Brazil, Manaus-AM, 2019

REALIZATION

UNIVERSIDADE DO ESTADO DO AMAZONAS (UEA-AM, BRAZIL)

FUNDAÇÃO DE MEDICINA TROPICAL DR. HEITOR VIEIRA DOURADO (FMT-HVD-AM, BRAZIL)

UNIVERSITY OF TEXAS MEDICAL BRANCH (TX,EUA)

INSTITUTO LEÔNIDAS E MARIA DEANE - FIOCRUZ AMAZÔNIA (ILMD)

SPONSOR

FUNDAÇÃO DE AMPARO A PESQUISA DO ESTADO DO AMAZONAS

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Administrative support

Mr. Eduardo Serrão

APRESENTATION

Universidade do Estado do Amazonas (UEA), Fundação de Medicina Tropical Dr. Heitor Vieira Dourado (FMT-HVD), Instituto Leônidas e Maria Deane - Fiocruz Amazônia (ILMD) and the University of Texas Medical Branch is organizing the a 3-day symposium on June 5-7, 2019, in Manaus, State of Amazonas, Brazil. The symposium is aptly titled “Virus by the River: Arbovirus emergence and re-emergence in the tropics”. It will take place on a riverboat cruise ship that will sail from the city of Manaus in the Amazon river. The purpose of this symposium is to bring together key stakeholders, such as public health officials and community leaders, vector biologists, modelers and vaccinologists, in order to foster the exchange of ideas as well as partnerships with the common goal of improving our understanding of emerging arboviruses.

PROGRAM

Wednesday, June 05

9:30 – Transfer at the lobby of Executive Tropical Hotel

10:00 - 12:00 - Institutional tour - Fundação de Medicina Tropical Dr. Heitor Vieira Dourado

12:00 - Transfer to the boat

12:30-14:00 - Lunch

14:00 - 16:00 – Nap time

16:00-16:30 – Opening remarks

16:30 – 18:00

SOLIMÕES RIVER SESSION

Robert Tesh (USA)

Oropouche Fever: A Common but Underreported Arboviral Illness in the Amazon

Pedro Vasconcelos (Brazil)

Experimental zika and yellow fever infections: contributions to pathogenesis

Paulo Pimenta (Brazil)

Vectorial competence, co-infection and vertical transmission of Zika and Dengue virus by the Brazilian Aedes population

18:00 – 22:00 - Sunset guitars and drinks

Thursday, June 06

8:00 – 9:00 – Breakfast by the meeting of two rivers

9:00 – 11:00

NEGRO RIVER SESSION

Mauricio Nogueira (Brazil)

Immunological interplay among flaviviruses

Felipe Naveca (Brazil)

Genomic, epidemiological and digital surveillance of Chikungunya virus in the Brazilian Amazon

Nikos Vasilakis (USA)

Establishment of an enzootic transmission cycle and consequences for public health

11:00 - 11:30 - Coffee et al. break

11:30 – 13:30

PURUS RIVER SESSION

Marcus Lacerda (Brazil)

What do we know about coinfections?

Luiz Tadeu Moraes Figueiredo (Brazil)

Human urban arboviruses can infect wild animals and jump to sylvatic maintenance cycles in South America

Turbo talks (15 min)

Adam Hendy (USA)

Effects of distance from edge and NDVI on mosquito vector communities in three urban forest parks in Manaus, Brazil

Sasha Azar (USA)

ZIKV Demonstrates minimal Pathologic Effects and Mosquito Infectivity in Viremic Cynomolgus Macaques

13:30 – 14:30 – Lunch

14:30 – 16:00 - Nap time

16:00 – 18:00

JURUÁ RIVER SESSION

Kathryn A Hanley (USA)

Forests as foci of Aedes-borne arbovirus spillover and spillback

Betânia Drumond (Brazil)

Yellow fever dynamics during recent outbreaks in Brazil, 2016-2018

André Siqueira (Brazil)

Addressing the gaps of knowledge on the natural history and therapeutics of chikungunya

18:00 - 18:30 – Coffee et al. break

18:30- 20:00

MADEIRA RIVER SESSION

Turbo talks (15 min)

