c mice challenged with DENV2. In order to investigate the role of the cellular immune response in the protection elicited by this vaccine, we first evaluated T-cell responses by depleting $CD4^+$ and $CD8^+$ cells in BALB/c mice immunized with pcTPANS1. Next, we identified immunodominant NS1-derived epitopes by using a peptide library covering the full length of the protein sequence, and evaluated production of INF-γ and TNF-α by $CD4^+$ and $CD8^+$ T cells upon stimulation with the selected peptides. Depletion of $CD8^+$ T cells in vaccinated animals caused a decrease in survival rates from 80% to 50%, while depletion of $CD4^+$ T cells almost completely abolished protection. We detected 3 peptides that were able to induce INF-γ production in vaccinated BALB/c mice as evaluated by ELISPOT assays, and 4 other peptides were identified after viral challenge. Production of INF-γ, as measured by intracellular cytokine staining assays, confirmed most of those peptides. On the other hand, levels of TNF-α remained ordinary after stimulation with most of the selected peptides. We also assessed the immunogenicity of those peptides in C57BL/6 mice due to the difference in MHC haplotype expression comparing to BALB/c animals, and identified two NS1-derived epitopes that featured prominently in the IFN-γ response in cells from both animal strains. To conclude, these results emphasize the importance of the cellular immune response in the protection against dengue induced by our DNA vaccine.

Financial support: CAPES, CNPq, INCTV, FAPERJ.

Abstract #061

DIRECT FLAVIVIRUS DETECTION BY PLASMON RESSONANCE

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The co-circulation of some *Flavivirus* and *Alphavirus* has drawn attention due to the similar symptoms they cause on infected patients, resulting on difficulties in clinical diagnosis and turning laboratory diagnosis essential. Direct diagnostic methods on the market are costly, difficult to use and poor accessible, making necessary for the development of new diagnostic methods for *Flavivirus* that could combine specificity, sensitivity and practicability. Due to their physicochemical characteristics, the gold nanoparticles can be used in new diagnostic tools as they have the necessary characteristics of new methods besides and have a lower production cost. Thus, the objective of this work is to evaluate the biotechnological potential of gold nanorods (AuNBs) to diagnose *Flavivirus*. For this, they were synthesized by the seed method and functionalized with polyethyleneimine (4mM). After functionalization with anti-*Flavivirus* monoclonal antibody in different concentrations (0.8, 0.4, 0.2, 0.1 μg/mL), AuNBs were incubated with 103 PFU/mL of *Dengue virus*, serotypes 1 (DENV1) and 2 (DENV2); *Zika virus* (ZIKV) and also for *Mayaro virus* (MAYV) which was used as negative control. The best antibody concentration and defined for use was 0.4 μg/mL. Regarding the time, with only 15 minutes of reaction, there was already a good detection of viruses. AuNBs were also challenged with virus samples (104,103,102 and 10 PFU/mL) diluted in human serum 1: 3200. UV-Vis spectra of the solutions were obtained for shift analysis at the maximum peak of the AuNBs extinction band. In each process, the observed shift indicated the effective binding of viruses to the nanorods. The proposed biosensor was able to detect 100 PFU/mL, both DENV2 and ZIKV. For all analyzed concentrations there was no shift considered positive for MAYV. Thus, the biosensor proposal using AuNBs seems to be is promising for *Flavivirus* detection.

Financial support: UFOP, CAPES, CNPq, FAPEMIG.

Abstract #062

CHARACTERIZATION OF DENGUE CASES AMONG PATIENTS WITH AN ACUTE ILLNESS, CENTRAL DEPARTMENT, PARAGUAY

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In 2018, Paraguay experienced a large dengue virus (DENV) outbreak. The primary objective of this study was to characterize dengue cases in the Central Department, where the majority of cases occur, and identify factors associated with DENV infection. Patients were enrolled from January-May 2018 if they presented with a suspected arboviral illness. Acute-phase specimens (≤8 days after symptom onset) were tested using rRT-PCR, a rapid diagnostic test for DENV nonstructural protein 1 (NS1) and anti-DENV IgM and IgG, and ELISA for IgG against NS1 from Zika virus (ZIKV). 231 patients were enrolled (95.2% adults) at two sites: emergency care and an outpatient clinical site. Patients included 119 (51.5%) dengue cases confirmed by rRT-PCR (n=115, 96.6%) and/or the detection of NS1 and anti-DENV IgM (n=4, 3.4%). DENV-1 was the predominant serotype (109/115, 94.8%). Epidemiologically, dengue cases and non-dengue cases were similar, though dengue cases were less likely to reside in a house/apartment or report a previous dengue case. Clinical and laboratory findings associated with dengue included red eyes, absence of sore throat, leucopenia and thrombocytopenia. At an emergency care site, 26% of dengue cases (26/100) required hospitalization. In univariate analysis, hospitalization was associated with increased viral load, anti-DENV IgG, and thrombocytopenia. Among dengue cases that tested positive for IgG against ZIKV NS1, the odds of DENV NS1 detection in the acute phase were decreased 10-fold (OR 0.1, 0.0-0.3). Findings from a predominantly adult population demonstrate clinical and laboratory factors associated with DENV infections and the potential severity of dengue in this group. The combination of viral load and specific IgG antibodies warrant further study as a prognostic to identify patients at risk for severe disease.

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Abstract #063

DISCOVERY OF *ZIKA VIRUS* NS5 METHYLTRANSFERASE INHIBITORS: A STRUCTURE-BASED VIRTUAL SCREENING AND MOLECULAR DYNAMICS SIMULATIONS APPROACH

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Zika virus (ZIKV) infections are a major public health problem worldwide. There are no specific vaccines and antivirals, so the search for antivirals is required. NS5 methyltransferase (MTase) is considered an attractive molecular target for drug development due its essential role in the ZIKV multiplication cycle. NS5 MTase promotes RNA methylation, improves genome stability and immune response evasion. Thus, this work aims to identify NS5 MTase inhibitors through structure-based virtual screening of a DTP AIDS Antiviral Screen Database from National Cancer Institute (USA). A total of 42,390 structures of this bank were download and structure with any Lipinski's rules violation were excluded. The structures were docked to NS5 MTase binding site (PDB 5ULP) by Glide and DOCK6 programs. Docking simulations were validated by crystallographic ligand re-docking and determination of the root-mean square deviation (RMSD) by DS Visualizer software. Molecular dynamics (DM) simulation was performed using the CHARMM force field implemented in the NAMD program at a 40ns simulation time. A total of 11,000 were docked to NS5 MTase (~ 26% of 42,390) and thiazolidinone derivative structure showed lower binding energy (Glide: -11.5 kcal / mol DOCK6: -48.4 kcal / mol) in comparison to the crystallographic ligand MS2042 (Glide: -10.8 kcal / mol DOCK6: -41.5 kcal / mol). DM simulations indicated conformational stability of Thiazolidinone-MTase complex during 40 ns and up to 7 hydrogen bonds to aminoacids of NS5 MTase active site. A thiazolidinone derivative has been identified as a promising candidate for NS5 MTase inhibitor. In order to validate these results, global antiviral activity against ZIKV will be performed.

Financial support: CAPES, UFSJ, FAPEMIG, CNPq.

Abstract #064

TRACKING HUMAN BLOOD MONONUCLEAR CELLS AFTER INFECTION WITH OROPOUCHE VIRUS

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Oropouche orthobunyavirus (OROV) is an Amazonian emerging virus with high potential of dissemination for several regions of the world, mainly due to deforestation, climate changes and expansion of population density in the Amazon. The major symptoms associated with OROV infection are headache, myalgia, arthralgia and exanthema. Moreover, hemorrhagic and neurological complications are also frequently associated with OROV infection in humans and other animals. As the infection of monocytes and dendritic cells are usually key events during arboviral infections, we decided to evaluate the viral replication and gene expression modulation in human peripheral blood mononuclear cells (PBMCs) during OROV infection. For this, PBMCs from healthy donors, THP-1 and Jurkat lineages were infected with OROV. Genome and antigenome of OROV were assessed by RT-qPCR and RNA PrimeFlow assay by flow cytometry and immunofluorescence using specific RNA hybridization probes. Productive infection was also evaluated by focus forming units (FFU) assay and the expression of antiviral innate immunity genes was evaluated by RT-qPCR. Interestingly, although OROV was not able to establish a productive infection in human PBMCs, significant levels of viral genome were maintained in a small proportion of these cells (mainly monocytes and lymphocytes), as demonstrated by RT-qPCR and RNA PrimeFlow. The OROV infection in PBMCs were followed by increased expression of type I and II IFNs and Interferon-stimulated genes (ISGs). Thus, the data indicate that even though the human lymphocytes and monocytes lineages are infected by OROV, human PBMCs cells are not normally permissive to OROV infection. However, the maintenance of viral genome in lymphocytes and monocytes points that these cells may act as a Trojan horse in specific situations or microenvironments, as observed during immunosuppression in the central nervous system.

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Abstract #065

THE USE OF A NEUROPROTECTIVE DRUG IN THE CONTEXT OF USUTU VIRUS INFECTION

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Usutu virus (USUV) is an arbovirus capable of causing encephalitis in humans and other vertebrates. This pathogen demands greater attention due to increased detection in mosquitos and birds throughout Africa and Central Europe. The lack of specific treatments or vaccines only heightens this need. Guanosine is a neuroprotective compound which has been used in the treatment of various neuropathologies. We hypothesized that the neuroprotective properties of guanosine may be beneficial against USUV infection. C57BL/6 mice (8-12 weeks old) intracranially infected with 101 (LD80) and 103 PFU (LD100) of USUV presented signs of neurological disease including hunched back, paralysis, and conjunctivitis. Mice succumbed to these doses of virus after 10 and 7 days, respectively. Increased viral loads and Inflammatory cytokines were only observed in the brain. Guanosine treatment to C57 WT mice infected with 101 PFU of USUV is not protective. We therefore evaluated guanosine's effects *in vitro*. USUV infection at a MOI of 0,1 was cytotoxic to human neuroblastoma cells (SH-SY5Y), leading to 107 viral PFU, 48h post infection (p.i.). Treatment with guanosine in SH-SY5Y cells did not improve cell viability or interfered in the viral load. Diversely, we were not able to recover virus from induced pluripotent stem cell-*derived microglia* (iPSC-MGLCs) infected with USUV. 104 viral units were recovered from USUV-infected microglia treated with guanosine, which indicates that guanosine treatment potentiates USUV replication in these cells. This finding correlates to our previous result, that guanosine is not protective *in vivo*. Overall summary, our data shows the intracranial infection with USUV causes encephalitis in adult C57 mice. We observed that guanosine does not prevent mortality *in vivo*. In accordance, guanosine does not improve cell viability or interferes in USUV viral load in neuroblastoma cells. However, the treatment led to increased susceptibility to infection in iPSC-MGLCs.

Financial support: FAPESP, CAPES.

Abstract #066

7-DEAZA-2'-C-METHYLADENOSINE (7DMA) TREATMENT IS PROTECTIVE AGAINST USUTU VIRUS INFECTION

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Usutu virus (USUV) is an arbovirus causing encephalitis in humans and other vertebrates. The increase detection in mosquitos and birds throughout Africa and Central Europe demands attention. The lack of specific treatments or vaccines only heightens this need. 7-Deaza-2'-C-Methyladenosine (7DMA) is a viral polymerase inhibitor characterized by its antiviral activity against *Zika virus*. We hypothesized 7DMA treatment is beneficial in USUV infection. To evaluate the antiviral and cytotoxic effects of 7DMA, VERO cells and human immortalized neuroblastoma cells (SH-SY5Y) were infected with USUV. 7DMA [50uM] was added every 2h during 24h corresponding to a complete USUV replication cycle. The supernatant was collected 24h p.i. and the viral load was accessed by plaque assay. In VERO cells, 7DMA [50Um] reduced the viral load in 2 logs when added during the first 2 hours p.i.. A reduction of 1 log was observed up to 12hr p.i.. In SH-SY5Y, a 1 log reduction was observed up to 16h p.i. indicating 7DMA is a potent inhibitor of USUV *in vitro*. Further, to investigate 7DMA's efficacy *in vivo,* type I e II knockout mice (IFN-α/βγR−/−) were subcutaneously infected with a lethal dose (10^4) of USUV. Mice were daily treated with vehicle solution or 7DMA [50mg/Kg] via gavage for 6 days p.i. We observed that the infected vehicle treated mice succumbed to infection on day 6 p.i. while mice treated with 7DMA survived up until day 9 p.i.. This data demonstrates that 7DMA is protective against USUV infection *in vivo.* After the characterization of the IFN-α/βγR−/− mice model we aim to investigate the protective potential of 7DMA in wild type mice infection model. Overall summary, our data indicates 7DMA efficiently reduced the viral load *in vitro*. Further, we observed that the subcutaneous infection with USUV causes mortality in adult IFN-α/βγR−/− knockout mice. Moreover, mice treated with 7DMA present delayed mortality, indicating 7DMA is a promising drug against USUV infection.

Financial support: FAPESP, CAPES.

Abstract #067

DENGUE AND ZIKA OCCURRENCE IN CHILDREN AND TEENAGERS IN A HIGH ENDEMICITY AREA

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For three decades dengue has been endemic in Brazil and in the last three years, circulated simultaneously with Zika in an extensive epidemic. Dengue (DENV) and Zika (ZIKV) are flavivirus with similar clinical manifestations, implying the need of a differential diagnosis. In this study we investigated Dengue and Zika infection in a cohort of children and adolescents with febrile cases in Goiânia, Goiás, Brazil Midwest, from 2015 to 2019. In total, 947 subjects had serum and urine samples (when suggestive of Zika infection) collected between the 1st and 5th day of disease. 59.3% subjects aged between two and nine years and 50.2% were female. Real time polymerase chain reaction followed by reverse transcription (RT-PCR) and serological techniques for NS1 antigen (NS1Ag) and antidengue and antizika IgM and IgG antibodies were performed for laboratory confirmation at BioTec Laboratory, Pharmacy Faculty- UFG. Results demonstrated that 135/947 (14.2%) samples were positive for dengue virus detected by one or more tests used. Viral RNA was detected in 68 (50.4%) samples, 120 (88.9%) were positive for NS1Ag and 72 (53.3%) for antidengue IgM. 80 (59,2%) samples were Dengue IgM positive without zika diagnosis confirmation. 37/947 (3.9%) samples were positive for ZIKV, which the viral RNA was detected in 24 (64.9%) serum samples, 13 (35.1%) in urine samples. Coinfection in Zika and Dengue cases was evidenced in seven cases which has PCR Zika results with NS1Ag and Dengue IgM positives. The antibody cross-reaction in Zika IgM and Dengue IgM was observed in four cases. Regions with DENV and ZIKV co-circulation the use of techniques with viral detection is enable differential diagnosis. Brazil Midwest is a region with high incidence resulting in significant epidemics, emphasizing the importance of understanding these diseases.