Mariana Sequetin (Brazil)

Epizootics due to Yellow Fever Virus in São Paulo State, Brazil: viral dissemination to new areas (2016-2017)

Ana Terzian (Brazil)

Zika Surveillance

Bárbara Chaves (Brazil)

HOSTZIKA study: an overview

20:00 – 22:00 - Dinner

Friday, June 07

8:00 – 9:00 – Breakfast by the beach

9:00 – 11:00

JAVARI RIVER SESSION

Arturo Reyes-Sandoval (UK)

Adenoviral platforms for the development of Chikungunya, Zika and Dengue vaccines: From design to clinical assessment

Shannan Rossi (USA)

In vivo chikungunya virus lineage phenotypes and implications for vaccine efficacy

Ricardo Palacios (Brazil)

Development of Butantan-DV, a live attenuated tetravalent dengue vaccine

11:00 - 11:30 – Coffee et al. break

11:30 – 13:00

JUTAI RIVER SESSION

Mauro Teixeira (Brazil)

Anti-inflammatory drugs for viral infections

Andrew Routh (USA)

Defective-RNAs: programmed vs spontaneous emergence, and host interference

13:00 – 13:15 – Closing remarks

13:15 – 14:00 - Lunch

14:00 – Transfer to the airport or Check-in in the Tropical Executive Hotel

Abstracts

Evidence of DENV antibody protection in a ZIKA virus infected population in an arbovirus endemic area

Ana Carolina Terzian
Faculdade de Medicina de São José do Rio Preto, Laboratório de Pesquisa em Virologia, São José do Rio Preto – SP, Brazil

Background: Dengue virus (DENV) comprises four antigenically-distinct serotypes. Infection with a given serotype confers lifelong homologous immunity. However antibody-dependent enhancement (ADE) following secondary infections among heterologous dengue virus (DENV) serotypes is well established and elevates risk for severe dengue disease. Based on in vivo enhancement among DENV serotypes and in vitro enhancement between different flaviviruses, the concern was raised that a ZIKV infection following a previous DENV infection may result in increased ZIKV pathogenesis. Conversely, studies conducted by our research group in the city of São José do Rio Preto (SP), Brazil, found no indication of ADE in patients. The aim of this study is verify the role of pre-existing DENB antibodies in Zika infection and disease.

Materials/methods We used a protocol to assess whether neutralizing antibodies from past DENV infection may be a protective factor or a risk for ADE during a secondary ZIKV infection. We performed a case-control study, analyzing whether pre-existing flavivirus infection represents a risk for disease enhancement during ZIKV infection. Second, we tested whether the immune responses to ZIKV infection were different in DENV exposed and unexposed individuals and whether DENV exposure influenced susceptibility to ZIKV infection and/or severity of ZIKV disease.

Results: Our analyses indicated that asymptomatic individuals had been exposed to multiple DENV infections, especially DENV-2, and that prior exposure to DENV offered protection against ZIKV infection and disease. Additionally, individuals who were not infected with ZIKV presented higher DENV IgG levels and avidity when compared to the ZIKV-infected patients.

Conclusions: Our findings supports the hypothesis that DENV immunity, especially associated with DENV 2, may reduce ZIKV pathogenesis, transmission and subsequently the disease. Thus, we did not find an association between previous DENV infection and the risk of ADE during a secondary flavivirus infection caused by ZIKV.

Adenoviral platforms for the development of Chikungunya, Zika and Dengue vaccines: From design to clinical assessment

Arturo Reyes-Sandoval
University of Oxford, Henry Wellcome Building for Molecular Physiology
Oxford, England, UK

The sudden presence of Zika and Chikungunya in the same geographical regions has overwhelmed health systems that were already challenged by Dengue, thus increasing the failure to provide treatment and preventive measures to their populations during the outbreak, while posing new challenges for treatment of both Zika and Chikungunya due to the long-term sequelae caused by ZIKV and CHIKV infections. These diseases are transitioning from an epidemic nature towards endemic diseases due to enabling drivers such as poor socioeconomic conditions, climate change and migration.

My group has recently developed vaccines against Zika, Dengue and Chikungunya viruses, and have demonstrated pre-clinical efficacy in a Zika virus challenge and induction of neutralising antibodies against Chikungunya virus. Both vaccines received in 2016 initial support for GMP production and Phase I clinical trials to confirm safety and immunogenicity in Oxford in 2018 and Mexico by 2020. The presentation will cover from early design to transition through pre-clinical models and results from early clinical trials.

Genomic, epidemiological and digital surveillance of Chikungunya virus in the Brazilian Amazon

Felipe Naveca
Instituto Leônidas e Maria Deane, Laboratório Ecologia de Doenças Transmissíveis na Amazônia Manaus, AM, Brazil

Since its first detection in the Caribbean in late 2013, chikungunya virus (CHIKV) has affected 51 countries in the Americas. The CHIKV epidemic in the Americas was caused by the CHIKV-Asian genotype. In August 2014, local transmission of the CHIKV-Asian genotype was detected in the Brazilian Amazon region. However, a distinct lineage, the CHIKV-East-Central-South-America (ECSA)-genotype, was detected nearly simultaneously in Feira de Santana, Bahia state, northeast Brazil. The genomic diversity and the dynamics of CHIKV in the Brazilian Amazon region remains poorly understood despite its importance to better understand the epidemiological spread and public health impact of CHIKV in the country. Roraima state in the North of Brazil faced a large CHIKV outbreak with 5,928 notified cases between August 2014 and August 2018 in Boa vista municipality, the capital of the state. A total of 20 novel CHIKV-ECSA genomes from the Brazilian Amazon region we generated using MinION portable genome sequencing. Phylogenetic analyses revealed that despite an early introduction of the Asian genotype in 2014 in Roraima, the large CHIKV outbreak in 2017 in Boa Vista was caused by an ECSA-lineage most likely introduced from northeastern Brazil. Epidemiological analyses suggest a basic reproductive number of R_0 of 1.66, which translates in an estimated 39 (95% CI: 36 to 45) % of Roraima's population infected with CHIKV-ECSA. Finally, we find a strong association between Google search activity and the local laboratory-confirmed CHIKV cases in Roraima. This study highlights the potential of combining traditional surveillance with portable genome sequencing technologies and digital epidemiology to inform public health surveillance in the Amazon region. Our data reveal a large CHIKV-ECSA outbreak in Boa Vista, limited potential for future CHIKV outbreaks, and indicate a replacement of the Asian genotype by the ECSA genotype in the Amazon region.

Human urban arboviruses can infect wild animals and jump to sylvatic maintenance cycles in South America

Luis Tadeu Figueiredo
Universidade de São Paulo, Faculdade de Medicina de Ribeirão Preto
Ribeirão Preto, São Paulo, Brazil

The most important human arboviruses worldwide (dengue viruses 1, 2, 3 and 4, Chikungunya virus, and Zika virus) can infect wild animals and jump from urban to sylvatic maintenance cycles in South America as did yellow fever virus in the past. All these viruses, are transmitted by the anthropophilic mosquito Aedes aegypti, and cause epidemics in Brazil. Yellow fever virus is the oldest example of an urban arbovirus that became sylvatic in South America. Presently, yellow fever has been a zoonosis of non-human primates that moves as a wave in forests of the Brazilian countryside traveling thousands of kilometers, killing many animals and eventually infecting man. However, since 2016, the zoonotic wave has reached the highly populated areas of the Southeast of the country producing the largest human outbreak in 60 years. As occurred with yellow fever virus, sylvatic cycles may occur with dengue, Chikungunya and Zika viruses. For becoming sylvatic the arbovirus requires an apparently unlikely conjunction of factors that surprisingly happen. These arboviruses could start to infect sylvatic primates and to be transmitted by Haemagogus mosquitoes of the canopy of trees. We mention here publications reporting evidences of sylvatic cycles of dengue, Chikungunya and Zika viruses in South America. Indeed, it is almost impossible to control sylvatic cycles of arbovirus. It is not possible to know where, when or why an arboviral spill-over would occur in wild animals. The sylvatic maintenance cycle could make impossible to eradicate an arbovirus. Moreover, an arbovirus in a sylvatic cycle could re-emerge anytime infecting humans and producing outbreaks. In case of reemergence of an arbovirus, it is important to impair that an urban cycle appears as a spill-back from sylvatic cycle.