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Abstract #068

MAYARO VIRUS INFECTION INDUCES INFLAMMATORY RESPONSE IN OSTEOCLAST AND OSTEOBLAST AND TRIGGERS BONE LOSS: ROLE OF CCL2 CHEMOKINE AND ITS RECEPTOR (CCR2)

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The Mayaro virus (MAYV) is an emergent arbovirus, member of the Togaviridae family, genus Alphavirus. He is responsible for sporadic outbreaks of acute febrile illness in countries of South America, including Brazil, where it is considered endemic. Mayaro Fever is a disease of a self-limiting nature, which can range from mild to moderately severe. As with other alphavirus infections, the rheumatic disease caused by the MAYV is characterized by disabling pain, arthritis and myositis. Our aim was to establish a murine model of Mayaro virus (MAYV) infection in immunocompetent mice and study the role of CCR2/CCL2 axis in bone loss induction. Four-week-old mice (wild type and $CCR2^{-/-}$) were infected with $1x10^6$ PFU of MAYV in the footpad. Clinical signs, viral loads and inflammatory parameters were assessed. Bone loss was measured by Micro-CT analysis. The potential of the MAYV to infect osteoclasts and osteoblasts was evaluated in vitro. Results demonstrated that MAYV induces paw edema at initial days after infection, prolonged hypernociception up to 28 dpi and massive viral dissemination to several tissues. Cytometry analysis revealed predominant migration of $CCR2^+$ cells in the muscle and increased expression of CCL2 by ELISA was observed in serum, maxilla, spleen and muscle. Micro-CT analyzes of tibia from MAYV-infected WT mice revealed increased bone loss from 14 to 21dpi that was reduced in $CCR2^{-/-}$ mice. Data from osteoblast and osteoclast cultures have demonstrated that MAYV is able to replicate in both cell types and induce the production of inflammatory mediators in later times. Overall, these data show that the C57BL/6 mice are a promising model for studying MAYV-induced arthritogenic disease specially the mechanisms involved with bone loss architecture.

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

Abstract #069

REMOTE SENSING FOR RISK MAPPING *AEDES AEGYPTI* INFESTATION: IS THIS A PRACTICAL TASK?

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Mosquito-borne disease affects million people in the world, and its transmission area continues to expand due to many factors linked to urban sprawl and global warming. The *Aedes aegypti* mosquito plays a central role in the dissemination of dengue, zika, chikungunya and urban yellow fever. Current preventative measures include mosquito control programs but unfortunately, identifying the mosquito habitats over a large geographic area based only on field survey is time-consuming and labour intensive. Thus, the objective of this study was to assess the potential of remote satellite images for determining land features associated with *A. aegypti* adult infestation in São José do Rio Preto/SP, Brazil. We used data from 60 adult mosquito traps; the remote sensing images were classified for landcover types using a supervised classification method. We modelled the number of *A. aegypti* females using a Poisson probability distribution in a geostatistical approach. The models were built in a Bayesian context using the Integrated Laplace Approximations (INLA) and Stochastic Partial Differential Equation method. We showed that *A. aegypti* adult females were positively associated with a large percentage of asbestos roof and flat slab. This may be related to several other specific features of the landscape, such as socioeconomic or environmental factors. Asbestos roof may be related to poor areas or because its ability to retain heat, optimizing the reproduction of mosquitoes. Regarding flat slab, this is a type of construction that can retain rainwater and increase the temporary mosquito breeding sites. Although preliminary, in this study we indicated the direction that future researches should follow, and demonstrated the utility of satellite remote sensing to identify landscape differences in urban environments in an epidemiological approach. Further analysis of site microclimatic factors may reveal more complex relationships between urban mosquito habitats and landcover features.

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Abstract #070

EVALUATION OF THE SPECIFICITY OF A COMMERCIAL NS1-TARGETED ANTI-ZIKA VIRUS IGM AND IGG ENZYME-LINKED IMMUNOSORBENT ASSAY

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Currently, many countries have concomitant transmission of several arboviruses that cause exanthematic febrile illness with confounding symptoms. Because of the global spread of Zika virus and the expected cross-reactivity among flaviviruses, accurate diagnostic immunoassays are needed. Recently, a commercial anti-ZIKV IgG and IgM ELISA assay (Euroimmun, Lübek, Germany) was developed and has shown high sensitivity for the serodiagnosis of ZIKV infections. In this study we evaluated the specificity of this test by using it in DENV and YFV positive samples. Sera were obtained from 232 patients with acute primary (n=101) or secondary dengue infection (n=131) (discriminated through avidity test) caused by the 4 serotypes and also from 79 pairs of serum samples from pre and post YFV vaccination. The evaluation using DENV primary samples resulted in no cross-reaction in anti-ZIKV IgG and 2 inconclusive results in the anti-ZIKV IgM. Conversely, 32/131 (24.4%) samples from patients with secondary acute dengue presented unequivocal positivity in the anti-ZIKV IgG ELISA test. For IgM, only 3 positives and one inconclusive result were observed. According to the serotype, 5.5% (1/18) of DENV-1, 16.6% (8/48) of DENV-4, 23.8% (5/21) of DENV-3 and astonishing 63.6% (28/44) of DENV-2 were positive for anti-ZIKV IgG. No cross-reaction was observed in YFV post-vaccination samples in both tests. Crucially, such cross-reactivity for anti-ZIKV IgG in DENV acute samples may be a problem in regions endemic for flaviviruses.

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Abstract #071

ANALYSIS OF THE EFFECT OF ANTIOXIDANT ACTIVITY OF A CARBON-BASED NANOMATERIAL ON ZIKA VIRUS INFECTIONS

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Zika virus (ZIKV) is one of the most important public health arboviruses. The increase of ZIKV cases in recent years has led to a worldwide concern due to the ease of dissemination, difficulty to combat vectors and related serious diseases. It is already known that during the viral infections occurs an increase of reactive species (RS) that leads an imbalance in redox homeostasis that causes biological damage important to viral pathogenesis. Thereby, to control RS may be a strategy to fight the infection. Some Carbon-based nanomaterial (CBNs), has antioxidant activity due to its high capacity for sequestrating the RS. In this sense, this study aimed to evaluate the antioxidant activity of one CBN during ZIKV infection. To evaluate CBN cytotoxicity, a MTT cell viability assay was performed on Vero cells. The antioxidant capacity of CBN was tested from ORAC assay using a standard Trolox curve. The antioxidant potential of CBN was measured by reactive oxygen species assay (ROS), U87MG cells were infected with ZIKV (MOI 1) and treated with CBN for 24 hours, after the incubation the Carboxy-DCFDA probe (Invitrogen™) was added and the reading was performed on the Victor X3 (Perkin Elmer) plate reader with wavelength of 485/535nm. The results showed that for all tested concentrations (up to 12.5 µM) are not cytotoxic. CBN presented an antioxidant capacity in low concentrations 50, 20, 5 and 10 nM, as expected since in higher concentrations occurs the formation of crystals, which could impair its activity. The reduction of ROS production of 17, 44, 43 and 57% was observed at concentrations 50, 25, 12.5 and 6.25 nM, respectively. Also, preliminary results have been shown a reduction of virus multiplication in cell treated with this CBN in lower concentrations. These results suggest that this CBN could be used against ZIKV in the context of infections.

Financial support: UFOP, CAPES, CNPq, FAPEMIG.

Abstract #072

ISOLATED EXTRACTS FROM *TONTELEA* SP. PROMOTE VIRUCIDA ACTION AGAINST ZIKA VIRUS

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Zika virus (ZIKV) is among the most important arboviruses and major public health problems in the Americas. Its transmission occurs through vectors or vertically in pregnant women and sexual transmission. ZIKV causes a variety of clinical manifestations and neurological complications that result in fetal malformation, microcephaly, and other snags. No vaccine or antiviral therapy against ZIKV is employed, so the search for antiviral is relevant. The aim of this study was to evaluate different extracts obtained of *Tontelea* sp. against ZIKV. Then, mammalian cells (VERO) were added into microplates ($5x10^4$cells/well), and treated with of four extracts at different concentrations to determinate the cytotoxic concentration to 50% of the cells (CC_{50}). The revelation was obtained after 48h by methyl-thiazol-tetrazolium (MTT) colorimetric method. We have detected the CC_{50} concentrations 182.0, 370.8, 225.0 and 351.5 µg/mL for twigs extracts (TGH and TGC) and for leafs extracts (TFAE and TFM), respectively. For antivirals assays, the same cells pre-treated with extracts were infected with ZIKV at multiplicity of infection (moi) of 0.1 virus/cell. 48hpi, we found the protective/effective concentration to 50% of cells (EC_{50}) and the results showed that TGH, TGC, TFAE and TFM were able to inhibit ZIKV at 38.7, 38.5, 74.2 and 83.0 µg/mL concentrations, respectively. Finally, the selective index (SI) was calculated which refers to the ratio between CC_{50} and EC_{50} each extract, which should be above 4.0. The SI were larger than 4 for the extracts TGH (SI=4.7), TGC (SI=9.6) and TFM (SI=4.2). The TFAE was considered toxic or non-selective. Then, we can determinate the virucidal concentration for TGH, TGC, TFAE and TFM extracts and its showed less than 15 µg/mL for all of them. Therefore, our data indicate potentials antiviral/virucidal actions against ZIKV in compounds present in the plant studied that belongs to *Tontelea* genus.

Financial support: UFSJ, FAPEMIG, CAPES, CNPq.

Abstract #073

ANTIVIRAL ACTIVITY OF AAK1 INHIBITORS AGAINST OROPOUCHE INFECTION

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Oropouche orthobunyavirus (OROV) is an arbovirus associated with frequent cases of acute febrile illness in the Amazon region. The high genetic diversity between orthobunyavirus generated by genetic reassortment or mutations, makes the design of broadly effective direct-acting antivirals a challenging task. Thus, host-targeted drugs looks a good alternative to develop antivirals against OROV. OROV infect host cells through clathrin-mediated endocytosis (CME), a highly orchestrated process dependent of activity of adaptor-associated protein kinase 1 (AAK1). Thereby, to investigate if chemical inhibition of AAK1 could inhibit OROV infection, we determined the half maximal inhibitory concentration (IC50) of three compounds (LP935509 and SGC-AAK1-1, both AAK1 inhibitors, and SGC-AAK1-N, a chemical negative control), in Vero E6 cells by focus formation assay after infection with 100 FFU of OROV. Unexpectedly, all compounds, including the negative control, had antiviral activity against OROV, with IC_{50}=4.7uM, 8.5uM and 1.8uM for LP, SGC-AAK1-1 and SGC-AAK1-N, respectively. Interestingly, results performed by a time-of-drug addition time approach showed higher OROV inhibition rate induced by SGC-AAK1-N when 5µM of this compound was added in the following time points: -2 and 0 hpi, using a MOI = 1. In contrast, LP and SGC-AAK1-1 treatments showed only a moderate antiviral activity by this assay, with no more than one log of reduction in any time points. In the end, the antiviral effect of SGC-AAK1-N during OROV infection was demonstrated after siRNA-transfection of Vero cells. In conclusion, although AAK1 inhibitors have had antiviral effect against OROV, the best candidate was our chemical negative control SGC-AAK1-N, a kinase inhibitor with no effect in AAK1. These results suggest than other kinases or other mechanisms of viral penetration are used by OROV in Vero cells.

Financial support: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

Abstract #074

IN-DEPTH CHARACTERIZATION OF CONGENITAL ZIKA SYNDROME IN IMMUNOCOMPETENT MICE: ANTIBODY-DEPENDENT ENHANCEMENT AND AN ANTIVIRAL PEPTIDE THERAPY

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Zika virus (ZIKV) infection during pregnancy can cause several birth defects to developing fetuses, and this disease state is termed Congenital Zika Syndrome (CZS). However, few studies have begun to investigate the potential long-term outcomes of affected offspring and possible pathogenic mechanisms. Our aims were to characterize the effects of congenital *Zika virus* (ZIKV) infection in immunocompetent mice and evaluate the potential role of antibody-dependent enhancement (ADE) in the exacerbation of those effects as well as the effects of an antiviral therapy against ZIKV. C57BL/6 pregnant dams were inoculated with $1x10^6$ PFU of ZIKV (HS-2015-BA-01 strain) by intraperitoneal route on gestational day (GD) 5.5 in the presence or absence of anti-envelope pan-flavivirus antibody (4G2), while negative control dams were injected with isotype control antibody (IgG2a) and PBS. Pregnant dams were euthanized 2 and 24 hours after infection to evaluate maternal immune activation (MIA) caused by ZIKV infection. Fetal alterations were analyzed on GD 15.5 and offspring investigated during adulthood. Congenital ZIKV infection induced MIA and fetal abnormalities in the offspring, as detected by higher viral loads, inflammatory mediators alterations and decreased fetal weight. Therapeutic administration of the AH-D antiviral peptide during the early stages of pregnancy prevented ZIKV replication and death in offspring. In the post-natal period, ZIKV infection resulted in neuropathological and ophthalmologic alterations, changes in intratubular morphology of testes and disruption of bone microarchitecture. Some alterations were enhanced in the presence of 4G2 antibody. Taken together, these results reveal that early maternal ZIKV infection causes several birth defects in immunocompetent mice, some of which can be enhanced by ADE phenomenon and are associated with MIA. Moreover, antiviral treatments, such as AH-D peptide therapy, may be beneficial during early maternal ZIKV infection.

Financial support: INCT Dengue, CNPq, CAPES, FAPEMIG, FINEP, National Research Foundation of Singapore and the Centre for Precision Biology at Nanyang Technological University.

Abstract #075

THIOSEMICARBAZONE INHIBITS CHIKUNGUNYA VIRUS *IN VITRO*

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Chikungunya virus (CHIKV) is a single positive strand RNA virus from the *Togoviridae* family that is transmitted through the bite of the female mosquito *Aedes* sp. Virus infected patients are affected by Chikungunya fever and present fever and joint pains as clinical symptoms. One of the main problems of CHIKV infections is that patients may develop a chronic disease that can lead to a disability. Currently, there is not antiviral treatment against CHIKV, demonstrating the need to develop noval antiviral agents. In this context, some studies have shown that thiosemicarbazones and their metal complexes possess pharmacological properties as antitumor, antiprotozoal, antibacterial and antiviral activity. Additionally, their synthesis is simple, clean, versatile and results in high yields. Therefore, this work aimed to evaluate the antiviral activity of two thiosemicarbazones (one and two) and their three metal precursors against CHIKV *in vitro.* For this, BHK 21 cells were infected with CHIKV *nanoluciferase* (MOI 0,01) in the presence or absence of compounds at non-cytotoxicity concentration for 16 hours. Through this assay, the thiosemicarbazone 1 at 50 µM inhibited 90% of CHIKV infection. Using a wider range of concentrations, it was determined that the compound has an EC_{50} of 20 µM, CC_{50} of 61 µM and SI of ≈ 3. Further analyses are in progress to assess the stage of virus replicative cycle inhibited and mechanism of action of this compound.

Financial support: CAPES, CNPq, Royal Society.