Oropouche Fever: A Common but Underreported Arboviral Illness in Amazonia

Robert B. Tesh
University of Texas Medical Branch, Departments of Microbiology & Immunology and Pathology, Galveston, Texas, US

Available evidence indicates that Oropouche virus (OROV) or one if its variants (genus: *Orthobunyavirus*) are widely distributed in tropical regions of South America, Panama and Trinidad. Human infection with OROV usually results in a brief (2-7 day) febrile illness, characterized by headache, myalgia, weakness, dizziness, nausea, vomiting, photophobia, conjunctivitis and retro-ocular pain. Some patients develop aseptic meningitis, and recrudescence of symptoms is not uncommon. Clinically, Oropouche fever is indistinguishable from dengue, chikungunya, Zika and number of other viral, bacterial and parasitic infections occurring in Amazonia. Consequently, many cases of Oropouche fever are misdiagnosed or not reported. Laboratory confirmation is required. This presentation will review the history, epidemiology, known geographic distribution and frequency of the disease as well as the ecology, modes of transmission, animal and vector associations, and control of OROV.

Defective-RNAs: programmed vs spontaneous emergence, and host interference

Andrew Routh, Elizabeth Jaworski, Stephen Kunkel, Rose Langsjeon, Stephanea Sotcheff, Yiyang Zhou

Department of Biochemistry and Molecular Biology, The University of Texas Medical Branch, Galveston, TX, USA and Sealy Centre for Structural Biology and Molecular Biophysics, University of Texas Medical Branch, Galveston, Texas, USA.

The role of defective RNAs (D-RNAs) is simultaneously a classical and an emerging topic. While D-RNAs have been well characterized for many viral models in cell-culture and their presence has long been appreciated, clear examples of their impact in disease and antiviral therapies are only now emerging. Nonetheless, D-RNAs have often been dismissed as an epiphenomenon unique to poor cell-culture practices and that they do not have a physiological role. Using model RNA viruses such as Flock House virus (FHV), and human pathogens such as chikungunya virus (CHIKV), we have characterized the formation and transmission of D-RNAs using novel sequencing technologies such as 'ClickSeq' and Nanopore sequencing. We find that while a range of diverse D-RNAs spontaneously arise during virus replication, a select few D-RNAs species are repeatedly seen and that these are present due to favored RNA recombination sites and/or selective encapsidation. We further demonstrate that these species elicit specific host transcriptional responses as a result of disrupting the host innate immune system. Therefore, while it is clear that nearly all viruses can generate D-RNAs as a by-product of their fickle and error-prone polymerases, we posit that from amongst this foggy mutational cloud, specific '*functional*' D-RNAs can emerge that perform an active roles in the replication cycles of viruses. Therefore, we aim to demonstrate that there exist two classes of D-RNAs: **1)** passive D-RNAs that spontaneously and purposelessly arise during replication and succeed only in reducing specific viral infectivity of inocula; and **2)** Functional D-RNAs whose formation has been evolutionarily selected to improve viral fitness.

ZIKV Demonstrates minimal Pathologic Effects and Mosquito Infectivity in Viremic Cynomolgus Macaques

Sasha R. Azar
University of Texas Medical Branch, Departments of Microbiology & Immunology and Pathology, Galveston, Texas, US