Abstract #076

IN VITRO ACTIVITY OF ANALOGUES OF PEPTIDES ISOLATED FROM INSECT POISON AGAINST THE ZIKA VIRUS

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Zika fever is an arboviruses caused by the Zika virus (ZIKV). The symptoms include fever, muscle aches, joint pains, skin rash, congenital malformation and neurological disorders associated to the infection, as microcephaly and Guillain-Barré syndrome. In 2016, the ZIKV outbreak had a worldwide impact and was considered a serious public health problem due to the increase number of newborns with microcephaly. Currently, there is no effective antiviral treatment against ZIKV infection, being necessary the development of noval therapies. Synthesized peptides based on natural bioactive molecules present as an alternative approach to the development of antivirals. Thus, the present work aimed to evaluate the effects of two synthetic peptides designed based on natural molecules isolated from *Oreumenes decoratus* insect venom on ZIKV infection. To this, Vero cells were infected with ZIKV-Nanoluc, a viral construct with the reporter gene Nanoluc, at the presence or absence of the synthetic peptides for 72 hours. Different concentrations of each peptide were evaluated by their citotoxicity (MTT) and effect on infectivity (Luciferase). The results showed that peptides 1 and 2 inhibited more than 90% of the virus infection at non-toxic concentrations. Additional analyses will be performed to evaluate the mode of action of these peptides.

Financial support: CNPq, CAPES, Royal Society.

Abstract #077

REDUCTION ON WEIGHT GAIN IN MICE INFECTED WITH DIFFERENTIATES BRAZILIAN ISOLATES OF ZIKA VIRUS

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Zika virus (ZIKV) is an arbovirus transmitted by mosquitoes of the genus *Aedes*. Phylogenetic analyses reveal the existence of two major lineages: one includes the African isolates, and the other the Asian and American isolates. ZIKV infection was characterized by causing a mild disease presented with fever, headache, rash, arthralgia, and conjunctivitis, with reports of an association with Guillain-Barré syndrome (GBS), microcephaly and meningoencephalitis. Our objective was to compare the immune response triggered by two Brazilian not mice adapted isolates of ZIKV (PE243 and SPH) when the central nervous system (CNS) was exposed. For this, 8 week age C57BL/6 wild-type and TLR2/9 and iNOS knockout mice were infected intracranially with 400 p.f.u. of PE243, SPH and MR766, an African mice adapted isolate, as positive control. Negative control mice were injected intracranially with C6/36 cell culture supernatant (mock). Mice were observed and weighed daily. C57BL/6 mice infected with MR766 lost weight, had conjunctivitis, paralysis, and died 8 to 12 days after infection as described in literature. C57BL/6 mice infected with Brazilian isolates did not die nor showed signs, but mice infected with PE243 gained less weight than mock, with statistical difference, and mice infected with SPH showed no statistical difference compared to mock. TLR2/9 KO mice infected with MR766 showed the same signs as C57BL/6 and died 7 to 9 days after infection. TLR2/9 KO mice infected with Brazilian isolates did not die nor showed signs but gained less weight than mock. iNOS KO mice infected with MR766 showed the same signs compared to wild type mice and died at 7 days after infection. iNOS KO mice infected with Brazilian isolates did not die, nor showed signs or lost of weight. We conclude that there was a reduction of weight gain in mice when infected with different Brazilian Zika isolates and TLR2/9 and iNOS in the model used do not impact on infection with ZIKV.

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Abstract #078

STANDARDIZATION FOR DETECTION OF SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) IN INNATE IMMUNE RESPONSE GENES THROUGH QPCR: EVALUATION IN ZIKA VIRUS MENINGOENCEPHALITIS

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Zika is an arboviruses caused by the Zika virus (ZIKV) that is transmitted by *Aedes* mosquitoes. With the spread of the virus to South America, there has been an increase in the occurrence of cases with nervous system (NS) complications associated with ZIKV infection, such as Guillain-Barré Syndrome (GBS), severe congenital malformations and meningoencephalitis. The mechanism of ZIKV immune response induction is not well understood. One hypothesis raised for the increase in cases of neurological manifestations may be due to host genetic factors. Polymorphisms are changes in the genome that may or may not compromise gene function. Single nucleotide polymorphisms (SNPs) are of great importance, especially when they occur in genes of the innate immune system, and may compromise the activity of these genes and, as a consequence, the individual's response to infections. The association of SNPs in the predisposition to severe clinical manifestations in ZIKV infections is not known. The aim of this work is to standardize assays for genotypic determination through allelic discrimination in CSF samples of children infected with ZIKV, which had manifestations in the NS. SNPs were selected according to the literature and are associated with genes encoding Toll-like receptors (TLR) and TLR activation pathway proteins. The SNPs selected for the study are: rs1024611 in the MCP-1 gene, rs387907272 in the MyD88 gene, rs8177374 in the TIRAP gene, rs3775291 and rs5743316 in the TLR3 gene, rs179008 in the TLR7 gene and rs6194223 in the OAS3 gene. These were evaluated by TaqMan probe assays using qPCR. CSF samples with different cellularities were used. DNA was extracted with Qiagen's DNeasy Blood & Tissue kit. Cellularity didn't affect the results, however samples with greater cellularity show better amplification on PCR. To confirm the results, the amplified DNA was sequenced. Sequences were analyzed in the Sequence Scanner and Mega softwares.

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Abstract #079

PREVIOUS CHIKV EXPOSURE INDUCES PARTIAL CROSS-PROTECTION AGAINST SECONDARY MAYV INFECTION IN MICE

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Chikungunya virus (CHIKV) and Mayaro virus (MAYV) are evolutionary closely related members of the Semliki Forest virus antigenic complex, classified into *Alphavirus* genus from *Togaviridae* family. These viruses can cause disease in humans, including symptoms as sudden fever and joint involvement that can persist for long periods. MAYV infection represents a growing concern for public health, which has caused sporadic and geographic limited outbreaks in regions of CHIKV circulation. Previous studies have shown that cross-protection between different alphaviruses can be mediated *in vitro* by broadly neutralizing antibodies specific to conserved epitopes. Given the close phylogeny, symptoms similarities and serological relationship, the aim of this study is to evaluate the cross-protective immunity developed by CHIKV exposure to subsequently MAYV infection. Therefore, we have pre-exposed 6 weeks old immunocompetent mice (C57BL/6) to $1x10^6$ PFU of CHIKV by intraperitoneal infection and after 4 weeks they were challenged with MAYV by subcutaneous footpad inoculation. We observed a partial reduction in disease severity and in viral tissue burden, which suggests the development of low cross-neutralizing antibodies against MAYV. Furthermore, a partial reduction of inflammatory monocytes recruitment was observed in the footpad and ankle, which correlates with the reduction of histological score and paw edema development. We are currently evaluating the influence of CHIKV pre-existing immunity against MAYV. In summary, our data suggests a relevant cross-protection between CHIKV and MAYV, which may be important to patient management in the case of MAYV emergence in areas of CHIKV circulation.

Financial support: Fundação de Amparo à pesquisa do Estado de São Paulo (FAPESP).

Abstract #080

NANOCOMPOSITE-BASED ELETROCHEMICAL PLATFORM FOR ZIKA VIRUS DETECTION

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The present work describes the development of electrochemical genossensor with graphite electrodes modified with reduced graphene oxide (rOG) and polythiramine (Poly-Tyr) to detect the gRNA of Zika virus (ZIKV). Zika virus (ZIKV) infection has become an emerging global health issue since the 2015 outbreak in Brazil and other countries, including the US. The control and monitoring of ZIKV infection are limited due to the unavailability of drugs, vaccines and rapid diagnosis. The most commonly used diagnostic test is the reverse transcriptase (RT-PCR) technique, which consists of a laborious method and can lead to indeterminate results. In this study, a novel biosensor based on graphite electrodes chemically modified was performed using electrochemical detection of hybridization between the virus-specific DNA probe (ZIK1) and the Zika RNA extracted from infected patients' serum (target). The electrochemical reduction of graphene oxide was conducted between 0.00 V to -1.50 V (*vs*. Ag/AgCl), 50 $mV.s^{-1}$, 10 scans. Next, tyramine 2.5 mmol L^{-1} was electrodeposited on graphite electrode by cyclic voltammetry, 60 scans, 0.00 to +1.10 V (*vs*. Ag/AgCl), 50 $mV.s^{-1}$.10 µL of different gRNA concentrations (10^{-6}, 10^{-8}, 10^{-9}, 10^{-12} and 10^{-14} µg/mL) were added onto modified graphite electrodes. The detection was conducted by differential pulse voltammetry using $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (5.0 mmol L^{-1}) solution containing KCl (0.10 mol L^{-1}). The analytical parameters were analyzed and the electrochemical genossensor showed detection limit of 10fg/mL. The platform was sensitive to variations in genetic material concentration and demonstrated efficient and cost-effective solutions in infection control and fills the gap of lack of diagnostic methods for the Zika virus.

Financial support: FAPEMIG, CAPES, CNPq.

Abstract #081

T CELL RESPONSES IN MICE IMMUNIZED WITH A DNA VACCINE ENCODING THE ECTODOMAIN OF THE DENV2 ENVELOPE PROTEIN

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The search for a safe and effective vaccine against all four serotypes of the dengue virus (DENV1-4) persists as a global health challenge. Our group constructed the pE1D2 DNA vaccine encoding the ectodomain of DENV2 Envelope (E) protein. The pE1D2 elicited a protective immune response against DENV2 in BALB/c mice, with production of neutralizing antibodies and activation of IFN-γ-producing T-cells. Although neutralizing antibodies have been considered the host's major defense mechanism against DENV, clinical and experimental data advise that T-cell response is also required for protection. Thus, we aimed to characterize the cellular immune response elicited by pE1D2. First we depleted $CD4^+$ or $CD8^+$ subsets of T lymphocytes in pE1D2-immunized BALB/c mice and subsequently challenged them with DENV2. Depletion of $CD4^+$ T cells almost completely abolished protection elicited by pE1D2, while about 80% of $CD8^+$ T cell-depleted mice survived viral challenge. However, 60% of pE1D2-immunized mice presented clinical signs of infection when depleted from $CD8^+$ T cells. In addition, E-derived epitopes involved in the immune response elicited by pE1D2 were screened using a peptide library covering the ectodomain sequence of the E protein. Peptides were used in ELISPOT and intracellular cytokine staining (ICS) assays with splenocytes isolated from pE1D2-immunized mice, challenged or not with DENV2. We identified 4 peptides involved in the immune response mediated by pE1D2 and 3 other peptides after viral challenge. ICS revealed that both $CD4^+$ and $CD8^+$ T cells were involved in IFN-γ and TNF-α production. The IFN-γ ICS confirmed reaction of almost all E-derived peptides. Besides, we observed an increase of TNF-α production by either $CD4^+$ or $CD8^+$ T cells from pE1D2-immunized mice after virus infection. These results accentuate the urgency of better understanding T cell response involved in protection against DENV and its application in vaccine strategies.

Financial support: CNPq, FAPERJ, INCTV, IOC/FIOCRUZ.

Abstract #082

IS THE ADP RIBOSE SITE OF THE CHIKUNGUNYA VIRUS NSP3 MACRO DOMAIN A TARGET FOR ANTIVIRAL APPROACHES?

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Several arboviruses that were previously restricted to geographic locations have been dispersed throughout the world in the recent years. Chikungunya virus (CHIKV) is an arbovirus of special concern due to cause the Chikungunya fever, characterized by an acute febrile illness, rash, and arthralgia that can progress to chronic and debilitating arthritic symptoms. Additionally, no commercial antiviral or vaccine are currently available against CHIKV infection. Therefore, the development of novel therapies that may lead to a future treatment is necessary. In this context, the ADP-ribose site into the macro domain of CHIKV nsP3 has been reported as an interesting target for the development of antiviral. Mutations in the ADP-ribose site demonstrated to decrease viral replication in cell culture and reduce virulence. Also, when the ADP-ribose binding of nsP3 macro domain was blocked, CHIKV recombinants were non-viable. In this study, 48,750 small molecules were screened *in silico* to its ability to bind to the ADP-ribose site at the macro domain of CHIKV nsP3. From in silico analysis, 12 small molecules were selected to perform *in vitro* analysis by using a CHIKV subgenomic firefly luciferase replicon in Huh-7 cells. Cell viability and CHIKV replication were evaluated and molecules C5 and C13 demonstrated to inhibit 53 and 66% of CHIKV replication, respectively. C5 and C13 did not inhibit CHIKV replication in C212 and RD cells. By using a CHIKV-Dual luciferase replicon contain two reporter genes, we also demonstrated that the treatment with either compounds are probably interfering in the early replication rather than after RNA replication has occurred.

Financial support: CAPES, Royal Society, Wellcome Trust Investigator Award.

Abstract #083

THE ROLE OF TAM RECEPTORS, AND THEIR LIGAND, GAS6, IN RESISTANCE AND SUSCEPTIBILITY DURING ZIKV INFECTION

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Zika virus (ZIKV) has gained worldwide attention as it has been correlated with severe fetal malformations, causing the Zika Congenital Syndrome (ZCS). However only 6-12% of mothers infected with ZIKV give birth to babies with malformations. These observations suggest that ZIKV infection during pregnancy is not deterministic for ZCS, but other susceptibility factors might be involved. It is possible that the essential targets of these differences are the viral entry receptors. *Tyro3*, *Axl*, *Mertk* (TAM receptors), and their ligands, Gas6 and Protein S are important candidates for ZIKV internalization. Still, their correlation with resistance or susceptibility to infection is unknown. Evaluate the immunobiology of TAM receptors, and their ligand, Gas6, in the context of a ZIKV infection. We observed that SJL, susceptible mouse lineage, showed higher levels in mRNA expression of TAM receptors compared with C57BL/6, resistant lineage. However, there are no differences in the expression of ISGs, suggesting no impairment of IFN 1 production. In this context, we demonstrated that the combination of rmGas6+ZIKV in SJL and C57BL/6 infection increased the viral load in spleen while the use of Axl kinase blocker, R428, decreased the number of viral particles. Interestingly, the use of rmGAS6+ZIKV led to the development of C57BL/6 affected offspring, turning this lineage susceptible to ZCS. Our results suggest the crucial role of TAM receptors**,** and the intracellular kinase portion of *Axl*, during ZIKV infection. These data contribute for a better knowledge about the biology of ZIKV, *in vivo*, that could be involved in the ZCS.

Financial support: FAPESP.

Abstract #084

THE ROLE OF mTORC COMPLEXES IN ASTROCYTES DURING ZIKV INFECTION

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Zika virus (ZIKV) has emerged as a global health problem since it was associated with the increase in microcephaly cases in Brazil between 2015 and 2016, however little is known about the mechanisms that trigger this condition. ZIKV is a *Flavivirus*, which presents a great tropism to the central nervous system (CNS), mainly by neuronal progenitor cells (NPCs) and glial cells. Astrocytes are abundant glial cells in the CNS and are fundamental in several physiologic functions within the CNS specially in the maintenance of neuronal metabolism by the production of lactate, via the glycolytic pathway, which is an important energetic substrate of neurons. Antiviral cytokines, as type I interferon (IFN), also activates glycolysis and is crucial for maintenance of the antiviral state. The IFN type I receptor (IFNAR) leads to the activation of AKT that activates protein complexes known as mechanistic target of rapamycin 1 and 2 (mTORC1 and mTORC2). These complexes are important in interferon stimulated genes (ISGs) translation and in glycolysis activation. It has been reported that ZIKV is able to block both the type I IFN pathway and AKT-mTOR activation. According to this, the aim of this work was to characterize the role of mTOR during ZIKV infection in astrocytes. Primary mouse astrocytes obtained from C57BL/6 WT mice, pre-treated or not with rapamycin (20nM) (mTOR blocker), were infected with ZIKV (*multiplicity of infection – MOI 1*) and evaluated by qPCR and plaque forming unit assay (PFU) 24 and 72 hours post infection. Preliminary data suggests that the impairment of mTORC1 activation during ZIKV infection by rapamycin, leads to a decrease in the viral load after 24h probably because the treatment with rapamycin can decrease the gene expression of *Ifit1* and *Oas2a* mRNA expression. Although preliminary, these data are important to support new studies focused on the role of mTORC in astrocytes and its relevance during neurogenesis and microcephaly during ZIKV infection.

Financial support: FAPESP.

Abstract #085

INHIBITITION OF NS2B/NS3 PROTEASE OF ZIKA VIRUS AND REPLICATION *IN VITRO*

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Zika virus (ZIKV) was not considered a dangerous human pathogen. However, this view has changed since 2015 when a large outbreak occurred in Brazil and rapidly spread to other countries in the region. During this widespread in South America, clusters of microcephaly and Guillain-Barré syndrome in newborns were reported, which were associated with Zika virus infections. Similar to other flavivirus, ZIKV expresses the serine proteinase NS3 that is responsible for viral protein processing and replication. Herein, we report the inhibition of the Zika virus Ns2B/Ns3 proteinase by three molecules using biochemical and biophysical methods. We extend our investigation using *in vivo* inhibitory effect on ZIKV replication in VERO E6 cells by plaque reduction assay. Cells were infected with 25 PFU of ZIKV for 1h at 37 °C, following overlay with MEM+1% carboxymethylcellulose (CMC) with or without molecules at a concentration of 5 µM and 15 µM. Treated cells showed efficient inhibition of the viral replication at concentrations that presented minimal toxicity to the cells. The assays showed that three molecules reduced around 50% of ZIKV replication. These data were also confirmed by qPCR and we performed viral replication growth curve, the data showed inhibition in the first 24h of treatment. Besides, we evaluated the virucidal effect of these molecules in Vero E6 cells, and at two different concentrations, there was no inhibiting effect on virus replication. Our findings pave way for the usage of these molecules as an inhibitor lead compound for ZIKV.

Financial support: CNPq, CAPES, FAPESP.

Abstract #086

OROPOUCHE VIRUS *IN VITRO* REPLICATION IS INHIBITED BY ACRIDONE FAC 06

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Over the decades, arboviruses have been responsible for important public health issues, *Oropouche orthobunyavirus* (OROV) is an emerging arbovirus associated with a fever illness called Oropouche fever in the Amazon region of South and Central America, is neurotropic in animal models and can cause encephalitis and meningitis in humans. However, the pathogenic determinants associated with neurological involvement is not fully understood and there is still no antiviral therapy specified for the treatment of these infections. In this study, we report the inhibition of virus replication in Vero E6 cells by plaque reduction assay. Cells were infected with 25 PFU of OROV for 1h at 37°C, following overlay with MEM+1% carboxymethylcellulose (CMC) with or without acridone FAC-06 at a concentration of 5µM. Treated cells showed efficient inhibition of the viral replication at concentrations that presented minimal toxicity to the cells. The assays showed that the acridone exhibited a >70% inhibition of OROV replication with no effect on cell viability. Our results suggest that this is a promising molecule to be studied with potential antiviral activity against the Oropouche virus.

Financial support: FAPESP.

Abstract #087

IDENTIFICATION OF ARBOVIRUSES AS NEUROLOGICAL DISORDERS PROMOTERS IN PATIENTS ADMITTED AT A COLLEGE HOSPITAL IN SÃO JOSÉ DO RIO PRETO, SÃO PAULO, BRAZIL

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Arboviruses are transmitted by several hematophagous arthropods, like *Aedes aegypti* e *A. albopictus.* Two arboviruses families are remarkable for their impact on public health-*Togaviridae,* as *Chikungunya (CHIKV)* virus; and the *Flaviviridae* family, as *Dengue (DENV)* and *Zika (ZIKV)* viruses. In Brazil, an increase in the arboviroses incidence rates has been observed in association with neurological disorders over the last years. Therefore, the work herein has aimed at retrospectively assessing the arboviruses incidence as neurotropic agents in cerebrospinal fluid (SCF) samples of patients showing neurological symptoms, seeking for a clinical and virologic diagnoses correlation to morbidity and mortality. We have selected, from arboviral disease-suspected patients with neurological symptoms attended in 2016-17, 281 samples of CSF, and 10 of serum. CSF and serum were screened by Enzyme-Linked Immuno sorbent Assay (ELISA) and/or reverse transcription polymerase chain reaction (RT-PCR) for DENV, CHIKV and ZIKV. Among all samples, 13 (4.47%; 13/291) have been positive to arboviruses. DENV infection has been detected in 53.8% (07/13) cases, ZIKV infection in 23.7% (3/13) and CHIKV infection in 15.38% (2/13). Guillain-Barré syndrome, encephalitis, meningitis and transverse myelitis have been the most frequent manifestations observed. Data, yet preliminary, have shown the importance of virologic identification of these agents in relevant clinical conditions. The association of arboviruses to neurological disorders has been shown to be increasing and evident. Literature offers few prospective analyses based on surveillance system. In a hyperendemic context of arboviruses co-circulation, the broad clinical impact of these one may only be established by constant and active surveillance of diagnoses. The present study will strongly contribute to the efforts for a differential diagnosis and characterization of etiological agents associated to central nervous system disorders.

Financial support: FAPESP.

Abstract #088

SCREENING OF THE ACRIDONES ANTIVIRAL ACTIVITY AGAINST OROPOUCHE VIRUS

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Oropouche virus (OROV) is an emerging arbovirus associated with epidemic of febrile disease in Amazonic region of Brazil and other countries of South and Central American. The major symptoms associated with OROV infection are headache, myalgia, arthralgia and exanthema. Moreover, hemorrhagic and neurological complications are also frequently associated with OROV infection in humans and other animals. There is still no antiviral therapy specified for the treatment of these infections. Thus, the present work aimed to evaluate the antiviral effects of 10 acridones against OROV. To this, different concentrations of each acridone were evaluated by their citotoxicity (MTT) and evaluated the inhibition of virus replication in Vero E6 cells by plaque reduction assay. Cells were infected with 25 PFU of OROV for 1h at 37°C, following overlay with MEM+1% carboxymethylcellulose (CMC) with or without acridone at a concentration of 5µM. Treated cells with FAC-15, FAC-22 and FAC-21 showed efficient inhibition of the viral replication at concentrations that presented minimal toxicity to the cells. The assays showed that the acridone exhibited a 50% (FAC-15 and FAC-22) and 60% (FAC-22) inhibition of OROV replication with no effect on cell viability. Our results suggest that these are a promising molecule to be studied with potential antiviral activity against the Oropouche virus.

Financial support: FAPESP.

Abstract #089

A CELL-BASED HIGH-CONTENT SCREENING ASSAY TO TEST FDA-APPROVED COMPOUNDS AGAINST OROPOUCHE VIRUS INFECTION

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Oropouche virus is an arbovirus present in Latin America, causing one of the most numerous mosquito-borne viral fevers in Brazil. Oropouche Fever has reached more than half million people, causing fever, body aches and inflammation of the central nervous system. Like other arboviruses, there is no specific treatment, neither vaccine, for the disease. High-content screening (HCS) is a method for massive testing of compounds, used mostly in drug discovery. We combined this technology with drug repurposing, which consists in giving a new purpose to a drug that is already approved for human use to treat a disease. At LNBio, we studied the characteristics of Oropouche virus (OROV) infection *in vitro* and developed an HCS assay to find compound candidates for a potential treatment. The aim of our project is to test and repurpose compounds using the NCC-NIH FDA approved drugs library for OROV treatment. We produced our viral stock with the OROV BeAn 19991 reference strain and established a high-throughput screening (HTS)/high-content screening assay for the identification of protective drugs based on counting HOESCHT-labeled nuclei using the Operetta microscope. The compounds were tested in 20μM concentration, and we screened over 700 drugs already approved for use, from which we selected 30 compounds capable of reducing the death of infected cells by at least 50% in comparison to uninfected controls. Most of the compounds that presented an reduction on cell death were related to neurotransmission, which is an interesting result, since the disease in some cases affects the SNC. Other compounds were from different classes, such as anthelmintic and anti-depressive. We successfully established an HCS assay for identification of compounds with protective properties against OROV infection. In the future, we hope to confirm our data with *in vitro* testing and characterize efficacy, mechanisms of action and move forward with in vivo testing. We intend to improve our assay by adding new fluorescent dyes for cytoskeleton and mitochondria.

Financial support:

Abstract #090

NEUROADAPTING A ZIKA VIRUS ISOLATED IN BRAZIL BY PASSING IN THE BRAIN OF NEWBORN MICE

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Zika virus (ZIKV) is the most recent arboviral infection to reach epidemic proportions in the western hemisphere. In general, the disease caused by ZIKV is clinically similar to those of other arboviruses (e.g. dengue), which hampers specific diagnosis, and occasionally it has been associated with neurological syndromes such as Guillain-Barré syndrome. Yet, ZIKV infections during pregnancy may cause the Congenital Zika Syndrome, with brain and systemic damages in the fetus and/or infants, including microcephaly. Most of murine models for ZIKV studies uses immunocompromised animals, which provides robust and lethal infections, but maybe not the ideal for vaccine tests. Therefore, in order to establish an immunocompetent murine model susceptible to the infection, our group has been working on the neuroadaptation of a clinical Brazilian ZIKV isolate sample by successive passages in the brain of newborn mice. Groups of newborn Swiss mice, ranging from three to eight days old, were infected by the intracerebral route with ZIKV. On the seventh day following infection, animals were euthanized and brains were collected. In order to assess the viral load curve, the brains were processed and samples were titrated by a plaque assay in Vero cell cultures. At each passage, the sample with the highest viral titer was used for subsequent inoculation in the brain of other newborn mice. Our initial results showed an alteration in the plaque morphology produced by infection with virus isolated after the second mouse passage comparing to those observed with the initial ZIKV sample, with an increase in the mean diameter of the plaques. Regarding viral load, there was a 4-log increase in the number of plaques during the first four passages followed by a slight reduction. Presently, we notice a new rise in the viral load in the brain of eight-day-old mice. Sequence of the virus genome revealed some point mutations and experiments will be continued by virus passages in older mice.

Financial support: FAPERJ, FINEP, INCTV, CNPq, IOC-FIOCRUZ.

Abstract #091

PRODUCTION OF A RECOMBINANT PROTEIN OF ZIKA VIRUS IN BACULOVIRUS EXPRESSION SYSTEM

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Zika virus (ZIKV) has become a public health emergency due to its possibility of congenital complications. The envelope protein of ZIKV (ZIKV-E) is the major protein involved in viral particle interaction with cell receptors and membrane fusion. It has been shown to be immunodominant and associated with the generation of antibodies and, thus, is strategically important for the serological diagnosis of the infection. The aim of this work is to produce a recombinant ZIKV-E in insect cells using the baculovirus expression system. Initially, genes coding from 148 ZIKV sequences were aligned to generate a consensus sequence, codon optimized and cloned into pET21. This vector was used as a template DNA for a conventional polymerase chain reaction (PCR) followed by analysis in agarose gel. The band corresponding to ZIKV-E gene was purified using the QIAquick gel extraction kit. Then, a blunt-end TOPO cloning reaction was performed between the insert (ZIKV-E) and pFastBac HBM-TOPO vector. The recombinant vector was used to transform bacteria from DH5α strain, with subsequent plating in selective medium. Positive clones were submitted to plasmid DNA extraction followed by an enzymatic digestion to confirm the correct orientation of the insert. Recombinant construct was used to generate baculoviruses containing the ZIKV-E gene by transposition in DH10Bac strain. A colony PCR was performed to confirm the transposition of ZIKV-E gene into the bacmid, which was then purified with the Pure Link Hi Plasmid Pure Kit and used to transfect Sf9 growing insect cells. Western blotting assays are being performed to verify the recombinant ZIKV-E expression in each viral passage. Afterwards, purification of the proteins will be carried out on Ni columns by affinity chromatography and the antigenicity of the recombinant protein will be accessed by an enzyme linked immunosorbent assay (ELISA) using serum samples from healthy and infected patients.

Financial support: CNPq, FAPEMIG.

Abstract #092

ACTIVITY OF A PHOSPHOLIPASE ISOLATED FROM *CROTALUS DURISSUS TERRIFICUS* ON THE REPLICATIVE CYCLE OF CHIKUNGUNYA VIRUS

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According to the Pan American Health Organization (PAHO), around 120,000 cases of Chikungunya fever in the Americas were confirmed in 2017. Unlike other arboviruses, the major problem of Chikungunya virus (CHIKV) infection is the possibility of chronification. There are no specific treatments or vaccines against CHIKV infection and infected patients are submitted to palliative care. In this context, natural compounds may provide an alternative source for the identification of molecules with therapeutic potential. Compounds isolated from animal venoms have shown antiviral activity against other viruses as dengue (DENV), yellow fever (YFV), and measles viruses. In addition, current treatments as hypertension have been based on isolated snake venom compounds. The present work aimed to evaluate the activity of proteins isolated from the venom of snake genera *Crotalus sp* and *Bothrops sp* on the replicative cycle of CHIKV *in vitro*. Cytotoxicity and antiviral activity of 5 proteins isolated from snake venoms were evaluated by MTT assay and CHIKV replicon systems in BHK-21 cells, respectively. PLA_2CB protein isolated from *Crotalus sp* showed significant antiviral activity, inhibiting 81% of CHIKV infectivity. The post entry stage of the CHIKV replication was evaluated, but no antiviral effect was observed by the treatment with the proteins. By our knowledge, this is the first description of the PLA_2CB activity on the CHIKV replicative cycle. Further analyses are in progress to evaluate the effect of the PLA_2CB on other stages of the replicative cycle.

Financial support: CAPES, CNPQ, FAPEMIG, The Royal Society.

Abstract #093

COMPARISON OF FLAVIVIRUS TITRATION TECHNIQUES TO ESTABLISH A FAST AND RELIABLE METHODOLOGY FOR SPECIFIC NEUTRALIZING ANTIBODIES IDENTIFICATION

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There is a huge overload of the Brazilian health system related to the occurrence of Flavivirus infections, especially those transmitted by *Aedes aegypti* bites, such as Dengue (DENV), Zika (ZIKV) and Yellow Fever (YFV). From those, Dengue infections represent the most important arboviral disease in the world in terms of epidemiologic impact. The WHO considers the plaque reduction neutralization test (PRNT) as the "gold standard" to characterize and quantify circulating levels of anti-flavivirus neutralizing antibodies. However, this method is time-consuming and not suitable for strains that do not plaque. Fluorescence-activated Cell Sorting (FACS) has been used to detect virus-infected cells, determining titers of virus in a rapid and effective manner. The goal of this study is to compare titers of DENV 1-4 clinical isolates obtained by plaque assay (PFU) and by FACS (FFU). Kinetics of infection by FACS was performed to observe the size and scattering of infected cells by flow cytometry. At first, positive samples were isolated and propagated in C6/36 and VERO cells lines. The strains were then titrated in VERO and BHK-21 cells using up to 10-5 dilution. In VERO, the titers obtained through plaque assay were 2.108 pfu and through FACS were 1.108 ffu. In BHK-21 cells, was not observed plaque formation, but the titration could be performed by FACS and the titer was 6.108 ffu. For the viral kinetics of cell infection, the same strains with the same dilutions were evaluated for viral presence in 24, 48, 72 and 96 hpi. The result could be identified in culture after 24 hpi, but there is a peak of viral presence at 48 hpi. This means that FACS assay is an improvement over the plaque assay because reduce the infection period from 5-7 days to 24-48 hours. Also, this technique can be used in any clinical isolates, regardless of whether or not this virus forms plaques. Further experiments are being conducted in order to use the same strains to perform PRNTs in FACS platform.