To evaluate the effects of ZIKV infection on non-human primates (NHPs), as well as to investigate whether these NHPs develop sufficient viremia to infect the major urban vector mosquito, *Aedes aegypti*, four cynomolgus macaques (*Macaca fascicularis*) were infected with 5.0 $\log_{10}$ focus-forming units (FFU) of DNA clone-derived ZIKV strain FSS13025 (Asian lineage, Cambodia, 2010). Following infection, the animals were sampled (blood, urine, tears, and saliva), underwent daily health monitoring, and were exposed to *Ae. aegypti* at specified time points. All four animals developed viremia, which peaked 3-4 days post-infection at a maximum value of 6.9 $\log_{10}$ genome copies/mL. No virus was detected in urine, tears, or saliva. Infection by ZIKV caused minimal overt disease: serum biochemistry and CBC values largely fell within the normal ranges, and cytokine elevations were minimal. Strikingly, the minimally colonized population of *Ae. aegypti* exposed to viremic animals demonstrated a maximum infection rate of 26% during peak viremia, with two of the four macaques failing to infect a single mosquito at any time point. These data indicate that cynomolgus macaques may be an effective model for ZIKV infection of humans and highlights the relative refractoriness of *Ae. aegypti* for ZIKV infection at the levels of viremia observed.

HOSTZIKA study: an overview

Bárbara Chaves

Fundação de Medicina Tropical Dr. Heitor Vieira Dourado, Manaus-AM, Brazil

Zika fever is an emerging disease with great power of expansion, caused by the Zika virus, an arbovirus transmitted by mosquitoes genus *Aedes*. Association of ZIKV infection with severe neurological conditions and cases of congenital malformation such as microcephaly caused government and society to pay attention to the disease and its consequences in the short and long term, leading to a greater demand for studies that elucidate the dynamics of this virus. This work aims to elucidate the dynamic between urban/wild cycle transition on human health. For this, serum surveillance of non-human primates and clinical investigation of ZIKV infections in humans were performed. This study recruited 221 patients, 141 were woman and 80 men, almost all primary clinical samples were screened by RT-PCR assay for zika. Of the 209 samples tested, 36 samples were zika positive. All patient's serum samples were screened by ZIKV DetectTM IgM 2.0 Capture ELISA and 68 was positive. Since March 2018 until March 2019 were collected 50 free-living monkeys of three different species, saki monkeys (*Pithecia pithecia*), squirrel monkeys (*Saimiri sciureus*), and bicolor tamarins (*Sanguinus bicolor*) and 20 collected samples of eight different captive species from the zoo. One Saguinus bicolor free-living monkey had positive ZIKV PCR result (Ct value= 36). Sorological ZIKV IgM Capture ELISA test was positive for this same monkey confirming ZIKV infection, in addition two more female were positive to ZIKV IgM, one free-living from UFAM also Saguinus bicolor, and other was captive from CIGS-zoo of *Sapajus apella* specie. These preliminary results show that ZIKV is circulating in non-human primates and It may be being maintained in sylvatic cycle. By exploring the potential zoonotic reservoirs of ZIKV through the sampling of human sera and monkey sera, we will be able to evaluate the genetic diversity and ecological dynamics of ZIKV that can contribute to the maintenance of the virus in the environment.

Epizootics due to Yellow Fever Virus in São Paulo State, Brazil: viral dissemination to new areas (2016-2017)

Mariana Sequetin Cunha[1], Antonio Charlys da Costa, Natália Coelho Couto de Azevedo Fernandes[3], Juliana Mariotti Guerra[3], Fabiana Cristina Pereira dos Santos[1], Juliana Silva Nogueira[1], Leandro Guariglia D'Agostino[1], Shirley Vasconcelos Komninakis[4,5], Rodrigo Albergaria Ressio[3], Adriana Yurika Maeda[1], Fernanda Gisele Silva Vasami[1], Ursula Mitsue Abreu Kaigawa[1], Laís Sampaio de Azevedo[1], Paloma Alana de Souza Facioli[1], Ester Cerdeira Sabino[2], Élcio Leal[6]