Financial support: FAPESP, CNPq.

Abstract #094

ARBOVIRUSES INVESTIGATION IN DENGUE SUSPECTED CASES DURING AN OUTBREAK IN SÃO JOSÉ DO RIO PRETO, SP

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Arboviruses are emerging viruses by nature. Transmitted by arthropods, they are often associated with outbreaks in humans and animals, representing a serious public health problem with significant economic and social impact. The main ones are Dengue (DENV), Yellow Fever (YFV), Mayaro (MAYV), Rocio (ROCV), Oropouche (OROV), Zika (ZIKV) and Chikungunya (CHIKV). Others such as Venezuelan Equine Encephalitis (VEEV), Eastern Equine Encephalitis (EEEV), Saint Louis (SLEV), West Nile (WNV), and Ilhéus (ILHV) are less frequent but no lesser important. The clinical manifestations can be easily mistaken with other diseases and range from asymptomatic or mild infections, which may progress to more severe forms and even lead to death. São José do Rio Preto, located in the northwest of the state of São Paulo, is an area endemic to DENV with a high rate of *Aedes aegypti* infestation, a possible vector for multiple arboviruses. Here, deaths from YFV, microcephaly in neonates due to ZIKV and increase of CHIKV cases were reported. Based on this, this study investigates the presence of these neglected arboviruses in samples from non-reactive DENV NS1 sera from 2018 and 2019 during outbreak. Approximately 100 samples from each year were randomly selected for confirmatory diagnosis by qPCR Multiplex for the 4 DENV serotypes, Lanciotti for ZIKV and CHIKV, and Bronzoni Duplex-RT-PCR and Multiplex-Nested-PCR for the remaining arboviruses. Of these, 35% DENV-2 positive samples were found in 2019, characterizing a serotype 2 prevalence compared to 25% in 2018. Suggestive concomitant infections by DENV-2 and other arbovirus, as well as suggestive samples of DENV-4, VEEV, YFV, MAYV, SLEV, and ILHV were found by Nested-PCR with specific primers and will be sequencing for confirmation. This preliminary survey helps us to draw an epidemiological profile of diseases in the region and improve advances in surveillance of these pathogens, which is crucial to prevent and/or mitigate future epidemics.

Financial support: FAPESP, CAPES, Pós-Graduação FAMERP.

Abstract #095

ELISA FOR DETECTION OF HUMAN ANTI YELLOW FEVER VIRUS IGG USING ANTIGEN OF INFECTED VERO CELL EXTRACTS

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Yellow fever is a disease caused by the yellow fever virus (YFV), a member of the Flaviviridae family, genera Flavivirus. From June/2016 to July/2018, there occurred in Brazil one of the largest outbreaks of yellow fever in recent history, without a clear reason. One of the methods that can be used to identify the IgG antiYFV is ELISA. This work had as a goal to standardize an ELISA to analyze the seroconversion of health care professionals. For the standardization, two serum samples were collected in different periods of time from the same patient. The first serum sample was negative as analyzed by seroneutralization test (PRNT), and the second was positive after vaccine booster, by PRNT. An indirect ELISA was made using as antigen extract of VERO cells (CI) infected with YFV at MOI 0,01 for 4 days. After lysis, the wells were sensibilized with 100µg/mL of total protein. Blocking of the reaction was made with 5% bovine albumin. The positive samples for YFV were diluted and applied in triplicates, including, also, the sample with low neutralizing titer, the negative and the blank control. Secondary antibodies conjugated with peroxidase and 3,3',5,5'- tetramethylbenzidine (TMB) was used to reveal the reaction. The analysis of the curve revealed equivalent optical densities (at 450 nm) in the triplicates, creating a linear relation with a R^2 varying from 0,954 to 0,946. A Pearson's correlation analysis was made, resulting in $p<0,05$. However, even the samples with low PRNT titers presented ELISA positive. However, other variables, such as avidity and the joining point in the antibody has been described as important variables in the process of neutralization and protection.

Financial support: CAPES, CNPq, FAPEMIG, DECIT-MS, PRPG-UFMG.

Abstract #096

IMPACT OF ZIKA VIRUS CIRCULATION IN PERCENTILE CURVES FOR NEONATAL ANTHROPOMETRIC MEASURES IN AN ARBOVIRUSES ENDEMIC REGION, BRAZIL

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Anthropometry is a simple way to get information about the health of a newborn. Although there are consistent patterns and curves, some species have singularities that must be considered. Infection by the virus during a pregnancy has brought a worldwide question about the debris of this arbovirose. Descriptive and retrospective study, based on anthropometric data obtained from live newborn records in 2014 and 2016 at the University Hospital. A total of 7033 newborn records were rebuilt. The anthropometric values of the study neonates were related to the values of the international standards, except for the lower head circumference above them. In the year 2016, the current Zika virus in the population is already present, the number of cases of microcephaly decreased, while the average head circumference increased in the study population. The differences between regions of teaching regions and international aspects have the same importance as the singularities of each population. The changes between the years 2014 and 2016 can be justified by the higher number of births and the lower consumption of medications and drugs, potentially teratogenic in the year 2016. To know the anthropometric profile of an auxiliary region in the way fetal development can be conducted. The differences between the years 2014 and 2016 may be associated with the improvement of the prenatal relationship in the region.

Financial support: FAPESP.

Abstract #097

RETROSPECTIVE ANALYSIS OF NEWBORNS IN ENDEMIC AREA FOR ARBOVIROSIS IN 2016

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The change in the occurrence of microcephaly records and the co-circulation of ZIKV and DENV arboviruses in São José do Rio Preto, an endemic area of DENV, attributed the knowledge of newborn anthropometry in *Hospital da Criança e Maternidade (HCM)* to the necessity of formulating a Head Circumference (HC) value considered normal in this population. The retrospective study analyzed the HC of alive newborns in HCM between January and December 2016, looking for factors that could influence it, especially in the occurrence of microcephaly. The study includes clinical, epidemiological and anthropometric data from HCM newborns. The authors compared newborns with altered HC to newborns exposed to ZIKV during pregnancy and made a correlation between HC values obtained in 2016 with values from population studies prior to ZIKV circulation in the region (2014) and with Intergrowth-21st project. The average HC obtained was 34.15cm and, for both boys and girls, it was mostly above P50 for the same sex and gestational age. In general, children with abnormality after the birth had a lower head circumference ($p < 0.0001$) and gestational age ($p < 0.0001$) than healthy ones. After the union with 2014 data, 6844 newborns could be analyzed. The percentile curves for weight followed almost the same as Intergrowth-21st, for males and females. However, newborns from São José do Rio Preto had shorter statures than the Intergrowth-21st study, but higher than expected head circumference considering gender and gestational age. Regarding height and head circumference, there was a statistically significant difference in almost all gestational age values, while it occurred only in some sectors for weight. Therefore, the increase in head circumference occurs isolated and this can justify the cases of ZIKV-associated brain malformations in the absence of microcephaly in this region. This microcephaly may be occurring in a subclinical way, as the method to diagnose it isn't the most appropriate yet.

Financial support: CNPq.

Abstract #098

LONG-TERM SYMPTOMS OF SYMPTOMATIC DENGUE IN A PROSPECTIVE COHORT IN BRAZIL

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Dengue virus (DENV) is the most important arbovirus in world, in terms of morbidity and acute clinical implications. Currently, we have a limited understanding of the long-term effects of symptomatic dengue infection as a limited number of studies utilizing case-control cohorts have been conducted. The aim of this study is to characterize the long-term symptoms in dengue cases after 60 days from acute disease. Employing a prospective case-control DENV cohort, we compared the presence of long-term symptoms 60 after days following symptomatic dengue infection against the control group. The prevalence of long-term symptoms was 31,8% in confirmed dengue fever patients while in control group it was 2,7%, with statistical significance ($p < 0,001$). The most important symptoms related after 60 days after the acute episode were headache, arthralgia, asthenia, anxiety, insomnia, depression and alopecia, with statistical significance between the control group and confirmed dengue fever and suspected dengue fever. There was not related to age, gender, acute clinical manifestations or severity. Dengue-suspected cases, including confirmed one, are associated with late symptom when comparable to general population, including both constitutional and neuropsychiatric symptoms.

Financial support: CNPq, FAPESP

Abstract #099

BIOLOGICAL AND MOLECULAR CHARACTERIZATION OF THE YELLOW FEVER VIRUS OF THE MINAS GERAIS OUTBREAK, IN 2018

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Sylvatic yellow fever (SYF) is an arbovirose caused by *Yellow fever virus* (YFV), which occurs in the Amazon basin, re-emerging outside this area with irregular periodicity. Between December/2016 and July/2017 a major SYF outbreak began in Brazil causing 777 human cases and 261 deaths, and another one struck the southeast region, between December/2017 and May/2018, causing 181 deaths in Minas Gerais (MG). Previous studies have already explored genomic characteristics of YFV circulating in Brazil and suggested that the outbreak was caused by a monophyletic group, divided into two subclades. However, investigations should be deepened considering the risk of the disease reurbanization. These findings reinforce the need to understand biological and molecular characteristics of circulating YFV, to support more studies involving pathogenesis, clinical and diagnostic aspects of SYF. The objective of this study was to perform a molecular and biological characterization of the YFV circulating in the 2018 MG outbreak. Thus, for the biological characterization, serum samples from SYF acute phase patients, hospitalized at Eduardo de Menezes, were confirmed by qPCR and then used for viral isolation. The characterization will be made through evaluation of the viral multiplication profile and cytopathic effects, in C6/36, Vero, BHK-21 and HEP-G2 cells, and defective particle production by comparing the viral multiplication in different cells followed by titration in Vero and tested by qPCR. For the molecular characterization, viral RNA extracted from isolated virus was amplified by RT-PCR for NS5, C/prM and envelope regions of YFV genome. The obtained fragments were sequenced by Sanger method and phylogenetic analysis were performed based on the maximum likelihood method. The sequences obtained in this study were grouped with other South American genotype I samples and these results corroborate with literature data, obtained from YFV sequences of the recent outbreaks in Brazil.

Financial support: FAPEMIG, CAPES, CNPq, Fiocruz Minas.

Abstract #100

ASSOCIATION OF ANTI-DENV IGG ANTIBODIES WITH DENGUE SEVERITY

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A rapid and potent plasmablast antigen-specific response is induced in dengue infection, predominantly with IgG-secreting cells, reaching maximum values between the 6th-7th days of the onset of patient's symptoms, a period that coincides with the development of the disease severity. In this study we determined DENV-specific IgG, IgG1 and IgG3 concentration in serum samples (n=65) from individuals with dengue distinct clinical manifestations, by enzyme-linked immunosorbent assay (ELISA), with in-house procedures. Serum samples were selected from two virologically- and/or serologically- well-characterized cohorts of DENV cases from health care units in Goiânia city, Gioás, Brazil, followed-up during two different DENV epidemics: October/2005-march/2006 and June/2012-july/2013, with the prevalence of DENV-3 (2005/2006) and DENV-1/ DENV-4 (2012/2013). DENV-specific IgG demonstrated a higher quantity in patients with dengue more severe clinical manifestation (p=0,001) and, by applying de ROC curve methodology, we observed an area under curve of 0,6729 (p=0,049) when compared data from patients with dengue fever (DC) versus dengue hemorrhagic fever (DHF). Analysis with DENV-specific IgG1 and IgG3 demonstrated no significant results. Our results suggest the participation of IgG antibodies in dengue pathogenesis and point for a future application of immunoenzymatic assays with DENV-specific IgG antibodies to monitor the disease severity in individuals with dengue from endemic areas.

Financial support: PRONEX.

Abstract #101

ZIKA VIRUS REPLICATION IN SKELETAL MUSCLE CONTRIBUTES TO AMPLIFICATION OF PERIPHERAL VIRAL LOAD AND DISSEMINATION TO CENTRAL NERVOUS SYSTEM

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The ZIKA virus (ZIKV) is a single stranded positive RNA enveloped virus that belongs to the *Flaviviridae*. The ZIKV infection promotes a mild fever with a high incidence of rash and muscle/joint pain. Viral replication in muscle tissue may be associated with tissue damage and contribute to viral spread, but the ability of ZIKV to replicate in muscle cells has not yet been demonstrated. Therefore, the objective is to investigate the susceptibility of myogenic precursors and myotubes by ZIKV *in vitro* and *in vivo,* using a neonatal mice model. In our *in vitro* study primary human myoblasts isolated from skeletal muscle were infected with ZIKV at multiplicity of infection of 5. At different hours post-infection the efficiency of replication was determined. Thus, we observed that ZIKV is able to replicate in human muscle cells with peak of replication after 36 hours. The viability of the myoblasts during infection was analyzed and ZIKV replication results in cell death. Apparently, myogenic precursors is more susceptible to infection, since viral replication in HSMM cell differentiated into myotubes resulted in lower amplitude of ZIKV release and less percentage of positive cells at immunofluorescence assay. We also performed *in vivo* studies using wild type SV129 newborn mice (3 days-old) infected subcutaneously with 10^6 PFU of ZIKV, resulting in in clinical signs such as weight loss. A temporal increase at ZIKV RNA detection was observed in muscle followed by higher levels at expression of inflammatory mediators in infected animals. Curiously the increase of viral RNA detection in central and peripheral nervous system occurs only after the peak of muscular replication. This study provides evidence that human myogenic precursor cells as well the muscle tissue of neonatal mice are susceptible to infection by ZIKV, with induction of muscle inflammation and probably contributing to viral load support until the virus spreads in nerve tissue.

Financial support: CNPq, FAPERJ, FINEP.

Abstract #102

CHALLENGES IN FLAVIVIRUS DIFERENTIAL DIAGNOSISIN REGIONS OF CO-CIRCULATION OF DENGUE AND ZIKA VIRUS

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Dengue (DENV 1-4) and Zika(ZIKV) are flaviviruses, family *Flaviviridae* that co-circulate in Brazil since 2015. These viruses clinical diagnosishave been a challenge due to antibody cross- reaction in serologic tests. In this study, we analyzed the immune response in pregnant women with suspected ZIKV infection during a Zika/Dengue epidemic in Goiania city, Goias, Brazil Midwest, from 2016 to 2017.We analyzed pregnant women with age varying from 16 to 37 years, in differential gestational period,with more than five days since the disease onset of symptoms and collectedserum samples for antibody antidengue and antizika(MAC-ELISA) detection, followed by plaque reduction neutralization test (PRNT), preliminarily with DENV-1, DENV-2 and ZIKV. In MAC-ELISA assay, 5 samples were ZIKV positive and 10 demonstrated cross-reactivity between DENV and ZIKV. When we apply PRNT50, 15 samples presented cross-reactivity, making impossible the determination of the infection etiologic agent. In PRNT90, 13 (n=15) samples confirmed ZIKV infection and two (n=15) confirmed DENV infection. The majority of positive samples for both, DENV and ZIKV, in MAC-ELISA (n=10), 80% were ZIKV positive in PRNT90 and, all MAC-ELISA ZIKV positive (100%) maintained the same result in PRNT90. Flaviviruses diagnosis in samples with cross-reaction was possible when applied the PRNT90 criteria. More rigorous titles in PRNT90 are useful in DENV endemic areas for diagnostic studies, decreasing flaviviruses serum background cross-reaction. This study proves the challenges for ZIKV and DENV diagnosis in patients with history of previous flavivirus infection.

Financial support: PROMEN.

Abstract #103

ROLE OF C-C CHEMOKINE RECEPTORS TYPE 2 AND 5 (CCR2/CCR5) DURING ACUTE PHASE OF CHIKUNGUNYA VIRUS INFECTION

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Chikungunya virus (CHIKV) associated disease has been linked to an increase in inflammatory chemokines levels. Thereby, the aim of this work was to study the role of the chemokine type CC receptors (CCR2 and CCR5) in the pathogenesis of CHIKV infection. To do so, WT mice of the C57BL/6 lineage were used along with and knockouts for CCR2 (CCR2-/-) and CCR5 (CCR5-/-) receptors. The expression profile of these receptors in different cell types, as well as their binding chemokines, in addition to several clinical and inflammatory parameters, were evaluated. Flow cytometry analyses showed increased expression of CCR2 and CCR5 receptors after the CHIKV infection predominantly in macrophages and lymphocytes. On the WT mice, were observed lymphopenia, chemokines increased and recovery of the viral load in several tissues, in addition to tissue damage and prolonged articular hypernociception. However, in the absence of the CCR2 and CCR5 receptors, the inflammation was more exacerbated, since in these groups a higher level of chemokines was found, as well as an increased neutrophil infiltrate and prolonged tissue damage in CCR2-/- mice. In the CCR5-/- mice, in addition to the mentioned findings, a higher viral recovery was also observed in initial post-infection time. However, the hypernociception observed in the groups of knockout animals happened only in the initial time with respect to the CCR2-/-, or nonexistent, in the CCR5-/- mice. As a final point, this work reinforces the importance of macrophages and lymphocytes in the control of the disease caused by CHIKV, since they are the main cells affected by the absence of the CCR2 and CCR5 receptors, besides demonstrating that the receptors studied seem to be important in the assembly of the appropriate immune response for fighting the virus and resolving the inflammation process. These receptors also interfere directly or indirectly in mechanisms associated with the hypernociception induced by the CHIKV infection.

Financial support: CAPES, CNPq, FAPEMIG, INCT.

Abstract #104

GENOMIC HISTORY AND DYNAMICS OF THE CHIKUNGUNYA VIRUS EAST-CENTRAL-SOUTH AFRICAN LINEAGE IN THE AMERICAS

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Since 2014, Brazil has been facing the largest Chikungunya virus (CHIKV) epidemic in a single country this decade, with over half a million of CHIKV reported cases, and two distinct genotypes, the East-Central-South African (ECSA) and the Asian lineages, circulating in the country. Despite the unprecedented magnitude of the CHIKV epidemic in Brazil, its evolutionary history, countrywide spread and adaptive dynamics remain to be elucidated. We conducted a detailed genomic analysis of 113 CHIKV ECSA sequences from the Americas, including 54 new CHIKV genomes from Brazil generated using portable genome sequencing. Our results show that the ECSA lineage, introduced in mid 2014 in Bahia, is now the only genotype circulating in Brazil. Spatial genetic analyses indicate that the northeastern Brazil acted as the main source of ECSA-America (ECSA-Am) transmission to other regions of Brazil and beyond. Molecular clock estimates show that ECSA-Am is evolving at pace of $4x10^{-4}$ substitutions per site per year, similar to other CHIKV epidemic lineages. However, comparative evolutionary analyses of the ECSA-Am reveals a significantly higher evolutionary rate in non-structural regions for the ECSA-Am compared to the Indian Ocean Lineage (ECSA-IOL), which is skewed towards envelope genes. We also identify two mutations under positive selection in the ECSA-Am non-structural regions, nsP2 P16L and nsP4 E280D, which are also present in all Asian genotype sequences. These mutations were predicted to be solvent exposed and might have an impact in polymerase activity and replication. Although no mutations that have been previously shown to increase fitness to *Ae. albopictus* were observed, we identified a single mutation, E1K211, that might be relevant for replication in *Ae. aegypti* mosquitoes and mammalian host cells. Genotype surveillance data from the Americas is needed to assess the burden, impact and monitor mutations associated with CHIKV transmission and disease severity.

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Abstract #105

MAYARO VIRUS REPLICATION RESTRICTION AND INDUCTION OF MUSCULAR INFLAMMATION IN MICE ARE DEPENDENT ON AGE, TYPE-I INTERFERON RESPONSE, AND ADAPTIVE IMMUNITY

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Mayaro vírus is an emergent arbovirus first described in forest regions of the American continent, with recent and increasing notification of urban area circulation. MAYV-induced disease shows a high prevalence of persistent arthralgia and myalgia. Despite this, knowledge regarding pathogenesis and characteristics of host immune response of MAYV infections are still limited. Using different ages of wild-type, adult Type I Interferon receptor deficient and adult recombination activation gene-1 deficient mice, we have investigated the dependence of age, innate and adaptive immunity for the control of MAYV replication, tissue damage and inflammation in mice. We have found that MAYV induces clinical signal and replicates in young WT mice, which gain the ability to restrict MAYV replication with aging. In addition, we observed that mice age and type I interferon response are related to restriction of MAYV infection and muscular inflammation in mice. Moreover, MAYV continues to replicate persistently in $RAG1^{-/-}$ mice, being detected at blood and tissues 40 days post infection, indicating that adaptive immunity is essential to MAYV clearance. Despite chronic replication, infected adult $RAG1^{-/-}$ mice did not develop an apparent signal of muscle damage in early and late infection. On the other hand, MAYV infection in young WT and adult IFNAR-/- mice triggers an increase in the expression of pro-inflammatory mediators, such as TNF, IL-6, KC, IL-1β, MCP-1, and RANTES, in muscle tissue, and decreases TGF-β expression, that were not significantly modulated in adult WT and RAG-/- mice. Taken together, our data demonstrated that age, innate and adaptive immunity are important to restrict MAYV replication and that adaptive immunity is also involved in MAYV-induced tissue damage. These results contribute to the comprehension of MAYV pathogenesis, and describe translational mice models for further studies of MAYV infection, vaccine tests and therapeutic strategies against this vírus.

Financial support: CNPq, FAPERJ.

Abstract #106

EFFECT OF ANTIVIRAL SOFOSBUVIR ON ZIKA VIRUS REPLICATION *IN VIVO*

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In February 2016 the World Health Organization declared a public health emergency due to the worsening of confirmed cases of ZIKA infections and its relationship to congenital malformations such as microcephaly and neurological syndromes. Therefore, the search for pharmacological strategies that have inhibitory activity against ZIKA is of vital importance. Initially used for the treatment of hepatitis C, Sofosbuvir has been shown to be effective in inhibiting ZIKA in vitro. The objective of this paper is to establish a model of ZIKA infection in vivo, and to analyze the antiviral effects of Sofosbuvir treatment. To this end, neonatal swiss mice 3 days postnatal were infected with the African or Brazilian ZIKA strains (2x107 and 2x104 PFU, respectively) by intraperitoneal injection (ip) and treated with sofosbuvir at a dose of 20mg / kg / day for 7 days. The animals were followed daily for weight, mortality and behavioral tests. Signs of encephalitis caused by the African strain and loss of balance and paralysis in the lower limbs of animals caused by the Brazilian strain between 10 and 13 days after infection (p.i) were observed. Animals infected with ZIKA did not show weight gain when compared to controls, besides poor performance in behavioral tests. Motor disorders, learning and long-term memory loss were prevented with treatment. The treatment also significantly reduced the mortality caused by the viral infection. We also evaluated viral load in different tissues and saw that at 96 hours pi, the virus remains only in brain tissue, where we observe an activated microglia, suggesting the presence of damage caused by viral infection. Thus, we conclude that sofosbuvir has great therapeutic potential in the treatment of ZIKA as it reduces mortality and prevents short and long term sequelae resulting from ZIKA infection.

Financial support: CNPq, FAPERJ.

Abstract #107

THE ROLE OF INNATE RECOGNITION PATHWAYS IN PLACENTAL CELLS AFTER OROV INFECTION

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The Oropouche virus (OROV) is an arbovirus with potential to cause epidemics in the most populated regions of Brazil. Individuals infected with OROV develop a febrile illness, which can progress to neurological and hemorrhagic complications. Furthermore, an increased incidence of abortion was reported during major OROV epidemics. However, the teratogenic potential of OROV and the pathogenetic mechanisms associated with the hematoplacental barrier breakdown have not yet been investigated. The immunological response and antiviral activity in the placenta are dependent on the expression of pattern recognition receptors (PRRs) and production of type I interferon (IFN), mainly on cytotrophoblast, syncytiotrophoblast cells and placental macrophages. However, some viruses are able to establish long-term placental infections, in part by mechanisms dependent on the antagonism of the type I IFN pathways. Thus, we intend to characterize the mechanisms and pathways by which type I and III IFNs modulate the placental immune response during infection by OROV. Preliminary results show that OROV replicates in human placental lineage and can induce the expression of IFN type I and III genes in addition to interferon regulatory genes such as IRF-1, IRF-3 and IRF-7. These results suggest an involvement of immunological recognition pathways and contribute to the follow-up of the next experiments.

Financial support: FAPESP, CNPq.

Abstract #108

THE PROTEIN COMPLEX MTORC MAY INFLUENCE CHIKV INFECTION IN MURINE DENDRITIC CELLS

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The Chikungunya virus (CHIKV) is an emerging arbovirus present in tropical and subtropical regions transmitted by the arthropod vector *A. aegypti*. Although CHIKV infection may be asymptomatic, it usually leads to the development of an acute febrile illness, joint pain and swelling. One of the major complications associated with CHIKV are chronic manifestations such as arthralgia and arthritis, these symptoms can last for months to years. The chronic disease resulting from CHIKV infection has similar characteristics to rheumatoid arthritis which there is an imbalance in the function of dendritic cells (DCs). Thus, we believe that the infection, activation and metabolic imbalance of dendritic cells may play an essential role in the pathogenesis of CHIKV and in the development of chronic inflammation in the joints. To analyze the effect of CHIKV on the function and metabolism of DCs in vitro, DCs differentiated from WT C57BL/6's bone marrow were pretreated with rapamycin (5, 10, 100 or 200 ng/mL) and infected with CHIKV (MOI 1) for 3, 6 and 24 hours. We characterize viral replication and expression of genes associated with the immune innate response. Preliminary results suggest that bone marrow-differentiated dendritic cells from wild type mice previously treated with inhibitor of the MTORC 1 and 2 metabolic pathways and infected with CHIKV may contribute to an increase in infection within 24 hours. In addition, we observed changes in the expression of IRF 3, 5 and 7, NFk-B and RNase L at different concentrations of inhibitor, suggesting an important role of these pathways during CHIKV infection.

Financial support: FAPESP.

Abstract #109

MOLECULAR SURVEILLANCE OF DENGUE, ZIKA AND CHIKUNGUNYA IN SYMPTOMATIC PATIENTS FROM SÃO JOSE DO RIO PRETO, SP, BRAZIL

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Aiming to detect patterns and mechanisms of viral circulation, various surveillance actions have been intensified, as well as molecular surveillance in different regions of the country. The objective of this study was to analyze the presence of *Dengue virus* (DENV), *Chikungunya virus* (CHIKV) and *Zika virus* (ZIKV), their subtypes and associated genotypes in clinical samples of patients with fever who sought the Health Services of the Municipality of São José do Rio Preto in an active epidemiological surveillance system. For the investigation of these arboviruses specific primers for the envelope gene (ZIKV), NsP1 (CHIKV) and NS5 (DENV) were used through real-time RT-PCR. Between February/2016 and May/2019, 3925 serum samples were analyzed. Two hundred and twenty three samples (5.68%) were confirmed as positive for ZIKV, 843 (21.48%) for DENV and three (0.076%) for CHIKV. The serotypes of DENV found were: 95.3% (803/843) DENV-2, 4.5% (38/843) DENV-1 and 0.2% (2/843) DENV-4. It is important to note that eight cases of DENV-2/ZIKV co-infection (2016 and 2018), three cases DENV-1/ZIKV (2018) and eight cases of DENV-1/DENV-2 co-infection (2019/outbreak) were observed. Samples of the DENV were submitted to the envelope gene (DENV-1:07; DENV-2:34) and complete genome (DENV-1:04; DENV-2:10) sequencing and were used for phylogenetic reconstruction. The analyses showed the circulation of the lineages L1 and L6 belonging to genotype V of DENV-1 and lineages BR3 and BR4, recently introduced in the municipality, belonging to genotype III (also known as American/Asian) of DENV-2. Our data showed DENV-2 as the predominant serotype and allow us to associate the introduction of this new strain with explosive epidemic reported in São Paulo state in 2019 may be due to differences in viral fitness between these lineages. This work demonstrates the importance of university-health system integration through molecular studies to understand the origin and evolution of arboviruses.

Financial support: FAPESP.

Abstract #110

COINFECTION OF DENGUE AND MAYARO VIRUSES IN AAG-2 AND C6/36 CELL LINES

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Mayaro virus (MAYV) was first isolated in 1954 in Trinidad and Tobago. It is a non-specific febrile illness, accompanied by skin rash and joint pain that can last for several months. Since its isolation, sporadic outbreaks have occurred across Central and South America, though it has been mostly isolated to rural areas, sustained in non-human primates with only incidental human infections. Dengue virus (DENV) however, has circulated in urban areas in human hosts for a much greater length of time. Concern has recently risen for the potential of MAYV to urbanize and present an additional challenge to the healthcare systems of large Brazilian cities. In 2017 and 2018, MAYV was detected circulating in Goiânia, Brazil along with DENV, with 65% of the MAYV infected patients also being coinfected with DENV. This suggests the viability of the DENV vector, *Aedes aegypti*, as a vector for MAYV, further suggesting its potential to urbanize. In 2019, three cases of locally contracted MAYV infection were confirmed in Niteroi, Rio de Janeiro, further supporting the hypothesis of urbanization. This work will seek to characterize coinfection of these two viruses in AAG-2 and C6/36 cell lines. We will infect at varying time points and intervals, with just DENV, just MAYV, DENV and then MAYV, and MAYV then DENV. We will run qPCRs on all of our infection conditions to determine how the viruses are affecting the expression of the other's viral genome. Finally we will perform immunofluorescence for DENV and MAYV to visualize where the different viruses are localizing in the cells and the environment. The project is still in its initial stages and we currently have no results, however, we anticipate to have finished this phase of the project, if not more, by the time of the conference in December.

Financial support: CAPES, CNPq.