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Beginning in late 2016 Brazil faced the worst outbreak of Yellow Fever in recent decades, mainly located in southeastern rural regions of the country. In the present study we characterize the Yellow Fever Virus (YFV) associated with this outbreak in São Paulo State, Brazil, describing its dissemination to areas free of vaccination. Blood or tissues collected from 430 dead monkeys and 1030 pools containing a total of 5,518 mosquitoes were tested for YFV by quantitative RT-PCR, immunohistochemistry (IHC) and indirect immunofluorescence. A total of 67 monkeys were YFV-positive and 3 pools yielded YFV following culture in a C6/36 cell line. Analysis of five nearly full length genomes of YFV from collected samples was consistent with evidence that the virus associated with the São Paulo outbreak originated in Minas Gerais. The phylogenetic analysis also showed that strains involved in the 2016-2017 outbreak in distinct Brazilian states (i.e., Minas Gerais, Rio de Janeiro, Espirito Santo) intermingled in maximum-likelihood and Bayesian trees. Conversely, the strains detected in São Paulo formed a monophyletic cluster, suggesting that they were local-adapted. The finding of YFV by RT-PCR in five *Callithrix* monkeys who were all YFV-negative by histopathology or immunohistochemistry suggests that this YFV lineage circulating in Sao Paulo is associated with different outcomes in *Callithrix* when compared to other monkeys.

Yellow fever outbreaks bring new insights on arboviruses infections

Betânia Paiva Drumond

Universidade Federal de Minas Gerais, Departamento de Microbiologia Belo Horizonte, MG, Brazil

Yellow fever virus (YFV) of the *Flaviviridae* family (genus *Flavivirus*) is the causative agent of yellow fever (YF). YFV is transmitted to humans by mosquitoes of the genus *Haemogogus*, *Sabethes* and *Aedes* and it is widely distributed in the tropics of South America and Africa. YFV is mainly maintained in a sylvatic transmission cycle involving non-human primates (NHP) as hosts. Highly susceptible to YFV infection, NHP are considered sentinel animals for the presence of YFV in a region. YFV transmission to humans is mostly incidental, but recent reports of massive yellow fever outbreaks causing deaths of thousands of people in Brazil in 2016-2019, Angola, and Democratic Republic of Congo in 2016, demonstrate that YF is still a serious public health concern despite preventive vaccination against the disease. In Brazil, YFV is endemic in the Amazon Basin, but since 1999, the majority of the human cases were reported beyond the limits/boundaries of the endemic area. The change in the epidemiological pattern of sylvatic YF in Brazil highlighted the importance of a better surveillance system, especially in the transition areas. Highly susceptible to YFV infection, NHP are considered sentinel animals for the presence of YFV, and since 1999. In December 2016, a huge sylvatic YF outbreak took place in Southeast Brazil, causing deaths of thousands of NHP and of people in rural or urban areas. The occurrence of YF outbreaks near big and populous urban centers is a worrying scenario, but also an unprecedented opportunity to study the dynamics of arboviruses. During the last YF outbreaks in Brazil, we have received hundreds (n=711) of NHP samples to investigate YFV. We have investigated the presence of YFV genome in NHPs carcasses from rural to urban areas of Minas Gerais state A total of 35% of NHP were YF positive and results confirmed YFV circulation in rural (48%), periurban (6%), and urban (46%)areas. We detected high YFV genomic loads, indirectly demonstrated by low Cts in the RT-qPCR. Alouatta sp and Callicebus sp had the higher positivity values compared to Callithrix sp. Following, the genetic characterization of YFV and phylodynamics analyses showed unique introduction of the virus in Southeast Brazil, followed by silent circulation before the epidemic in 2016, and the persistence of YFV in the Southeast region, causing yearly epidemics from 2016 to 2018. This suggested that this region may present suitable ecological and climatic conditions for YFV maintenance during an epidemic and interepidemic seasons. Negative YFV samples were then tested for Chikungunya and Zika viruses. Chikungunya virus was not detected, by Zika virus (ZIKV) was detected in 12% of tested NHP (n=152). The data showed the extensive occurrence of YFV in NHP in Minas Gerais in 2017 and 2018, and the circulation of ZIKV in 2017 in NHP from urban areas. The detection of ZIKV in NHP points to the possibility of these hosts to play a role in the maintenance cycle of the virus. The detection of YFV in NHP in urban and periurban arenas poses a risk of urban transmission cycle of YF, although no human cases were linked to urban transmission of YFV during the last outbreaks in Brazil. The role of NHPs in arboviruses cycle in Brazil, not only in urban, but also in sylvatic areas, deserves further investigation.