Abstract #111

CONGENITAL ZIKA SYNDROME NEUROPATHOLOGIC FINDINGS REPRODUCED IN NEONATAL MOUSE INFECTION WITH DIFFERENT ZIKA VIRUS STRAINS

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Zika virus (ZIKV) is an arbovirus member of the *Flaviviridae* family. ZIKV emerged as an international public health concern when viral infection was first linked to microcephaly and other fetal malformations. It is now well established that ZIKV can reach the fetus, causing a broad spectrum of neurodevelopmental defects referred to as Congenital Zika Syndrome (CZS). However, establishment of a model which reproduces brain damage patterns observed in CZS cases is a current challenge to understand the mechanisms underlying ZIKV pathogenesis in the developing brain. To characterize histopathological findings in the developing brain exposed to ZIKV, we analyzed cerebral tissue from Swiss-Webster neonate mice infected intraperitoneally with three ZIKV strains: the mouse-adapted neurovirulent African strain (MR766) and two clinical isolates from the epidemics in Brazil - one from the state of Pernambuco (ZIKV-PE) and other from Espírito Santo (ZIKV-ES). MR766 was highly virulent, as all infected subjects died between 5 and 6 days post-infection (dpi), showing serious body growth impairment and disseminated parenchymal edema and neuronal degeneration in the brain. Clinical onset and time-to-death was delayed in animals infected with the Brazilian ZIKV isolates compared to MR766. Nevertheless, both ZIKV-ES and PE generated severe clinical outcomes, such as seizures, hind limb paralysis and imbalance. All subjects infected with both Brazilian isolates died within 14 to 25 dpi. Neuropathologic findings observed in ZIKV-ES and PE infected mice brain resembles the patterns previously described in CZS cases, such as cortical, hippocampal and cerebellar calcification, cerebellar dysplasia and gliosis, highlighting the applicability of this model in future studies on therapeutic intervention against ZIKV, pathophysiological characterization and viral attenuation tests.

Financial support: CNPq, FAPERJ's Rede Zika.

Abstract #112

DETECTION OF NEUTRALIZING ANTIBODIES AGAINST FLAVIVIRUSES IN FREE RANGING BIRDS, PARAGUAY (2016-2018)

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Saint Louis encephalitis virus (SLEV), West Nile virus (WNV) and Ilheus virus (ILHV) are flaviviruses maintained in nature by enzootic transmission networks between mosquitoes and birds. These viruses have been detected in South America and identified as causative of neurological diseases. In Paraguay there is no data regarding enzootic activity of these flaviviruses. The present study aimed to determine the activity of SLEV, WNV and ILHV in free wild birds collected between 2016-2018 in different habitats in Paraguay (rural: Itacurubí de la Cordillera; periurban: San Lorenzo and Atlantic forest: San Rafael National Park). A total of 222 birds sera were screened by plaque reduction neutralization test against the aforementioned viruses. Four positive samples were detected, all with homotypic serological responses. One anti-SLEV positive (Rufous-bellied thrush, collected in a rural area during winter, title: 1:20), one anti-WNV positive (Barred Antshrike, collected in a periurban site during autumn, title: 1:80) and two anti-ILHV positive samples (one White-tipped dove collected in the Atlantic Forest during spring and a Shiny Cowbird, collected in the periurban site during autumn, both showing titters of 1:20). All infected birds are resident species that could indicate the possible autochthonous enzootic activity for the analyzed flaviviruses. The low seroprevalences detected might be a result of the dilution effect of the viral activity due to avian host and mosquito vector diversity. Previous studies reported SLEV and WNV infection in Rufous-bellied thrush, Shiny Cowbird and White-tipped dove in South America, however there are no previous reports for WNV infection of Barred Antshrike. ILHV has been detected in Northeastern Argentina and in urban and forest areas of Brazil (Sao Paulo state), confirming that this virus is endemic in the region. These results bring new information about enzootic activity of flaviviruses in Paraguay and will serve as a basis for future studies.

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Abstract #113

STUDY OF THE INVOLVEMENT OF THE MACROPHAGE MIGRATION INHIBITOR FACTOR IN ZIKA VIRUS' PATHOGENESIS IN AN EXPERIMENTAL MICE MODEL

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The Zika virus (ZIKV) is a flavivirus transmitted by Aedes aegypti mosquito. Sexual and vertical transmission is also possible. The infection usually causes mild fever, however there have been reports severe neurological complications. Macrophage migration inhibitory factor (MIF) is a cytokine produced by several cell types and is related to the severity of various diseases, including West Nile virus (WNV) encephalitis and hemorrhagic dengue. In addition, elevated levels of MIF were found in the plasma of rhesus monkeys infected by ZIKV. Our hypothesis is that MIF could contribute to the exacerbation of tissue damage induced by ZIKV. The aim of this study is to investigate the involvement of MIF in ZIKV pathogenesis. For this we used a neonatal murine model in wich the active replication of ZIKV in the brain promotes histological changes such as areas of necrosis and neuroinflammtion. Wild-type C57BL/6 (WT) and MIF-deficient (MIF-/-) mice were infected with 106 or 104 PFU of ZIKV or Mock with 3 (P3) or 6 (P6) days old. Analyzes of MIF expression in the brain of the infected WT showed that its expression was dose and age dependent being increased in younger animals. Our results also show that in the P3 infection the WT animals, with both viral loads, presented signs of neurological and motor disorders and evolved to death, while the MIF-/- this was observed only in animals that received the highest viral load and those that were infected with 104 PFU did not show any clinical signs of infection. In the P6 infection, none of the groups presented clinical signs. We also analyzed the viral load in the brains of the animals and observed that all groups had high levels of viral RNA and had no significant differences between them, except in the animals infected with 104 PFU where the MIF-/- maintained the viral load between 6 and 12 dpi while the WT had an increase. These results suggest that the absence of MIF may play a protective role in a less severe infection model.

Financial support: CNPq, FAPERJ, FINEP.

Abstract #114

MOLECULAR INVESTIGATION OF FLAVIVIRUSES IN SAMPLES OF NON-HUMAN PRIMATES FROM THE WILDLIFE CENTER OF THE FEDERAL UNIVERSITY OF UBERLÂNDIA

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More than two thirds of emerging human pathogens are from zoonotic origin and over 70% of them originate from wildlife species. Unplanned urbanization, deforestation, habitat fragmentation, animal trade, ecological changes and modern transports result in the spread of pathogens and their vectors. Mosquito-borne flaviviruses are some of the most important examples of emerging diseases with global significance, infecting humans and animals. Targeting surveillance to identify flaviviruses present in its natural sources as mosquito vectors enable to identify and deal with potential infection hotspots before problems occur in human populations. Therefore, the aim of this study was to investigate the presence of flavivirus by the RT-PCR technique in the blood samples of non-human primates treated from 2013 to 2018 at the wildlife center of the Federal University of Uberlândia. Twenty-eight samples of black-tufted marmoset (Callithrix penicillata), one of black-striped capuchin (Sapajus libidinosus) and one of black howler (Alouatta caraya), obtained from animals captured from urban areas of the Triângulo Mineiro, were investigated. All samples tested in this study were negative for flaviviruses. These results did not exclude the previous infection, but demonstrate that the animals were not in the viremia phase and, therefore, were not a source of infection for vectors. This result represent relevant data since some animals were reintroduced to the wild life. The surveillance of flavivirus by RT-PCR as a pre-release assessment at wildlife centers would be critical for surveillance better understand of the circulation of these arboviruses.

Financial support: CNPq, Royal Society – Newton project, FAPEMIG.

Abstract #115

PLANT EXTRACTS AS POTENTIAL SOURCES OF ANTIVIRAL DRUGS AGAINST DENGUE AND ZIKA VIRUS

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Plant extracts are potential sources for the development of antiviral drugs for the treatment of diseases caused by *Dengue virus* (DENV) and *Zika virus* (ZIKV). To date, our group screened more than 1,000 extracts from plants for their *in vitro* antiviral activity against both viruses in Vero cells (ZIKV) and BHK-21 cells (DENV-2). The antiviral effect was analyzed by observing the degree of inhibition of the viral cytopathic effect (CPE) and by MTT colorimetric assay. Five active extracts from 4 different families were fractionated using an UPLC (Nexera-Shimadzu) coupled to a HRMS (MaXis-Bruker). Analysis of the HRMS spectra of the active fractions against DENV-2 and/or ZIKV allowed the identification of some of the active compounds, which structures were confirmed by comparison with authentic samples or by isolation and analysis of its NMR spectra. Active fractions of extracts obtained from plants of *Hippeastrum genus* (*Amaryllidaceae*) afforded antiviral compounds against both viruses such as narciclasine, pretazettine and lycorine. We also identified a new alkaloid narciclasine–like which showed EC_{50} of 3.5 µg/mL and SI of 4.9 and 6.5, for DENV-2 and ZIKV, respectively. To our knowledge, this is the first report on the antiviral activity of this compound against any virus. Active fractions of extracts from others families as *Orchidaceae* (*Habenaria petalodes)*, *Fabaceae* (*Chamaecrista sp.*) and *Rubiaceae* (*Palicourea sp.*) are being investigated to identify other compounds with antiviral properties.

Financial support: IRR - Fiocruz, PROEP/P3D CNPq, CAPES, FAPEMIG.

Abstract #116

RETROSPECTIVE COHORT OF PREGNANT WOMEN EXPOSED TO ZIKA VIRUS IN SÃO JOSÉ DO RIO PRETO IN 2016

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In 2015 the Zika Virus (ZIKV) was introduced on Brasil and ever since represented a constant concern due to its teratogenic actions, which affect specially the fetal nervous system.However, other maternal and gestational factors also reflect on the fetal formation. A regional analysis of maternal factors became essential for a better understanding of the current epidemiological panoraman and its developments. The objective was to analyze the profile of pregnant women who gave birth at *Hospital da Criança e Maternidade* (HCM) in 2016 and the consequent implications on their newborns (NB), highlighting the influence of the contact with the ZIKV. This is a retrospective study using data from medical records of pregnant women who gave birth at the HCM .The study has found that the average maternal age was 27.5 years. Of all deliveries analyzed,3234 occurred through the abdomen;another 421 through the vagina.The NB who were born by cesarean had a average head circumference (HC) of 34.25cm,while the average number of the vaginal births was 33.38cm.Related to maternal pathologies, was observed that diseases with reduced space for fetal development (intrauterine growth restriction) or compromised supply of oxygen and nutrients to the fetus (arterial hypertension) resulted in a decrease in HC and gestational age.Yet, the increase in space (polyhydramnios) was accompanied by an increase in HC. In mothers who had addictions, the average HC was decreased by all addictions presented on pregnant women, being even lower in the group of pregnant women addicted to illicit drugs.ZIKV infection was diagnosed in 40 pregnant,in which the NB had an average HC of 34.38 cm, 5 of which had neurological affection and no fetus had microcephaly.The values and analyzes presented by the study allow identifying a major influence not only of exposure to ZIKV but especially by factors involving the pregnancy,like maternal pathologies with growth restriction and pregnancies with maternal addictions.

Financial support: CNPq.

Abstract #117

CHEMOKINE AND GROWTH FACTORS IN DENGUE HOSPITALIZED PATIENTS

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Dengue is a viral disease caused by four dengue serotypes. Endemic in about 100 countries, dengue threatens about 3.9 billion people in the world. In Brazil, dengue has been affecting the population since 1980's. Although dengue is a dynamic disease with a wide clinical spectral, it is generally characterized by fever, polyarthralgias, diarrhea, rash and bleeding. However, despite short time plasma leakage is considered the most critical event in dengue patients, endothelial permeability is a fact that must be better understood. It is believed that soluble immune mediators interact with endothelial cells and alter its integrity, which provoke disorders such as pleural and abdominal effusion, bleeding and shock. Thus, in order to corroborate available data and contribute, chemokines and growth factors were quantified in serum samples of dengue hospitalized patients. Therefore, serum samples were submitted to ELISA assays in order to quantify CCL2/MCP-1, CXCL10/IP-10, CCL5/RANTES, EGF, HGF and PDGF-bb in patients classified as dengue without warning signs (DwoWS), dengue with warning signs (DwWS) and severe dengue (SD). As result, chemokines showed statistical significance between healthy controls and dengue groups. High levels of MCP-1 and IP-10 were found in patients DwWS/SD in comparison to DwoWS and healthy controls, while low levels of RANTES were found in patients DWWS/SD in comparison to DwoWS and healthy controls. High levels of HGF were found in patients DWWS/SD in comparison to DwoWS and healthy controls. Low levels of EGF were found in patients DwWS/DG in comparison to healthy controls. There was no statistical significance in levels of PDGF-bb between dengue groups and healthy control, although there was a trend to low levels between healthy controls and DwWS/SD. These findings corroborate described literature and suggest the role of chemokines and growth factors in dengue immunopathogenesis.

Financial support: FAPERJ, CNPq.

Abstract #118

IMPACT OF PRIOR ZIKA VIRUS EXPOSURE ON ACUTE DENGUE INFECTION: CLINICAL AND VIOLOLOGICAL ASPECTS

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Dengue is a viral infectious disease and is considered the most important arbovirus in the world in terms of morbidity and mortality. Historically, more severe cases of the disease have been observed following a heterologous serotype infection at the first infection, resulting in a cytokine storm capable of promoting antibody-dependent enhancement (ADE). Recent studies following the emergence of Zika as a public health impact arbovirus have hypothesized that prior immunity triggered by an infection with another flavivirus could promote such a pathophysiological mechanism with exacerbation of immune response and, consequently, a more severe evolution to the disease. Dengue. Thus, this study aimed to analyze the dengue epidemic in 2019, after the Zika virus circulation in São José do Rio Preto. A total of 312 suspected dengue cases were evaluated by viral antigen screening (detection of non-structural protein type 1, NS1, and polymerase chain reaction, PCR), with 277 confirmed for DENV-2, with 77 cases without alarm signals, 186 with alarm signs and 14 cases of severe dengue. Comparing the data obtained between 2016 and 2019, a significant increase in the frequency of dengue cases with alarm and severe dengue cases was observed, 29.2% and 4.7% respectively. Several factors may influence the occurrence of severe forms of dengue, including those associated with the host as the pathogen, including occurrence of ADE in patients who were not exposed to DENV-2 in 2016; the viral fitness change of circulating strains between the two years; or the change in pathogenesis of Dengue when associated with a previous Zika infection. To clarify which of these factors were responsible for the change in the clinical profile in the population, we will perform the sequencing of these samples to detect possible mutations and seroneutralization (PRNT) analysis to verify previous infections by one or more DENV serotypes and/or ZIKV. Considering the current epidemiological context of arboviruses in the country, the data presented demonstrate the crucial need to elucidate the role of the factors mentioned in the pathophysiology of severe forms of dengue.

Financial support: FAPESP, CNPq.

Abstract #119

DEVELOPMENT OF PEPTIDE-BASED SEROLOGIC NANODEVICES TO DIFFERENTIALLY IDENTIFY DENGUE AND ZIKA INFECTIONS

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Dengue is one of the most important infectious diseases nowadays and early diagnosis is a determining factor for disease outcome. Co-circulation of viruses that have serological cross-reactivity with Dengue virus (DV), such as Zika virus (ZV), further complicates diagnosis. One approach towards creating diagnostic tests able to differentiate between such viruses is to determine peptides that would lack immune response cross-reactivity. However, the immobilization of small proteins or peptides on surfaces has been a barrier to the development of tests. The goal of this work is to identify specific peptides in the non-structural protein 1 (NS1) of DV and ZV and evaluate their use in serological diagnostic platforms. For this, we screened DV and ZV peptide libraries with NS1 monoclonal DV1-4 and ZV antibodies (NS1 mAbs). Three peptides were identified with specific binding: one that is ZV-specific and two that are DV-specific. We tested our peptide ELISA assay with 170 human samples from patients who had known a prior diagnosis of DV and/or ZV infection. The pepELISA DV-specific showed a sensitivity of 97% and a specificity of 96,3%. The pepELISA ZV-specific showed a sensitivity of 77,9% and a specificity of 97,7%. With the success of pepELISA, we evaluated a lateral flow-based assay using gold nanoparticles (GNP), in two immobilization variation. For the first, biotinylated peptides were conjugated with streptavidin and spotted onto a nitrocellulose membrane and GNP conjugated with anti-human IgG were ran with the human samples. For the second test, peptides synthesized covalently linked to lipoic acid were conjugated to the surface of GNP. Both strategies were able to differentiate ZV and DV mAbs and patient samples. These techniques presented here are effective, fast and inexpensive tests and would allow in near future the rapid assessment of the exposure - very necessary in a vaccine campaign of both viruses.

Financial support: FAPESP, CAPES, CNPq.

Abstract #120

CHIKUNGUNYA'S NATURAL HISTORY AND THERAPEUTIC RESPONSE MULTICENTER STUDY: FOCUS ON ACUTE AND CHRONIC MUSCULOSKELETAL MANIFESTATIONS (REPLICK)

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Chikungunya virus (CHIKV) is an arbovirus, in which clinical presentation is divided into three stages: acute, post-acute and chronic. Joint pain and musculoskeletal manifestations are the most classic symptoms, which can persist for up to years causing physical impairment and significative impact of health quality of patients. Although CHIKV has become an important public health problem in epidemic countries, literature data on infection progression, chronification rate and definition of risk factors for severity are still limited, especially considering systematic and harmonized studies addressing the breadth of disease burden caused by this infection in distinct geographical locations. By initiative of a group of scientists from different fields, the *Clinical Research Network Applied in Chikungunya*, REPLICK, a Brazilian network was created with support from the Ministry of Health to elucidate and produce relevant knowledge on this infection. The REPLICK group include at least 30 investigators from 11 research centers, located in nine national states. The first study conducted by REPLICK is a multicenter prospective cohort to follow the natural history of CHIKV, with the main objective is to describe the chronification and complication rate and associated risk factors by CHIKV in Brazilian territory. Analysis on translational medicine, immunological response and genetic profile to define biomarkers and therapeutic targets will also be accomplished. Patients diagnosed with CHIKV, at any phase, will be followed on protocol-scheduled visits for until 1180 days, including laboratory, clinical and therapeutic evaluations. Pain, musculoskeletal compromise, considering different domain core sets, health related quality of life, work productivity, anxiety and depression will be also assessed. Our results will be useful to enhance future management guide, diagnosis and treatment of CHIKV, with systematic and reliable data that can serve as a model for other studies.

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Abstract #121

CLEAVAGE OF E3 PROTEIN OF CHIKUNGUNYA VIRUS IS REQUIRED FOR EFFECTIVE INFECTION, BUT THIS PROTEIN DOES NOT PREVENT VIRUS BINDING AND INTERNALIZATION

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The recent outbreak caused by chikungunya virus (*Alphavirus, Togaviridae*) worldwide has shown that, despite being first isolated in the 1950s, this infection still has several points to be elucidated. This especially includes the correct diagnosis of the disease caused by the virus, but also treatment and prevention of infection. The virus, which in spite of short-lived viremia, produces symptoms such as high fever, myalgia, rash, and arthritis/arthralgia, which may persist for months or years, causes a major public health impact. In Brazil, since its introduction in 2014, there were more than 600,000 reported cases of chikungunya virus infection, with approximately 475 confirmed deaths in the country. The aim of this project was to evaluate the infectivity profile of immature chikungunya particles in furin producing or deficient cells, as well as to test an infectious clone with specific mutation for the furin protease cleavage region. Immature particles (without E3 cleavage) were produced in furin deficient LoVo cells, in addition to transfection of viral RNA produced by infectious clone into HeLa cells. Infectivity assays were performed on Vero, C6 / 36, LoVo cells, and furin protease transfected LoVo cells. The results show that immature particles are infectious only in the presence of furin protease, and the E3 protein, although covering the surface of the E2 protein responsible for cell receptor binding, does not prevent virus-cell binding and internalization, but Virus entry into the cell does not occur without the prior cleavage of this protein. These results can elucidate some mechanisms of chikungunya virus infection and gives new insights for vaccine production and new drugs for infection treatment.

Financial support:

Abstract #122

YELLOW FEVER VIRUS FOUND IN SYSTEMIC INFECTION IN NONHUMAN PRIMATES FROM SÃO PAULO STATE SHOWS SIMILARITIES WITH EPIZOOTIC STRAIN CIRCULATING IN BRAZIL

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Yellow fever (YF) is a zoonotic disease, characterized by acute and hemorrhagic manifestations whose transmission occurs through mosquito bites. The virus is a reemergent infectious agent with an important impact on public health. Many humans and animals deaths were reported between the end of 2016 and the beginning of 2018. By the end of 2016, monkeys carcasses were reported in the northeast region of São Paulo State. The most animals reported, marmosets, howler and capuchin monkeys were necropsied, and tissue and blood samples were collected. Some sylvatic YF cases were confirmed by laboratory techniques, in Ribeirão Preto, Jardinópolis, Jaboticabal, and Monte Alto. Samples of these animals were sent to the Molecular Virology Laboratory from Research Center of Virology of Medical School of Ribeirão Preto/USP. RNA was extracted from the tissues to perform the RT-qPCR. Four animals were positive for YF and were subjected to Sanger sequencing analysis, with detection of a 1000 bp fragment from Capsid, prM and partial E region of the genome. Also, *in vitro* isolation was performed from a serum sample collected before a baby monkey death, in the acute phase. Different sample specimens were tested by RT-qPCR, resulting in positive in blood, serum, brain, heart, lung, liver, stomach, spleen, large intestine, mesenteric lymph node, adrenal, kidneys, urinary vesicle, and gonads. Positive detection in different organs demonstrated a systemic spread of the virus. Sequencing alignment showed that the virus that infected all animals were from the same virus strain and is very similar to other strains previously published in Espirito Santo State in 2017. The accurate detection of the virus by molecular techniques allied to the study of the molecular epidemiology can provide early public health measures and help to prevent human deaths. Additionally, it can help to understand better the outcomes of the epidemic, providing practical improvements in the surveillance health service.

Financial support: Ministério da Saúde/MEC, CNPq.

Abstract #123

GENETIC CHARACTERIZATION OF DENV-3 FROM IRANDUBA, AMAZONAS STATE

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Dengue is the most common arboviral infection worldwide and it can be caused by four distinct serotypes of dengue virus (DENV 1-4). Phylogenetic studies have classified DENV-3 into five genotypes, namely, I, II, III, IV, and V, on the basis of their genetic diversity. In Brazil, DENV-3 was first isolated from an autochthonous case in 2000, in the state of Rio de Janeiro. DENV-3 genotype III is prevalent in Brazil. In Manaus, Amazonas State, circulation of the four serotypes has been presenting in a hyper endemic circulation of DENV since 2008. In this study, we report DENV-3 isolation from a 39-years-old male patient serum from an urban area in Iranduba, Amazonas State, in July 2015. Patient presented fever, myalgia, chills, rash itching, eyeball pain and asthenia. The viral RNA was extracted from serum sample and a RT-PCR using genus-specific *primers* was performed targeting the Flavivirus NS5,Alphavirus nsP1 gene. The sample was also tested to other arboviruses. Two *Multiplex-Nested*-PCRs were performed with species-specific *primers* for DENV 1 to 3, YFV, MAYV, EEEV, WEEV, and VEEV. A *Nested*-PCR was performed with species-specific *primers* for SLEV, DENV4, ROCV, ILHV, WNV, BSQV, IGUV and OROV. The DENV-3 positive sample was isolated in *Aedes albopictus* C6/36 cells and after the viral genome was deep sequenced using the Illumina platform. The phylogenetic tree was constructed by the Neighbor-Joining test analysis supported that DENV-3 strain 91_15_AM_BR_2015 belongs to genotypes III, even cluster with Venezuelan samples.

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Abstract #124

ACRIDONES INHIBITS MAYARO VIRUS REPLICATION *IN VITRO*

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Mayaro virus (MAYV) is an arbovirus that was endemic in the Amazon region and recently have emergence in different regions of Brazil, including Southeast. Besides, the potential of A. aegypti in transmit it contributes to the virus being able to install an urban cycle in the near future. There is no therapy or vaccine available against this virus and antiviral research becomes relevant. Acridones, a group of compounds extracted from natural sources, showed potential antiviral actions against virus. Thus, this study aimed to evaluate the effect of a panel of 08 synthetic acridones on the MAY life cycle in vitro. The compounds were screened using a Vero EG by plaque reduction assay. Cells were infected with 20 PFU of MAYV for 1h at 37°C, following overlay with MEM+1% carboxymethylcellulose (CMC) with or without acridone at the highest non-toxic concentration for cells with arbitrary 80% cell viability threshold. The assays showed that two acridones, FAC 21 and FAC 22, exhibited an 80 % and 70%, respectively, inhibition of MAYV replication with no effect on cell viability. These molecules inhibited replication in a concentration-dependent manner, showing significant differences between two different concentrations in virus replication, but these data should be further studied. Besides, we evaluated the virucidal effect of in Vero E6 cells in the same concentration and the molecules had no virucidal effect. Our results suggest that these molecules act in the virus replication and not in the viral particle and they are promising molecules to be studied with potential antiviral activity against the Mayaro virus.

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Abstract #125

IN-SILICO SCREENING OF EPITOPES PAN-FLAVIVIRUS FOR VACCINE AND DIAGNOSTIC

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Dengue, Zika and Yellow Fever viruses are very similar agents, both consisting of 3 structural proteins and 7 nonstructural proteins (NS). The NS1 is strongly associated with the process of immune system escape and viral tropism. NS1 dimer form is found in plasma membrane-bound and hexamer is secreted. It has been a main targets for the production of diagnostics and vaccine. However, the structural similarities between the epitopes of these viruses, as well as the wide variability of existing polymorphisms for each species make the development of efficient therapies difficult. The present study made a broad analysis of Dengue1-4, Yellow Fever and Zika virus NS1 polyprotein sequences found in Brazil and deposited in GenBank, aiming to compare epitopes already known for each of the species, with prediction of specific linear and structural epitopes for humoral or cytotoxic cellular responses, considering the variability found in the country. 352 protein sequences were collected, the optimal consensus for each species was made on the MergeAlign server (s). The determination of conserved blocks, as well as polymorphic residues, was done using MEGA. Structural modeling of the protein for each virus was done using I-Tasser(monomer) and Gramm-x(dimer). Structural refinement with GalaxyWeb and ModRefiner (s). The quality of the models has been checked with the MolProbity (s). Immunogenic linear epitopes were predicted through the IMED (s). Structural epitopes were predicted with the ElliPro (s), and linear B and T cell epitopes were predicted through the BepiPred2.0 and NetCTL (s) respectively. NS1 antigenic epitopes already reported were collected (ViPR server). The antigenicity and allergenicity of the epitopes were checked (VaxiJen2.0 and AllerTOP servers). Good targets were confronted with the simulated immune system through C-ImmSim. The appointment of good conserved epitopic targets could lead to the possibility of producing a universal vaccine against Flavivirus.

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Abstract #126

BENZOIC ACIDS DERIVATIVES AGAINST MAYARO VIRUS

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Mayaro virus (MAYV) is an arbovirus member of the genus Alphavirus, family Togaviridae. MAYV is enzootic to tropical South America and endemic to rural areas. It is maintained in a sylvan cycle involving wild vertebrates, including nonhuman primates and Haemagogus mosquitoes. Birds can act as secondary hosts, being important for the dissemination of the virus. Most of MAYV infections are sporadic and occur in persons with a history of recent activities inside or around forests; but several small outbreaks have been reported in the Amazon Region, usually limited to rural areas near or inside forests, where the vector is found. Although high antibodies rates are found in some rural communities, it is difficult to isolate MAYV, because of the relatively short period of viremia. Mayaro fever is a non-fatal, typically dengue-like, acute febrile illness, characterized by frontal headaches, epigastric pain, myalgias, incapacitating arthralgias, maculopapular rash, chills, nausea, photophobia and vertigo. There is still no antiviral therapy specified for the treatment of these infections. Thus, the present work aimed to evaluate the antiviral effects of 7 esters derived from benzoic acids against MAYV. For this, different concentrations of each acid were evaluated by its cytotoxicity (MTT) and the inhibition of virus replication in Vero E6 cells by plaque reduction assay was evaluated. Cells were infected with 25 MAYV PFU for 1 h at 37°C after overlap with MEM+1% carboxymethylcellulose with or without benzoic acid at a specific concentration. Treated cells with DAB-16, DAB-22 and DAB-23 showed efficient inhibition of viral replication at concentrations that showed minimal toxicity to cells. Assays showed that benzoic acid exhibited an inhibition of 80% (DAB-16), 40% (DAB-22) and 60% (DAB-23) of MAYV replication without affecting cell viability. Our results suggest that these molecules are promising to be studied with potential antiviral activity against Mayaro virus. We want to improve our findings by adding real time PCR and immunofluorescence assays.

Financial support: FAPESP.

Abstract #127

ARBOBIOS: A BRAZILIAN COHORT OF DENGUE WARNING SIGNS PATIENTS DURING 2019 EPIDEMICS.

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It is known that 3.6 billion people worldwide live in areas that place them at risk of DENV infection, 400 million overall are exposed to DENV infection. Around 2 to 5 % of infected individuals progress to Severe Dengue (SD). The mortality can be reduced to less than 1% if robust early predictor of progression to SD exists. To establish a warning signs cohort for identification and validation of prognostic biomarkers for the severe DENV infection. During the 2019 epidemic in Brazil, a prospective longitudinal cohort of warning signs (WS) DENV patients was constitute in the following cities: Araraquara, SP; Arcos, MG; Campo Grande, MS; Nova Serrana, MG; Palmas, TO and São José do Rio Preto, SP. Two following-up visits were programmed at days 7 and 14. The presence of RNA DENV virus were done by in-brew qPCR after RNA extraction with EasyMag (bioMérieux) and serological responses were evaluate by IgM ELISA (PanBio) only for samples negative in qPCR. For this study, we used SMS data banking. A total of 1117 patients were enrolled and 48 refused to participate. 1069 were analyzed and from those 1016 were adults. The virus RNA was detected in 36.2% of the samples (387) most of them were serotype 2, and out of 682 negative for virus RNA, 444 were reactive in the serological test (65.10%), it is assumed that 77.7% (831) of the participants presented DENV infection at the time of the study. Only 1.59% (17) were not detected by both tests. The follow-up were realized in 984 and 960 patients for days 7 and 14, respectively. This is the biggest WS DENV clinically well characterized cohort in Brazil. As expected, we detected the presence of DENV virus in a low number of WS DENV patients, however IgM serological test were positive in most of negative samples for qPCR. The follow-up were concluded but each medical record should be analyzed and revised to estimate the number of SD. The next step of this study is process the samples for transcriptomic and micro RNA analyses.

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